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POINT OF CARE TECHNOLOGY: EXPEDITING DIAGNOSIS TO IMPROVE CLINICAL OUTCOMES FOR HIV-INFECTED CHILDREN

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Original published work submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2019

DECLARATION:

Student's contribution to article(s) and agreement of co-author(s)

I, Karl-Günter Technau, student number 9400042X, declare that this thesis is my own work and that I contributed adequately towards research findings published in the articles stated below which are included in my thesis.

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Agreement by co-authors:

By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his thesis.

Article 1:

Title: Improving early identification of HIV-infected neonates with birth PCR testing in a large urban hospital in Johannesburg, South Africa: Successes and challenges

Journal name, year, volume and page numbers: Journal of the International AIDS Society, 2017, 20:1-8.

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2nd Author	Louise Kuhn		11/13/17
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5th Author	Gayle Sherman		30/7/18

Article 2:

Title: Xpert HIV-1 point-of-care test for neonatal diagnosis of HIV in the birth testing programme of a maternity hospital: A field evaluation study

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14th Author	Louise Kuhn		7/16/18

To my children

Simphiwe and Carmen

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Technau K, Kuhn L, Coovadia A, Sherman G. Gender differences emerging during birth HIV diagnosis in Johannesburg, South Africa. 19th International Workshop on HIV Observational Databases (IWHOD), 26-28th March 2015. Catania, Sicily. Abstract 0075 (poster).

Technau K. Linking mothers with the diagnosis and care of HIV-exposed infants, 15th April 2016, SAHIVSOC 3rd biennial conference 13-16 April 2016. Oral presentation.

Technau K, Sherman G, Bhowan K, Murnane P, Coovadia AH, Kuhn L. Comparing Point of Care to Laboratory HIV PCR testing at birth in a hospital setting in Johannesburg, South Africa. 8th International Workshop on HIV Pediatrics. 16-18 July 2016; Durban, South Africa. Abstract O_14 (oral).

Carmona S, Wedderburn C, Macleod W, Hsaio M, Jani I et al. Field performance of point-of-care HIV testing for early infant diagnosis: pooled analysis from six countries from the EID Consortium. 21st International AIDS Conference; Durban, South Africa; July 18–22, 2016. Abstract LBPE008 (poster).

Carmona S, Wedderburn C, Macleod W, Hsaio M, Jani I et al. Updates from the EID Consortium network: field evaluations and moving towards implementation - Implementing Point-of-Care Technologies for Early Infant Diagnosis of HIV. African Society of Laboratory Medicine (ASLM), 2016. 3-8 December 2016; Cape Town, South Africa. Oral presentation.

Kuhn L, Technau K, Strehlau R, Shiau S, Patel F, Sherman GG, Tiemessen C, Aldrovandi GM, Coovadia A, Abrams EJ. Treatment of Acute HIV infection in Neonates. Conference on Retroviruses and Opportunistic Infections (CROI), 13 -16 February 2017, Seattle, USA. Abstract 1205 (oral).

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Kalimashe M, Ledwaba J, Technau K, Kuhn L, Sherman G, Hunt G. Rates of HIV drug resistance in HIV-infected infants who were diagnosed at birth. International Workshop on HIV Drug Resistance and Treatment Strategies 2017, 6-8 November 2017, Johannesburg, South Africa. Abstract 35 (poster).

Mazanderani AH, Kufa T, Technau KG, Strehlau R, Patel F, Shiau S, Burke M, Kuhn L, Abrams EJ, Sherman GG, for the LEOPARD Study Team Early Infant Diagnosis HIV-1 PCR tests identify infants with high viral loads at birth: findings from a cohort study in Johannesburg, South Africa. 10th International Workshop on HIV Pediatrics 2018. 20-21 July 2018, Amsterdam, the Netherlands. Abstract 92 (poster).

Strehlau R, Patel F, Burke M, Paximadis M, Technau KG, Shiau S, Abrams EJ, Sherman GG, Tiemessen C, Kuhn L for the LEOPARD Study Team. Postnatal transmission or rebound of in utero HIV infection treated by ARVs in maternal breastmilk? 10th International Workshop on HIV Pediatrics 2018. 20-21 July 2018, Amsterdam, the Netherlands. Abstract (poster).

Other: Reports to DOH during the time relevant to birth testing and maternal identification of HIV (Appendix C)

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Technau KG, Kuhn L, Coovadia A, Murnane PM, Sherman G. Xpert HIV-1 point-of-care test for neonatal diagnosis of HIV in the birth testing programme of a maternity hospital: A field evaluation study. Lancet HIV. 2017 Oct;4(10):e442-e448.

Technau KG, Mazanderani AH, Kuhn L, Hans L, Strehlau R, Abrams EJ, Conradie M, Coovadia A, Mbete N, Murnane PM, Patel F, Shiau S, Tiemessen CT, Sherman GG. Prevalence and outcomes of HIV-1 diagnostic challenges during universal birth testing - an urban South African observational cohort. J Int AIDS Soc. 2017 Aug 29;20(Suppl 6):21761.

Technau KG, Strehlau R, Patel F, Shiau S, Burke M, Conradie M, Sorour G, Sherman GG, Coovadia A, Murnane PM, Abrams EJ, Kuhn L. Twelve month outcomes of HIV-infected infants identified at birth: An observational cohort study at one maternity site in Johannesburg, South Africa. Lancet HIV. 2018 Dec:5 (12):e706-e714.

ABSTRACT

Only half the HIV-infected children in South Africa access antiretroviral treatment (ART), despite it being available for over 15 years. Translating infant diagnosis into early treatment and retention in care remains a key problem. This project aims to evaluate the new strategy of birth HIV polymerase chain reaction (PCR) testing in the context of point-of-care (POC) technology, thus enabling early treatment and linkage to care in the days after birth. The first paper of the thesis describes the advent of birth HIV PCR testing in a large maternity/delivery unit in Johannesburg, South Africa. High coverage (93%) and excellent uptake (>99%) were achieved in implementing the Roche COBAS® TaqMan® (Version 2.0) HIV-1 Qualitative central laboratory-based test (LABT) between June 2014 and December 2016. Overall, maternal HIV prevalence was 23%, and 1.6% of tested infants were found to be HIV-infected at birth. Of these, 16% were born to women newly diagnosed at delivery. The second paper evaluated the concurrent implementation of the Cepheid Xpert HIV-1 Qualitative POC test (POCT), run together with the LABT when staff capacity allowed. While only achieving 52% coverage, the POCT performed at sensitivity of 100% (95% CI 88·4–100) and specificity of 99·9% (95% CI 99·7–100) with a Cohen's κ coefficient of 0·967 (95% CI 0·922–1·00). It enabled a higher proportion of result return ($p<0.0001$) than LABT alone (96% vs. 53%) in all infants and earlier initiation of ART (1 day of age vs. 6 days of age, $p=0.0001$) in infected infants. The third paper (diagnostic challenges) showed that 24% of initial non-negative birth tests (i.e. positive or indeterminate) were indeterminate, and in 5% of cases a significant diagnostic uncertainty remained despite a protracted course of testing. The final paper outlines the one-year outcomes of confirmed HIV-infected infants diagnosed at birth. The diagnosis enabled 98% of infants to commence ART. The 12-month mortality was 0·14 (95% CI 0·08-0·24) and retention in care of those initiated on site, excluding deaths, was 78%, with 71% achieving viral suppression to <400 copies/ml. Although diagnostic dilemmas are a key challenge and keeping the cohort in care requires significant effort, this study nevertheless demonstrates that diagnosis at birth serves as an important avenue to improve ART coverage in children. In conclusion, birth HIV testing together with POC testing, if implemented strategically, can effectively increase access to ART for HIV-infected infants.

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ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral treatment
AZT	Zidovudine, antiretroviral drug
CDC	Centers for Disease Control and Prevention
CE-IVD	Conformité Européene In Vitro Diagnostics
CHER	Children with HIV Early Antiretroviral Therapy study
EDTA	Ethylenediamine tetraacetic acid
EID	Early infant diagnosis of HIV
ELISA	Enzyme-linked immunosorbent assay
ESRU	Empilweni Services and Research Unit
HIV	Human immunodeficiency virus
IeDEA	International epidemiological Databases to Evaluate AIDS
LABT	Laboratory-based HIV PCR test
LEOPARD	Latency and Early Neonatal Provision of Antiretroviral Drugs study
LTFU	Loss to follow-up
NAT	Nucleic acid test for HIV
NHLS	National Health Laboratory Service
NIH	National Institutes of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NPV	Negative predictive value
NVP	Nevirapine, antiretroviral drug
PACTG	Pediatric AIDS Clinical Trials Group
PCR	Polymerase chain reaction
PEPFAR	US President's Emergency Plan for AIDS Relief
PMTCT	Prevention of mother-to-child transmission of HIV
POC	Point-of-care
POCT	Point-of-care test
PPV	Positive predictive value
RMMCH	Rahima Moosa Mother and Child Hospital
SDG	Sustainable Development Goal
TNA	Total nucleic acid

UNAIDS	Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
VL	HIV viral load
WHO	World Health Organization

OVERVIEW

1. Introduction

In South Africa, the human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) epidemic remains a leading cause of child and adult mortality.(1) Although under-5 mortality, which peaked in 2003 (78.1 per 1000 live births) largely due to HIV, had declined to the lowest levels in two decades (40.5 per 100 live births) in 2015 (2), this is still almost double the 25 per 1000 live births target of the United Nations Sustainable Development Goals (SDG 3) by 2030.(3, 4) This is a clear indicator that, despite the many advances in reducing the impact of HIV, the challenge is far from over.

In the context of the South African paediatric HIV epidemic, this project was undertaken from 2013 onwards and assimilates research on a new strategy of early infant diagnosis of HIV (EID) implemented at the birth time point. It also assesses point-of-care (POC) technology in this setting, and the implications of early diagnosis, such as diagnostic challenges and one-year outcomes. While the project commenced at a time when national EID protocols were in a state of flux, the work was predicated by extensive research in EID and prevention of mother-to-child transmission (PMTCT) at the site in question (5-12), namely Rahima Moosa Mother and Child Hospital (RMMCH). The site is an academic paediatric referral centre which also functions as the main delivery facility and obstetric referral site for complicated cases of a sub-district of Johannesburg, South Africa.

This chapter provides critical contextual information on the HIV epidemic affecting women and children, the diagnostic issues affecting infants, and the programmatic challenges that exist around EID and subsequently providing HIV-infected infants with access to life-saving antiretroviral treatment (ART). This is followed by a brief discussion of each of the four manuscripts that form the basis of the thesis. Recommendations and lessons learnt conclude.

2. History and present status of the problem

2.1 HIV and AIDS in children and the current response

Increasing understanding, new discoveries, and developing technology are gradually changing the face of HIV and AIDS, and with it, global public health, as the world continues to improve the available modalities of dealing with the disease – igniting debates and creating awareness that challenge and transform the way the world thinks about sexuality, discrimination, and health care along the way. But still, the plight of children infected with HIV, particularly in lower-resourced settings, and the destructive effects of AIDS, both directly and by means of parental illness and death, are matched only by widespread war and famine.

The first indications of what was to become the worldwide AIDS pandemic were recorded in 1981.(13-15) Soon it was noted that the condition was not limited to adults, but also affected infants.(16) The term “AIDS” was coined in 1982 (17, 18), and the actual virus was isolated in 1983 (19-21), but it was only named HIV in 1986 by the International Committee on the Taxonomy of Viruses. Cases of transplacental and breastmilk transmission were first published in 1984 and 1985 (22, 23), and in 1987, the Ministry of Health in Uganda declared a national emergency, which is seen as the first large-scale African response and acknowledgement of the problem.(24) In 1988, the Joint United Nations Programme on HIV and AIDS (UNAIDS) announced that there were more women infected than men in sub-Saharan Africa.(25)

The first guidelines on the prevention of perinatal transmission of the virus causing AIDS were issued in 1985.(26) At this time, a total of 217 cases of paediatric AIDS had been reported in the USA and available data suggested that the mother-to-child transmission rate lay between 0% and 65%. The guidelines recommended counselling and testing, a repeat test towards the end of pregnancy, couples counselling and testing, delaying pregnancy in the event of a positive result, and the avoidance of breastfeeding. In 1994, the Centers for Disease Control and Prevention (CDC) published guidelines formalising the use of zidovudine (AZT) to prevent mother-to-child transmission following the findings of the Pediatric AIDS Clinical Trials Group (PACTG) 076 trial.(27, 28) Since then, many ground-breaking trials have been conducted, making the hope for virtual

elimination of paediatric disease a possible aspiration, as well as making safe conception and pregnancy a reality for HIV-infected women.

Presently in South Africa, maternal HIV prevalence is around 31% (30.8% point-estimate in 2015, highest in past five years), and prevalence in the population as a whole lies above 12%.(29, 30) The mother-to-child transmission rate has steadily declined from 20-40% in the absence of prevention, to under 5% in 2010 and under 3% in 2013 (at four to eight weeks).(31-34) South Africa has adopted the stance of promoting breastfeeding, and no longer makes free breast milk substitutes available, except in isolated cases.(35) While previously highly risky, pregnancy in the context of HIV has become a manageable process that can be concluded safely for mother, father and child.

Despite this progress in PMTCT, there are currently an estimated 2.1 million children (0-14 years) living with HIV globally, 63% of which live in eastern and southern Africa (Figure 1.1). South Africa alone is reported to be home to 320,000 children with HIV; more than 10% of the global total. Although coverage estimates of interventions for paediatric care and prevention are slightly better for eastern and southern Africa compared to globally, paediatric ART coverage remains at only 51% in 2017 (Figure 1.2) and 55% for South Africa.(36)

The key to paediatric treatment access is diagnosis. If a neonate acquires HIV, they have a 40% mortality risk at one year of life if they are not diagnosed and then treated.(37-39) In comparison, a new adult infection carries at least a ten-fold lower mortality risk.(40) Without an early diagnosis, the infected infant may often only be identified when presenting symptomatically ill, often with severe or life threatening diseases.(41) Up to a third of HIV-positive mothers may not know about the exposure as yet and themselves be newly diagnosed when the infant presents ill.(41) In the early years of the epidemic, a diagnosis of having HIV was tantamount to a death sentence, in that there was no effective treatment. Decades later, many South African infants and children face a similar fate; not because of the absence of treatment, but because of the failure to diagnose them in time. Nevertheless, amongst countries in eastern and southern Africa, South Africa has the highest percentage (80%) of infants born to

pregnant women living with HIV receiving a virological test for HIV within two months of age (Figure 1.3).(36)

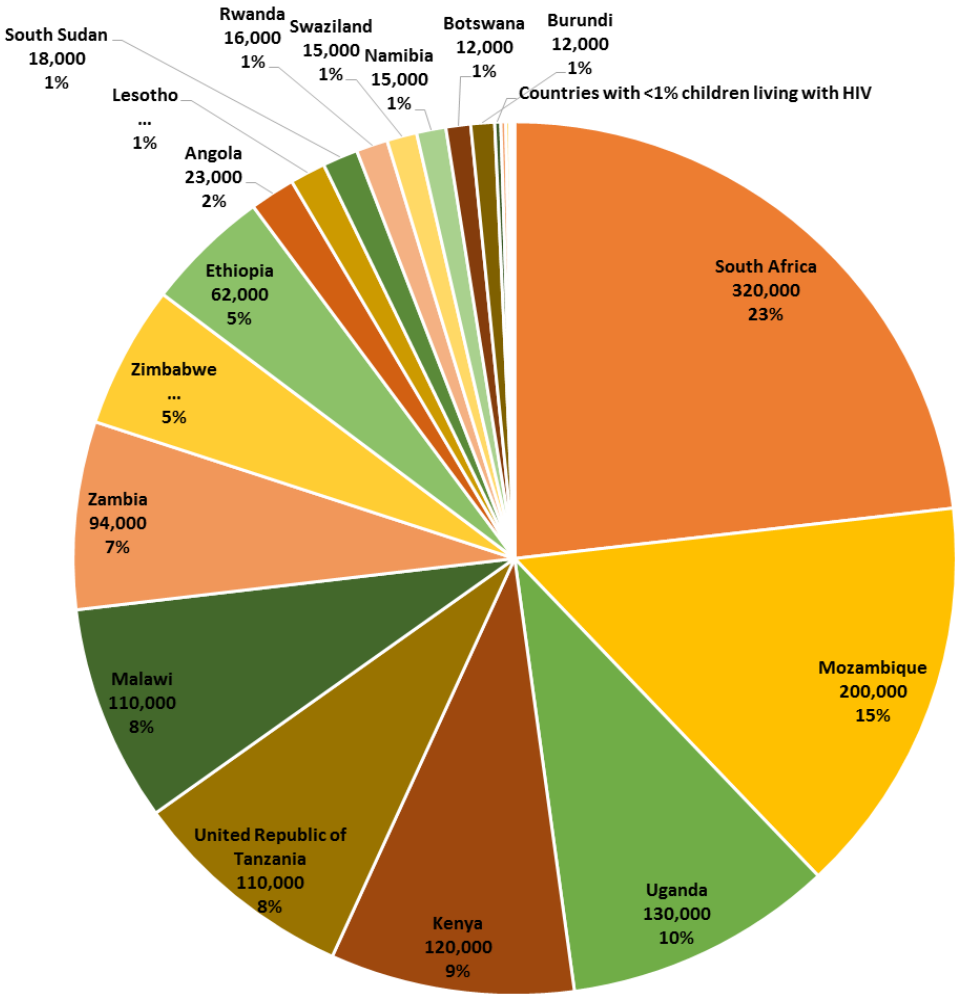


Figure 1.1 Estimated numbers of children with HIV in eastern and southern Africa

Source: UNICEF analysis of UNAIDS 2017 estimates

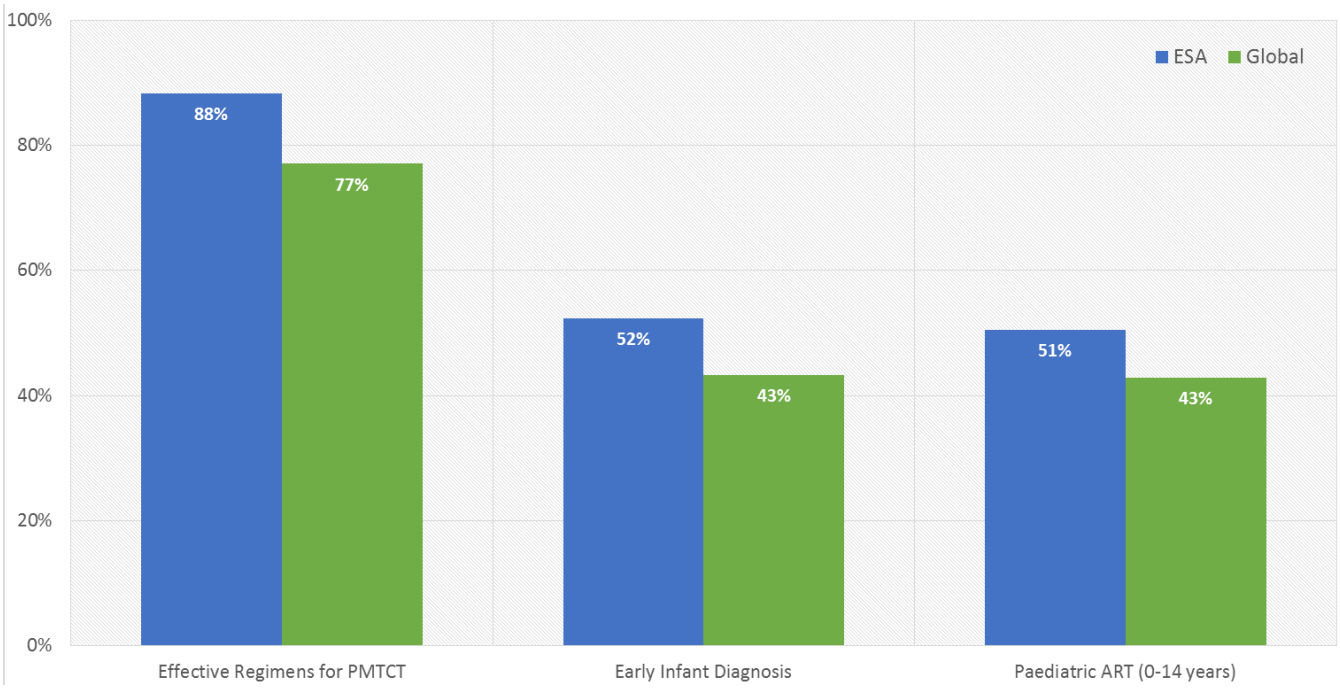


Figure 1.2 Coverage of key interventions for preventing mother-to-child transmission of HIV and for paediatric care and treatment globally and in eastern and southern Africa (ESA), 2016

Source: UNAIDS/UNICEF/WHO Global AIDS Response Progress Reporting and UNAIDS 2017 estimates

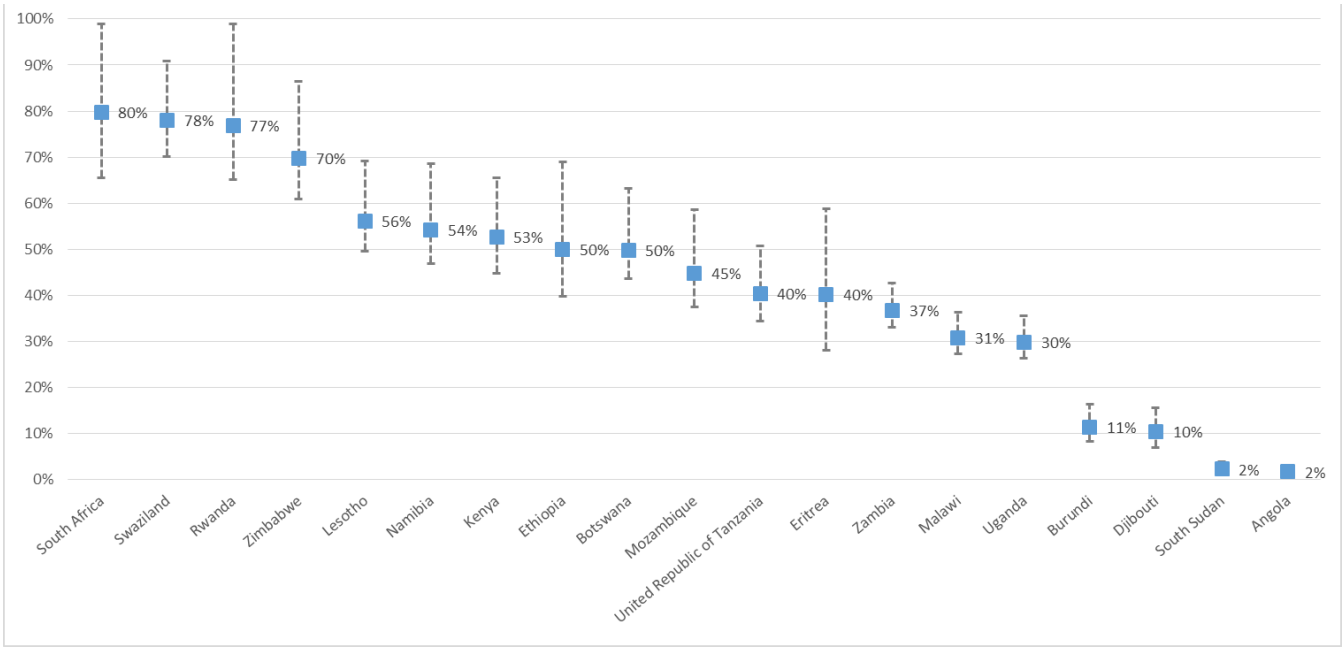


Figure 1.3 Percentage of infants born to pregnant women living with HIV receiving a virological test for HIV within two months of birth, eastern and southern Africa, 2016

Source: UNAIDS/UNICEF/WHO Global AIDS Response Progress Reporting and UNAIDS 2017 estimates

2.2 The evolution of HIV diagnosis – The birth test as a new strategy

The first test for HIV was approved by the United States Food and Drug Administration in 1985. The test was based on the enzyme-linked immunosorbent assay (ELISA), a technique which was established in 1971.(42, 43) In 1987, the Western Blot was approved as a more specific test for HIV. In 1992, the first “rapid” HIV test kit was approved, followed by the first non-blood (oral) test in 1994. In 1996, the first home testing kit became available. These tests all detect antibodies in a person’s blood or oral fluid.

Polymerase chain reaction (PCR) HIV tests, on the other hand, enable the qualitative and quantitative detection of virus directly, rather than antibodies, by detecting viral genetic material. PCR testing as a new technology for targeting genetic material was first discovered in 1983 by Mullis and Smith, who were awarded the Nobel Prize for Chemistry in 1993 for this work.(44, 45) The technology has grown over the past 20 years, paving the way for a multitude of diverse medical work.(46) For example, viral load (VL) PCR testing, which provides a quantitative result of HIV, became available in 1996, and has since become a tool for both diagnostic confirmation and HIV treatment monitoring.

Whereas HIV diagnosis in adults is primarily based on the detection of antibodies, this cannot be applied to children under 18 months of age due to persistence of maternal antibodies. In the first months of life particularly, a definitive diagnosis can only be made by means of virological testing. Current South African and World Health Organisation (WHO) guidelines recommend the use of nucleic acid tests (NAT) for infant diagnosis, such as PCR or VL testing.(47-49) In more recent years, evidence has emerged that there may be even later seroreversion in HIV-exposed infants, and therefore virological testing, or at least confirmation, may need to be extended up to 24 months of age.(50)

Apart from requiring virological testing rather than antibody testing, infants require multiple tests. Mother-to-child transmission of HIV occurs either *in utero* (HIV detectable in the first 48 hours of life), intrapartum (not detectable at birth, but detection possible by six weeks), or postpartum during the course of breastfeeding.(51) Given the high early

mortality of HIV, testing should not be delayed until all risks, such as breastfeeding, have ceased. It is therefore critical to develop EID protocols that accommodate these factors, enabling early detection of *in utero* infected infants as well as later detection in cases where the infection may only have happened intra- or postpartum.

