# EARLY DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION STATUS IN VERTICALLY EXPOSED INFANTS IN A LOW RESOURCE SETTING

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, in fulfilment of the requirements for the degree of Doctor of Philosophy.

This thesis is presented as a series of publications and unpublished data.

Johannesburg, 2006

## DECLARATION

I declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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# DECLARATION OF CO-AUTHORS

For all 6 publications and the submitted manuscript detailed hereunder,

Gayle G. Sherman's contributions were as follows:

study conception

study design and implementation

monitoring and co-ordination of clinical and laboratory aspects of the study

analysis and interpretation of data

drafting and revising all manuscripts

All authors read and approved the final version of each manuscript.

# PUBLICATION 1

**PMTCT from research to reality – results from a routine service.**

S Afr Med J 2004;94(4):289-292.

Sherman GG, Jones SA, Coovadia AH, Urban MF, Bolton KD.

SAJ's major contribution was in execution of the study at the clinical level including data collection and daily management of the clinical team. She assisted with data analysis and critical review of the manuscript.

AHC, MFU and KDB were integrally involved in establishing the Prevention of Mother to Child Transmission and Paediatric HIV Clinic service at Coronation

Hospital and provided valuable insights for improving the study design and creating an environment conducive to the study. They were involved in the clinical execution of the study and participated in critical review of the manuscript. AHC was an integral member of the core clinical study team throughout the study period.

## PUBLICATION 2

**HIV-1 DNA polymerase chain reaction for diagnosis of HIV infection in infancy in low resource settings.** Pediatr Infect Dis J 2005;24(11):993-997.

Sherman GG, Cooper PA, Coovadia AH, Puren AJ, Jones SA, Mokhachane M, Bolton KD.

GGS and PAC initiated this publication that combined distinct study cohorts viz. from infant 'diagnostic' and 'feeding' studies.

PAC, AJP and MM participated in the 'feeding' study but were in no way involved with the 'diagnostic' study that is the subject of this thesis. GGS, AHC and SAJ made no contribution to the 'feeding' studies. KDB contributed towards both study cohorts and his involvement for the purposes of this manuscript is as for PUBLICATION 1. The contributions of SAJ and AHC are as for PUBLICATION 1. PAC assisted in preparation of the manuscript. PAC and AJP performed the data analysis on the 'feeding study' for this publication. All authors assisted with critical review of the manuscript.

## PUBLICATION 3

**Dried blood spots improve access to HIV diagnosis and care for infants in low-resource settings.** J Acquir Immune Defic Syndr 2005;38(5):615-617.

Sherman GG, Stevens G, Jones SA, Horsfield P, Stevens WS

## PUBLICATION 4

**Affordable diagnosis of human immunodeficiency virus infection in infants by p24 antigen detection.** Pediatr Infect Dis J 2004;23(2):173-176.

Sherman GG, Stevens G, Stevens WS.

GS, PH and WS contributed intellectually towards the laboratory component of the publications. GS & PH modified the assays before analyzing the samples and assisted with data analysis. All authors contributed towards critical review of the manuscript. SAJ contributed as outlined under PUBLICATION 1.

## PUBLICATION 5

**Oral Fluid Human Immunodeficiency Virus Tests. Improved Access to Diagnosis for Infants in Poorly Resourced Prevention of Mother to Child Transmission Programs.** Pediatr Infect Dis J 2005;24(3):253-256.

Sherman GG and Jones SA.

## PUBLICATION 6

**Is early HIV testing of infants in poorly-resourced PMTCT programs unaffordable?** Trop Med Int Health 2005;10(11):1108-1113.

Sherman GG, Matsebula TC, Jones SA.

TCM assisted with study design and data analysis of the costing component and participated in the preparation and critical review of the manuscript. The contributions of SAJ are as for PUBLICATION 1.

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To Clive, Alexander and Natasha

# PUBLICATIONS AND PRESENTATIONS

## PUBLICATIONS PRESENTED FOR THIS THESIS

1. Sherman GG, Jones SA, Coovadia AH, Urban MF, Bolton KD. **PMTCT from research to reality – results from a routine service**. S Afr Med J 2004;94(4):289-292.

2. Sherman GG, Cooper PA, Coovadia AH, Puren AJ, Jones SA, Mokhachane M, Bolton KD. **HIV-1 DNA polymerase chain reaction for diagnosis of HIV infection in infancy in low resource settings**. Pediatr Infect Dis J 2005;24(11):993-997.