At the outset of this project, the National Department of Health guideline was limited to testing infants at six weeks of age. Testing before the age of six weeks was only recommended if infants were symptomatic, which included the "symptom" of prematurity, low birth weight, or symptomatic illness at birth.(52) The six-week time point was chosen because it presented a single test that could identify virtually all infections in the era of exclusive formula feeding. Before the advent of widespread PMTCT interventions, the majority of HIV-infected infants were infected in the intrapartum rather than *in utero* period (only an estimated 27%) (51, 53), which meant that the sensitivity of tests before six weeks of age was low. However, subsequent evidence suggested that it would be more effective to test HIV-exposed infants at birth because a higher proportion were infected *in utero* than intrapartum, even though the absolute number of infant HIV-infections was declining.(9, 10, 41, 54-57) The question of whether this should become a national policy and guideline had therefore not yet been agreed at the outset of the project, but was integrated into the national policy in 2015 (Figure 1.4).(47)

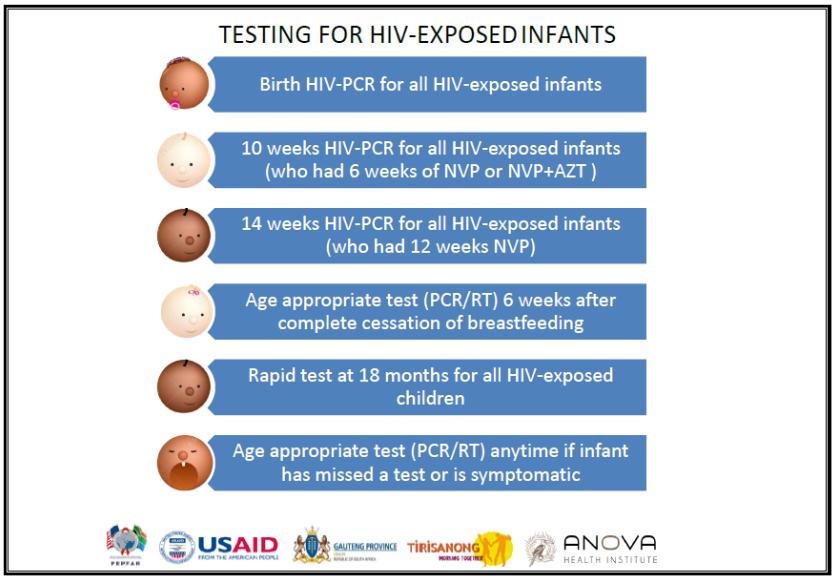


Figure 1.4 Current recommended algorithm of testing in HIV-exposed infants in Gauteng province, South Africa

Apart from being able to identify a higher proportion of cases at birth, reducing high mortality was a further reason to adopt birth testing. In a study conducted at RMMCH and surrounding facilities, mortality among 289 HIV-infected infants and children under 24 months of age identified in the course of 2011 remained above 20%.(6) In this cohort, more than half the infants were ill at the time of diagnosis, even if this diagnosis was at six weeks of life. Marston et al estimated 20% mortality in perinatally-infected infants by 90 days of life.(58) In the earlier infant diagnostic study at RMMCH, 25% of infants known to have been infected at birth died by 14 weeks of life.(9) Reducing this mortality seemed conceptually only possible if the age of diagnosis was reduced.

Another crucial aspect of birth testing is that it may prevent future drug resistance to non-nucleoside reverse transcriptase inhibitor (NNRTI)-treatment. This is because HIV-infected infants are at high risk of developing resistance to nevirapine (NVP), an NNRTI class of antiretroviral agent which is used as part of infant prophylaxis in the PMTCT programme. Resistance may even occur after a single dose, which is the standard recommendation (59), and as NVP is usually commenced immediately following birth in HIV-exposed infants, this compromises future treatment with NNRTI-based therapy.(60) However, if the diagnosis of HIV takes place at birth, and therefore before NVP commencement, this class of agents can be preserved for future treatment. With more than 90% of deliveries in South Africa occurring in institutions, this may be an optimal time to present this testing modality to HIV-exposed infants (61), although whether this will in fact prevent NVP exposure in infected infants remains to be seen.

The case of the “Mississippi Baby”, named after the American state in which she was born, has raised hopes of an even greater benefit of very early initiation of treatment – that of a possible “functional cure” of HIV.(62-64) The HIV-infected newborn was started on aggressive ART 30 hours after birth, and treatment was continued for 18 months, after which she was lost to follow-up. However, despite treatment cessation, she maintained an undetectable viral load for over two years, at which point she experienced a viral rebound and was put back on ART. Nevertheless, the case sparked research into whether very early treatment (within hours of birth) may not only hold a key to lowering

or eliminating viral reservoirs and reducing HIV mortality and morbidity, but also open pathways for cure strategies. This served as further motivation to study very early diagnosis and initiation of ART.

2.3 Point-of-care testing modalities

Considering a standard PCR test requires a central laboratory step with a turnaround time of days, rather than hours, very early initiation of ART is often not possible. In some settings, the delays from time of phlebotomy to result return to mother/parents may even be weeks, depending on proximity and transport resources.(65, 66) The advent of POC PCR testing devices may yet provide a practical and feasible solution to this problem by enabling an immediate diagnosis and action. However, before recommending any devices for routine clinical care, rigorous testing both in the laboratory and in the field are required.

There are a variety of POC testing platforms available, and four modalities have achieved Conformité Européene In Vitro Diagnostics (CE-IVD) approval: Alere™q HIV-1/2 Detect, Cepheid Xpert®HIV-1 Qual, Diagnostics for the Real World's SAMBA I HIV-1 Qual Test, and SAMBA II HIV-1 Qual Whole Blood Test. The Alere™q HIV-1/2 Detect and Cepheid Xpert®HIV-1 Qual have also achieved WHO pre-qualification status (67), which aims to ensure that global standards of quality, safety and efficacy are met, and resources are used effectively to improve outcomes. Other modalities include the Liat HIV Quant (IQuum). Although there is laboratory evaluation data for a number of these assays (68-72), little data was available for field evaluation in neonates (73, 74), particularly at the time of birth.

A new technology like POC, that changes the dynamics of result return, may also significantly affect the emotional response and improve coping of mothers in the context of infant HIV testing. Maternal stress in HIV-positive mothers is high, not only related to the actual result, but also because of the waiting time for the result.(5, 75-78) Acceptance of this technology by mothers and healthcare providers therefore needed consideration when assessing the performance of POC devices.

The term “point-of-care” theoretically suggests a bedside test that can be concluded in the ward. Running the above assays generally takes at least 60-90 minutes and the devices, although compact and available in different configurations, are sophisticated instruments that require maintenance. The practical implications of testing infants at delivery needs careful assessment to determine where devices should be placed and how they could be best maintained. This was important in order to strike a balance between having processes unfold close to the patient location while maintaining control of the instrument and monitoring its performance.

2.4 Management of HIV-infected infants – Diagnostic challenges and treatment outcomes

HIV PCR testing technology relies on the presence of virus in the blood. While high coverage of maternal ART during pregnancy and infant prophylaxis after birth has achieved major reductions in transmissions overall, this level of drug exposure is thought to mask some infections and may delay diagnosis.(10, 79, 80) Anecdotal reports suggest that some infected infants may have been missed at the six-week test after NVP exposure.(81) Timing of EID in relation to drug exposure is therefore another important consideration. The sensitivity of the six-week HIV PCR test in the context of daily dose NVP is not known, but there are strong indications that peripartum ART exposure can affect the sensitivity of the PCR test during the first six weeks.(79) In the earlier infant diagnostic study, the sensitivity of the two-week test was reduced to 83% by single dose NVP exposure at birth.(9, 10) These reports speak to the problems encountered at the six-week diagnostic time point. With the introduction of birth testing it was critical to investigate the prevalence and nature of diagnostic dilemmas and possible missed diagnoses.

Left untreated, HIV will eventually progress to AIDS, and ultimately lead to AIDS-related death, in the vast majority of cases. Disease progression is more rapid in children, with mortality highest in the first months of life.(57, 82-84) However, when managed properly, HIV becomes a chronic illness that need not shorten life expectancy. Treatment typically consists of three antiretroviral drugs, which are now widely available in most countries. Although initial treatment guidelines for children were based on CD4 cell counts and

clinical staging as determinants of ART eligibility, having evolved over the last two decades, current guidelines recommend immediate treatment of all infected individuals.(47, 52, 85, 86) The landmark Children with HIV Early Antiretroviral Therapy (CHER) study was a key turning point in this regard, and saw the beginning of treatment upon diagnosis becoming the standard for children.(82)

Most literature on infected infants consists of cohorts that commence at the time of diagnosis or the time of treatment initiation, and not the time of birth. There are therefore very limited cohort descriptions of infected infants identified at birth. There are some clinical trial cohorts, but these were largely gathered at a time when very early ART was not available. More data is available for infant cohorts in general from the time of diagnosis or treatment initiation and the literature review of this is presented in Table 1.1. Since gauging the outcomes of infants routinely diagnosed at birth is challenging in this context, it is critical to establish a clear description of such a cohort in a context where birth testing became national policy.

Studies of HIV natural history before early ART found that high peak viral loads were associated with more rapid disease progression, but that *in utero* infection was not necessarily associated with higher rates of rapid progression.(87-89) With birth testing, it would be possible to follow up and assess larger cohorts to distinguish *in utero* from later infection but, importantly, infant mortality would need to be assessed in the context of current PMTCT regimens and transmission rates. In addition, it will be crucial to better understand outcomes associated with the different timing of infection (*in utero* vs. later).

Birth testing holds the promise of being able to identify the majority of perinatally HIV-infected infants at birth. If these infants could be initiated on ART immediately, the could provide an important avenue of improving paediatric ART coverage.

Table 1.1: Cohorts of HIV-infected infants followed from birth and later

Cohort category	Author & years of cohort	Cohort location	Cohort description	Guideline
Includes diagnosis at birth	Lilian et al (90) 2008-2010	Johannesburg, South Africa	38 infected infants (29 tested positive at birth, 9 negative at birth and positive at 6 weeks) Enrolment shortly after delivery at one site. Enrolment depended on availability of study staff. Study occurred during the era before birth testing and was focussed on diagnostic strategy.	WHO stage 3 or 4 or CD4 criteria, South Africa 2008 (86)
	Frigati (91) 2013-2015	Cape Town, South Africa	26 infected infants Cohort of infected infants managed at one site. Allowed for referral from other sites and entry during the first month of life. The study occurred before introduction of routine birth testing and represents a descriptive cohort study.	Treat on diagnosis. South Africa 2015 (47)
	Prendergast, Mphatswe (57, 92) 2007-2008	Durban South Africa	63 infected infants randomised to 43 (30 in utero infected) infants received immediate ART And 20 (14 in utero infected) infants received deferred ART Enrolment started during antenatal care of the mother at two sites and focussed on ART strategy.	Clinical Trial
Diagnosis before 8 weeks	Sheahan (93) 2008-2012	Malawi, DR Congo	121 infants diagnosed before 12 weeks Enrolment occurred from 4 sites over three years. The focus was on ART initiation following diagnosis.	Treat upon diagnosis. WHO 2008 (94)
	Violari (82) 2005-2007	Johannesburg and Cape Town, South Africa	377 infected infants (252 infants started immediate ART, 125 deferred ART) This study excluded infants who were clinically symptomatic at the time of potential enrolment and focussed on ART strategy and outcomes.	Clinical Trial
ART before 6 months	Shiau, Coovadia, Kuhn (95-97) 2005-2007	Johannesburg, South Africa	54 infected infants started ART before 6 months of age The study focussed on ART strategy and occurred before birth testing was introduced.	Clinical Trial
	Shiau (98) 2004-2011	Johannesburg, South Africa	Three cohorts including infants started <6 months of age: 104 (cohort 1), 252 (Cohort 2), 102 (Cohort 3). This study focussed on ART outcomes before the introduction of birth testing and involved three cohorts.	WHO stage 3 or 4 or CD4 criteria, South Africa
	Porter (99) 2004-2012	Southern African collaboration	4945 children initiating ART during infancy This was a large cohort study involving multiple sites. It focussed on characteristics at ART initiation and outcomes.	Multiple National Guidelines
ART before 12 months	Palumbo, Violari (60, 100)	Southern Africa, India	164 children, 123 <12 months of age (Cohort 1) 287 children, 77 <12 months of age (Cohort2) This study focused on ART outcomes comparing different populations. Enrolment required diagnosis.	Multiple National Guidelines

ART: antiretroviral treatment, WHO: World Health Organisation

3. Problem statement

HIV PCR POC testing platforms are new to the field of HIV diagnosis and monitoring. They offer hope of reducing turnaround times and potentially improving infant outcomes. However, little large-scale experience exists, particularly at the birth time point, and therefore thorough evaluation in the field is required to assess performance and feasibility of implementation.

Likewise, birth testing as a new strategic component in the EID protocol also requires piloting and evaluation. As a new time point of testing, significantly different to the previous large out-patient clinic six-week testing, it is critical to evaluate coverage and uptake, as well as outcomes and implementation issues relating to the test. In addition, the prevalence and outcomes of diagnostic dilemmas and difficulties must also be evaluated.

There are few cohorts of HIV-infected infants followed from birth in large numbers or in a routine clinical setting. In the context of earlier (birth) diagnosis and POC technology, it is vital that cohorts are followed carefully to assess outcomes beyond the time of diagnosis. Furthermore, the effect of a routine birth diagnosis, with or without POC testing, must be described. This will enable historical and future comparisons to establish if routine birth diagnosis can improve ART initiation and retention in care rates, and thereby reduce mortality.

4. Aims and objectives

4.1 Aim

The overall aim of this project was to implement birth HIV PCR testing and assess its outcomes. Linked to this, the accuracy and outcomes of POC testing were assessed in order to evaluate performance and draw implementation experience from the process. Following this, the aim was to describe diagnostic difficulties and one-year programmatic outcomes of infected infants.

4.2 Objectives

1. Assess the implementation metrics of coverage, uptake, test outcomes, and ART initiation rates of birth testing at the RMMCH delivery setting.
2. Assess the performance of the Cepheid Xpert® HIV-1 Qualitative assay (Cepheid, Sunnyvale, CA) POC test (POCT) in terms of accuracy, turnaround time, and effect on timing of ART initiation when implemented at the birth time point.
3. Describe the diagnostic challenges encountered in the process of large-scale birth testing at RMMCH.
4. Describe the one-year mortality, retention in care, and virological suppression outcomes in a cohort of confirmed HIV-infected infants identified at the time of birth.

5. Overview of the research

5.1 Article 1: Improving early identification of HIV-infected neonates with birth PCR testing in a large urban hospital in Johannesburg, South Africa: Successes and challenges

5.1.1 Source of data and methods

Based at RMMCH, this component of the work used routinely-collected data in the postnatal services shortly after birth, and spans the period June 2014 to December 2016. Permission from the Department of Health was granted to trial routine universal birth HIV PCR testing at RMMCH specifically (Appendix A). This was subsequently introduced as national policy in June 2015.(47) HIV-exposed neonates were identified through maternal records and individual interviews and, where the documented maternal HIV status was negative or unknown, mothers were tested. Maternal testing was conducted pre-delivery where possible but mostly occurred in the immediate postnatal period. All women presenting with negative HIV status were offered retesting from January 2015, irrespective of when the last test had occurred but before 2015 only if the last test was done more than six weeks before. Infant HIV PCR tests were conducted using the Roche COBAS® TaqMan® HIV-1 Qualitative Test (Version 2.0).

Maternal diagnosis and testing outcomes are presented as a basis for calculating the coverage of HIV PCR birth testing of HIV-exposed neonates. Details are then provided about the HIV PCR test outcomes, followed by result return rates (to mothers of their neonate's test result) and ART initiation rates of neonates found to be infected.

5.1.2 Summary of results

Among 30,591 women who delivered in the time period, 7,085 (23.2%) were found to be HIV-infected, comprising 6,864 women known to be infected before arriving for delivery and 221 women newly diagnosed at the time of delivery. A total of 6,695 (92.9%) infants were tested (6,377 enrolled and 318 not enrolled), with coverage improving rapidly over time from 72.9% in June 2014 to 99.1% in the second quarter of 2015. Reasons for not

enrolling tested infants included inability of research staff to reach mothers before testing/discharge. Uptake of testing when the test was offered was >99% throughout. Weekend and public holiday cover was an important factor determining birth testing coverage.

Infants were tested at a median age of 14 hours (IQR: 8-21) and laboratory turnaround time was 44 hours (IQR: 32-58). Final diagnostic outcomes of the 6,377 enrolled infants were 99 (1.6%) infected, 6,261 (98.4%) uninfected, one infant who posed a diagnostic dilemma, and 16 infants with an error result. Of the 99 infected infants, 88 (89%) returned to the site at a median age of 8 days (IQR: 6-13 days) and 7 followed up elsewhere. In total, 95 (96%) of the 99 infected infants started ART, in four cases lost to follow-up from the site it was uncertain if ART was ever started. Of note is that the 221 mothers newly diagnosed as HIV-infected at delivery gave birth to 16 (16%) of the 99 infected infants.

5.1.3 Main discussion points and contribution to the field

This study showed that within two and a half years of implementation, an HIV PCR birth testing coverage of >90% could be achieved. This success went hand-in-hand with an effective maternal HIV testing programme at delivery. Maternal testing and identification of newly diagnosed mothers was a critical component, since almost a fifth (16%) of infected infants were born to newly diagnosed mothers. These achievements were a major public health success and showed that birth testing was feasible and could provide an avenue for very early diagnosis of *in utero* infected infants to start ART.

We showed our *in utero* transmission rate to be ~1.6%, which confirmed findings of others and demonstrated that major strides in PMTCT had been achieved. This rate did not change significantly during the study. This population of infants is often considered very high risk for early mortality and it was reassuring that >90% of infected infants were able to receive their diagnosis and start ART largely within the first two weeks of life.

We noted that indeterminate first HIV PCR results were common, making up almost a quarter of combined positive and indeterminate results. More detailed discussion about this phenomenon is provided in the third paper.

Important questions remained. What would the effect of POC technology be on these result return and ART initiation dynamics? What were the diagnostic challenges encountered? And how would very early ART initiation translate into one-year outcomes?

5.2 Article 2: Xpert HIV-1 point-of-care test for neonatal diagnosis of HIV in the birth testing programme of a maternity hospital: A field evaluation study

5.2.1 Source of data and methods

This component fell within the above mentioned study period and, as such, was a sub-study, which aimed to investigate the performance of the Cepheid Xpert HIV-1 Qualitative POCT run concurrently with the Roche COBAS TaqMan HIV-1 standard laboratory-based test (LABT). From October 2014 to April 2016, POCT was run from an additional ethylenediamine tetraacetic acid (EDTA) sample collected at the time of birth testing when staff capacity allowed.

The paper describes the field evaluation of the performance of the POCT in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's κ coefficient. Although the POCT was not run with the intention of testing every HIV-exposed infant, there was an attempt to include as many as possible. Weekend coverage was not possible throughout. Testing of infants who were admitted due to neonatal problems immediately after birth, such as very premature infants, was also not always possible. Their first blood tests usually occurred at the time of their admission compared to well neonates, whose mothers were interviewed and consented for neonatal testing during their postpartum recovery. This created a group of 1) POCT and LABT tested infants, and 2) LABT only tested infants. Result return, ART initiation, and coverage between the two groups were then compared.

5.2.2 Summary of results

Of 18,268 women delivering between 1 October 2014 and 30 April 2016, 4,336 HIV-exposed infants were identified, of which 3,970 were enrolled on the study and tested. In total, 2,238 (56%) had a POCT and LABT birth HIV PCR, and 1,732 (44%) only had a LABT. Within the POCT/LABT group, we found POCT sensitivity of 100% (95% CI 88·4–100) and specificity of 99·9% (95% CI 99·7–100), with a Cohen's κ coefficient of 0·967 (95% CI 0·922–1·00). Although we found a 5% error rate on the first run of POCTs, this could be reduced to <1% on re-running error samples. The error rates were operator dependant, with the laboratory technician achieving the lowest (4.3%), followed by the project manager (7.0%) and the nurses (9.2%, p=0.022).

When we compared result return rates between the POCT/LABT vs. LABT only groups, we found that overall result return rates were much higher with the POCT/LABT group, at 96% vs. 53% (p<0.0001). When disaggregating this by infant birth test result, the difference remained for negative PCR results, but result return rates to mothers of PCR-positive infants was high in both groups (100% vs. 89%, p=0.10) as positive results without POCT prompted active tracing. We found ART initiation rates for PCR-positive infants to be equivalent between the groups (p=0.10), but infants with POCT results were initiated significantly earlier (1 day of age vs. 6 days of age, p=0.0001).

Even though full POCT coverage was not the primary aim, important implementation data was collected. Doing POCT on 55% of the study days, we achieved an overall coverage of 52%. The highest monthly coverage achieved was 81%, but end-of-year periods saw rates of <40%. While implementing the POCT, we ran a median of six (IQR: 5-8) tests a day and learnt that the median duration required (blood sampled to result returned to mother) was 170 minutes. This meant that the 90-minute process within the instrument made up only half the turnaround time, with transport to the lab and back to the mother being major components (40 minutes and 16 minutes) of the average turnaround time, with the rest made up of time processing in the satellite laboratory.

5.2.3 Discussion points and contribution to the field

This study showed near perfect concordance between POCT and LABT at birth and demonstrated that the POCT performed reliably within a busy postnatal maternity setting of birth testing, a context which had not previously been evaluated in this manner for this technology. This was therefore a significant contribution to the field.

However, the study also found that the practical introduction of POCT into a maternity/neonatal setting was challenging, and required significant staff and operational support. Nevertheless, error rates and false positive results could be mitigated by repeating samples that yielded positive or error results on the same specimen, thus reducing the overall error rate and improving the PPV. The fact that we observed inter-operator differences was an important finding and, together with the reasons for the errors, highlighted that ongoing training and support are needed when implementing this technology at scale.

Although POCT provided higher immediate result return rates and earlier access to ART for infected infants, the ultimate rate of ART initiation was similarly high among those infected infants not tested by POCT. So while the ability to shorten the cascade from test to ART initiation by eliminating an additional visit was beneficial, it was not clear if this would carry long-term benefits or adverse consequences.

POCT did, however, improve overall (positive and negative) result return rates significantly and enabled >95% of mothers to receive their infant's result, whereas only half the mothers whose infants did not have POCT received their infant's negative results back. This meant that we were able to provide more holistic care, including reassurance and counselling of the mother on adherence, infant feeding, how to keep her infant negative, and were able to remind her to return for her infant's next HIV PCR test.

5.3 Article 3: Prevalence and outcomes of HIV-1 diagnostic challenges during universal birth testing – an urban South African observational cohort

5.3.1 Source of data and methods

While implementing and evaluating birth testing, every infant required individual management. Within this context, multiple cases of diagnostic dilemmas emerged, and understanding these and providing guidance for their management was a critical component of EID at this time point. The paper provides a detailed account of these cases.

This component of the work involved analysis of all non-negative PCR results received, which included positive and indeterminate birth results. According to national and WHO guidelines the diagnosis of an infant as HIV-infected requires two conclusive positive results on separate samples. Confirmatory testing was therefore conducted using the qualitative (HIV total nucleic acid [TNA] PCR) and quantitative (VL) versions of the COBAS® AmpliPrep/COBAS® TaqMan (CAP/CTM) HIV-1 Test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA). A positive result could include a positive qualitative result or a detectable quantifiable quantitative result. All infants with a non-negative result were actively traced and followed up on site as far as possible. Infants were categorised as:

- Positive HIV-1 infection status, based on two clearly positive virological results from samples taken at two different time points;
- Negative HIV-1 infection status, defined as an isolated indeterminate result followed by at least two negative confirmatory virological results taken at two different time points while not on combination ART;
- Uncertain HIV-1 infection status, defined as neonates with non-negative virological results that did not meet criteria for confirmed positive or negative infection status after repeat testing;
- Loss to follow-up (LTFU), defined as patients who did not have sufficient follow-up testing to meet any of the above criteria.

We assessed whether screening or final results were associated with factors such as maternal VL, CD4 count, or treatment duration or with infant factors such as age at test, VL, and NVP exposure.

5.3.2 Summary of results

Following 5,743 (91%) tests in 6,309 HIV-exposed infants born between June 2014 and August 2016, we found 104 (1.8%) with an initial non-negative result. Of 102 with laboratory data, 78 (76%) were positive and 24 (24%) were indeterminate on their first result. Upon confirmatory testing and further follow-up, these infants were categorised as 83 (81%) infected, 8 (8%) uninfected, 5 (5%) uncertain status, and 6 (6%) LTFU.

Infants with an initial indeterminate result had a significantly longer time to final diagnosis than those with an initial positive result (31 vs. 2 days, $p<0.0001$), and had only a 43% chance of achieving a clear diagnosis compared to 99% of infants with an initial positive PCR result. Both the infant and maternal VL results were significantly lower in infants with initial indeterminate results compared to those with initial positive results.

5.3.3 Discussion points and contribution to the field

This study highlighted that initial and final diagnostic uncertainty are important and relatively frequent problems. While a clear diagnosis could be made for the majority of infants with initial indeterminate results (24%), a minority (5%) remained with uncertainty despite a prolonged diagnostic phase of management. We identified that maternal and infant VL were important factors associated with diagnostically uncertain cases.

Our findings raise important questions about the effects of infant antiretroviral prophylaxis and maternal treatment within the context of our currently available testing modalities. Despite the overall transmission rate at birth (1.4%) demonstrating marked improvements compared to past eras, significant support for dealing with diagnostic challenges is still required as EID expands, and includes birth testing. The WHO's 2018 guidelines for EID recognise and address these EID diagnostic dilemmas and have called for additional data on their definition and management. In particular, their concern

centres around children testing indeterminate that are uninfected and the propensity for initiating them on lifelong ART.

5.4 Article 4: Twelve month outcomes of HIV-infected infants identified at birth: An observational cohort study at one maternity site in Johannesburg, South Africa

5.4.1 Source of data and methods

All confirmed HIV-infected infants identified during the period from September 2013 until June 2016 where included. This period was subdivided into three eras: September 2013 to May 2014 (Era 1) was a period where only “high risk” HIV-exposed infants were offered HIV birth testing. During the period June to October 2014 (Era 2), universal birth HIV testing occurred and included all HIV-exposed infants born at RMMCH. Between October 2014 and June 2016 (Era 3), POC testing was available and added to universal birth testing where staff capacity allowed. Time to ART initiation, mortality, retention in care, and viral suppression after one year were reported.

5.4.2 Summary of results

Era 1 included 13 confirmed HIV-infected infants, while 11 infants were confirmed HIV-infected in Era 2 and 64 in Era 3. Of the latter, 35 were POC co-tested and 29 were not. Of 88 confirmed infected infants, the majority (98%) started ART, and as birth testing became more established over the three eras, so time to ART initiation reduced from a median of nine days to two days. POC testing enabled ART initiation before 48 hours after birth in >80% of cases with co-testing.

By 12 months, the mortality was 0·14 (95% CI 0·08-0·24) overall, and there were no significant differences between eras of testing and POC co-testing. An important association of higher mortality and pre-ART VL of >100,000 copies per ml was described. Infants with VL >100,000 copies/ml had a mortality of 0·28 (95% CI; 0·14, 0·49) vs. 0.07 (95% CI; 0·02, 0·19, p=0.0105) mortality in those with pre-treatment VL <100,000 copies/ml.

Among 72 infants who initiated ART at the site, retention in care was 77·8%, and 71·3% of those retained had a VL <400 copies/ml at 12 months. No significant differences were noted when comparing eras to each other and when stratifying by POC testing.

5.4.3 Discussion points and contribution to the field

In the context of >90% birth testing coverage, we achieved >95% ART initiation in HIV-infected infants identified at birth. This is a significant finding as it shows how birth testing (thought to identify the majority of HIV-infected infants at a single time point in South Africa) can result in ART access for most infants. Contrasted with the national 55% rate of overall infant ART coverage, this is a very encouraging achievement, which, if brought to scale, may make high paediatric ART coverage in a setting like South Africa a reality.

We found an overall mortality at one year of 14% and 13% in those started on ART, with no significant effect of POCT. An important finding was the association with a high pre-treatment VL, which resonates with early natural history work suggesting that high VL and not primarily timing of infection result in poor outcomes and rapid progression. The mortality of 13-14% is still high and may yet be an underestimate due to loss to follow-up. It is at similar to lower levels to what had been observed previously, and shows that early diagnosis and ART are not able to prevent all mortality. Nevertheless, at the very least a larger proportion of infected infants are able to access the opportunity when they may often have been missed, progressed without ART or died in the past.

In our cohort, pre-ART VL levels were lower than previously observed, consistent with reports of declining baseline viraemia in the EID programme.(101)

Retention in care on site was 77.8%, excluding the known deaths. We found that maintaining the cohort in care was challenging, and was only possible with relatively individualised efforts of tracking and support. Many mothers experienced socio-economic difficulties and this, together with the low transmission rate, suggested to us that the mothers who had transmitted HIV to their infants would be at higher risk of problems such as poor health-seeking behaviour. Many mothers had to travel in the

months after delivery and it became apparent that accurate hand-held transfer notes and better links with surrounding facilities may be able to provide support for mothers and better follow-up information.

Overall, we did not find that POCT made a significant difference in one-year outcomes, but this may be a reflection of the high level of tracking that was done in the absence of POCT. The main benefit of POCT was the earlier initiation of ART. Facilities without our resources to track and recall patients may yet benefit from POCT, which warrants further research.

6. Limitations

The study was conducted at a single site, which limits generalisability and makes it difficult to draw recommendations for national guidelines. However, although a limitation, it is also a strength in that the site represents a single large birth cohort of HIV-exposed and HIV-infected infants.

The site is a university teaching hospital that is relatively well resourced, including a team able to do tracing telephonically and, occasionally, where required, to do home visits; a research unit with a satellite laboratory facility and, more importantly, funding for additional staff support; and a convenient location in an urban area, close to the large reference laboratories and the university. All of these factors make generalisability difficult, particularly to rural settings. At the same time, the benefit of this particular setting was that it has the third highest number of deliveries in Johannesburg and accounts for ~5-7% of deliveries in Gauteng; thus, catering for a large routine population.

The presence and advocacy of local “champions” has previously been noted as a limitation further impeding generalisability. This is a double-edged sword, as larger-scale research at a public facility is not possible without direct local leadership and involvement. Doing the work on site and gaining the necessary research outputs capacitates the site as a whole to function as a centre of excellence and provide advice and guidance to sites further afield.

As a central delivery facility, the site did not offer follow-up testing for the HIV-exposed birth-uninfected infants, and this made it difficult to present the important numbers of later infections. However, work on gathering this data is ongoing, although it was beyond the scope of this thesis.

7. Conclusion and recommendations

7.1 Conclusion

While high coverage (>90%) and excellent uptake (>99%) of laboratory-based birth HIV PCR testing are achievable in a large routine clinical setting in South Africa, it is important to bear in mind that the nature of birth testing as a strategy is different to routine testing at out-patient well baby clinics, in that it must cover seven days a week and all hours within the delivery unit. This has significant implications for existing staff members who have to accommodate this function. Birth testing requires additional counselling and phlebotomy to what was done before. Any additional activity must be reported correctly in order to assess performance and outcomes and accurate collection of routine data is an important further task. Given the numbers involved this can be time consuming. Weekend and public holiday cover are challenges which, in the absence of contingency planning, may result in lower coverage.

POC testing is more labour intensive, and in our setting we achieved just above 50% coverage with the resources we had in place. The main additional resource was staff and important functions included counselling, phlebotomy, data collection and conducting the tests. We learnt important implementation lessons and developed confidence in the technology because of the concordance with LABT results. Importantly, our work highlighted that the name “point-of-care” does not simply mean bedside care, but required a monitored and supervised environment on site.

A major benefit of POCT was the return of results to mothers; >95% of mothers whose infants were tested received the results before discharge, while only half the mothers of infants who underwent only LABT returned for their results. These findings were recognized by the WHO in 2017.(102) Whereas laboratory PCR testing requires a second visit for results return or for results return to be done at the referral health facility by other staff, POCT makes it possible for the same staff to return results on the same day. Results were usually returned by the same individual who performed the phlebotomy. Not only was this useful for both HIV-positive and HIV-negative babies, and their mothers, it also provided an opportunity for access to further same day counselling and support. We did not have formal data on the perception of mothers but found

anecdotally that mothers were relieved to hear their infant's result on the same day. There were no costs to mothers, occasionally mothers had to remain in hospital for a short while longer but waiting for results was often concurrent with waiting for other midwife/obstetric activities and neither mothers nor hospital staff were delayed significantly. HIV-infected infants could be initiated on the same day of result return, again obviating the need for a separate result return date. Our site had excellent tracking capacity, something unique that may not be available at other sites, and therefore even the majority of infected infants who did not have POCT were started on ART, albeit a few days later.

The birth time point includes a proportion (15-25%) of neonates requiring admission straight after birth, for example, in cases where they are premature, low birth weight, or have congenital infections. This requires attention of the admitting clinicians to accommodate birth testing. We observed that these neonates were thus less likely to have the POCT done, given that their PCR testing usually occurred immediately at the time of admission, something that could not be accommodated in our study due to the need for informed consent required for research. POCT could potentially still be done if samples collected at night are stored to be run in the morning or POCT is conducted afterhours. However, even though these infants were harder to reach with POCT, they had a high chance of still being admitted at the time when the LABT result became available. This was generally within two to three days and therefore did not unduly delay ART initiation.

The transmission rate at birth of 1.4-1.6% reflects success of PMTCT over time. Despite this low prevalence, the POCT performed exceptionally well. In the process of this evaluation, we established that re-running the test for samples with positive or error results was advisable to improve the PPV and the final error rates. The final PPV was excellent, and makes POCT an equal option to consider as a tool within the EID protocol in terms of performance compared to LABT. The final error rates, reduced by re-running of initial error yielding samples, were acceptable and comparable to error rates observed with LABT done in the reference laboratory. An important caveat is operational

feasibility. More work is required to understand whether the additional resources a site needs to run POC testing effectively would be a cost-effective intervention.

A very important finding of this study was that the majority (>75%) of HIV-infected infants were well enough for discharge after birth and were not severely ill. This made it possible for HIV-infected infants identified at birth to be treated as relatively healthy outpatients. The concept that EID for the majority of infected infants can be moved to the birth time point where most of them are well is an important and encouraging opportunity for addressing the problem of low ART coverage in infants (currently 55% in South Africa) and infant survival.

Given that diagnostic dilemmas were common, largely due to indeterminate LABT results, a prolonged diagnostic phase in infants was necessary. It is therefore vital that this phase be considered in the training of health care professionals to both equip them technically, and to support them and the families of affected infants with the necessary counselling skills.

A minority of infants remained with an uncertain diagnosis, despite multiple later tests. As this is a critical component of EID, which can result in a complicated course for individual infants, clinicians and families, clear protocols should be in place to manage it, including later treatment cessation protocols if the diagnosis is still not confirmed but ART has been started. Difficulties of managing these cases include the extensive counselling that is required due to the complex nature of the cases as well as the important treatment decisions, e.g. starting ART without a confirmed diagnosis or stopping ART without having ruled out HIV definitively. In addition, we identified a link between lower VL values in infants with diagnostic dilemmas and mothers who are on ART, highlighting that the drawback to improved PMTCT and maternal ART coverage is that they significantly affect diagnostic sensitivity, which may worsen as preventative efforts improve.

Another dilemma is that even though overall birth testing was achieved at a median of 12-14 hours after birth, infants had generally received at least one to two doses of the

NVP prophylaxis by the time their first HIV PCR test was run anyway. This places HIV-infected infants at significant risk of developing NVP resistance, with subsequent implications for their future regimen choices. On the other hand, it was felt that delaying the administration of NVP for all infants until POC testing was done was not in the interest of the majority, who were HIV uninfected, and that NVP administration immediately after birth should not change in order to avoid confusion and delays, particularly in busy settings.

High rates of ART initiation were achieved irrespective of POC testing, reflecting excellent tracing ability of the site. POC testing did, however, enable earlier ART initiation, making it possible to start ART in >80% of infected infants before 48 hours of life. It will therefore be important to continue studying POC testing in sites without this tracing capacity, as it may yet show benefit with regard to ART initiation rates.

Infants confirmed to be HIV-infected at birth had a mortality of 14% by one year, and we observed significant loss to follow-up. As previously described in the literature, a high VL, and not necessarily the timing of infection, was an important risk factor. More work is required to understand the effect of pre-treatment VL of infants and other factors that lead to poor outcomes.

Due to the low transmission rate in this era of high maternal PMTCT coverage, the cohort of HIV-infected infants that were identified at birth does appear to represent a group at risk of poor socio-economic and health-seeking behaviour outcomes. Mothers who did not follow the full PMTCT protocol, experienced treatment interruptions during pregnancy, or were only diagnosed late in pregnancy were also likely to experience difficulties in infant ART adherence and supervision. Although the cohort is heterogeneous, we found that it was not an easy group to keep in care. Many infants were born to non-South African citizens, and obstacles in securing the correct documentation from authorities placed them under additional pressure, making access to some resources, like grants and food parcels, difficult. Patient mobility was another factor impeding continuity of care. Currently, there are a lack of links between sites and tracking infants across the country is challenging. This indicates a clear need for better

tracing facilities and technical advances to support tracking, such as unique patient identifiers. In addition, mothers and families require intensive support, ranging from help with partner disclosure and relationship problems, to socio-economic and administrative assistance.

Efforts to completely eliminate paediatric HIV transmission continue and are of primary importance, but until such time as this is achieved, this project shows that EID at birth can significantly change the paediatric HIV landscape. In South Africa, where most deliveries occur in facilities and where PMTCT coverage is high, birth represents the single time point of identifying the largest number of infected infants. By incorporating birth testing, and possibly POC technology, this single time point presents the opportunity for most HIV-infected infants in South Africa to be diagnosed and treated at a time when they are still healthy, rather than await a later diagnosis and possible illness.

7.2 Key recommendations

1. Implement training of healthcare workers:
    - for EID and birth testing in general;
    - on the diagnostic phase of managing infants with unclear results (both the immediate protocol of testing, as well as for cases of protracted testing and monitoring where there is persisting uncertainty);
    - to support ART initiation and management in neonates (this must include counselling and support of mothers, families and health workers).
2. Improve communication and linkage through better use of existing inter-facility and health worker communication about patients. This should emphasise correct use and completion of patient-held records and use of the unique identifier where possible (such as the new Road to Health Book barcode).
3. Develop protocols that incorporate POCT into EID, and consider merging with the other uses of POCT, including monitoring of treatment (VL tests) and tests for other illnesses.

7.3 Important research questions that remain

1. Describe the ratio of *in utero* vs. intra- and postpartum infections and their characteristics and outcomes.
2. Analyse the risk factors of mortality and poor clinical outcomes to develop strategies to further reduce mortality and improve HIV-infected infants' outcomes.
3. Assess maternal outcomes in relation to infant outcomes in the context of their social-economic circumstances.
4. Explore POCT in different settings, specifically those less resourced, to assess whether the benefits of POCT are not in reality greater than what could be shown in our setting.
5. Study innovative strategies of POCT use, including multiplatform and targeted approaches to make it more feasible and development of protocols (such as well baby vs. sick baby, high risk vs. low risk).
6. Full cost-effectiveness study of POCT vs. LABT.
7. Qualitative work on maternal, family and healthcare provider wellbeing and coping in the context of EID and infant ART.

ARTICLES

Article 1:

Title: Improving early identification of HIV-infected neonates with birth PCR testing in a large urban hospital in Johannesburg, South Africa: successes and challenges.

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Research article

Improving early identification of HIV-infected neonates with birth PCR testing in a large urban hospital in Johannesburg, South Africa: successes and challenges

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Abstract

Introduction: Timely diagnosis is necessary to avert early death in HIV-infected neonates. Birth PCR testing may improve early identification and facilitate access to care. We implemented a birth HIV diagnosis programme in Johannesburg, South Africa and present successes and challenges of the first two and a half years of operation.

Methods: Between June 2014 and December 2016, we sought to identify all HIV-exposed births and offer newborn HIV PCR testing before discharge after delivery. The programme identified newly delivered women who had tested positive during pregnancy and provided post-partum HIV antibody testing for women without recent negative results. HIV-positive women were required to consent for neonatal birth testing and asked to return a week later to obtain their results. Neonatal venous blood was sampled and tested at the national laboratory using Roche COBAS® TaqMan® HIV-1 Qualitative Test (Version 2.0). Non-negative results triggered active follow-up for confirmatory testing and appropriate treatment.

Results: Of 30,591 women with live births, 6864 (22.4%) were known to be HIV positive and an additional 221 women (1.4% of those tested) were identified during maternal postnatal testing. Of 7085 HIV-positive women, 6372 (89.9%) were interviewed and agreed to data collection, 6358 (99.8%) consented to birth testing for 6467 neonates and a blood sample was collected for 6377 (98.6%). If tested, 6210 (97.4%) tested negative, 91 (1.4%) positive, 57 (0.9%) revealed errors and 19 (0.3%) were indeterminate . Seven of the 19 neonates with indeterminate results and one with initial error result were found to be infected on subsequent testing yielding an intrauterine transmission rate of 1.6% (95% CI: 1.3–1.9). Sixteen (16%) of 99 infected infants were born to women (n = 221) identified during postnatal testing. With active outreach, 95/99 (96%) infected infants were initiated on antiretroviral therapy. Of 6261 neonates with negative results, 3251 (52%) returned to receive their test results.

Conclusion: Our programme successfully achieved high coverage and uptake of birth PCR testing and was able, with active tracking, to start almost all identified HIV-infected neonates on antiretroviral therapy. Implementation required additional staff for counselling, quality control and outreach. Return for negative results was low and neonates with indeterminate results required multiple repeat tests.

Keywords: HIV; diagnosis; neonate; polymerase chain reaction; birth; in-utero

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Introduction

Timely diagnosis is necessary to avert early death in HIV-infected infants as these infants progress rapidly and almost half will die in the first two years of life without antiretroviral therapy (ART) [1]. Prevention of Mother-to-Child Transmission (PMTCT) programmes have been able to reduce the incidence of new HIV infections in infants dramatically [2,3]. Nevertheless transmissions continue to occur and, given the persistently high prevalence of HIV among child-bearing women, the numbers of new infant infections remain of major concern. There are several technical, biological and social reasons that hamper implementation of effective infant diagnosis programmes [4,5]. We, and others, have shown that HIV-infected infants are often identified late when presenting with advanced disease [6–8]. Failure to identify HIV-exposed infants earlier at scheduled follow-up testing has been found to be an important gap in the PMTCT cascade [9].

Diagnosis of HIV infection in infants and young children requires follow-up testing using HIV polymerase chain reaction (PCR) since infection can be acquired *in utero*, intrapartum and during breastfeeding. Until now, most programmes have selected six weeks as the age to offer the first PCR test [4]. This age was selected on pragmatic grounds to fit with the immunization schedule, at a point where almost all intrauterine and intrapartum infections should be detectable by PCR and aiming to minimize the number of PCR tests given the cost associated with the high

volumes of tests required predominantly in low- and middle-income countries [4,10].

In settings, such as South Africa, with high rates of institutional deliveries (national rate 84%, City of Johannesburg >90%) [11] birth testing, now part of national guidelines, could achieve high coverage of the HIV-exposed births and avoid the loss to follow-up that has occurred with later testing [12]. Birth testing is expected to identify the majority of infected children as, with more effective PMTCT, intrapartum infections are less common [13–15]. Importantly, birth testing allows for identification of intrauterine-infected neonates who may die before later testing [1,6,14,16].

We implemented a universal birth HIV diagnosis programme at Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa, in June 2014. Here, we present the successes and challenges of the first two and a half years of the programme to highlight the logistical challenges of implementation.

Methods

We implemented a pilot universal birth HIV PCR testing programme at RMMCH in order to diagnose HIV-infected neonates as early as possible and initiate ART. At this time, the South African public health services supported PCR testing at the six weeks immunization visit at primary health care clinics. In 2013, guidelines expanded to recommend that PCR testing also be offered at maternity services for high-risk HIV-exposed neonates, including those who were preterm or low birthweight and in June 2015, this was amended to universal birth testing of all HIV-exposed neonates [12]. At this time, PMTCT guidelines in South Africa supported Option B + i.e. initiation of ART for all HIV-positive pregnant women upon diagnosis and advised six weeks of daily nevirapine prophylaxis for all HIV-exposed infants.

We aimed to offer HIV PCR testing for all HIV-exposed neonates born at RMMCH or presenting hours after birth if birth had occurred at home or on the way to the hospital (born before arrival). Our site had already established a maternal HIV testing programme at delivery for non-positive women in the antenatal, delivery and postnatal wards [17]. We define "postnatal" as the period in hospital after birth (well and with mother in obstetric ward or under observation/admitted to nursery). Post-Caesarean section mothers stayed for three days while post-vaginal delivery stays ranged from 6 to 24 h. Well neonates born overnight were seen the following day in the postnatal wards while ill neonates were attended to by the treating clinicians. Prior to January 2015, women of unknown HIV status or with negative HIV test results more than 6 weeks earlier were offered testing [12]. From January 2015, all non-positive women presenting for delivery were offered testing. Two counsellors screened all women in the postnatal wards to identify known HIV-positive women and to offer maternal HIV testing for those who qualified. Maternal testing was done using ADVANCED QUALITY™ rapid anti-HIV 1&2 test (InTec Products Inc., Xiamen, China) for screening, and ABON™ HIV 1/2/O Test (Abon Biopharm, Hangzhou, China) for confirmation.

Two additional counsellors interviewed all HIV-positive women regarding their medical and obstetric history and HIV treatment before discussing informed consent for neonatal PCR testing. These data were recorded on paper forms that were captured electronically into a REDCap database maintained to provide routine data on HIV-related services at RMMCH [18]. Separate signed informed consent for both routine data collection and for neonatal testing was obtained. Blood was drawn from neonates within hours after delivery by venipuncture. Two paediatric phlebotomy-trained nurses were responsible for Ballard scores to assess gestational age and venesection [19]. Partial weekend and public holiday cover by the study staff occurred from August 2014 to June 2016.

Neonatal venous whole blood was collected in 0.5 ml ethylenediaminetetra-acetic acid (EDTA) tubes and sent to the National Health Laboratory Service's central HIV PCR laboratory for testing on Roche COBAS® TaqMan® HIV-1 Qualitative Test Version 2 (Roche Molecular Systems, Inc., Branchburg, NJ). Whole blood was stored as dried blood spots (DBS) at −80°C as a precaution if additional testing was required. All samples were recorded on a sample tracking log sheet by the phlebotomist using a unique tracking barcode that matched each individual patient record. The laboratory alerted the clinical team electronically to all non-negative HIV PCR results, including errors, as soon as these results became available. Indeterminate HIV PCR results were defined by standardized laboratory test criteria and represent either low-level viremia or false positives.

Mothers were invited to the hospital's out-patient HIV clinic on a Monday one week post-delivery to receive their neonate's test result. Two data capturers prepared all patient files in advance of the return date by printing results. Mothers of neonates with negative birth PCR results were advised to go for follow-up testing at their local primary health care clinic as per national guidelines. Active outreach efforts were made to ensure that all mothers of neonates with non-negative results received the results.

For infants with positive and indeterminate initial PCR results, repeat qualitative PCR and viral load testing using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 test (Roche Molecular Systems, Inc., Branchburg, NJ) was done on a second blood sample to confirm the neonate's HIV status. ART was offered through the hospital's paediatric HIV service for all presumed HIV infected neonates. ART was initiated as soon as possible after one positive result prior to confirmation. Where a neonate's initial result was unavailable due to a sample or laboratory error, they were re-bled where possible or their stored DBS sample was tested.

De-identified data from June 2014 through December 2016 were extracted for analysis. Data entry was verified and cross checked and data cleaning queries were run regularly. Descriptive statistics of the coverage and outcomes of the programme were analyzed using SAS (Version 9.4, SAS Institute Inc., Cary, NC).

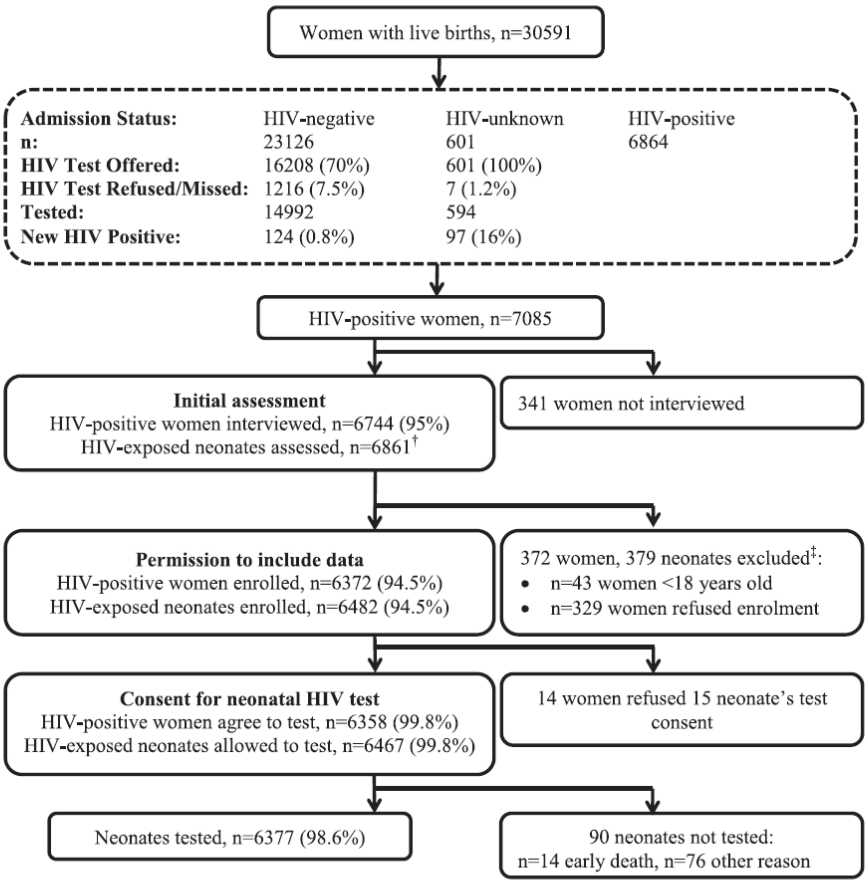


Figure 1. Identification of HIV-positive mothers and subsequent coverage and uptake of neonatal testing. †includes 111 second twins and three second and three third triplets. ‡ 318 (83.9%) of the 379 excluded neonates had a birth HIV PCR test performed with three testing positive, 312 negative, one indeterminate and two error results.

The National Department of Health provided permission for universal HIV PCR testing of all HIV-exposed neonates at RMMCH in May 2014 prior to the inclusion of this recommendation in the national guidelines. The Human Research Ethics Committee of the University of the Witwatersrand approved the use of data for observational evaluation of birth testing (M140555 and M140639).

Results

Between June 2014 and December 2016, 30,591 women and their neonates were admitted to the postnatal wards after having delivered at the hospital or being born before arrival. Of the total, 6864 (22.4%) women were known to be HIV positive. HIV antibody tests were offered to 16,809 (68.3%) of the 23,727 non-positive women and 15,586 (92.7%) of these agreed to test. An additional 221 (1.4%) of 15,586 women tested were found to be HIV positive as part of postnatal testing. Thus, the programme identified 7085 HIV-positive women, 3.1% of whom were newly identified during postnatal maternal testing (Figure 1). The average age of the HIV-positive women was 30.4 years (interquartile range [IQR] 26–34) and 22% had <12 weeks of ART prior to delivery.

Counsellors interviewed 6744 (95.2%) of the 7085 HIV-positive women and 6372 (94.5%) met eligibility and agreed to routine data collection. Of these, 6358 (99.8%) agreed to birth PCR testing for their 6467 neonates (109 neonates part of multiple birth) of whom 6377 (98.6%) actually had blood drawn for the test. Of the 90 neonates not tested, early death (median age 1 day [IQR: 0–2]) due to prematurity precluded testing in 14 neonates (median birthweight 745 g [IQR: 665–830]) and the remainder were due to logistical limitations including lack of phlebotomy capacity or inability to wait for phlebotomy (Figure 1). Taking all these steps in the cascade into account, we estimate that 6695 (97.6%) of the 6861 HIV-exposed neonates had a

blood sample collected for PCR testing. Coverage of the birth testing programme (defined as the proportion of HIV-positive mothers interviewed each quarter) varied over time (Figure 2A). Coverage improved rapidly at the onset of the programme from 72.9% reaching 99.1% in the second quarter of 2015. However, coverage at the end of 2014 declined before improving in the second quarter of 2015. This is most likely explained by reduced study staff capacity to cover birth testing. The decline did not occur towards the end of 2015 or 2016 because staff capacity was boosted by routine ward staff taking responsibility for the programme. Figure 2B displays the number of weekend or public holiday days in each quarter as well as the total number of days in each quarter not covered by study staff. Uptake (defined as the proportion of HIV-exposed neonates whose mothers consented to HIV testing if interviewed) remained high and fairly consistent across all months. However, the proportion of consented neonates who had blood taken declined at the end of 2014 and again towards the end of the study period (Figure 2C).

Blood was drawn from the 6377 HIV-exposed neonates at a median age of 14 h (IQR 8–21) and results were released to the site at a median age of 59 h (IQR 47–76 h; Figure 3). In total, 95% of neonates were tested before 30 h and 99% before 61 h of life. The age at the time of sample collection did not differ significantly between HIV-infected and -uninfected neonates. Most neonates (97.6%) had blood drawn for PCR after the first dose of nevirapine prophylaxis was administered at median age of 25 min (IQR: 10–114). The median time between phlebotomy and result release from the laboratory to the clinical site was 44 h (IQR: 32–58 h), with non-negative results released a median of five hours later than negative results ($p = 0.0005$).

Of the 6377 neonates tested, 6210 (97.4%) samples tested negative, 91 (1.4%) positive, 57 (0.9%) yielded errors and 19 (0.3%) were indeterminate. For the 57 neonates with birth tests that failed to yield results, 41 (72%) had repeat tests performed on the stored DBS sample in 24 (59%) and on repeat sampling during a later visit in 17 neonates. Forty tested PCR negative, one was positive. Indeterminate results comprised 19 of 110 (17%) non-negative PCR results and required repeat testing to establish a definitive diagnosis. In total, 7 of the 19 neonates who initially tested indeterminate were subsequently classified

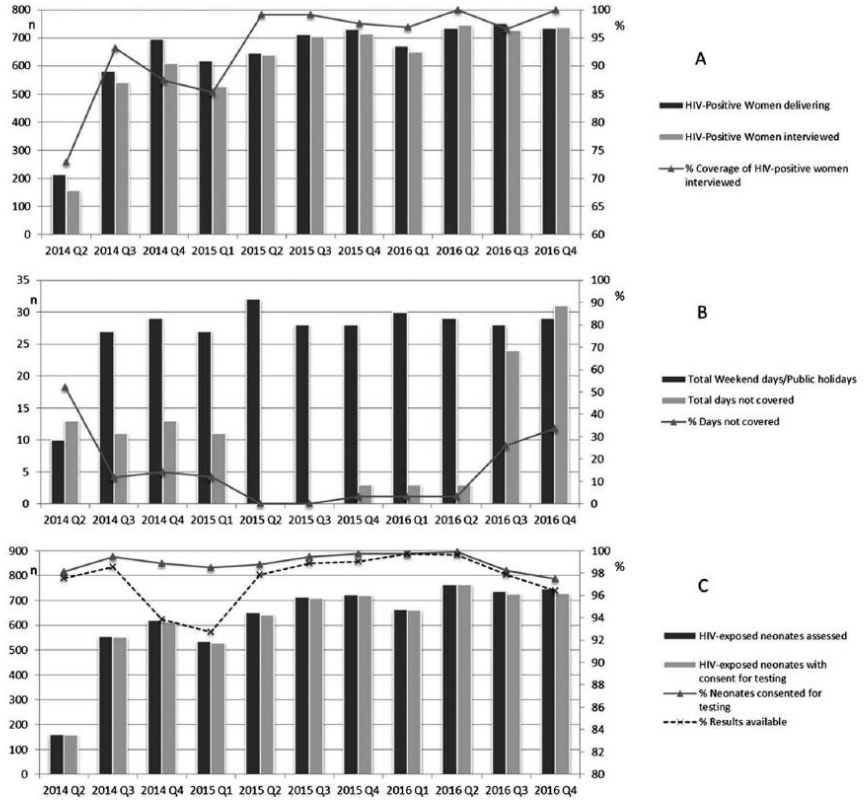


Figure 2. Enrolment trends over time – Panel A: Coverage of HIV-positive mothers interviewed, Panel B: Study staff capacity to cover birth testing daily, Panel C: Coverage of birth testing. Q = quarter; Q1 = January-March; Q2 = April-June; Q3 = July-September; Q4 = October-December.