3. Sherman GG, Stevens G, Jones SA, Horsfield P, Stevens WS. **Dried blood spots improve access to HIV diagnosis and care for infants in low-resource settings**. J Acquir Immune Defic Syndr 2005;38(5):615-617.

4. Sherman GG, Stevens G, Stevens WS. **Affordable diagnosis of human immunodeficiency virus infection in infants by p24 antigen detection**. Pediatr Infect Dis J 2004;23(2):173-176.

5. Sherman GG, Jones SA. **Oral Fluid Human Immunodeficiency Virus Tests. Improved Access to Diagnosis for Infants in Poorly Resourced Prevention of Mother to Child Transmission Programs.** Pediatr Infect Dis J 2005;24(3):253-256.

6. Sherman GG, Matsebula TC, Jones SA. **Is early HIV testing of infants in poorly-resourced PMTCT programs unaffordable?** Trop Med Int Health 2005;10(11):1108-1113.

**INVITED PUBLICATIONS and GUIDELINES**

1. Sherman GG. **Infant HIV Diagnostic Guidelines to facilitate Adoption**. S Afr J HIV Med 2003:24-26.

2. Sherman GG, Napier G, Stevens WS. **Infant testing and outcomes**. Section 6.3, pages 25-32 in Doherty T, Besser M, Donohue S, et al. September 2003. An Evaluation of the Prevention of Mother-To-Child Transmission of HIV Initiative in South Africa. Lessons and Key recommendations. Available at: www.hst.org.za/uploads/files/pmtct_national.pdf Accessed December 28, 2005

3. National Department of Health. **Guidelines for the management of HIV-infected children**. 1st edition 2005, Department of Health, Pretoria, South Africa. Or available from: http://www.doh.gov.za/docs/factsheets/guidelines/artguide04-f.html. [Accessed 10 January 2006].

4. Stevens W, Sherman G, Cotton M, Gerntholtz L, Webber L. **Revised guidelines for diagnosis of perinatal HIV-1 infection in South Africa**. S Afr J HIV Med 2006 (in press).

## PRESENTATIONS ARISING FROM THIS STUDY

1. *The clinical context of HIV diagnosis in infancy*. Pediatric Diagnostic and Monitoring Working Group meeting of the HIV Collaborative Forum, Denver, Colorado, USA. 9 February 2006.

2. *Early diagnosis of HIV: A Public Health Model.* UNICEF-WHO Pediatric Care Consultation, UNICEF house, New York, USA. 11-13 Jan 2006.

3. *Diagnosis of HIV in infants in South Africa.* HIV Collaborative Forum invitation to George Washington University, Washington D.C., USA. 24 Oct 2005.

4. *Improving access to infant and paediatric diagnosis*. Roll-out Comprehensive Care for HIV-infected and –exposed Children: National Department of Health meeting with Concerned Paediatricians, Birchwood Hotel, Johannesburg, 21 July 2005.

5. *Country/program infant diagnosis projects: South Africa* and *Economic benefits of MTCT diagnosis*. Consultation on Infant HIV Diagnosis, Centers for Disease Control, Atlanta, Georgia, USA. July 14-15, 2005.

6. *Scaling up infant diagnosis in South Africa.* World Health Organisation, Geneva, Switzerland. July 12, 2005.

7. *Early infant diagnosis of HIV.* Symposium Session: Affordable HIV diagnosis & monitoring for scaling up of ARV treatment programs. 2nd South African AIDS conference, Durban, 7-11 June 2005.

8. *Accurate and affordable diagnosis of HIV in infants*. Satellite session: Treatment and research options for paediatric HIV infection in South Africa. 2nd South African AIDS conference, Durban, 7-11 June 2005.

9. *Diagnosing infants in low resource settings: getting more for less.* Roche Satellite symposium, 2nd South African AIDS conference, Durban, 7-11 June 2005.

10. *Counseling and diagnosis in HIV affected children*. Population Council, USAID and Department of Social Development's workshop to develop operations research and program priorities on orphans and vulnerable children (OVC) in South Africa. Caesers Palace, Gauteng, 22 January 2004.

11. *Diagnosis of HIV infection in infancy in South Africa*. Guidelines for the Continuum of Care for HIV/AIDS and related diseases, Chief Directorate: HIV/AIDS and TB, Department of Health. Paediatric Section: Working Group Meeting, Sun Intercontinental Hotel, JHB International Airport. 25 September 2003.