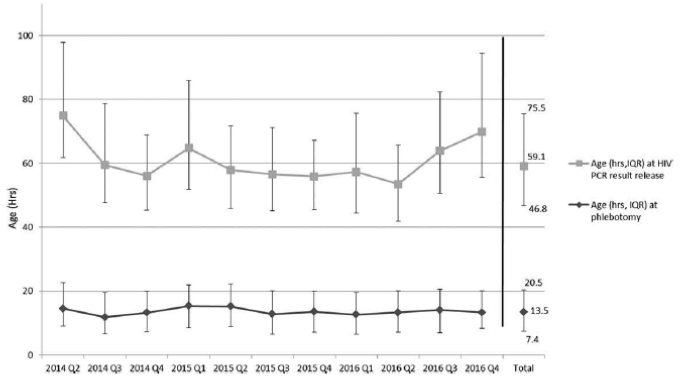


Figure 3. Age (hours) of neonate at phlebotomy for first HIV PCR test and at HIV PCR result release. hrs = hours, IQR: interquartile range. Q = quarter.

as HIV infected. Five of them had viral load >1000 cps/ml (copies per millilitre) and qualitative PCR-positive results on repeat testing, two had repeat qualitative PCR-positive results. One neonate died due to complications of prematurity before repeat testing could be performed. The remaining 11 neonates with initial indeterminate results tested HIV PCR negative on subsequent tests, had no detectable viral load on any sample and were subsequently classified as HIV uninfected (Figure 4). In sum, we estimate the intrauterine transmission rate in this cohort to be 1.6% (99/6360–95% CI: 1.3–1.9). Sixteen (16%) of these 99 infected neonates identified at birth were born to 221 women newly diagnosed as part of the postnatal maternal testing programme.

Of the 99 HIV-infected neonates, 88 (89%) returned to the site and underwent confirmatory testing and eight (8%) received follow-up testing at other clinical sites. Three neonates were lost to follow up with no records of confirmatory HIV testing or contact with the health service. Of the 96 infected neonates who underwent confirmatory testing at any site, all but three were confirmed either with a subsequent positive PCR or with a detectable viral load test. These three neonates tested indeterminate or negative on confirmatory PCR tests and are being monitored closely. The median viral load among 94 neonates with results was 4.4 log cps/ml (IQR: 3.3–5.3 log cps/ml, range: 1.8–6.7 log cps/ml).

Of the 99 HIV-infected neonates, 88 (89%) returned to site to receive their test results at a median of 8 days (IQR 6–13 days, range 2–103 days) at which time ART was initiated and blood was drawn for confirmatory testing. Of the eleven neonates who did not follow-up on site,

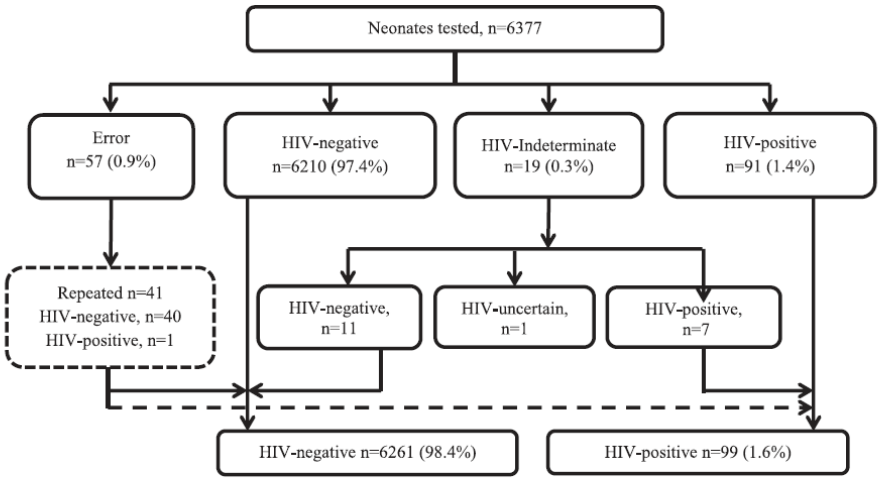


Figure 4. Initial birth HIV PCR test results and subsequent HIV infection status after repeat testing of neonates.

seven are known to have initiated ART at other clinical sites between 40–99 days. Thus, 95 of 99 (96%) HIV-infected neonates identified as part of the birth testing programme initiated ART. Of the 6261 neonates with negative birth HIV PCR results, 3251 (52%) received their test results on site at a median age of 10 days (IQR: 8–12, range 1–381).

Discussion

In the first two and a half years of operation, our universal birth PCR testing programme was able to achieve >90% coverage of the HIV-exposed neonates delivered at Rahima Moosa Mother and Child Hospital. Almost all (99%) HIV-positive women consented for neonatal testing and drop-offs in the cascade were primarily due to our capacity to reach the identified HIV-positive women prior to discharge. Our capacity to reach the identified HIV-positive women varied considerably by quarter and was determined by our ability to ensure that study staff were present on all days in the month, including weekends and public holidays. In quarters where we had study staff coverage on weekends and public holidays, coverage exceeded 98% and it is encouraging that coverage was maintained at a high level despite the study staff withdrawing on the weekends as a result of routine staff conducting birth testing. In June 2015, universal birth testing of all HIV-exposed neonates was adopted by the South African PMTCT guidelines [12,20]. While the majority of women deliver in a healthcare facility in South Africa, birth PCR testing programmes will need to ensure that they can provide adequate staff coverage on all days, including weekends and public holidays, to achieve the full potential of birth testing for early infant diagnosis.

Our site had already established a maternal HIV testing programme at the time of delivery as advised by South African policy [12,20]. We were able to leverage this programme to identify the HIV-exposed neonates who needed testing. Maternal testing coverage for previously negative women was only 70% because during the initial study period guidelines did not require women with recent negative results to undergo testing. Although a relatively small proportion (3.1%) of HIV-exposed neonates was identified from newly diagnosed mothers, this group contributed 16% of all HIV-infected neonates. This highlights the importance of maternal testing on newly delivered women with non-positive results to identify high-risk infants, either HIV infected and in need of ART or HIV exposed and in need of PMTCT. Many of these newly identified HIV-positive women may represent incident infections at high risk of transmitting and, by definition, none will have accessed PMTCT [17,21–23].

We were pleased to observe a low intrauterine transmission rate of 1.6%. This is reassuring confirmation of the excellent PMTCT coverage in this population with the effective Option B+ strategy of initiation of maternal ART upon diagnosis. Given the logistics in the postnatal wards of RMMCH, it was not possible to collect a blood sample from neonates prior to the first nevirapine prophylaxis dose. Nevertheless we used the current state-of-the-art qualitative HIV PCR test from Roche (COBAS® TaqMan® HIV-1 Qualitative Test Version 2) which has been optimized with improved sensitivity compared to prior versions [24]. A limitation of our programme was the inability to provide systematic follow up of neonates who tested negative at birth to determine the rates of infections detected later as well as the coverage rates of later tests.

Indeterminate HIV PCR results accounted for almost a quarter of non-negative birth results and required follow-up with multiple subsequent tests to determine the infant's infection status. Indeterminate PCR results may signify true infections with low levels of HIV available or may be false positives [13]. The additional tests and consultations to resolve these diagnostic dilemmas added costs and complexity to the programme.

We were able to ensure that almost all (95/99) neonates who were found to be HIV infected initiated ART, most in the first two weeks of life. However, this entailed active outreach efforts as well as liaison with other clinical sites. Without active tracking, only 52% of the mothers of infants with birth PCR-negative results returned to receive the results. This was despite clear counselling offered as part of the programme. Further efforts are required to facilitate return of results. "Results for Action" reports listing HIV PCR results for a facility are emailed weekly and being used to trace infants with non-negative results. Point-of-care testing offers an opportunity for immediate result return before discharge. To reduce the pressure on staff at the time of delivery and reduce the counselling required, antenatal visits should be used to prepare pregnant women for neonatal birth and follow-up testing. Additional counselling at delivery will be required to reinforce the importance of early infant diagnosis.

In our programme, turnaround time of the PCR results was a median of 2 days and at a median neonatal age of 59 h. The 5-h delay in turnaround time for non-negative results is likely due to quality assurance procedures for positive results and repeat test runs for indeterminate results and unlikely to impact on clinical management. At our site, like in most maternity services in South Africa, the majority of neonates will have been discharged from the healthcare facility by at least 48 h of age. Hence, neonates with non-negative results need to be urgently located for confirmatory testing and ART initiation. Point-of-care testing at delivery may mitigate losses that occur due to failure to return for results. In the meantime, active outreach efforts are essential to ensure that infants with non-negative results are located and engaged in care.

For the infants we were able to manage at our site, we elected to initiate ART presumptively based on a single positive PCR result. We aimed to draw a second blood sample on the day of ART initiation for confirmation. The motivation for this is the growing appreciation of the importance of early initiation of ART in infants [25]. All of the infants we managed were confirmed on at least one qualitative PCR or with detectable HIV RNA on at least one viral load test.

Since HIV PCR testing on a blood sample collected <48 h of life is thought not to be able to detect an intrapartum infection, follow-up testing is necessary to detect these and postnatal breastfeeding-acquired infections. Given the cost of PCR tests particularly at high volumes, decisions need to be made about the frequency and timing of these subsequent tests whilst balancing the need for this vulnerable population of exposed and infected neonates to receive appropriate interventions[10].With the addition of birth testing, the South African national programme now advises that the second PCR test be scheduled at 10 weeks of age, 4 weeks later than previously recommended for the first PCR. With PMTCT, the ratio of intrauterine:intrapartum infection shifts [14], with an increasingly small proportion of infections occurring intrapartum. In 2014, the 6-week HIV transmission rate for Gauteng, the province where RMMCH is situated, was 1.6% (National Health Laboratory Service). Since our presumed intrauterine transmission rate was 1.6%, we estimate that we detected the majority of the combined toll of intrauterine and intrapartum infections. Therefore, a birth testing programme combined with 10-week repeat testing should not undermine the gains of the earlier 6-week testing programme despite the delay in detecting intrapartum infections. High birth testing coverage may prevent losses to follow-up and mortality of infected neonates. Better understanding of the extent to which PMTCT interventions (e.g. extensive maternal ART and daily dose nevirapine) may affect the performance of different infant diagnostic tests would be helpful to select optimal time points for later testing.

An important limitation is that our birth testing programme was implemented in the context of a study where additional resources were provided for counselling, follow-up and quality control. Therefore, our outcomes may not be generalizable to routine rollout at other hospitals in South Africa and elsewhere. We worked closely with the routine ward staff in the maternity service but provided additional counsellors and phlebotomists as well as a study team who provided management and quality control. The challenge to maintain coverage of the birth testing programme over weekends and public holidays reduced over the study period as the programme was incorporated into routine care. Efforts to incorporate the tracking and data capturing systems into routine care are ongoing whilst shifting study staff away from full service delivery and into training and supervisory roles. Innovative ways of incorporating other existing staff into the programme are being explored. We required written informed consent for neonatal birth testing which arguably might not be required in routine service. Consent for neonatal testing was extremely high (99%) but even were formal verification of consent not required, the process of explaining birth testing and the necessity for further follow-up and engagement in care is essential in a routine programme as well. The other staff-intensive components of the study, including quality control (e.g. ensuring bloods and results matched to the correct infant) as well as tracking the infants with non-negative results, engaging and retaining them in care, are also essential components of any routine programme.

Conclusions

We have demonstrated that universal birth PCR testing of HIV-exposed neonates can be successfully implemented in a large urban hospital and can lead to early identification of HIV-infected infants and early initiation of ART. In our experience, it is the human resources that are critical to implementing the programme and achieving retention in the PMTCT cascade. Important staffing considerations include a strong counsellor presence covering seven days a week including public holidays, to provide three tiers of counselling: (1) maternal screening and testing for all delivering women, (2) maternal interviewing and counselling for all known and newly diagnosed HIV-positive women including taking consent for neonatal HIV testing and (3) follow-up counselling to provide results and encourage follow-up tests. Staff to perform neonatal phlebotomy, or collect dried blood spots, liaise with the laboratory, assemble results, and maintain adequate records are also required. Systems to encourage further postnatal testing of infants with negative results as well as to ensure timely follow-up and linkage to care of all neonates with non-negative results are crucial.

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Competing Interests
The authors have no conflicts of interest to disclose.

Authors' contribution
KT, LK, AC, SC, GS: study design, data interpretation, writing. KT, GS, LK: data analysis. KT: data collection. All authors have read and approved the final version.

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Xpert HIV-1 point-of-care test for neonatal diagnosis of HIV in the birth testing programme of a maternity hospital: a field evaluation study

Karl-Günter Technau, Louise Kuhn, Ashraf Coovadia, Pamela M Murnane, Gayle Sherman

Summary

Background Point-of-care testing (POCT) among HIV-exposed infants might improve linkage to care relative to laboratory-based testing (LABT). We evaluated HIV-1 POCT at birth in the context of universal LABT in a maternity hospital and describe our implementation experience.

Methods We did a field evaluation study between Oct 1, 2014, .and April 30, 2016, at the urban Rahima Moosa Mother and Child Hospital (RMMCH), Johannesburg, South Africa. We aimed to sample consecutive neonates at birth with POCT (Cepheid Xpert HIV-1 Qualitative test) and compared results with those of LABT (Roche COBAS TaqMan HIV-1 Qualitative test) with respect to performance in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's κ coefficient, result return, antiretroviral treatment (ART) initiation, and coverage.

Findings 18 268 women delivered livebirths at RMMCH and 4267 (23%) were HIV-positive with 4336 HIV-exposed neonates delivered. Mothers of 4141 (96%) HIV-exposed neonates were offered infant birth testing. Mothers of 4112 (99%) neonates consented. In 78 neonates with consent (2%), a test was not done due to early neonatal death (n=13), mother departing before venesection, or staff unavailability. Among 3970 infants who had LABT, 57 (1%) tested positive, 3906 (99%) tested negative, two (<1%) were indeterminate, and five (<1%) had an error result. 2238 (56%) of these infants had concurrent POCT. POCT detected all 30 HIV-infected neonates (sensitivity 100%; 95% CI 88·4–100) with two additional false-positive results (specificity 99·9%; 99·7–100). All positive and 96·2% of negative POCT results were returned compared with 88·9% of positive and 52·8% of negative LABT results. Although every POCT required 90 min of instrument time, 2·6 h (IQR 2·3–3·1) elapsed between phlebotomy and result return. In days, median time of result return for POCT was 1 day, significantly earlier than 10 days for LABT (p<0·0001). ART was initiated in 30 neonates (100%) with positive POCT compared with 24 (88·9%, p=0·10) of 27 infants who had LABT only, with initiation occurring a median of 5 days earlier in the POCT group (p<0·0001). POCT implementation required additional staff and weekend cover.

Interpretation Compared with LABT, POCT was associated with good performance, improved rates of result return, and reduced time to ART initiation. Resources needed to integrate POCT into a routine birth testing programme require further evaluation.

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Introduction

Infant HIV infection requires early diagnosis and antiretroviral treatment (ART) initiation before the first peak in mortality at 2–3 months of age.[1–3] Birth HIV PCR testing for all neonates born to HIV-infected mothers was added to South Africa's early infant diagnosis (EID) algorithm in June, 2015, and later adopted as a conditional recommendation by WHO.[4,5] Improving early diagnostic and ART coverage is challenging and might be strengthened by innovative approaches like point-of-care testing (POCT).[6,7]

Most births in South Africa occur at centralised maternity units, while postnatal well-baby follow-up and subsequent HIV testing occur at peripheral clinics.[8] HIV PCR testing within the public sector, including birth testing, is centralised in nine laboratories in the country and turnaround times range from 24 h to weeks for remote peripheral clinics. Thus, after delivery, neonates and mothers are usually discharged before birth HIV results are released. In high maternal HIV prevalence settings like South Africa, where provincial neonatal HIV-exposure rates range from 17·5% to 40·1%,[9] large volumes of HIV birth test results from bloods sampled at maternity units have to be returned at peripheral clinics responsible for postnatal care. This delay poses logistical challenges that threaten the usefulness of birth HIV testing.[10] Non-negative results should prompt urgent tracing of the neonate for repeat HIV testing and appropriate care. HIV negative result return is also important and provides the opportunity to counsel mothers regarding the necessity of further testing for intrapartum and postnatal infection.

POCT offers the potential of returning the HIV PCR results to the mother before she and her neonate are discharged from the delivery unit. This reduces the need to trace neonates with non-negative results, and enables immediate initiation of ART in neonates with positive

Research in context

Evidence before this study

HIV PCR point-of-care testing (POCT) technology has recently become part of the range of tests for early infant diagnosis (EID) of HIV and might be considered a game changer for paediatric HIV diagnosis and treatment access. Only two EID POCT platforms have WHO prequalification status. We searched PubMed and conference proceedings using the search terms "point-of-care", "HIV", "early infant diagnosis", and "birth for English language articles published up to April 30, 2017. Few field evaluation data exist for performance of POCT at birth, particularly with Cepheid POCT technology or for implementation of birth testing with POCT in high prevalence settings. Universal birth HIV PCR testing with standard laboratory-based testing (LABT) of HIV-exposed neonates has recently become standard of care in South Africa and is endorsed by a conditional WHO recommendation.

Added value of this study

This field evaluation shows good performance of the Cepheid Xpert HIV-1 qualitative POCT at birth comparable to the standard of care, laboratory-based test. The field evaluation was done in the context of a universal birth testing programme for all HIV-exposed neonates in an urban hospital setting in Johannesburg, South Africa. Logistical challenges experienced while implementing POCT in a study context are described. The study observed benefits of POCT, including improved result return rates and earlier initiation of antiretroviral treatment (ART) in HIV-infected neonates.

Implications of all the available evidence

The results offer guidance locally and globally to policy makers, implementers, and public health managers on a priority paediatric programme. While field performance is good, further consideration will be required to translate the lessons from this well resourced setting into standard routine practice. Universal HIV birth testing with linkage to early ART can be successfully achieved in an urban setting in sub-Saharan Africa albeit with additional resources. It is encouraging that retention in the prevention of mother-to-child transmission of HIV care cascade was improved and EID POCT has the potential to improve outcomes for HIV-infected children. Further work is required on routine implementation and costing of EID POCT in different settings, particularly efforts to achieve full POCT coverage or assessment of targeted POCT approaches.

results. Although there are some laboratory evaluation data available,[11–14] field evaluations of POCT platforms for EID, particularly at birth, are required before implementation. The Alere Q NAT test (Alere Technologies, Jena, Germany) has shown sensitivities of 98·5% (no neonates),[15] 96·5% overall (93·3% in 92 neonates),[16] and 99% in 291 neonates,[17] with specificity higher than 99·5%. Few published data exist for the Xpert HIV-1 Qualitative assay (Cepheid, Sunnyvale, CA, USA) used for EID POCT in infants and neonates.[18]

We did a field evaluation of birth POCT at a busy maternity hospital in Johannesburg, South Africa. Here, we present a comparison of the performance of the POCT (Xpert HIV-1 Qualitative assay) with the standard of care laboratory-based testing (LABT) in neonates, heavily exposed to maternal ART. We report on implementation of POCT within a birth testing programme albeit that POCT occurred within a study setting.

Methods

Study design and participants

We did the field evaluation study at Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa, where about 1000 births occur per month of which around 23% are HIV exposed.[19] This urban public hospital is the subdistrict's main delivery facility and referral site for complicated cases. At the time of the study, the standard intervention for prevention of mother-to-child transmission (PMTCT) was option B+ (ie, life-long maternal ART for all infected women).[4] Counsellors screened all new deliveries to identify known HIV-positive women and those newly diagnosed at delivery, and LABT was offered for all exposed infants since June 5, 2014. HIV-positive women were interviewed about obstetric and ART history, HIV disclosure, partner testing, and ART adherence, and data for the neonate (anthropometrics, gestation, Apgar scores[20]) and method of delivery were collected. From Oct 1, 2014, to April 30, 2016, all identified HIV-positive women were invited to enrol their neonates in an observational cohort study of routine universal birth testing including this field evaluation of POCT. LABT was not dependent upon enrolment in the study.

Written informed consent was obtained from all mothers and the study was approved by the Human Research Ethics Committees of the University of the Witwatersrand (Johannesberg, South Africa; M140639) and the institutional review board of Columbia University (New York, NY, USA) who also approved the study and use of data.

Procedures

Neonatal whole blood was sampled by venipuncture in the postnatal ward or during neonatal admission. Cord blood was never sampled. The sample for LABT was collected into a 0·5 mL EDTA (edetic acid) tube and sent to the national laboratory (Johannesburg, South Africa) for HIV PCR testing (Roche COBAS TaqMan HIV-1 Qualitative test, version 2·0, Roche Molecular Systems, Branchburg, NJ, USA) where processing was done by routine, non-study staff. From the same blood draw, an additional identical 0·5 mL whole blood sample was

collected for POCT (Xpert HIV-1 Qualitative assay, Cepheid, Sunnyvale, CA, USA) for processing by study staff in a small satellite research laboratory on site.[21] The minimum volume of blood required for this assay is 0·1 mL but extra volume was drawn to allow for repeat tests on the same sample if needed. Neonates were not rebled for POCT if their initial LABT blood draw had been taken by the routine clinical staff before study consent could be obtained.

The on-site satellite laboratory housed three Cepheid instruments, each with four modules capable of running four POCTs independently. Technical support from Cepheid was readily available telephonically and on-site assistance was provided for troubleshooting and instrument maintenance (about once or twice a month as required). The POCT instruments were operated by a laboratory technician or study nurse supervised by a laboratory-trained project manager. Each test required adding 0·75 mL buffer followed by 0·1 mL whole blood to a cartridge, loading the cartridge into a module and running for 90 min. Two staff members verified results and conveyed them to the mother before discharge. Maternal admissions post-caesarean section lasted 3 days, while post-vaginal delivery stays ranged from 6 h to 24 h. Staff coverage for POCT was available on weekdays (during office hours) until September, 2015, when Sunday coverage was added. Public holiday coverage was limited, particularly over December and January. The research funded study team consisted of four cousellors, one phlebotomist, two study nurses, one laboratory technician, two data capturers, one project manager, and a medical doctor.

POCT provided the following results: HIV detected, HIV not detected, error, invalid, and no result. The final three options were combined as errors and analysed according to associated standardised instrument error codes. LABT provided the following results: HIV positive, HIV negative, indeterminate (defined by laboratory cut-offs and representing either low-level viraemia or false positives), and various error results.

A positive LABT or POCT result prompted ART initiation and confirmatory testing by means of repeat LABT and an HIV viral load test (COBAS AmpliPrep/COBAS TaqMan HIV-1 test, version 2·0 [Roche Molecular Systems Inc, Branchburg, NJ, USA]). We initiated ART on a positive POCT before receiving the LABT result from the concurrent blood draw only after the POCT was positive on re-run. Indeterminate LABT prompted further testing to establish HIV status. Management of infected neonates was done on site. All mothers received an appointment to collect their neonate's LABT result within 1 week. If the LABT result was positive or indeterminate, a study nurse telephonically recalled the mother for an earlier visit. Results, tracing barcodes, and the hospital's contact details were recorded in the neonate's patient-held record with the mother's permission.

Statistical analysis

Staff recorded interview and laboratory data on paper forms that were captured electronically into REDCap.[22] We calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and Cohen's κ coefficient assuming the final diagnostic outcome of the concurrent LABT was the gold standard. Rates and timing of results return and initiation of ART for infected neonates were described. The coverage of LABT and POCT was calculated as the percentage of identified HIV-exposed neonates in whom testing occurred and correlations were calculated (Spearman's correlation). To compare groups for continuous variables, the Student's *t* test or Wilcoxon rank-sum tests were used, and for categorical variables the χ^2 test or Fisher's exact test. De-identified data were analysed with SAS (version 9.4, SAS Institute Inc, Cary, NC, USA).

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. We received a reduced price per test cartridge into which the blood and buffer are inserted from the manufacturer (Cepheid). The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

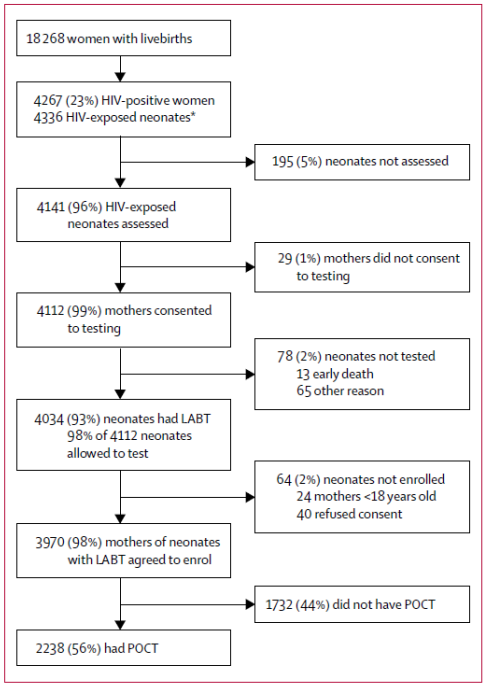


Figure 1: **Participating HIV-positive mothers**
LABT=laboratory-based testing. POCT=point-of-care testing. *Includes 67 twins and two triplets.

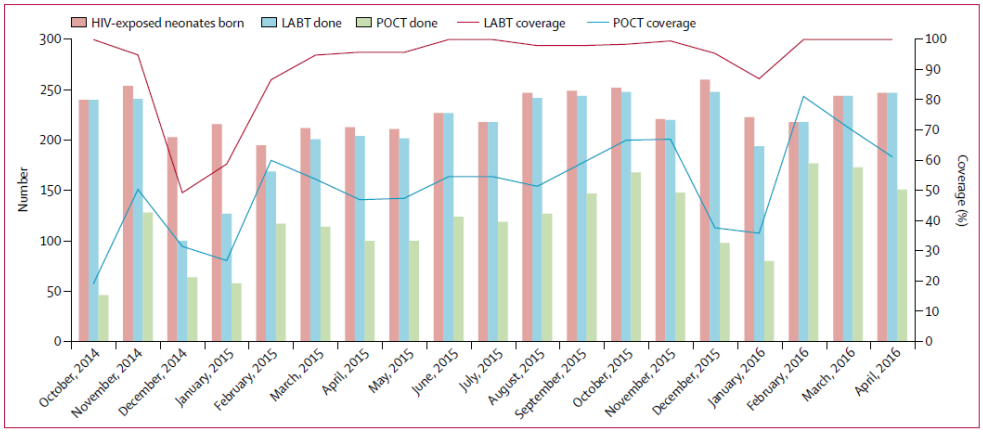


Figure 2: **Monthly coverage of birth HIV PCR testing by laboratory-based testing (LABT) and point-of-care testing (POCT)**
Coverage was defined as the percentage of HIV-exposed neonates with either LABT or POCT out of all HIV-exposed neonates identified in each month. LABT=laboratory-based testing. POCT=point-of-care testing.