12. *National PMTCT programs determine HIV status at 12 months of age: Which HIV test is best?* Sherman GG, Jones SA, Coovadia AH. 1st South African AIDS conference, Durban, 3-6 August 2003.

13. *Diagnosing HIV in infancy in South Africa*. Roche Satellite symposium, 1st South African AIDS conference, Durban, 5 August 2003.

14. *Diagnosing Infant HIV infection: Towards realistic guidelines for Prevention of Mother to Child Transmission (PMTCT) programs in low resource settings*. 43rd Annual Conference of the Federation of South African Societies of Pathology, Path Renaissance, Johannesburg 1 July 2003.

15. *Determining HIV infection status of abandoned children.* The Abandoned Children's Forum Conference at Johannesburg City Hall, Johannesburg,18 June 2003.

**POSTER PRESENTATIONS AT CONFERENCES**

1. HIV-1 DNA polymerase chain reaction for diagnosis of HIV infection in infancy in low resource settings. Sherman GG, Cooper PA, Jones SA, Puren AJ, Mokachane M, Bolton KD. 2nd HIV/AIDS conference, Durban, South Africa. 7-10 June 2005.

2. The cost of early infant diagnosis in PMTCT programs in low resource settings. Sherman GG, Matsebula T, Jones SA. XV International AIDS Conference, Bangkok, Thailand, 11-16 July 2004.

3. Death before diagnosis: IMCI clinical algorithm fails to identify HIV infected infants under the age of 12 months. Jones SA, Sherman GG. XV International AIDS Conference, Bangkok, Thailand, 11-16 July 2004.

# PREFACE

Healthcare provision in the developing world is plagued by limited resources and high disease burdens. The notion that inexpensive, partially accurate, surrogate diagnostic markers can advance care in this already chaotic environment retards progress.

Accurate diagnosis is a vital first step in treating disease.

## ABSTRACT

Sub-Saharan Africa is the eye of the HIV epidemic. This study was conducted when treatment for the majority of HIV-infected patients in low resource settings was considered unattainable and the risks of diagnosing HIV often outweighed the benefits. Coupled with the complexities of HIV diagnosis in infancy, children typically were only diagnosed once already ill or not at all. Key strategies to address the paediatric epidemic focused on preventing mother to child transmission and reducing mortality and morbidity of infected children predominantly with co-trimoxazole prophylaxis. Both strategies required early diagnosis of HIV infection in infancy for monitoring prevention programs and identifying infected children respectively. The diagnostic algorithm for resource limited settings recommended the use of inexpensive, technically simpler HIV antibody detection assays that are unsuitable for use in HIV-exposed children under 12-months of age. Paradoxically this algorithm provided a barrier to HIV diagnosis in children because of high loss to follow-up rates and death in the first year of life.

The objective of this study was to establish an accurate, affordable diagnostic algorithm for early diagnosis of HIV infection that could be rapidly implemented in South Africa and benefit other resource limited settings. The HIV infection status of 300 vertically exposed infants was determined according to first world criteria in a prospective, cohort study at Coronation Hospital, Johannesburg over 21 months. This status was used to assess the accuracy of clinical examinations and HIV assays in diagnosing HIV at 6-weeks, 3-, 7- and 12-months of age. The average cost of determining an infant's HIV infection status was measured.

A single HIV DNA PCR test at 6-weeks of age proved highly accurate in determining HIV status at a marginally increased cost to government and was incorporated by the South African Department of Health into national policy. The

ultrasensitive p24 antigen assay and HIV antibody detection assays on serum and oral fluid were identified as valuable candidates where PCR testing is unavailable. Dried blood spot samples from heelpricks are critical for policy to be translated into practice since skills to perform venesection in 6-week old babies are limited. The next challenge lies in operationalising these findings at a clinical and laboratory level to the benefit of the 300 000 South African children annually exposed to HIV at birth. The urgency of early diagnosis has been increased by the availability of highly effective antiretroviral therapy.

## ACKNOWLEDGEMENTS

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# NOMENCLATURE

| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| ANECCA | African Network for the Care of Children Affected by HIV/AIDS |
| Ag | Antigen |
| CDC | Centers for Disease Control |
| CWCH | Coronation Women and Children Hospital |
| DNA | Deoxyribose Nucleic Acid |
| DBS | Dried Blood Spots |
| ELISA | Enzyme Linked Immunosorbant assay |
| FDA | Food and Drug Adminstration |
| HIV | Human Immunodeficiency Virus |
| IMCI | Integrated Management of Childhood Illness |
| RNA | Ribonucleic Acid |
| PCR | Polymerase Chain Reaction |
| PMTCT | Prevention of Mother to Child Transmission |
| UNAIDS | The Joint United Nations Programme on HIV/AIDS |
| UNICEF | United Nations Children's Fund |
| WHO | World Health Organisation |

# 1. INTRODUCTION

By 2005 UNAIDS estimates were that 25 million people had died from HIV/AIDS since 1981 and 40.3 million people, the highest total yet, were living with the disease (1). The latter includes 2.3 million children under the age of 15 years, 85% of whom are located in sub-Saharan Africa. In 2005, 700 000 children were newly infected and 570 000 died worldwide.

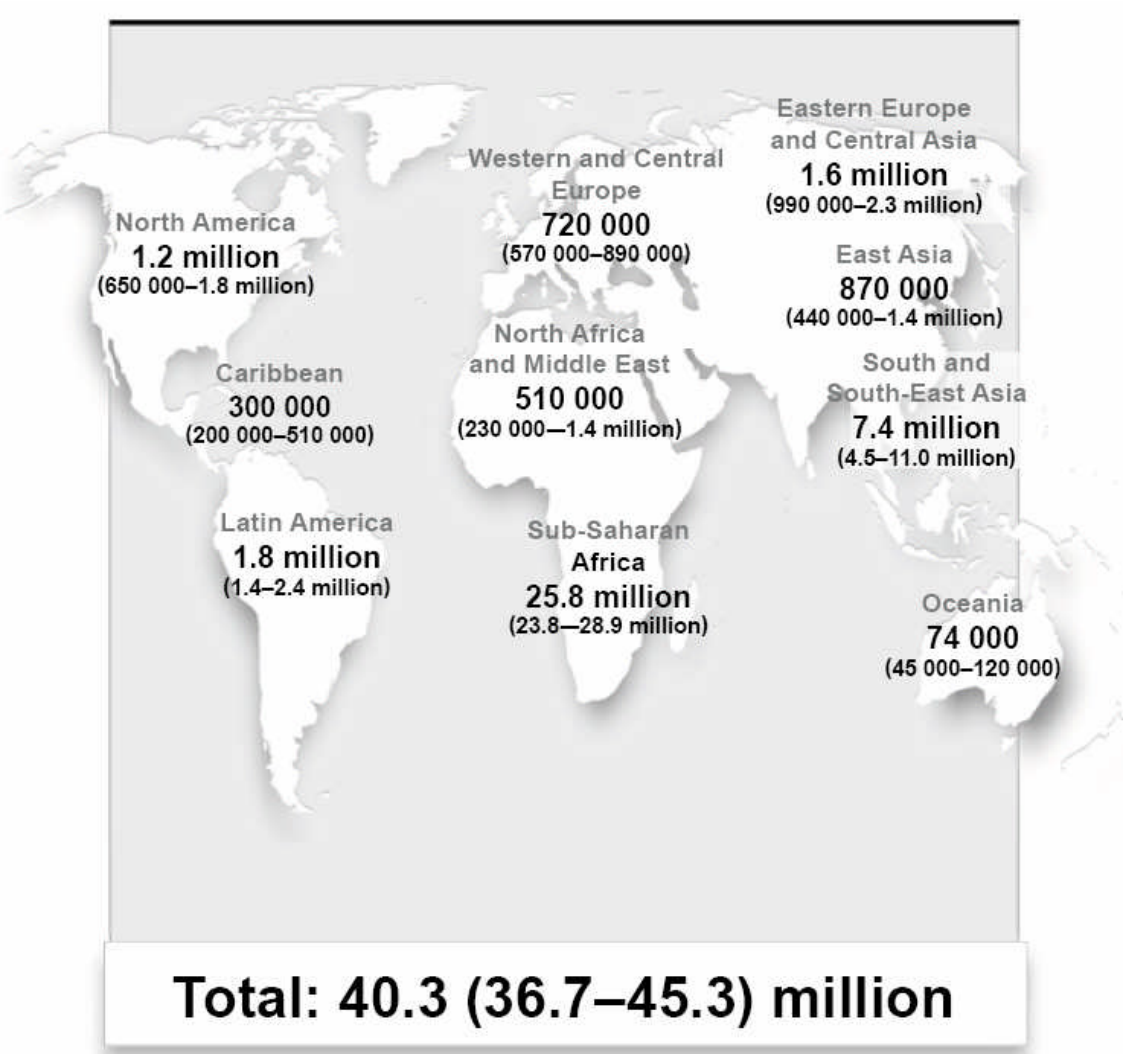



**Figure 1.** Adults and children estimated to be living with HIV in 2005 (1)

The South African National Department of Health's HIV Sero-prevalence survey of pregnant women aged 15 to 49 years demonstrated a prevalence of 29.5% in 2004 (Figure 2) (2). Considering the country's estimated birth rate of approximately one million per annum, this means that 29.5% of all babies born in South Africa are vertically exposed to HIV amounting to 295 000 babies per annum.

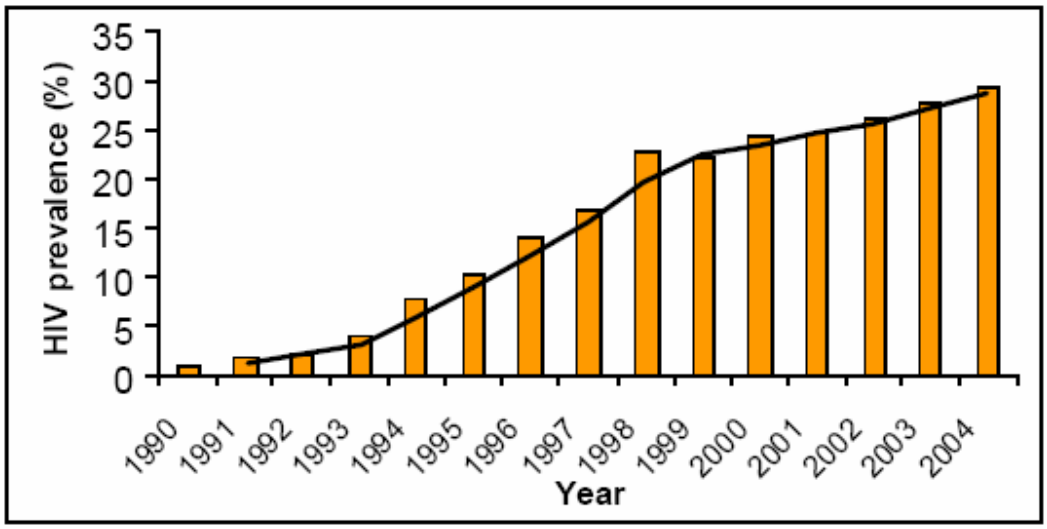