Results

Between Oct 1, 2014, and April 30, 2016, 18 268 women delivered livebirths at RMMCH and 4267 (23%) were HIV-positive with 4336 HIV-exposed neonates delivered (figure 1). Mothers of 4141 (96%) HIV-exposed neonates were offered infant birth testing. LABT uptake was high with mothers of 4112 (99%) neonates consenting to testing. In 78 neonates with consent (2%), a test was not done because of early neonatal death (n=13), mother departing before venesection, or staff unavailability. Thus, 4034 (93%) of 4336 HIV-exposed neonates born had LABT. Mothers of 3970 (98%) neonates with LABT agreed to study participation of whom 2238 (56%) had concurrent POCT (appendix p 1). Therefore, POCT coverage of all HIV-exposed neonates born (n=4336) was 52%. Mothers of neonates who had POCT had marginally longer ART exposure during pregnancy (23 weeks *vs* 21 weeks, p=0·010) and were more likely to have attended antenatal care (97% *vs* 95%, p=0·0053), but had similar rates of being on ART by delivery (93%; appendix p 1). Neonates who had POCT were less likely to have required admission (5% *vs* 23%, p<0·0001) and had lower rates of birthweight less than 2·5 kg (15% *vs* 25%, p<0·0001) and prematurity (20% *vs* 28%, p<0·0001).

See Online for appendix

Coverage of both tests was reduced during year-end holiday periods (figure 2). Coverage of LABT was maintained at more than 90%. LABT coverage during the holidays in December, 2015, to January, 2016, was low at (87%), but was improved compared with the previous year (December, 2014, to January, 2015) during which time it was 49%. A median of seven LABT tests were done every day (IQR 5–9, range: 1–16). Coverage of POCT peaked at 81% in February, 2016, but was less than 40% at both year-ends. POCT was done on 317 (55%) of the 578 study days, during which POCT coverage was 83%. A median of six POCT tests were done every day (IQR 5–8, range 1–13). Overall, monthly coverage of the two tests were moderately correlated (Spearman's r=0·53, p=0·014).

LABT of 3970 neonates produced 54 positive (1%; 95% CI 1·0–1·7), 3876 negative (98% 97·2–98·1), 12 indeterminate (<1%; 0·1–0·5), and 28 error (1%; 0·4–1·0) results. Confirmatory HIV testing for the 54 positive neonates showed repeat positive HIV PCR results or a viral load higher than 1000 copies per mL on subsequent samples in 51 cases. In three neonates confirmation was not possible. Repeat sampling and testing of the 12 neonates with indeterminate results confirmed HIV infection in three cases, showed no evidence of HIV infection in seven cases, and the diagnosis remained indeterminate in two cases. Of 28 neonates with LABT errors, 23 had repeat testing all with negative results. Thus, final HIV diagnostic outcome by LABT for 3970 neonates tested at birth was 57 positive (1%; 95% CI 1·1–1·8), 3906 negative (99%; 98·1–98·9), two indeterminate (<1%; 0·0–0·1), and five with no results because of inability to repeat the sample. POCT results for 2238 neonates tested concurrently comprised 32 positive (1%; 95% CI 0·9–1·9), 2098 negative (94%; 92·7–94·7), and 108 error (5%; 3·9–5·7) results.

The Cohen's κ coefficient of 0·967 (95% CI 0·922–1·00) supports strong agreement between the tests (figure 3). With the final diagnostic outcome on the LABT as the gold standard, the sensitivity of POCT was 100% (exact CI 88·4–100) because all LABT-identified infected neonates were also identified by POCT. The specificity of POCT was estimated to be 99·9% (exact CI 99·7–100) because two false positive POCT results occurred, however, on repeat POCT of the

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same sample, results were negative. The PPV was 0·938 (95% CI 0·792–0·992) and the NPV was 0·995 (0·991–0·998). Of the 108 (5%) errors on the first POCT test, 103 samples were rerun yielding 94 (91%) negative results and nine (10%) persisting errors. When three of the latter samples were repeated for a third time, all tested negative. Therefore, 97 of 108 initial POCT errors tested negative on repeat POCT reducing the final POCT error rate to 11 (<1%) of 2238 tests.

Mechanical or cartridge-related errors were the most frequent (n=48) followed by sample volume errors (n=44; appendix p 2). Half of the sample volume errors occurred during a period when incorrect pipettes were supplied. Around half (54%) of the the mechanical or cartridge errors occurred in the months before modules required replacement. Most POCT (1864 [83%]) were run by the laboratory technician with an error rate of 4·3%. The remaining tests included 287 (13%) run by the project manager with an error rate of 7·0% and 87 (4%) tests run by nurses with an error rate of 9·2% (p=0·022).

Blood was sampled at a median of 14 h (IQR 8–21) after birth. The median turnaround time from phlebotomy to result release for LABT was 43 h (IQR 31–54) and for POCT was 2·6 h (2·3–3·1). In addition to the 90 min to run the POCT in the instrument, 64 min between phlebotomy and result release and 16 min for result return to the mother were required (figure 4). Results were returned on the day of venesection except in 20 cases (1%) when result return occurred the following morning where the mother was due for discharge the day before.

The overall rate of POCT plus LABT result return (96%) was significantly higher than for LABT result return (53%, p<0·0001). POCT results were also returned significantly earlier at a median of 1 day compared with 10 days for LABT results (p<0·0001, table). For 30 neonates diagnosed HIV positive with POCT all mothers received results and all neonates started ART. For 27 diagnosed with LABT alone, 24 mothers received results and 24 neonates started ART (p=0·10). Time to result return was significantly earlier for POCT at a median of 1 day compared with a median of 8 days for LABT results (p<0·0001). Infected neonates identified by POCT initiated ART at a significantly earlier age than did infected neonates with only LABT (median 1 day *vs* 6 days, p<0·0001; table). Six (25%) of 24 HIV-infected neonates with LABT only initiated ART before their test results being returned to their mothers because of maternal illness or separation. This accounts for the discrepancies between median age at ART initiation and time to results return in the LABT only group.

		LABT				
		Positive	Negative	Indeterminate	Error	Total
POCT	Positive	30	2*	0	0	32
	Negative	0	2097	1†	0	2098
	Error	0	108	0	0	108
	Total	30	2207	1	0	2238

Figure 3: **Comparison of the initial result of POCT with the final diagnostic outcome from LABT**
POCT=point of care testing. LABT=laboratory based testing. * Because these two false positive POCT results re-tested as negative, if the final POCT diagnosis is used sensitivity, specificity, PPV, and NVP are all 1. †This neonate had further indeterminate and later negative results and the diagnosis remains uncertain.

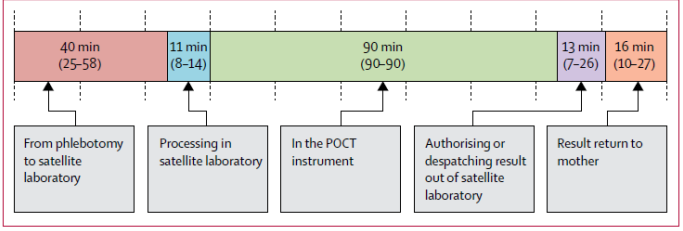


Figure 4: **Median (IQR) time in min per component between phlebotomy of neonate and POCT result return to mother**
POCT=point of care testing

	Total	POCT and LABT	LABT only	p value
Final HIV test results	3970	2238	1732	..
Positive	57	30	27	0·077*
Negative	3906	2207	1699	..
Indeterminate	2	1	1	..
No result	5	0	5†	..
Results returned to mother	3076 (77%)	2155 (96%)	921 (53%)	<0·0001
Age, days	1 (1–8)	1 (0–1)	10 (9–13)	<0·0001
Positive results	54 (95%)	30 (100%)	24 (89%)	0·10‡
Age, days	2 (1–8)	1 (1–1)	8 (6–12)	<0·0001§
Negative results	3021 (77%)	2124 (96%)	897 (53%)	<0·0001
Age, days	1 (1–8)	1 (0–1)	10 (9–13)	<0·0001
ART initiated in infected neonates	54 (95%)	30 (100%)	24 (89%)	0·10‡
Age, days (min–max)	3 (1–8; 0–104)	1 (1–2; 0–104)	6 (5–10; 2–78)	<0·0001§

Data are n, n (%), or median (IQR), unless otherwise shown. POCT=point-of-care testing. LABT=laboratory-based testing. ART=antiretroviral treatment. *p=0·56 when excluding indeterminate results and errors. ‡In these five cases it was not possible to repeat the test after an initial error was encountered. ‡Fisher's exact test. §Wilcoxon rank sum test.

Table: **Results return and ART initiation in neonates with POCT and LABT results compared with those with LABT results only**

Discussion

In this field evaluation of the Xpert HIV-1 Qualitative Assay within a large universal birth testing programme in South Africa, we showed near perfect concordance between POCT and LABT in routine use. POCT facilitated result return to mothers by enabling more mothers to receive results and to receive them earlier. Active tracing ensured that infected neonates both with and without POCT received ART, but POCT enabled earlier treatment. While weekday coverage of POCT was high, overall POCT coverage reached only 50% because the study was not set up to achieve full implementation.

The sensitivity of 100% and specificity of 99% at birth is comparable to or better than that reported for Alere

POCT at birth and for POCT (Xpert HIV-1 Qualitative assay) by the Early Infant Diagnostic Consortium.[15–18] The less than perfect specificity we noted was eliminated by an algorithm requiring a repeat test on all POCT positive results. Our two false positive POCT results tested negative on repeat testing of the same sample and on LABT. Because positive results have significant clinical implications, confirmatory testing on a separate sample is strongly recommended.

POCT results were received by 96% of mothers before discharge leading to significantly more mothers learning their child's HIV status (96%) than for LABT alone (53%). Although not directly measured, mothers generally agreed to have their neonate's test information and results documented in their patient-held record. Because we actively traced all mothers whose neonates had positive results, result return rates for positive neonates were good in both scenarios. All mothers of infected neonates in the POCT group received results and agreed to their neonates starting ART, whereas only 89% in the LABT only group did. Three mothers whose infected neonates had a LABT only were discharged before results were available and never returned despite active tracing. Enhanced counselling and collection of more comprehensive contact details might mitigate this loss to follow-up. Neonates with a POCT were started on ART significantly earlier. Whether very early initiation of ART, within 48 h of birth, leads to improved outcomes or increased risks is unknown, but current clinical consensus supports the benefit of ART initiation upon diagnosis.[23–25]

Further work on the psychological effect of HIV diagnosis at birth is required. While dealing with their own diagnosis (commonly during the current pregnancy), POCT requires mothers to navigate coping with a diagnosis of their neonate at delivery. During EID, the focus of health care is more often on the infant than the HIV-infected mother's health.[26,27] During the era of testing at 6 weeks in South Africa, EID was associated with beneficial and detrimental psychological effects.[28] Less is known about the effect of an even earlier diagnosis. In some instances, maternal illness and incapacitation after delivery prevented neonatal test results being communicated to the mother before initiating neonatal ART, highlighting the vulnerability of mothers and their infected neonates.

Privacy and space to do large volumes of POCT precluded placing the instrument in close proximity to the postnatal discharge ward. Instead it was situated in a separate satellite laboratory on another floor in the hospital complex. This arrangement explains the relatively long delay between blood draw and results return to mothers. The high daily case load and staff constraints made it practical for two to three blood samples to be collected before proceeding to the POCT instrument, accounting for the median of 40 min between phlebotomy and samples reaching the instrument (figure 3). The logistics of how POCT will be integrated into any clinical site needs careful consideration of these factors. In high case load settings, clinical staff in the postnatal wards might require at least one or more dedicated staff members for POCT implementation. Another potential solution, is implementation of POCT by building a so-called network of testers within an institution.[29]

The initial error rate observed with POCT (5%) was higher than with LABT (1%), higher than the 3% reported in the WHO pre-qualification report,[30] and lower than the 10% recently reported for the Alere Q POCT for birth testing.[16] Repeating the POCT on the same sample reduced the error rate to less than 1%. Closer analysis of the POCT errors identified many modifiable factors. These included site-specific problems, including a dusty environment and power interruptions during renovations, but also manufacturer and instrument-related issues such as faulty modules and incorrect pipette supply. Achieving 3% error rates or less will require ongoing training, supervision, and monitoring, and engagement of the manufacturer's support.[29,31]

Birth testing coverage with LABT as standard of care exceeded 90% within 23 months of implementation; however, POCT coverage averaged around 50% after 19 months of implementation because of constraints imposed by the study setting. These included a finite study team unable to provide POCT outside of office hours and having to obtain informed consent before venesection for POCT, whereas only LABT was possible without study consent. Because we opted not to rebleed neonates, our ability to enrol admitted neonates was restricted. Therefore, the POCT cohort generally comprised healthier neonates at birth, whereas sick neonates might have been prioritised for POCT if it were available outside of the study setting. Although the study setting was a barrier to assessing routine implemention of POCT at birth, the experience gained is described to pre-empt challenges that sites might face, such as requiring additional resources to integrate POCT into standard of care. Another limitation of this study is that it was done at a single stidy site, which could limit generalisability.

POCT is an accurate and useful instrument for birth HIV testing that increases overall result return rates, reduces time to result, and enables earlier ART initiation for HIV-infected neonates. Implementation is challenging and requires careful consideration and innovative approaches, particularly for busy sites in high maternal HIV prevalence settings. Further research is required to assess the benefits of very early ART initiation in infected children to establish whether this specific consequence of POCT warrants the additional resources required to implement birth POCT. More information is needed about the psychosocial effect on mothers of very early diagnosis of their infants as well as a cost–benefit analysis of additional resources needed for full implementation.

Contributors

K-GT, LK, AC, PMM, and GS contributed to the study design, data interpretation, and writing. K-GT, GS, and LK contributed to the data analysis. K-GT was responsible for data collection.

Declaration of interests

We declare no competing interests.

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Research article

Prevalence and outcomes of HIV-1 diagnostic challenges during universal birth testing – an urban South African observational cohort

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Abstract

Introduction: HIV-1 polymerase chain reaction (PCR) testing at birth aims to facilitate earlier initiation of antiretroviral therapy (ART) for HIV-infected neonates. Data from two years of universal birth testing implementation in a high-burden South African urban setting are presented to demonstrate the prevalence and outcomes of diagnostic challenges in this context.

Methods: HIV-exposed neonates born at Rahima Moosa Mother and Child Hospital between 5 June 2014 and 31 August 2016 were routinely screened at birth for HIV-1 on whole blood samples using the COBAS® AmpliPrep/COBAS® TaqMan (CAP/CTM) HIV-1 Qualitative Test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA). Virological results were interpreted according to standard operating procedures with the South African National Health Laboratory Service. All neonates with non-negative results were actively followed-up and categorized according to HIV infection status as positive, negative, uncertain and lost to follow-up (LTFU).

Results: 104 (1.8%) of 5743 HIV-exposed neonates received a non-negative birth PCR result, for which laboratory data were available for 102 (98%) cases – 78 (76%) tested positive and 24 (24%) indeterminate. HIV infection status was confirmed positive in 83 (81%) infants, negative in 8 (8%), uncertain in 5 (5%) and LTFU in 6 (6%) cases. The positive predictive value (excluding cases of uncertain diagnosis and inadequate testing) following a non-negative HIV-1 PCR screening test at birth was 0.91 (83/91; 95% confidence interval: 0.85–0.96). Neonates testing positive at birth had significantly higher viral load (VL) results than those testing indeterminate at birth of 4.5 and 3.0 log copies/ml ($p = 0.0007$), respectively. Similarly, mothers of neonates with positive as compared to indeterminate birth test results had higher VLs of 4.5 and 2.7 log copies/ml ($p = 0.0013$), respectively. Half of neonates with an indeterminate birth test were shown to be HIV-infected on subsequent confirmatory testing, with time to final diagnosis 30 days longer for these neonates ($p < 0.0001$).

Conclusion: Indeterminate HIV-1 PCR results accounted for a quarter of non-negative results at birth and were associated with a high risk of infection in comparison to the risk of *in utero* transmission. Indeterminate birth results with positive HIV PCR results on repeat testing were associated with later final diagnosis. The HIV-1 status remains uncertain in a minority of cases because of repeatedly indeterminate results, highlighting the need for more sensitive and specific virological tests.

Keywords: HIV-1 PCR; early infant diagnosis; birth testing; indeterminate

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Introduction

Routine HIV-1 polymerase chain reaction (PCR) testing at birth for all HIV-exposed neonates was introduced into the South African Consolidated Guidelines in June 2015 in order to enhance access to care and thereby reduce HIV-related morbidity and mortality [1,2]. Although targeted birth testing amongst high-risk HIV-exposed neonates had been part of the national testing guidelines since 2013 [3], implementation of the new guidelines has seen the volume of testing amongst neonates less than seven days of age increase by more than 40 times, with approximately 20,000 birth tests performed each month [4]. Importantly, whereas the rapid scale-up of birth testing has been associated with earlier diagnosis of intrauterine-infected neonates, there has also been an increase in the number of diagnostic challenges arising during the neonatal period.

Early infant diagnosis (EID) and treatment of HIV-1 is important due to the rapid progression of disease and early HIV-related morbidity and mortality [5–7]. Accurate diagnosis is critical to ensure that infected infants start

antiretroviral therapy (ART) early and that uninfected infants are not unnecessarily exposed to life-long treatment. The recent case of the "Mississippi baby", and other similar cases, further highlights the importance of very EID on account of the potential for functional cure and loss of detectability associated with very early ART initiation. These cases also highlight the possibility that diagnostic difficulties at birth may hold important information for this field [8–10].

Although PCR testing methods used for EID have reported sensitivities and specificities nearing 100% [11,12], important limitations exist. High coverage of maternal ART and early infant prophylaxis, as well as early initiation of treatment in infants prior to receiving confirmatory test results, are associated with high-level exposure to ART at the time of testing. This in turn has been associated with suppression of viraemia in infected infants and loss of detectability and uncertain results when using diagnostic assays [13–17]. Although the association between ART and indeterminate HIV-1 PCR results has been highlighted, and recommendations made regarding the management of such infants, further research is required to ensure timely definitive diagnosis and successful linkage to care [18,19]. Waning antibody levels and seroreversion following early ART initiation are additional phenomena that make later diagnostic confirmation difficult [20–22]. In contrast to the possibility of loss of detectability, there is also concern that on account of the dramatic reduction in mother-to-child transmission in South Africa there will necessarily be a drop in the positive predictive value of all infant diagnostic testing methodologies, thereby increasing the risk of treating uninfected infants [23–25].

Hence, research is urgently needed to inform evidence-based management of infants with uncertain and indeterminate HIV-1 results during early infancy. We describe the prevalence and outcomes of diagnostic challenges associated with HIV-1 PCR testing at birth within a single health facility in a high-burden setting over a two-year period.

Methods

Neonates born to HIV-infected mothers were enrolled at Rahima Moosa Mother and Child Hospital (RMMCH), a tertiary institution situated in Johannesburg, South Africa, with approximately 1000 deliveries per month and an antenatal HIV prevalence of 23% [26]. The cohort of all infants born between 5 June 2014 and 31 August 2016 who had an HIV-1 PCR test at birth were included in the analysis. During this period, all HIV-exposed neonates were routinely screened at birth for HIV-1 using ethylenediaminetetraacetic acid (EDTA) anti-coagulated whole blood samples obtained by phlebotomy in the hours following delivery and sent to a diagnostic laboratory for testing. Those with a non-negative result were actively traced and followed up either at RMMCH or referred to local facilities if unable to return. Initially all neonates with a non-negative laboratory HIV-1 PCR result were initiated on combination ART and samples taken simultaneously for confirmatory testing on follow-up. This practice was based on findings (from the previous version of the current assay) that neonates with an HIV-1 PCR result at birth, whether positive or indeterminate, were invariably found to have a confirmed HIV-1-positive infection status [27]. However, after it was found that some neonates who tested indeterminate at birth had negative confirmatory testing, this practice was stopped and only neonates with a clearly positive virological result were initiated on ART prior to awaiting confirmatory results. For those neonates following up at RMMCH, confirmatory testing was performed using the same qualitative HIV-1 PCR assay and/or a viral load (VL) test on EDTA anti-coagulated whole blood and plasma, respectively. Neonates with indeterminate or discordant results were retested at each clinic visit until a definitive HIV-1 status was determined. Qualitative PCR testing outside of RMMCH was performed on either EDTA anti-coagulated whole blood or whole blood dried blood spot (DBS) samples, depending on access to phlebotomy services. All mothers of neonates who underwent birth testing and received a negative result were recommended to follow up for additional testing to detect possible intra-partum and post-partum infection according to national guidelines [1].

Laboratory methods

Qualitative HIV-1 PCR and VL testing were performed at accredited (ISO 15189:2012) diagnostic laboratories using the qualitative and quantitative versions of the COBAS® AmpliPrep/COBAS® TaqMan (CAP/CTM) HIV-1 Test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA). The CAP/CTM is a total nucleic acid real-time reverse transcriptase PCR assay that detects HIV-1 proviral DNA and RNA on whole blood, and HIV-1 RNA only on plasma, with a limit of detection of approximately 300 RNA copies/ml and limit of quantification of 20 RNA copies/ml, respectively [11,28]. All non-negative virological results were interpreted according to standard criteria used within the National Health Laboratory Service (NHLS) to distinguish clearly positive from indeterminate results. All qualitative HIV-1 PCR results with a cycle-threshold value of ≤33 and a relative fluorescence intensity ≥5, and VL results with a quantified or higher than the linear range result (>7 log RNA copies/ml) were defined as clearly positive. Hence, all non-negative virological results with a cycle-threshold of >33 and/or relative fluorescence intensity of <5 and VL results where RNA was detected but below the linear range of the assay (i.e. <1.3 log RNA copies/ml or <2.0 log RNA copies/ml for those samples that required a 1:5 dilution due to inadequate volume) were interpreted as indeterminate [29,30]. As a means for controlling for sample swap, genetic profiling of short tandem repeat loci was performed using the PowerPlex® 16 HS System (Promega Corporation, Madison, WI, USA) on the birth and subsequent samples of a patient who had a clearly positive virological result at birth followed by negative results on subsequent clinic visits.

Classifying HIV-1 status

The final diagnostic status of infants who received a non-negative HIV-1 PCR result was classified as follows:

- Positive HIV-1 infection status was based on two clearly positive virological results from samples taken at two different time points.
- Negative HIV-1 infection status was defined as an isolated indeterminate result followed by at least two negative confirmatory virological results taken at two different time points whilst not on combination ART.
- Uncertain HIV-1 infection status was defined as neonates with non-negative virological results that did not meet criteria for confirmed positive or negative infection status after repeat testing.
- Lost to follow-up (LTFU) was defined as patients who did not have sufficient follow-up testing to meet any of the above criteria.

We examined whether maternal factors, including VL, CD4 cell count or duration of ART pre-delivery, or infant factors, including age at screening test, age at diagnostic confirmation, VL and relation to commencement of daily dose nevirapine (ddNVP), were related to the screening or confirmatory testing outcomes in any way. All mothers identified as HIV-infected who delivered at RMMCH between June 2014 and August 2016 were invited to sign a data sharing informed consent form, approved by the Human Research Ethics Committee of the University of the Witwatersrand (M130653, M140760, M140555 and M140639). Clinical and laboratory data, recorded on paper, were captured into a routine REDCap database [31]. Data were analysed using SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA), and descriptive methods were used to present frequencies of events, medians and interquartile ranges (IQRs), the Cochran–Armitage Trend test to assess trends of indeterminate result outcomes and Kaplan–Meier method to assess time to diagnosis. We describe the events along the diagnostic process. Cases with complex or uncertain diagnostic events had file reviews and are presented as brief case reports.

Results

A total of 5743 (91%) of the 6309 HIV-exposed neonates born at the hospital were enrolled in the study of which 104 (1.8%) received a non-negative HIV-1 PCR result at birth. Of 102 (98%) neonates with laboratory data available, 78 (76%) were classified as positive and 24 (24%) were indeterminate according to laboratory criteria. After confirmatory testing, 83 (81%) infants were confirmed HIV-1 infected, amounting to an intrauterine transmission rate of approximately 1.4%, and 8 (8%) infants were assigned a negative HIV-1 infection status. The HIV-1 status of an additional 5 (5%) infants remains uncertain and 6 (6%) were LTFU. The positive predictive value (excluding cases of uncertain diagnosis and inadequate testing) following a non-negative HIV-1 PCR screening test at birth (i.e. all detected virological results) was 0.91 (83/91; 95% confidence interval: 0.85–0.96), and this increased to 1.0 when using NHLS cutoff values to distinguish positive from indeterminate results.

Amongst the 83 infants who were confirmed HIV-1 infected, 74 had a positive and 9 had an indeterminate HIV-1 PCR result at birth (Table 1). Of the 74 neonates

Table 1. Steps in establishing final HIV-1 infection status of 102 infants with non-negative birth PCR results

First PCR result		Second PCR result			Earliest VL result		Final HIV-1 infection status,† *N* (%)			
Birth HIV-1 PCR test	*n*	Result	*n*	Age (days)‡	*n*	VL (log RNA copies/ml)‡	Age (days)‡	Positive	Uncertain	Negative	LTFU
Positive	78	Positive	68	2 (1–9)	66	4.48 (3.4–5.4)	2 (1–8)	68			
		Indeterminate	4	4, 4, 8, 40	4	<1.3[e], 2.58, 4.09, 4.56	68, 8, 4, 4	3	1[e]		
		Not tested	6		3	4.30, 5.04, 6.62	1, 4, 94	3			3
		Total birth HIV-1 PCR positive results						74 (95)	1 (1)		3 (4)
Indeterminate	24	Positive	5	6 (6–12)	4	2.29, 2.96, 3.05, 4.45	12, 0, 6, 1	5			
		Indeterminate	7	8 (6–24)	6	TND[i], 3.1 (3.0–3.2)	2 (1–8)	4[a–d]	2[g,i]		1
		Negative	11	7 (3–11)	10	TND (*n* = 9), 2.82[f]	8 (4–10)		2[f,h]	8	1
		Not tested	1		0						1
		Total birth HIV-1 PCR indeterminate results						9 (38)	4 (17)	8 (33)	3 (13)
Total, *n* = 102								83 (81)	5 (5)	8 (8)	6 (6)

†Positive HIV-1 infection status was defined as two positive virological results from samples taken at two different time points. Negative HIV-1 infection status was defined as an isolated indeterminate result followed by at least two negative virological results taken at two different time points whilst not on combination ART. Uncertain HIV infection status was defined as initial non-negative virological results that did not meet either of the above criteria. Lost to follow-up (LTFU) was defined as insufficient follow-up testing to meet the above criteria.
‡Individual results are displayed for ≤4 cases and median (interquartile range (IQR)) for ≥5 cases.
PCR: polymerase chain reaction; VL: viral load; TND: target not detected.
The superscripts [a–d] in column Positive and [e–i] in column Uncertain indicate the cases presented in more detail in Figure 1(a,b), respectively.

who screened positive, 72 had a retrievable confirmatory VL result, with a median value of 4.5 log copies/ml (IQR: 3.4–5.4), which was significantly higher than the 9 neonates with indeterminate results at birth who had a median VL of 3.0 log copies/ml (IQR: 2.8–3.2, p = 0.0007). All eight neonates who were diagnosed as uninfected had an isolated indeterminate screening result with at least two subsequent undetected virological results. Three of these infants repeatedly tested negative following all ART exposure cessation (including ddNVP prophylaxis and potential ingestion in breast milk of maternal ART) and five tested negative whilst still on ddNVP prophylaxis. Excluding the six infants who were LTFU, infection status could be clearly confirmed in the majority of cases (87 (91%) of 96 cases) by repeat virological testing on follow-up. However, in 9 (9%) of 96 cases a clear diagnosis could not be made on immediate follow-up on account of testing yielding repeatedly indeterminate virological results or negative results within the context of combination ART pressure. In four of the nine cases (cases a–d, Figure 1(a)), ongoing testing eventually confirmed a positive HIV-1 infection status while in five cases (cases e–i, Figure 1(b)) the diagnosis remains uncertain despite further testing.