**Figure 2.** Prevalence of HIV among antenatal care attendees in South Africa 1990-2004 (2)

Prevention of Mother to Child Transmission (PMTCT) is the major prevention strategy for paediatric HIV infection and has proved extremely successful in the developed world in virtually eradicating vertically acquired paediatric HIV infection. The HIV transmission rate, established by diagnosing HIV-exposed babies, is a fundamental indicator of the efficacy of a PMTCT program and therefore crucial to ensuring successful PMTCT programs for eliminating this route of infection in South Africa and elsewhere. Moreover, early HIV diagnosis is essential to identify HIV-infected children to receive comprehensive care including antiretroviral therapy.

The diagnosis of HIV infection in infants is more complicated than in older children and adults where diagnostic algorithms comprising a single group of tests viz. HIV antibody detection tests such as HIV enzyme linked immunosorbent assays (ELISA) are used. Transplacental transfer of maternal HIV antibodies means that all babies born to HIV infected women have HIV antibodies (3). This precludes the use of HIV antibody detection assays for diagnosis early in life. Instead viral detection assays which are technically more complex and expensive are required. The maternal HIV antibodies have been documented to persist for up to 18 months of age therefore a positive HIV ELISA result up to this age may signify HIV exposure rather than HIV infection. The process whereby uninfected, exposed infants gradually lose maternal HIV antibodies and revert to having a negative HIV ELISA test is termed seroreversion. Most exposed infants will serorevert much earlier than 18 months of age, often by 9-12 months of age. Diagnosis in children is further complicated in breastfed children by the ongoing risk of postnatal transmission of HIV.