Samples for HIV-1 PCR screening at birth were taken at a median age of 14 h (IQR: 7–23) and were not significantly different (p = 0.52) between neonates with a positive birth result as compared to neonates with an indeterminate result (Table 2). However, time to diagnosis was significantly later for infants with an indeterminate screening result (p < 0.0001), which was confirmed as either infected or uninfected on a sample taken at a median age of 32 days (IQR: 14–180) by Kaplan–Meier analysis (Figure 2). For infected infants the time to confirmation was also significantly longer (p = 0.0004) for infants who had indeterminate HIV-1 PCR tests at birth (31 days (IQR:14–43)) compared to infants with a positive birth test (2 days (IQR: 1–8)). Duration of maternal antenatal ART exposure was not significantly different

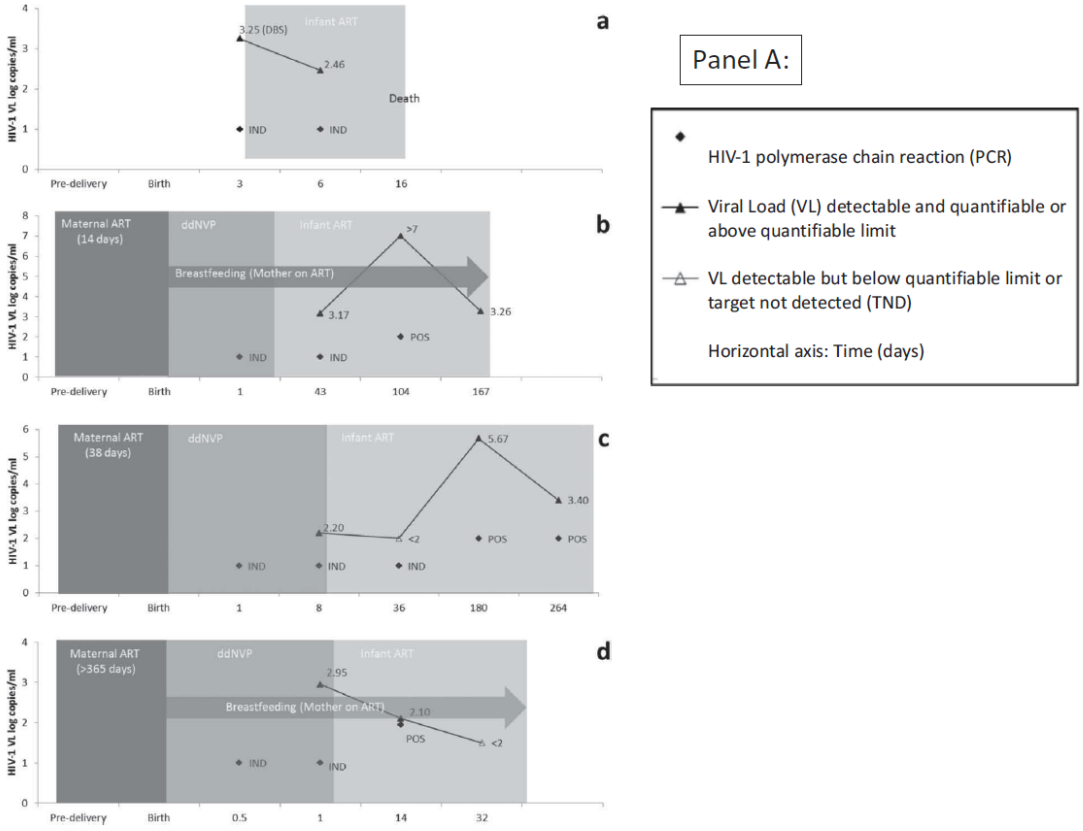


Figure 1. (A) and (B): HIV-1 PCR and viral load (VL) results in cases with positive (a–d) and uncertain HIV infection status (e–i), respectively. The time periods for which maternal antiretroviral therapy (ART), infant prophylaxis of daily dose nevirapine (ddNVP) and infant ART were given are represented by progressively lighter shades of grey. HIV-1 PCR tests were all done on whole blood and VL tests performed on plasma except where DBS is indicated. Due to space constraints some later repeat PCR negative or VL TND results were omitted (cases f–h).

DBS: dried blood spot; POS: positive; IND: indeterminate; NEG: negative; ART: antiretroviral therapy.

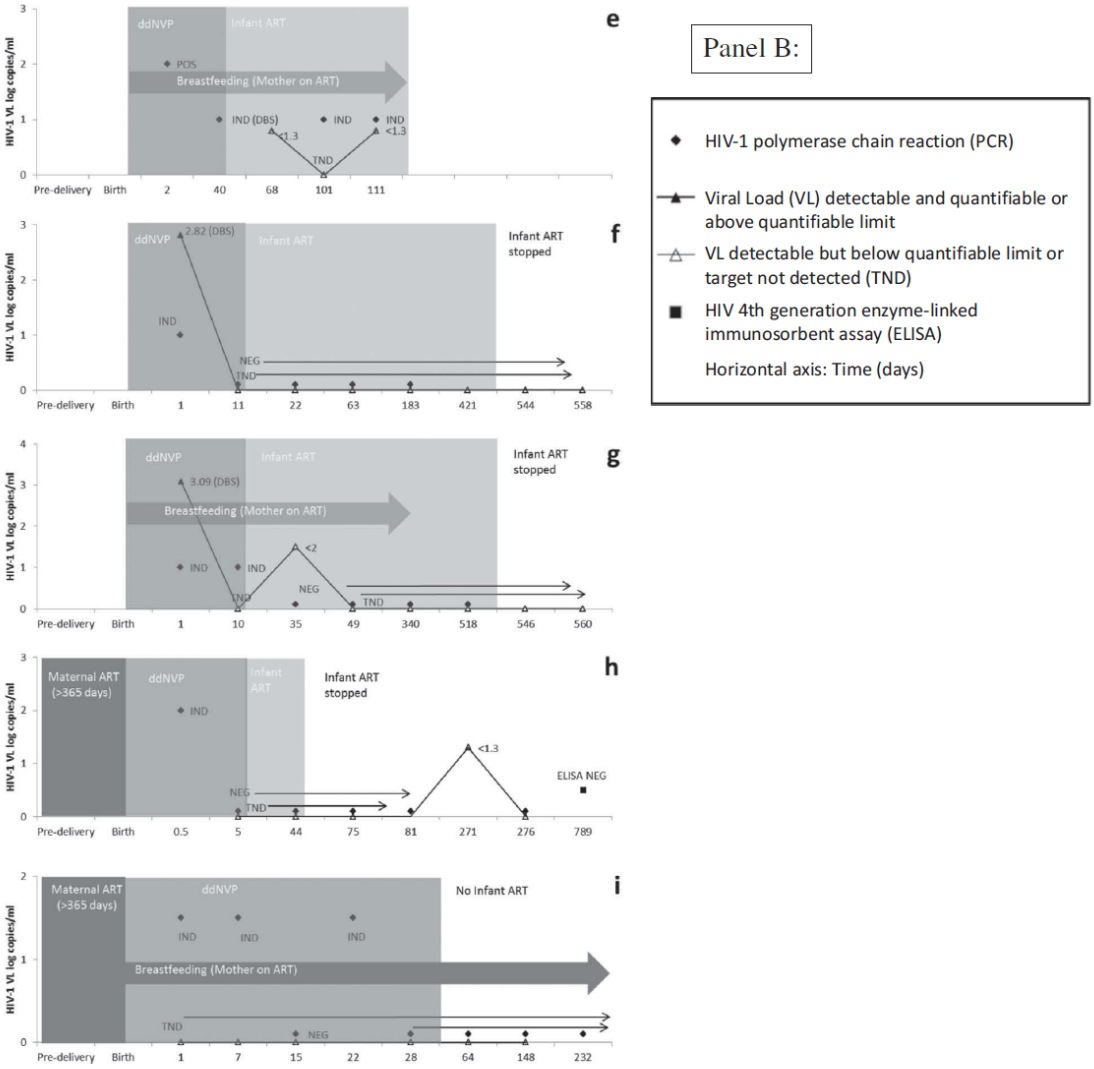


Figure 1. (continued).

between infants with a positive birth test as compared to infants with an indeterminate birth test, but maternal VL values were significantly different (p = 0.0013) with mothers of neonates with screening indeterminate results having a lower median baseline VL result (2.7 vs. 4.5 log copies/ml). There was a trend towards a higher CD4 count in mothers of neonates who tested indeterminate at birth but this was not statistically significant (p = 0.059). The probability of being confirmed as infected was 99% for infants with an initial screening positive result (one case remains with an uncertain diagnosis) versus 43% for infants with a screening indeterminate result (p < 0.0001). When stratifying neonates by final diagnostic status (positive, negative or uncertain), there was a significant difference only in maternal VL (p = 0.0008) when comparing those with a confirmed positive HIV-1 status (n = 68) median 4.5 (IQR: 3.4–5.1), uncertain HIV-1 status (n = 4) median 2.8 (IQR: 2.4–3.4) and confirmed negative HIV-1 status (n = 7) median 1.9 copies/ml (target not detected-3.9). Four (17%) of 24 neonates who tested indeterminate and 7 (9%) of 78 neonates who tested positive at birth (p = 0.28) died during the course of this study.

Cases a–d (Figure 1(a)) are all examples of confirmed infected infants where the diagnosis took longer to make on account of confirmatory indeterminate test results that were

Table 2. Associations between screening birth HIV-1 PCR results and maternal and infant factors

	HIV-1 PCR positive	HIV-1 PCR indeterminate	*p*
N	78	24	
No maternal ART exposure, *n* (column %)	25 (32)	5 (21)	0.29
Maternal ART exposure pre-delivery, *n* (%)	53 (68)	19 (79)	
0–12 weeks	21 (40)	6 (32)	0.48
12–26 weeks	20 (38)	6 (32)	
>26 weeks	12 (23)	7 (37)	
Median (IQR) weeks ART exposure	16 (7–23)	18 (3–135)	0.61
Maternal viral load (VL) data available, *n* (%)[a]	61 (78)	19 (79)	0.92
Median (IQR) log copies/ml	4.5 (3.7–5.0)	2.7 (1.9–4.3)	0.0013
Maternal CD4 cell count data available, *n* (%)	75 (96)	22 (92)	0.37
Median (IQR) maternal CD4 cell count (cells/μl)[a]	280 (168–472)	406 (264–608)	0.059
Median (IQR) age (hours) when birth sample taken	13.7 (8.6–19.7)	10.4 (5.3–20.9)	0.52
Nevirapine timing data available, *n* (%)	59 (76%)	18 (75%)	0.84
Blood for PCR was collected before NVP, *n* (%)	4 (7)	3 (17)	0.34
Median age (days) at final confirmation of HIV status (IQR)	2 (1–8)	32 (14–180)	<0.0001
Lost to follow-up, *n* (%)	3 (4)	3 (13)	0.14
Final status, *n* (%)			
Confirmed infected	74 (99)	9 (43)	<0.0001
Confirmed uninfected	0	8 (38)	
Uncertain	1 (1)	4 (19)	

[a]Median time of maternal VL (0.2 weeks after delivery (IQR: 0–2)) and CD4 (5 weeks before delivery (IQR: 15 weeks before–0.2 weeks after)) blood draws relative to delivery were not significantly different between the groups for each test. PCR: polymerase chain reaction; ART: antiretroviral therapy; IQR: inter-quartile range.

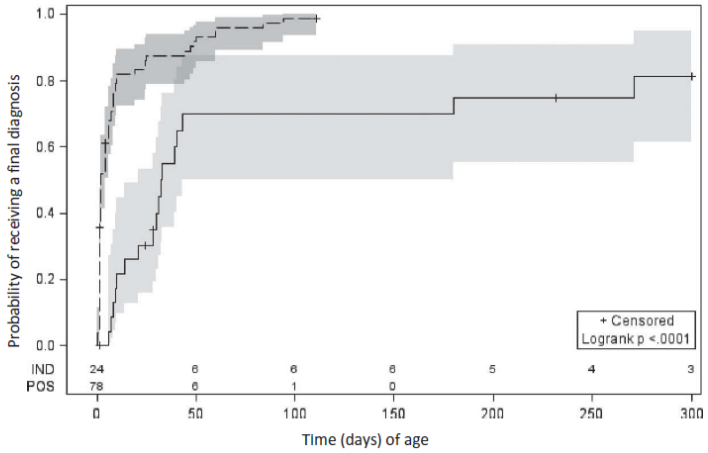


Figure 2. Kaplan–Meier curves of time to successful diagnosis (infected or uninfected) comparing infants with screening birth PCR positive (*n* = 78) to indeterminate (*n* = 24) results.
POS: positive (dashed line), IND: indeterminate (solid line) HIV-1 polymerase chain reaction (PCR) screening result with shaded areas representing 95% confidence intervals and numbers at risk above the *x*-axis. Censoring occurred at last test where diagnosis remained uncertain or at last visit date where loss to follow-up occurred.

unable to provide an immediate definitive diagnosis. In all four cases, infants repeatedly tested HIV-1 PCR indeterminate associated with low-grade viraemia (around 2–3 log copies/ml).

The HIV-1 status of cases e–i remains uncertain (Figure 1(b)) because virological detection was noted in at least two separate samples, by virtue of a combination of positive or indeterminate HIV-1 PCR results and low but quantifiable or indeterminate VL results. However, the virological test results have not fulfilled the criteria for a positive HIV-1 infection status. The exception is case f who demonstrated virological detection on a single sample at birth that tested HIV-1 PCR indeterminate with a VL of 2.82 copies/ml on DBS. On account of the uncertain diagnosis associated with a single quantifiable virological result in this case, genetic profiling was performed which confirmed that the birth sample belonged to the correct patient and ruled out the possibility of a sample swap or contamination with another patient's sample. Cases f and g are similar in that both had an HIV-1 PCR indeterminate result at birth that was associated with a quantifiable VL, using leftover whole blood stored on a DBS card from the birth sample. Both cases were not exposed to maternal ART during the antenatal period. In case g the indeterminate result at birth was followed by an indeterminate result at 10 days of age (during ddNVP exposure) and an indeterminate VL at 35 days of age that was detectable but not quantifiable (<2 log copies/ml). All subsequent virological testing was negative. In both cases f and g, ART was stopped at 76 weeks of age and both have since had at least two undetected virological test results at ±4 weeks off treatment. In case h, a birth indeterminate result was followed 270 days later by a single indeterminate VL and this case is being monitored closely. Case e, who was not followed up on site, had three indeterminate results after the birth PCR positive result, the first of which was tested on a DBS card and occurred during ddNVP exposure while the latter two occurred during combination ART exposure. To date, the patient has never had a quantified VL result on ART. Whereas case i is the only case with an uncertain diagnosis who was not initiated on combination ART, treatment was subsequently stopped for cases f–h on account of inadequate evidence to confirm a positive HIV-1 infection status. None of these cases had shown any evidence of rebound.

Discussion

Whereas HIV-1 status could be confirmed in the majority of neonates who received a non-negative HIV-1 PCR result, there were significant delays and challenges associated with infants who tested indeterminate at birth, comprising 24% of all non-negative screening tests. This group of neonates were found to carry a significant risk of having a positive HIV-1 infection status, confirmed in 43% of cases, and required extensive follow-up beyond a standard once off confirmatory test. Furthermore, four neonates who tested indeterminate at birth remain with an uncertain diagnosis. Overall, 25–30% of infants with a non-negative result required a diagnostic phase of management that extended beyond a single repeat test.

Considering the high morbidity and mortality associated with HIV-1 infection amongst infants, and considering 4 out of 24 (17%) infants who tested indeterminate at birth died during the course of this study, the need for a rapid definitive diagnosis is clearly of paramount importance. However, with similar proportions of neonates who tested indeterminate at birth found to have a positive and negative HIV-1 status on subsequent testing, and initiation of treatment prior to confirmatory testing known to confound diagnosis, a balance needs to be struck between effectively managing suspected HIV-infection and unnecessarily committing a patient to ART. Although NHLS cutoff values used to distinguish positive from indeterminate results are associated with an improved positive predictive value of the assay, they are also necessarily associated with an increase in delayed diagnosis and uncertain HIV-1 status that requires close monitoring with repeated testing. Furthermore, indeterminate results pose difficulties not only for clinicians but also for primary caregivers and the family of infants given an uncertain HIV-1 diagnosis. Importantly, these caregivers may lose confidence in clinicians and the healthcare system in general if clinical staff are unable to provide a clear and timely diagnosis.

Although it remains to be determined whether indeterminate HIV-1 PCR results are more common at birth, indeterminate results are not a phenomenon associated solely with birth testing. Rather they occur within all age groups in which HIV-1 PCR testing is performed and have been described as a leading cause of non-negative results within South Africa's EID programme prior to the introduction of birth testing, comprising on average 16% of all non-negative results [18,32]. More data are needed to assess how birth testing affects the rates of indeterminate results. Whereas concerns surrounding sample swap and contamination are valid, and possibly account for some indeterminate results, they do not comprise the majority of such cases. Similarly, indeterminate results cannot simply be accounted for by citing a reduction in the positive predictive value of diagnostic assays within the context of declining mother-to-child transmission rates. Clearly, other factors are associated with uncertain and delayed diagnosis amongst HIV-infected neonates. Our study demonstrates a correlation between lower maternal and infant VL results in relation to indeterminate HIV-1 PCR results, suggesting that mechanisms of virological control, including ART and immunological factors, need to be considered when dealing with EID challenges. This further highlights the importance of time of testing, especially considering cases which tested positive at birth but received indeterminate confirmatory results during ART exposure. Sensitivity and specificity of HIV-1 PCR assays for EID were not measured in our study but sensitivity appears to be decreased by maternal and infant prevention of mother-to-child transmission prophylaxis [15–17], and the high proportion of indeterminate results in our study suggests a need for more sensitive and specific assays.

A total of 5 infants (5%), out of 102 neonates with a non-negative HIV-1 PCR result at birth, remain with an uncertain diagnosis. Three of these cases (f, g and h) tested indeterminate at birth and were started on ART on the day of confirmatory testing. This practice was based on findings from the previous version of the current assay that neonates with a non-negative HIV-1 PCR result at birth, whether positive or indeterminate, were invariably found to have a confirmed HIV-1 positive infection status [27]. However, once it was determined that this was not the case with the more sensitive CAP/CTM v2.0 assay, this practice was stopped. All three of these infants have since followed up on site, and treatment has been interrupted under close clinical supervision. The diagnosis of these infants remains uncertain as it has yet to be determined what the required length of time is for monitoring post-treatment cessation in order to exclude HIV-1 infection [24]. Case e represents the only infant with an uncertain HIV-1 status where combination ART has not been stopped and is also the only case, amongst those with an uncertain diagnosis, that tested HIV-1 PCR positive at birth. It is worth noting that this infant is being followed up outside of the study setting and that confirmatory testing was performed on a DBS sample only, without a simultaneous VL test, and that the volume of blood tested on a DBS sample (approximately 60 µl) is less than that used to test EDTA anti-coagulated whole blood samples (100 µl) and this may have had an impact on the confirmatory result.

As a collective, the diagnostic challenges described in this study raise important questions concerning EID, including the potential for antiretroviral prophylaxis to be associated with virological control and even "functional-cure"-type scenarios [9]. Furthermore, infants with multiple indeterminate virological results followed by loss of detectability raise fundamental questions regarding the mechanism of post-exposure prophylaxis and the possibility of transient or abortive infectious processes. Similarly, it remains to be determined whether isolated indeterminate results represent false detection or true infection associated with faster virological control.

Conclusions

Whereas the majority of neonates with a positive HIV-1 PCR test at birth were confirmed to be HIV-1 infected, indeterminate results were associated with uncertainty and diagnostic delay. Although indeterminate results comprised 24% of all non-negative birth tests in this study, true ongoing diagnostic dilemmas were rare with most cases resolving on repeat testing, and approximately half of these having a confirmed positive diagnosis and half confirmed negative. In some of these cases a quantifiable VL result confirmed the diagnosis whereas the repeat HIV-1 PCR test was indeterminate, suggesting a combination of virological testing methods may be beneficial when confirming HIV-1 infection. Essentially, the clinical requirements and social consequences of managing an infant with an indeterminate HIV-1 result make it critical that a timely and unequivocal diagnosis is established by the treating clinician and effectively communicated to the primary caregiver.

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Competing interests
The authors have no conflicts of interest to disclose.

Authors' contribution
KT, LK, AC, GGS, RS, CTT, EJA: study design; KT, RS, FP, MC, NM, LH, AHM: data collection; KT, AHM, LK, GGS, RS, CTT, PMM, SS, EJA, FP, LH: data interpretation and analysis; KT, AHM, GGS, AC, LK, PMM, SS, LH, RS, FP, MC, NM, CTT, EJA: writing.

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Article 4:

Title: Twelve month outcomes of HIV-infected infants identified at birth: An observational cohort study at one maternity site in Johannesburg, South Africa

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12-month outcomes of HIV-infected infants identified at birth at one maternity site in Johannesburg, South Africa: an observational cohort study

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Summary

Background Initiation of antiretroviral therapy (ART) following diagnosis of HIV infection at birth is an emerging area of paediatric HIV care. We present outcomes of HIV-infected infants identified at birth at Rahima Moosa Mother and Child Hospital in Johannesburg, South Africa.

Methods From September, 2013 (era 1), only high-risk HIV-exposed infants were offered diagnostic HIV PCR tests at birth. From June, 2014 (era 2), all HIV-exposed infants were offered laboratory-based diagnostic PCR tests. From October, 2014 (era 3), point of care (POC) diagnostic PCR tests were also done if staff availability allowed. We describe time to ART initiation, mortality, retention in care, and viral suppression among the HIV-infected infants identified across these eras.

Findings We tested 5449 HIV-exposed infants who were born between Sept 1, 2013, and June 30, 2016. 88 neonates with confirmed HIV infection were identified and included in the study, of which 86 (98%) started ART. Median age at ART initiation decreased from 9 days (IQR 6–25) in eras 1 and 2 to 2 days (1–8) in era 3. In era 3, more neonates who were co-tested with POC testing started ART within 48 h of birth (29 [83%] of 35; median 1 day [IQR 1–2]) than infants who were not co-tested (one [4%] of 29; median 6 days [5–10]). The probability of mortality by 12 months across the eras was 14% (95% CI 8–24) and did not differ by era. Of the 72 infants who survived and initiated ART at the site, 56 (78%) were retained at 12 months. Of the 56 infants retained in care, 40 (71%) had a viral load less than 400 copies per mL at 12 months, with no differences between eras (p=0·23).

Interpretation HIV-infected infants can be identified at birth and ART can be initiated within hours to days. Although most infants in our cohort started ART, mortality remained unacceptably high with suboptimal retention and viral suppression. Reducing mortality and improving retention and viral suppression remain urgent priorities.

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Introduction

In the absence of antiretroviral therapy (ART), HIV-infected infants are at high risk of early mortality. Estimates show that almost a quarter of these infants die before 3 months of age and up to half by 2 years of age.[1] South African guidelines have recommended immediate initiation of ART in all HIV-infected infants upon diagnosis regardless of immunological or clinical status since the landmark Children with HIV Early antiRetroviral therapy (CHER) study.[2] However, in 2017, ART coverage among HIV-infected children in South Africa was estimated to be only 55%.[3] Care for HIV-infected infants through the cascade of diagnosis, result return, ART initiation, and successful retention in long-term care is challenging.[4–6] We previously showed that even though birth testing misses intrapartum and postnatal infections, it identifies a larger total number of infected infants than when testing is done later, because of high rates of death and loss to follow-up.[7]

Early initiation of ART for neonates requires consideration of many practical and biological challenges inherent in early infant diagnosis (EID). We assessed whether birth testing for HIV improves EID coverage, leading to increases in rates of early ART initiation and reductions in HIV-related mortality.[8,9] Here, we assess whether the introduction of different birth testing approaches was associated with improved infant outcomes (time of ART initiation, survival, retention in care, and viral suppression).

Methods

Study design and participants

In this observational cohort study, we describe 12-month outcomes among infants confirmed to have HIV infection at birth at Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa. RMMCH is an academic paediatric referral centre and

Research in context

Evidence before this study

We searched PubMed and conference proceedings using the search terms "HIV", "ART", and "early infant treatment" for English language articles published up to March 30, 2018. HIV infection in infants has high mortality in the absence of suppressive antiretroviral therapy (ART). Early infant diagnosis has evolved to include diagnosis at birth, which enables identification of infants at birth who are presumed to have acquired infection in utero and are thought to be at risk of poorer outcomes.

Added value of this study

This study presents a large cohort of HIV-infected infants identified at birth at a single site in the context of high maternal ART coverage and a maturing prevention of mother-to-child transmission programme. Previous cohorts have generally described HIV-infected infants identified later, which inevitably excludes early mortality. Improvement of EID services and judicious use of point of care (POC) technology enables very early access to ART, but ART initiation in the absence of POC testing is also achievable within days of birth. Mortality, retention, and suppression require specific attention particularly in infants with high pretreatment viral loads.

Implications of all the available evidence

ART can be initiated very early in life, but reduction of mortality and long-term retention in care and viral suppression remain challenging. This difficulty is probably due to both biological and psychosocial factors, including health-seeking behaviour characteristics of these mothers and infants. POC technology and improvements in EID enable earlier initiation of ART, but further efforts are needed to strengthen long-term success.

functions as the main delivery facility and obstetric referral site for complicated cases of a subdistrict of Johannesburg. Maternal HIV prevalence at RMMCH was 23% during the study period.[8] We compared infant outcomes by changes in the EID programme that were implemented over this time.

From Sept 1, 2013, the EID programme expanded beyond the 6-week PCR HIV test, which was the recommendation at the time, to include a PCR test as close to birth as possible for high-risk neonates (era 1: targeted testing). High risk was defined as preterm (<37 weeks), low birthweight (<2500 g) or neonatal admissions, or a combination of these. Thus, during era 1, only a subset of the HIV-exposed neonates were eligible for birth testing. From June 5, 2014, the EID programme expanded further to test all HIV-exposed neonates born at the maternity service (era 2: universal testing).[8] From Oct 16, 2014, when staff availability allowed, point of care (POC) testing was added (era 3: universal testing, POC testing available).[9] POC staffing was limited during weekends and public holidays. EID coverage (proportion of eligible HIV-exposed infants tested) was about 66% during era 1 and 93% for birth testing overall in eras 2 and 3, and 51% for POC testing during era 3.[8,9]

The Human Research Ethics Committee of the University of the Witwatersrand and the Institutional Review Board of Columbia University approved the study. Mothers or legal guardians signed written informed consent for their own and their infant's participation.

Procedures

HIV PCR testing was done on whole blood sampled by venepuncture and tested at the national laboratory using the COBAS TaqMan HIV-1 Qualitative Test, version 2.0 (Roche Molecular Systems, Branchburg, NJ, USA) during eras 2 and 3. Version 1 of this assay was used during era 1. We used the Xpert HIV-1 Qual qualitative assay (Cepheid; Sunnyvale, CA, USA) for POC testing.[9] In all eras, neonates with positive or indeterminate results on the standard laboratory-based PCR were actively traced and repeat tested to confirm diagnosis with a repeat diagnostic PCR and a quantitative HIV RNA viral load (COBAS AmpliPrep/COBAS TaqMan HIV-1 test, version 2.0). HIV infection was considered confirmed when a second sample resulted in a positive diagnostic PCR or viral load of more than 20 copies per mL.[10]

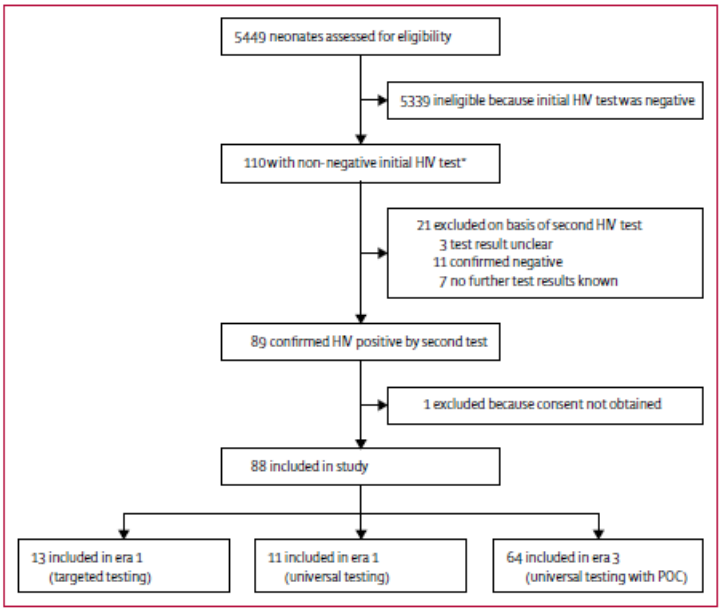


Figure 1: **Study population**

POC=point of care. ART=antiretroviral therapy. *First HIV total nucleic acid PCR result was either indeterminate or positive; confirmation of infection required two detectable quantifiable HIV viral loads or two positive total nucleic acid test results on two separate samples.

	Era 1: targeted testing (n=13)	Era 2: universal testing, no POC testing available (n=11)	Era 3: universal testing, POC testing available (n=64)	p value	Era 3: universal testing, not POC co-tested (n=29)	Era 3: universal testing, POC co-tested (n=35)	p value
Sex							
Female	6 (46%)	9 (82%)	33 (52%)	0·14	18 (62%)	15 (43%)	0·13
Male	7 (54%)	2 (18%)	31 (48%)	..	11 (38%)	20 (57%)	..
Birthweight (g)							
Range	1080–2438	2110–3425	905–3805	..	905–3805	1860–3710	..
Mean (SD)	1846 (470)	2776 (385)	2664 (653)	0·0003	2419 (760)	2867 (468)	0·008
Missing data	1 (8%)	0	0	..	0	0	..
Antiretroviral therapy during this pregnancy							
Yes, started before pregnancy and continued	2 (15%)	1 (9%)	6 (9%)	0·89	4 (14%)	2 (6%)	0·42
Yes, during pregnancy, treatment duration ≥12 weeks	4 (31%)	2 (18%)	19 (30%)	..	8 (28%)	11 (31%)	..
Yes, during pregnancy, treatment duration <12 weeks	5 (38%)	6 (55%)	21 (33%)	..	9 (31%)	12 (34%)	..
Yes, during pregnancy, unknown when	0	0	1 (2%)	..	0	1 (3%)	..
None	0	1 (9%)	10 (16%)	..	3 (10%)	7 (20%)	..
Unknown	2 (15%)	1 (9%)	7 (11%)	..	5 (17%)	2 (6%)	..
Gestational age							
≥37 weeks (term)	5 (38%)	9 (82%)	47 (73%)	0·066	16 (55%)	31 (89%)	0·011
<37 weeks (preterm)	7 (54%)	2 (18%)	12 (19%)	..	9 (31%)	3 (9%)	..
Missing data	1 (8%)	0	5 (8%)	..	4 (14%)	1 (3%)	..
First PCR							
Positive	9 (69%)	9 (82%)	61 (95%)	0·012	26 (90%)	35 (100%)	0·051
Indeterminate	4 (31%)	2 (18%)	3 (5%)	..	3 (10%)	0	..
Pretreatment viral load							
Median (IQR)	190 546 (1995–467 735)	2045 (530–36 180)	34 357 (7602–262 493)	0·12	40 015 (1778–229 087)	32 027 (9849–284 175)	0·92
Range	240–1 122 018	155–65 760	60–4 950 000	..	220–4 861 620	60–4 950 000	..
Pretreatment viral load (copies per mL)							
<100 000	6 (46%)	9 (82%)	35 (55%)	0·061	14 (48%)	21 (60%)	0·037
>100 000	7 (54%)	0	21 (33%)	..	8 (28%)	13 (37%)	..
Missing	0	2 (18%)	8 (13%)	..	7 (24%)	1 (3%)	..
Admission immediately after delivery							
Discharged to mother after delivery	7 (54%)	10 (91%)	43 (67%)	0·40	13 (45%)	30 (86%)	0·0019
Required admission due to neonatal condition	5 (38%)	0	16 (25%)	..	13 (45%)	3 (9%)	..
Required admission due to maternal condition	0	0	2 (3%)	..	2 (7%)	0	..
Missing	1 (8%)	1 (9%)	3 (5%)	..	1 (3%)	2 (6%)	..

Data are n (%) unless otherwise specified. POC=point of care.

Table 1: **Characteristics of the cohort of 88 neonates with confirmed HIV infection**

ART was started presumptively as soon as possible after the first available positive PCR result. The confirmatory sample was taken at this time but ART was not delayed to wait for the result. During era 1, lopinavir–ritonavir, lamivudine, and zidovudine was generally the preferred first-line regimen. In light of growing concerns about lopinavir–ritonavir use in preterm infants, hospital guidance was revised to recommend zidovudine, lamivudine, and nevirapine as first-line ART for neonates. Nevirapine was replaced with lopinavir–ritonavir after 42 weeks post-menstrual age and 14 days of calendar age or later based on patient readiness as assessed by the clinician in charge of the patient. Co-trimoxazole prophylaxis was initiated at 4–6 weeks of age. Follow-ups were done weekly or fortnightly in the first month of life, monthly until 6 months of age, and then every 3 months, unless clinical concerns necessitated more frequent visits. Loss

	Era 1: targeted testing (n=13)	Era 2: universal testing (n=11)	Era 3: universal testing, POC testing available (n=62)	p value	Era 3: universal testing, not POC co-tested (n=27)	Era 3: universal testing, POC co-tested (n=35)	p value
ART start location							
On site	12 (92%)	9 (82%)	60 (97%)	0·31	25 (93%)	35 (100%)	0·07
Not on site	1 (8%)	2 (18%)	2 (3%)	..	2 (7%)	0	..
Age at start of ART, days							
Mean (SD)	59 (150)	21 (24)	12 (25)	0·0002	18 (26)	8 (24)	0·15*
Median (IQR)	9 (6–25)	9 (6–25)	2 (1–7)	..	6 (5–10)	1 (1–2)	..
Range	3–555	2–74	0–104	..	2–95	0–104	..
Age category at start of ART							
<48 h	0	1 (9%)	30 (48%)	0·0007	1 (4%)	29 (83%)	<0·0001
48 h to 7 days	5 (38%)	2 (18%)	17 (27%)	..	14 (52%)	3 (9%)	..
≥8 days	8 (62%)	8 (73%)	15 (24%)	..	12 (44%)	3 (9%)	..
Starting regimen							
Lopinavir–ritonavir, lamivudine, and abacavir	0	0	5 (8%)	<0·0001	3 (11%)	2 (6%)	0·73
Lopinavir–ritonavir, lamivudine, and zidovudine	6 (46%)	0	1 (2%)	..	0	1 (3%)	..
Nevirapine, lamivudine, and zidovudine	5 (38%)	9 (82%)	51 (82%)	..	21 (78%)	30 (86%)	..
Nevirapine, lamivudine, and stavudine	0	0	2 (3%)	..	1 (4%)	1 (3%)	..
Other	1 (8%)	1 (9%)	1 (2%)	..	1 (4%)	0	..
Unknown	1 (8%)	1 (9%)	2 (3%)	..	1 (4%)	1 (3%)	..

Data are n (%) unless otherwise specified. Two of the 88 infants with confirmed HIV infection in era 3 who were not POC co-tested were not known to have started ART, so are not included here. POC=point of care. ART=antiretroviral therapy. *Wilcoxon two-sample test p<0·0001.

Table 2: **Initiation of ART among the 86 neonates with confirmed HIV infection who started ART**

to follow-up was defined as not returning to the site within 90 days of the last appointment.

Outcomes

We compared infant outcomes between the three EID programme eras. The primary focus was on era 3, which was further stratified into those who were and were not co-tested with POC testing. We compared baseline characteristics, age at ART initiation, and mortality and retention in care up to 12 months. Viral suppression was examined at visits closest to 6 months (between 3 and 8 months) and 12 months (between 9 and 14 months) after ART initiation.

Statistical analysis

We present characteristics of infants using descriptive statistics (median, IQR, proportions, and 95% CIs). Comparisons were tested using χ^2 and Fisher's exact tests for proportions, and Student's *t* test and Wilcoxon signed-rank tests for continuous variables. Survival was analysed by Kaplan-Meier analysis and log-rank tests. Analyses were done using SAS (version 9.4).

Role of the funding source

The funder was involved in the study design and monitoring but was not involved in analysis and interpretation of data, writing of the report, or the decision to submit the paper for publication. The corresponding author had access to the data and takes final responsibility for the decision to submit.

Results

5449 infants born between Sept 1, 2013, and June 30, 2016, were assessed for eligibility for the study. During era 1, 201 HIV-exposed neonates were tested, of which 15 (7%) had either positive or indeterminate initial results. One infant with an initial indeterminate result was confirmed negative, and for one infant, no consent was available. 13 infants had confirmed HIV infection and were included in analyses for era 1 (figure 1). During era 2, 798 HIV-exposed neonates were tested, of which 15 (2%) had either positive or indeterminate initial results. Two infants with an initial indeterminate result were confirmed negative, and two with initial positive results did not return for confirmatory testing. 11 infants had confirmed HIV infection and were included in analyses for era 2. During era 3, 4450 HIV-exposed neonates were tested, of which 80 (2%) tested positive or indeterminate initially. Five infants did not have confirmatory testing, three had a second indeterminate result, and eight who had an initial indeterminate result were confirmed negative. 64 infants had confirmed HIV infection and were included in analyses for era 3. Of the 64 infants with confirmed HIV infection in era 3, 29 (45%) were not co-tested with POC testing and 35 (55%) were.

Mean birthweight in the era 1 population was low (1846 g), with a high percentage preterm (seven [54%] of 13 infants; table 1). In era 3, infants not co-tested with POC testing had lower mean birthweights than those who were co-tested. Furthermore, a higher proportion of infants who were not co-tested were premature or

	Era 1: targeted testing (n=13)	Era 2: universal testing (n=11)	Era 3: universal testing, POC testing available (n=64)	p value	Era 3: universal testing, not POC co-tested (n=29)	Era 3: universal testing, POC co-tested (n=35)	p value
At 6 months (180 days)							
Retained in care on site	8 (62%)	8 (73%)	45 (70%)	0·53	21 (72%)	24 (69%)	0·17
Started or moved to care elsewhere before 6 months	2 (15%)	2 (18%)	3 (5%)	..	2 (7%)	1 (3%)	..
Died	2 (15%)	0	8 (13%)*	..	5 (17%)*	3 (9%)	..
Lost to follow-up	1 (8%)	1 (9%)	8 (13%)	..	1 (3%)	7 (20%)	..
At 12 months (365 days)							
Retained in care on site	6 (46%)	7 (64%)	43 (67%)	0·27	19 (66%)	24 (69%)	0·23
Started or moved to care elsewhere before 12 months	3 (23%)	2 (18%)	3 (5%)	..	2 (7%)	1 (3%)	..
Died	2 (15%)	0	9 (14%)*	..	6 (21%)*	3 (9%)	..
Lost to follow-up	2 (15%)	2 (18%)	9 (14%)	..	2 (7%)	7 (20%)	..

Data are n (%). POC=point of care. ART=antiretroviral therapy. *One infant in this group died at another site at age 99 days shortly after starting ART during a hospitalisation having not returned to site to start ART after birth; one infant died on site at age 286 days before ART start having been lost to follow-up after birth.

Table 3: **Retention in care of the 88 neonates with confirmed HIV infection**

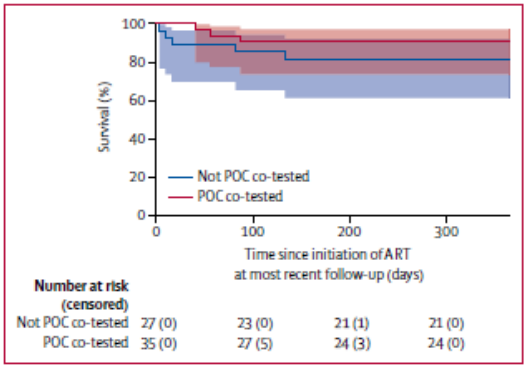


Figure 2: **Mortality in era 3 (universal testing) in the HIV-infected infants who were co-tested (n=35) and those who were not co-tested (n=27) with POC testing**

Lines show number at risk and shaded areas show 95% CI. ART=antiretroviral therapy. POC=point of care.

See Online for appendix

required hospital admission at delivery than those co-tested. Median pretreatment viral load was highest in the HIV-infected neonates identified in era 1 (table 1; p=0·12). In era 3, pretreatment viral loads were similar between those with and those without POC co-testing.

86 (98%) of 88 neonates confirmed to be infected with HIV initiated ART with no differences in uptake across eras. One neonate died before starting ART and one was lost to follow-up before ART could be initiated. Because confirmation of diagnosis required at least one further test, this statistic overestimates the success of starting ART given that six neonates had no follow-up testing. Among those with a confirmed diagnosis who initiated ART, the median age of initiation was 9 days (IQR 6–25) in eras 1 and 2, and 2 days (1–8) in era 3 (table 2). In era 3, 25 (86%) of 29 infants who did not have POC co-testing initiated ART at the site and two (7%) of 29 initiated ART elsewhere; whereas all 35 of the infants who did have POC co-testing initiated ART at the site. Among those initiating ART during era 3, the median age at initiation was 6 days (IQR 5–10) in those who were not co-tested compared with 1 day (1–2) in those who were co-tested. In era 3, of those initiating ART, 15 (56%) of 27 of those not co-tested started ART within 7 days compared with 32 (92%) of the 35 infants who were not co-tested; one (4%) of the infants who was not co-tested started ART within 48 h compared with 29 (83%) of those who were not co-tested (p<0·0001; table 2).

By 12 months of age, 11 (13%) of the 88 infants with confirmed HIV infection had died, and the probability of mortality by 12 months of age was 14% (95% CI 8–24; table 3; appendix). Of the 86 infected infants who initiated ART, ten have died by 12 months of age (nine infants who initiated ART at the site and one who had initiated elsewhere). One of the two infants with confirmed HIV infection who did not initiate ART died by 12 months of age. The estimates censor those lost to follow-up at their last visit and might be underestimates. The median age at death of infants after start of ART was 73 days (IQR 33–99; range 16–139; appendix). Probability of death by 12 months was higher in those with pretreatment viral load greater than 100 000 copies per mL (28%, 95% CI 14–49) than those with pretreatment viral load less than 100 000 copies per mL (7%, 2–19; p=0·01).

Probabilities of mortality by 12 months were 19% (95% CI 5–58) in era 1, no deaths in era 2, and 14% (7–26) in era 3. Although mortality was not significantly different whether or not the child had been co-tested with POC testing (a factor that changes the timing of ART initiation), the probability of death was double in those not co-tested, 19% (8–39), than in those co-tested, 9% (3–27; p=0·31; figure 2). Two of five deaths in infants not co-tested were in those born prematurely, whereas none of the three deaths in infants co-tested were in those born prematurely (appendix).

	Era 1: targeted testing (n=10)	Era 2: universal testing (n=9)	Era 3: universal testing, POC testing available (n=53)	p value	Era 3: universal testing, not POC co-tested (n=21)	Era 3: universal testing, POC co-tested (n=32)	p value
Measurement closest to 6 months (copies per mL)							
<400	4/8 (50%)	1/7 (14%)	27/47 (57%)	0·31	10/21 (48%)	17/26 (65%)	0·35
400-1000	1/8 (13%)	2/7 (29%)	5/47 (11%)	..	2/21 (10%)	3/26 (12%)	..
≥1000	3/8 (38%)	4/7 (57%)	15/47 (32%)	..	9/21 (43%)	6/26 (23%)	..
Measurement closest to 12 months (copies per mL)							
<400	4/7 (57%)	3/7 (43%)	33/42 (79%)	0·23	13/19 (68%)	20/23 (87%)	0·27
400-1000	0/7	0/7	1/42 (2%)	..	1/19 (5%)	0/23	..
≥1000	3/7 (43%)	4/7 (57%)	8/42 (19%)	..	5/19 (26%)	3/23 (13%)	..

Data are n/N (%). Denominators are number of infants for whom viral load results were available. POC=point of care.

Table 4: HIV viral load of the surviving 72 neonates with confirmed HIV infection at 6 and 12 months after antiretroviral therapy initiation on site

56 (64%) of 88 confirmed HIV-infected infants were retained in care at the site by 12 months (table 3). Of the 32 infants not retained in care on site at 12 months, five (16%) were reported to have initiated ART at other clinics (one of these five died), nine (28%) infants initiated ART on site and died (not having disengaged from care), three (9%) started ART at the site but were formally transferred to another site before 12 months, 13 (40%) infants started ART at RMMCH and were not known to have died but were lost to follow-up without transfer, and two (6%) did not start ART. If only the 72 infants who initiated ART on site and were not known to have died are included in the denominator, the proportion retained increases to 78%. In era 3, overall proportions of infants retained on site were similar between the era 3 subsets: 19 (66%) of 29 infants who were not co-tested with POC testing versus 24 (69%) of 35 infants who were (p=0·23; table 3).

12 months after the start of ART, 40 (71%) of the 56 infants who had an available viral load measurement had achieved viral suppression to less than 400 copies per mL (table 4). The proportion did not differ significantly across the three eras but was highest (33 [79%] of 42 infants) in era 3. Focusing on era 3, 13 (68%) of 19 infants who were not co-tested versus 20 (87%) of 23 who were co-tested achieved a viral load of less than 400 copies per mL at 12 months (p=0·18).

Discussion

Between September, 2013, and June, 2016, we strengthened the EID programme at one large hospital in Johannesburg, South Africa; first by adding birth testing to high risk HIV-exposed neonates only, second by expanding the birth testing to all HIV-exposed neonates and third, by introducing POC testing in addition to routine laboratory-based diagnostic testing. POC EID permitted earlier ART initiation in infants confirmed to be infected with HIV. However, even though a smaller proportion of infants who were co-tested with POC and had early ART died by 12 months compared with infants who were not co-tested, the 9% probability of mortality remains high. Retention in care in the POC co-tested infants was similar (69%) to the proportion in the infants who were not co-tested (66%) during the same era. These results highlight that the advantages of the resource-intensive efforts for very early diagnosis and treatment are substantially limited by infants who are lost to follow-up, many of whom probably died.

Some deaths, not known to us, might still have occurred in infants who were lost to follow-up. We excluded infants with unconfirmed HIV status, which included two cases of very early death both presumably due to prematurity. The non-significantly lower proportion of deaths in those co-tested with POC testing might be explained by differential selection of healthier children in the co-tested group. We previously reported that healthier infants (higher birthweight, fewer preterm, and fewer neonatal admissions) were more likely to be co-tested with POC testing,[9] because the logistics of screening the HIV-exposed births at the hospital mean that the less healthy neonates are more difficult to access and their fragile health condition often precluded additional laboratory testing. Comparison of deaths with other cohorts is complicated because our cohort probably comprises only of infants who were infected in utero, who have higher mortality rates.[11–13] Other cohorts that include only those testing at older ages might underestimate mortality because infected infants might have died before inclusion in those cohorts. The 14% probability of mortality in our cohort was higher than the 4% reported in CHER.[2] However, CHER only included infants who did not meet the clinical and immunological criteria to initiate ART that were in place at the time, and therefore were likely to be healthier. The proportion of deaths in our study is similar to the 14% that we observed in a previous study of young children initiating ART at age younger than 2 years at the same site,[14] in a large combined analysis of South African cohorts of infants initiating ART at a median of 6 months,[15] and in a small cohort of neonates in Cape Town.[16] The proportion is lower than the 24% that we observed in a previous surveillance study at several sites in Johannesburg,

conducted in 2011 among newly identified HIV-infected infants.[17] Our study shows that earlier diagnosis and treatment are not sufficient to prevent all mortality in HIV-infected children. Mortality was still found to peak at an early age, consistent with pre-ART literature.[1]

Attrition, to which many factors contributed, occurred at every step in the HIV care cascade, reducing the net benefit of early diagnosis. It needs to be recognised that these HIV-infected neonates were identified in the context of a less than 1·7% intrauterine transmission rate. Prevention of mother-to-child transmission coverage is very high in the region in which our study took place. In cases where prevention failed, a clustering of social and biological factors might have contributed to poor outcomes in HIV-infected neonates. For example, no access or late access to antenatal care and inadequate maternal ART adherence contribute to increased risk of HIV transmission and are the same factors that contribute to challenges sustaining ART for the infant.[18–20] In our study, attrition before starting ART was greater in those not co-tested with POC testing, but attrition after ART had been started was somewhat greater in those co-tested. This resulted in a net similarity in the overall rates of retention regardless of access to POC testing. The reasons for these differences are unclear. The short timeframe between testing and treatment initiation might leave families less well prepared for initiating and maintaining lifelong ART for their infants. Also, this very early test and treat approach might simply be shifting the timing of loss to follow-up to a later point along the cascade. We previously showed that coverage of birth testing was high (>90%) but that programme factors, including weekend or holiday cover and availability of phlebotomists before the time of discharge of mother and child limited our ability to reach all infants with an initial diagnosis.[8] These were not related to follow-up of infected infants and result return after the diagnosis, where maternal psychosocial factors seemed to play a larger role. The same complex factors that lead to loss between testing and receipt of results or ART initiation probably also contribute to high rates of loss after ART initiation. We also hypothesise that the subgroup initiating ART in the non-POC group might be a selection of more adherent families who had voluntarily returned to site. Many of the individual and structural factors preventing families from staying in care with no POC testing continue to affect retention and adherence with POC testing. Additionally, inadequate time for counselling in the immediate post-partum period might also contribute. Strategies to improve retention in care are urgently needed.

The low proportion (71%) of infants treated and retained through 12 months in our study who achieved viral suppression is probably because of suboptimal ART regimens or formulations for infants as well as caregiver difficulties in maintaining high levels of adherence to twice-daily administration of multiple drugs, often in poorly palatable formulations. Rates of preterm birth and low birthweight were high, even in the most recent era, highlighting the importance of improving treatment options for neonates who are born prematurely and with low birthweights, for whom drug dosing, disposition, and safety might be different than for full-term and average-weight neonates.[21] The low proportions of neonates with viral suppression occurred despite unexpectedly low pre-ART viral loads, consistent with findings of declining baseline viraemia within the EID programme in South Africa.[22] High pre-ART viral load was strongly related to mortality. Although this relationship was described before early ART was available, it seems to persist even with very early ART and this requires further study that takes into account other possible associations to reduce mortality more effectively.[13,23,24]

Our study has several limitations. Although we screened 5449 HIV-exposed neonates, we identified only 88 who had confirmed HIV infection; thus, numbers are small and CIs wide for all estimates. Our findings are further limited by the study taking place at a single relatively well resourced site, which might limit generalisability. Standard laboratory-based HIV PCR testing turnaround time in our setting is 2–3 days, which is faster than in many other settings. Although mothers and neonates are generally discharged by this time even at our site, we put in place active outreach to trace those with positive results (by telephone and by home visit where needed). We might therefore have biased the study against POC EID. POC tests have the advantage of yielding results before discharge, which avoids delays in disclosure and ART initiation inherent in EID systems that rely on return visits. Given our ability and experience of actively tracing families not POC co-tested, we are concerned that less well resourced settings, when reliant on routine laboratory-based EID, might have worse outcomes if only passive systems are in place to engage families in care. Our estimates of retention in care on site might underestimate overall retention in care because at least some of the infants were formally transferred to other services, where data on retention in care, loss to follow-up, and mortality were difficult to obtain. Several factors (maternal and child viral load interaction and effect on outcomes, adherence, social circumstances, and comorbidities) are possibly related to the outcomes we include here, but larger numbers are required to investigate independent associations with confidence.

In the light of these findings, attention needs to be given to how to improve outcomes in this high-risk population of HIV-infected neonates. Sustained maternal adherence with infant ART is well known to be taxing. Support of mothers to provide ART for their infants must be linked with support for the mother for her own treatment and wellness. Adequate maternal counselling is essential and needs to include clear explanation about EID and infant ART starting during antenatal care and continuing through each of the steps in the care cascade. Because of

scarce alternative drugs, we used nevirapine as part of the ART regimen in the first few weeks. However, all these infants had also been given nevirapine as prophylaxis before ART initiation and a high proportion had been exposed to maternal antiretroviral drugs, usually including efavirenz during pregnancy. Single-point mutations confer resistance to nevirapine and are known to be selected in almost all those exposed even to a single dose of this drug.[25,26] Infants were transitioned to lopinavir–ritonavir at 42 weeks postmenstrual age, but this drug is known to present substantial adherence challenges because of poor palatability. Better formulations of antiretroviral drugs with better tolerability and simplified dosing for infants, and particularly neonates, is an urgent research priority.[16] The approval of raltegravir for use during the neonatal period brings hope that better regimens can be constructed.[27,28] The high attrition in our study is related to a highly mobile population and a fragmented health service. Improved patient-held records might help mothers to continue care elsewhere should the need arise and assist providers at different sites to coordinate care.

In conclusion, HIV-infected infants can be identified at birth and initiated on ART within hours or days. However, they are a challenging population to retain in care and we observed a high rate of mortality and loss to follow-up and suboptimal viral suppression. Although testing at birth can lead to earlier treatment, more research is needed on strategies to reduce poor outcomes and enhance viral suppression. We could not show that POC testing improved retention in care or viral suppression at 12 months. Substantial efforts beyond ART initiation are required and should include adherence support, monitoring of maternal and infant health, and better social services. Mothers require support with acceptance of results, family mobility, and transfer to other health facilities.

Contributors

K-GT, GGS, LK, AC, EJA, and RS contributed to study design and all coauthors contributed to data interpretation and writing of the report. LK, SS, and K-GT contributed to data analysis and K-GT, RS, FP, MB, MC, PMM, and GS were responsible for data collection.

Declaration of interests

FP and MB report grants from National Institutes of Health, non-financial support from South African National HIV programme, and non-financial support from Cepheid during the conduct of the study. All other authors declare no competing interests.

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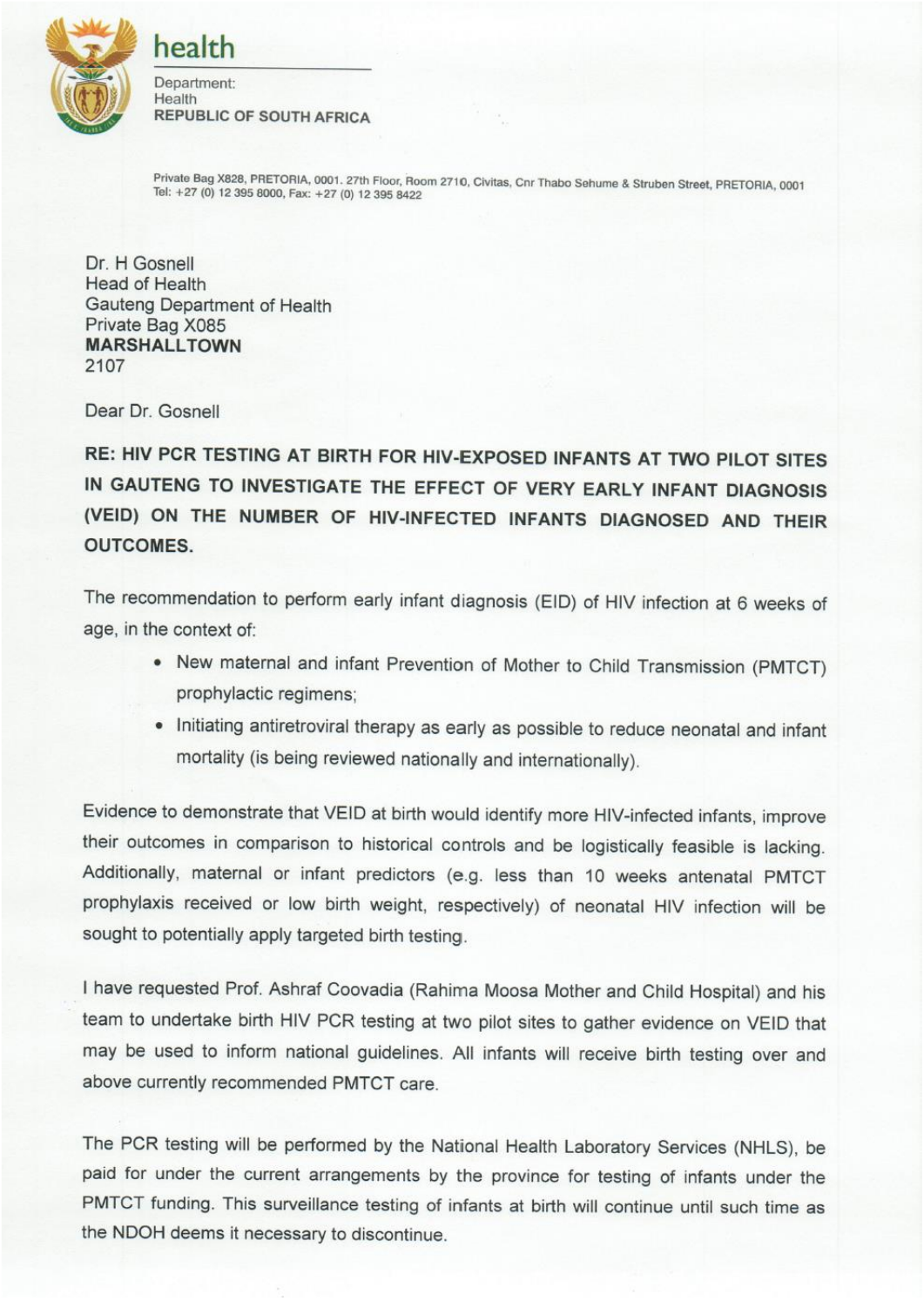
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APPENDIX A: Permission from National Department of Health to pilot universal birth testing



health

Department:
Health
REPUBLIC OF SOUTH AFRICA

Private Bag X828, PRETORIA, 0001. 27th Floor, Room 2710, Civitas, Cnr Thabo Sehume & Struben Street, PRETORIA, 0001
Tel: +27 (0) 12 395 8000, Fax: +27 (0) 12 395 8422

Dr. H Gosnell
Head of Health
Gauteng Department of Health
Private Bag X085
MARSHALLTOWN
2107

Dear Dr. Gosnell

RE: HIV PCR TESTING AT BIRTH FOR HIV-EXPOSED INFANTS AT TWO PILOT SITES IN GAUTENG TO INVESTIGATE THE EFFECT OF VERY EARLY INFANT DIAGNOSIS (VEID) ON THE NUMBER OF HIV-INFECTED INFANTS DIAGNOSED AND THEIR OUTCOMES.

The recommendation to perform early infant diagnosis (EID) of HIV infection at 6 weeks of age, in the context of:

- New maternal and infant Prevention of Mother to Child Transmission (PMTCT) prophylactic regimens;
- Initiating antiretroviral therapy as early as possible to reduce neonatal and infant mortality (is being reviewed nationally and internationally).

Evidence to demonstrate that VEID at birth would identify more HIV-infected infants, improve their outcomes in comparison to historical controls and be logistically feasible is lacking. Additionally, maternal or infant predictors (e.g. less than 10 weeks antenatal PMTCT prophylaxis received or low birth weight, respectively) of neonatal HIV infection will be sought to potentially apply targeted birth testing.

I have requested Prof. Ashraf Coovadia (Rahima Moosa Mother and Child Hospital) and his team to undertake birth HIV PCR testing at two pilot sites to gather evidence on VEID that may be used to inform national guidelines. All infants will receive birth testing over and above currently recommended PMTCT care.

The PCR testing will be performed by the National Health Laboratory Services (NHLS), be paid for under the current arrangements by the province for testing of infants under the PMTCT funding. This surveillance testing of infants at birth will continue until such time as the NDOH deems it necessary to discontinue.

Precautions to prevent birth testing from negatively impacting on general service delivery of the EID program should be taken at both sites.

The results of the birth testing will be made widely available.

Yours sincerely

DR Y PILLAY
DEPUTY DIRECTOR-GENERAL: HIV/AIDS, TB AND MCWH
DATE: 12/5/14

Cc: Dr. Zukiswa Pinini: HAST Directorate
Ms Susan Jordaan: CEO - Rahima Moosa Mother and Child Hospital

APPENDIX B: Ethics approvals relevant to the project

M140555

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140555

NAME: (Principal Investigator)	Dr Karl-Gunther Technau et al
DEPARTMENT:	Department of Paediatrics & Child Health Rahima Moosa Mother and Child Hospital
PROJECT TITLE:	HIV PCR Testing at Birth for HIV-Exposed Infants at two Pilot Sites in Gauteng to Investigate the Eeffect of Very Early Infant Diagnosis (VEID) on the Number of HIV-Infected Infants Diagnosed and Their Outcomes
DATE CONSIDERED:	30/05/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	

APPROVED BY:

Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 30/05/2014

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned researc and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from th research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit yearly progress report**.

Principal Investigator Signature M140555Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Dr Karl-Gunter Technau et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140639

NAME: (Principal Investigator) Dr Karl-Gunter Technau et al

DEPARTMENT: Paediatrics and Child Health
Rahima Moosa Hospital

PROJECT TITLE: Operational Evaluation of Standard and Point-of-Care Birth Testing of HIV-Exposed Infants

DATE CONSIDERED: 27/06/2014

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR:

APPROVED BY: _____
Professor P Cleaton-Jones, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/07/2014

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

DECLARATION OF INVESTIGATORS

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

_____ _____
Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Dr Karl G Technau

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140760

NAME: (Principal Investigator)	Dr Karl G Technau
DEPARTMENT:	Paediatrics Rahima Moosa Mother and Child Hospital
PROJECT TITLE:	Cohort Study of HIV-Infected and Exposed Children Receiving Care at Rahima Moosa Mother and Child Hospital Supported by Empilweni Services and Research Unit (IeDEA) (Previously M090501)
DATE CONSIDERED:	25/05/2009 (Initial Approval) 13/01/2017 (Recertified)
DECISION:	Approved unconditionally
CONDITIONS:	Annual Recertification July 2016 - July 2017
SUPERVISOR:	
APPROVED BY:	Professor P Cleaton-Jones,Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/01/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

31 January 2017
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

M140748

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140748

NAME: (Principal Investigator) Dr Karl G Technau et al

DEPARTMENT: Department of Paediatrics & Child Health
Rahima Moosa Mother and Child Hospital

PROJECT TITLE: Latency and Early neOnatal Provision of Anti-Retroviral Drugs Observational Cohort (LEOPARD-O)

DATE CONSIDERED: 25/07/2014

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR:

APPROVED BY:

Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 25/07/2014

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature M140748Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Dr Karl-Gunter Technau

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130355

NAME: (Principal Investigator) Dr Karl-Gunter Technau

DEPARTMENT: Department of Paediatrics and Child Health
Rahima Moosa Mother and Child Hospital

PROJECT TITLE: Infant HIV diagnosis in South Africa - Improving outcomes through point of care testing at birth the SAMBA Baby and Mothers Study (BAMS)

DATE CONSIDERED: 05/04/2013

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof Ashraf Coovadia

APPROVED BY: _____
Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 05/04/2013

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

DECLARATION OF INVESTIGATORS

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

_____ _____
Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX C: Reports generated to National Department of Health during PhD period

Empilweni Services and Research Unit
Rahima Moosa Mother and Child Hospital, JHB

South Africa (Private Bag X20 Newclare 2112) • Tel: +27(0)11 470 9421 Cel: +27 (0)82 687 3633 Fax: +27(0)86 553 5046 •
E-mail:karltechnau@gmail.com

29th January 2014

Attention:
Dr Yogan Pillay
Dr Sanjana Bhardwaj
Dr Nonhlanhla Dlamini
Ms Precious Robinson

Dear Colleagues

Initial report on prevalence of HIV infection in low birth weight and premature infants at RMMCH

Background:
We would like to report our concerning finding of an unexpectedly high prevalence of HIV infection in a cohort of infants tested early for HIV. Over the last years there has been documented improvement of the Prevention of Mother to Child Transmission (PMTCT) programme nationally.[1,2] Recent evidence from the Earlier Infant Diagnostic Study at RMMCH demonstrated that a large proportion (76%) of HIV transmission occurring by the time of birth could be diagnosed at birth.[3,4] An electronic record system is used to track the routine PMTCT related activities at the hospital. All women delivering at RMMCH are routinely counseled about their HIV status and offered HIV testing if their HIV status is unknown or if their last negative HIV result was more than 6 weeks prior. The 2013 South African PMTCT Guidelines include testing of premature, low birth weight or otherwise symptomatic infants at the time of birth or at the time of presentation even if this occurs before six weeks of age.

Services offered:
Since mid-August 2013 we have been providing an HIV PCR to low birth weight or premature infants at the time of birth in accordance with the guidelines referred to above. Similarly all neonates requiring admission directly after delivery on medical grounds (e.g. congenital infection, prematurity, very low or extremely low birth weight) undergo early testing for HIV if they are found to be HIV exposed. Infant diagnostic tests are processed at Charlotte Maxeke Johannesburg Academic Hospital's NHLS PCR laboratory using the Roche CAP/CTM HIV version 1 qualitative platform. All samples sent from RMMCH are collected as whole blood EDTA samples. All neonates who test PCR negative have a 2nd PCR test at 6 weeks of age.

Results:
Since mid August 2013 (5 months), 98 early tests have been documented. We have noticed a higher than expected prevalence of HIV at birth in this group. **Ten out of 98 (10%; 95% Confidence Interval: 4%-16%)** HIV-infected cases were found amongst neonates tested close to the time of birth (median 1 day; IQR: 1-1; maximum 10 days). Three of the ten mothers of infected neonates had no PMTCT exposure as they were only diagnosed around the time of birth. The seven other mothers had received either triple ART (FDC) from the time of booking or shortly after an initial course of AZT.

Table 1: Characteristics of HIV-positive mothers and tested infants

	Whole Group	HIV-Infected Infants*	PCR-negative Infants*	p
Number	98	10	88	NA
Percentage (95% CI)	100	10 (4-16)	90 (84-96)	NA
Maternal Age (yrs)	30 (26-34)	28 (23-33)	30 (26-34)	0.28
Parity	2 (2-3)	2 (2-3)	3 (2-3)	0.39
Maternal PMTCT, n (%)				
None	4 (4)	3 (33)	1 (1)	0.0029 (Fisher)
ART (+/-AZT)	94 (96)	7 (67)	87 (99)	
ART before delivery (weeks)	18 (9-25)	16 (5-19)	18 (9-25)	0.26
Maternal antenatal CD4	322 (207-458)	225 (63-363)	328 (220-459)	0.84
Birth weight (kg)	2.3 (2.0-2.4)	1.9 (1.4-2.3)	2.3 (2.1-2.4)	0.056
Gestation at Birth (weeks)	37 (34-39)	37 (32-37)	38 (34-40)	0.089

All continuous variables are reflected as median (inter-quartile range), compared by means of t-test for normally distributed and Wilcoxon for non-normal distributed data. *At the time of birth

The infected infants include one set of twins which could arguably be counted as one transmission in terms of HIV prevalence at birth. Maternal compliance was found to be similar in both groups but is more difficult to quantify. One transmitting mother (mother of twins) later was found to have NNRTI and 3TC resistance with a high viral load (VL>100,000 copies/ml) and she explained that she had been on triple therapy on and off a few years prior and then restarted it in pregnancy (FDC). This mother's treatment has since been optimized. Four mothers with infected infants had VL data available, two had VL <50 copies/ml by the first two months post-partum.

All infants have been confirmed to be HIV infected by means of HIV viral load (VL) testing. Important diagnostic challenges included two cases having an "Indeterminate" PCR result at birth, both were conclusively shown to be infected during later testing. VL assays performed close to the time of birth for confirmation may yield low values (we found values of 240 copies/ml in one case that rose to >10,000,000 within days, a further case of a VL of 830 and 2010 copies/ml). The diagnoses have been reviewed in consultation with the virology team of the NHLS. All infants are receiving care and nine are receiving ART (one recently diagnosed infant is being prepared) and are being closely observed by the treatment service at RMMCH in consultation with a group of experts in the field to help direct important treatment decisions.

Conclusions and Recommendations:

This number of newly diagnosed infants is of concern and raises the questions: 1) is this a particularly at risk population that is missed or may be at risk of not reaching the six week test either due to early neonatal mortality 2) or due to the mother's diagnosis not being made? It is important to establish whether the trend will remain in the same range statistically when the denominator increases.

The limitations of these data include: 1) Only low birth weight cases were tested at birth, not the whole exposed cohort, this excludes any perinatal transmission cases and therefore underestimates the transmission rate; low birth weight infants and previously undiagnosed mothers are a high risk group and may therefore overestimate the transmission rate; 2) although dedicated data systems are in place, data is from a busy high throughput service setting, all possible attempts are being made to ensure rigorous data.

We recommend ongoing testing in this group of HIV exposed infants and would motivate that broadening the early testing to the whole exposed birth cohort may be beneficial for the sake of monitoring this occurrence. This could be done at one or two sites and carefully monitored, then reassessed (e.g. RMMCH where the tools are in place to monitor this intervention). We recommend that mothers are carefully counseled antenatally to assess and uncover possible risk factors for virological failure (e.g.

prior ART exposure, interruption or poor adherence to ART). Finally we recommend an urgent assessment of maternal VL at birth with consequent resistance studies for women on ART with high VL in order to establish both the potential causes but also to identify at risk infants.

We report this formally to the National Department of Health as this may not otherwise be picked up in routinely collected statistics and it is a finding that concerns us. We would like to invite comment and advice and would like to discuss the best way forward.

Yours faithfully



Karl Technau
Senior Medical Officer, Paediatrics and ARV Services, Rahima Moosa Mother and Child Hospital



Ashraf Coovadia
Adjunct Professor, Paediatrics and Child Health, Rahima Moosa Mother and Child Hospital

References:

1. Goga AE, Dinh TH, Jackson DJ, group ftSs. Impact of the national prevention of mother-to-child transmission of HIV (PMTCT) program on perinatal mother-to-child transmission of HIV (MTCT) measured at six weeks postpartum, South Africa (SA). In: XIX International AIDS Conference. Washington; 2012.
2. Goga AE, Dinh TH, Jackson DJ, group ftSs. Evaluation of the Effectiveness of the National Prevention of Mother-to-Child Transmission (PMTCT) Programme Measured at Six Weeks Postpartum in South Africa, 2010.: Medical Research Counsil, National Department of Health, PEPFAR, US Centres for Disease Control and Prevention; 2012.
3. Lilian RR, Kalk E, Technau KG, Sherman GG. Birth Diagnosis of HIV Infection on Infants to Reduce Infant Mortality and Monitor for Elimination of Mother-to-Child Transmission. The Pediatric infectious disease journal 2013.
4. Lilian RR, Kalk E, Bhowan K, et al. Early diagnosis of in utero and intrapartum HIV infection in infants prior to 6 weeks of age. Journal of clinical microbiology 2012;50:2373-7.

Empilweni Services and Research Unit
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E-mail:karltechnau@gmail.com

23rd January 2014

National Department of Health:
Att: Dr Joseph Honwani
NHLS
Att: Prof Adrian Puren
CC: Sr Annie Jordan, Prof Ashraf Coovadia, Prof Gayle Sherman, Prof Keith Bolton, Mrs S Jordaan

Dear Colleagues

Report of Sensitivity and Specificity of Rapid Test Kits experienced at Rahima Moosa Mother and Child Hospital September-November 2013

Following the concerns voiced earlier during the year 2013 regarding the sensitivity of the rapid HIV test kits in use I would like to provide you with the following update. After documenting that we experienced problems with the sensitivity, particularly of the ABON test kit but also of the First Response kit, we proceeded to perform parallel testing on all samples and you kindly provided us with Determine test kits that we ran in parallel as well. We undertook to keep detailed records and to provide you with feedback on the sensitivity and specificity.

All HIV rapid tests performed at Empilweni Clinic (HIV clinic within Rahima Moosa Mother and Child hospital) were recorded carefully and all rapids were performed in parallel (ABON, First Response, Determine) under close supervision and according to the protocol applicable to each kit, extensive training having been received. We calculated sensitivity, specificity, positive and negative predicative values. The gold standard was: concordant rapid kits (all three kits) or in the event of discordance, an HIV ELISA result. Calculations were performed for each kit individually against the gold standard and in a fourth analysis for the current national series protocol against the gold standard. Data are confidential and this analysis is for service auditing and quality control only and may not be published unless prior ethics approval is granted.

In the period September to November 2013, 232 rapid tests were performed. The total comprised 57 (25%) children (defined as <15 years old). One teenage patient was excluded from analysis for ABON and First response as the adolescent was on ART and known HIV-positive and should ideally not have undergone a rapid test (it was done as she was part of a group of three undisclosed peers who all underwent rapid testing and she was included to avoid stigmatisation). Her ABON and First Response were negative, while Determine and ELISA were positive. One patient was excluded who had a positive ABON and a negative First Response and negative Determine but who did not proceed to have his ELISA taken. Out of the remaining 230 individuals, two did not have a Determine test performed for unknown reason and they are excluded from the Determine analysis. Apart from the two excluded cases, there were 6 discrepant results (Table 1).

Table 1: Cases of Discrepancies

	ABON	First Response	Determine	ELISA
1 – Age: 59 years	Pos	Neg	Neg	Pos
2 – Age: 25 years	Neg	Pos	Pos	Pos
3 – Age: 3 years	Neg	Pos	Neg	Neg
4 – Age: 1.5 years	Neg	Neg	Pos	Neg
5 – Age: 41 years	Neg	Neg	Pos	Pos
6 – Age: 23 years	Pos	Pos	Neg	Pos

The overall HIV prevalence was 12%. Figure 1 demonstrates the detailed parameters.

Figure 1: Detailed Statistics for HIV rapid Diagnostic Kits



The results for this three month period under evaluation demonstrate that each kit reached a maximum sensitivity of 92.9% (95 % CI: 83-100). **This implies that the current series protocol that is part of National Guidelines may also only achieve that level of sensitivity and would have missed 2 of 28 (7.14%) infected persons. If this is indeed a generalised occurrence it translates to thousands of missed opportunities in the current high prevalence PMTCT/HCT era in South Africa.** In our setting, the use of the kits in parallel helped to eliminate some potential missed diagnoses and given the current HIV prevalence in our tested cohort it appears that a significant number of cases would be missed if we reverted to the series protocol and we would suggest to continue using the protocol of testing in parallel with multiple kits, even for adults. The use of the determine kit as a third kit helped identify one additional HIV-positive adult who would have been missed.

We would like to thank you for your ongoing support. I would like to acknowledge our dedicated staff especially Sr Estelle Smith. We request and would welcome any feedback and would like to continue working towards improving the services provided.
Yours faithfully



Karl Technau, Senior Medical Officer, Rahima Moosa Mother and Child Hospital

APPENDIX D: Permission to Incorporate Original Articles

Karl Technau

From: Elisa de Castro Alvarez <elisa.alvarez@iasociety.org>
Sent: 20 November 2017 12:20
To: Karl Technau; JIAS Editorial Office
Subject: RE: Request for use of articles

Dear Karl,

Thank you for email.

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So, please feel free to include your articles within the PhD thesis in the format in which they have been published.

Best luck with your PhD defense and warm regards,

Elisa de Castro Alvarez PhD
Support Editor

Elisa de Castro Alvarez, PhD

JIAS Support Editor
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Avenue de France 23 | CH-1202 Geneva | Switzerland

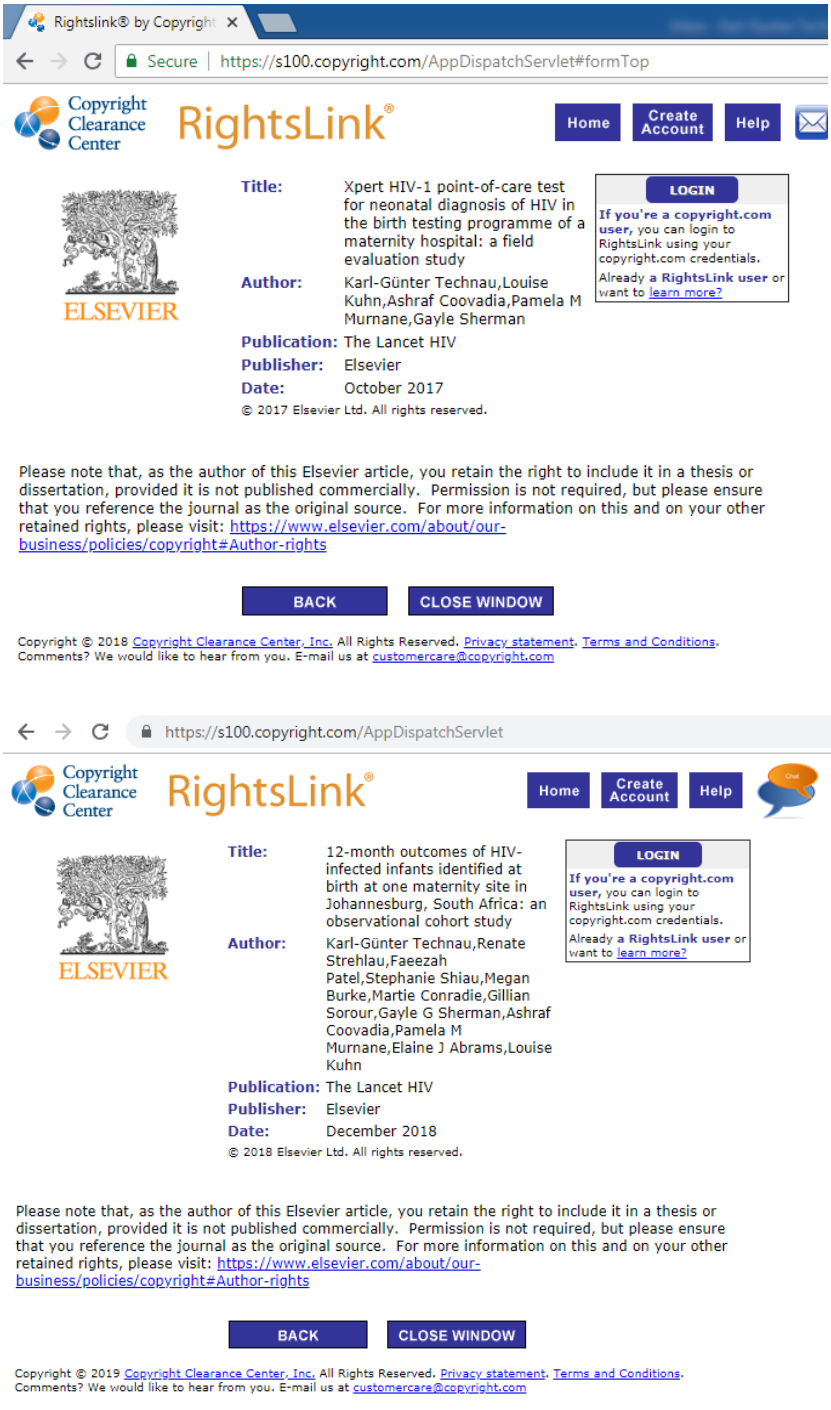
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From: Karl Technau [mailto:karltechnau@gmail.com]
Sent: Sunday, November 19, 2017 8:58 AM
To: JIAS Editorial Office <editorial@jiasociety.org>; Elisa de Castro Alvarez <elisa.alvarez@iasociety.org>
Subject: Request for use of articles

Dear Drs Bras and Alvarez
I hope the email finds you well
Two articles where recently published in the JIAS, please see attached.
With the permission of my co-authors I would like to include both of these articles in my PHD. I would like to request your permission to use the papers within the PHD thesis in the format in which they have been published.
Please let me know if this would be possible and what steps I need to take
Kind regards
Karl Technau

Karl Technau

Senior Medical Officer
Empilweni Service and Research Unit



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Title: Xpert HIV-1 point-of-care test for neonatal diagnosis of HIV in the birth testing programme of a maternity hospital: a field evaluation study

Author: Karl-Günter Technau,Louise Kuhn,Ashraf Coovadia,Pamela M Murnane,Gayle Sherman

Publication: The Lancet HIV

Publisher: Elsevier

Date: October 2017

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ELSEVIER

Title: 12-month outcomes of HIV-infected infants identified at birth at one maternity site in Johannesburg, South Africa: an observational cohort study

Author: Karl-Günter Technau,Renate Strehlau,Faeezah Patel,Stephanie Shiau,Megan Burke,Martie Conradie,Gillian Sorour,Gayle G Sherman,Ashraf Coovadia,Pamela M Murnane,Elaine J Abrams,Louise Kuhn

Publication: The Lancet HIV

Publisher: Elsevier

Date: December 2018

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Appendix E: Turn-it-in report

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