In 1992, following an international workshop on "Early Diagnosis of HIV Infection in Infants", the HIV DNA Polymerase Chain Reaction (PCR) test was recommended for routine infant diagnosis although HIV culture remained the 'gold standard' assay (4). Detection of proviral DNA by PCR was first described in 1988, rapidly validated for clinical use (5-12) and a commercial HIV DNA PCR test kit developed and tested (13-16). HIV DNA PCR testing on dried blood spot (DBS) samples was achieved as early as 1991 (17-22). Other, less accurate HIV tests for infant diagnosis under investigation at the time were *in vitro* antibody production tests (6, 7, 23), detection of HIV antibodies of immunoglobulin A and M subtype since neither isotypes are transferred transplacentally to the foetus (24-27) and p24 antigen assays (5, 10, 12, 28). Subsequently HIV RNA assays were shown to be highly sensitive and specific for early diagnosis of HIV (29-32) as was reverse transcriptase activity (33). More comprehensive accounts of these and later studies are summarized elsewhere (34, 35).

Attempts to improve early identification of HIV-infected children in low resource settings have centered on surrogate immunological (e.g. low CD4 counts and hyperimmunoglobulinaemia) and clinical markers of disease (36-39) as well as modifying the use of antibody detection assays to exclude HIV infection earlier in infancy (40-45).

Although the concept of using HIV antibody titers to facilitate an earlier diagnosis of HIV in infancy has been widely investigated (40, 42-48), it has not been validated in clinical practice. Seroreversion marks the time after which the simpler antibody detection assays are as accurate in diagnosing infants as the more complex and costly viral detection assays but the age at which seroreversion occurs is highly variable. This has been attributed to different patient populations and testing systems (42). At 12-months of age the sensitivity of an HIV ELISA test approaches 100% but the reported specificity ranges from 70% to 95% as a consequence of false positive results from declining maternal antibodies in HIV-uninfected infants (43, 47, 49). The clinical utility of antibody detection assays, including rapid tests, for excluding HIV infection has not been explored in infants younger than one year of age and is vital in settings where viral detection assays are not yet available.

Currently, widely accepted guidelines from the Centers for Disease Control (CDC) require at least two concordant viral detection assay results on blood sampled on two separate occasions to establish HIV infection status in infancy (3). The HIV DNA PCR test, a qualitative test that detects proviral DNA is recommended for routine clinical practice. The guidelines state that the HIV tests should be performed at more than 1 month of age and again at more than 4 months of age. The rationale for delaying the second HIV PCR test is to ensure diagnosis of all in-utero and intrapartum (during labour and delivery) HIV transmission. It applies only when the first test is negative and stems from a concern that a small percentage of infections, especially those occurring intrapartum, may not be detectable by PCR until 4 months of age (3, 50-52).

However, if the HIV PCR test is positive then the second confirmatory test need not be delayed.

The guidelines published by the American Academy of Pediatrics for the diagnosis of HIV infection in infancy also require at least two but preferably three HIV PCR tests and testing for seroreversion in uninfected children at age 12 and 24 months (52). The cost effectiveness of this approach in the developed world, estimated to be US $24 million extra to identify one additional HIV-infected child has been questioned (51).

Guidelines for infant diagnosis in South Africa recommending at least two HIV DNA PCR tests per patient where 'ideal conditions' existed were published in 2001 by the Southern African HIV Clinicians Society (53). These applied to very few children whose families could afford access to private healthcare. In the same year and in line with national policy, the Gauteng Department of Health's HIV paediatric guidelines recommended the archetypal 'low resource setting' infant diagnostic protocol viz. that all vertically exposed children be followed up on co-trimoxazole prophylaxis until their HIV infection status was determined at 12 months of age by an HIV ELISA test (3, 54, 55). This strategy entailed initiating PMTCT follow-up services for approximately 250 000 babies per annum (2), 70% of whom were HIV-exposed but uninfected. With limited public healthcare resources to accommodate this policy, efforts expended to identify HIV-infected pregnant women in PMTCT programs failed to translate into improved care for HIV-exposed babies. This became apparent in 2002 when the first report of the 18 South African PMTCT pilot sites initiated in 2000 was unable to provide HIV transmission rates because of high loss to follow-up rates of infants prior to HIV testing and concluded that the "policy of postpartum care of children was largely unrealistic" (56).

In 2004 The Star newspaper printed a retraction of a front page article claiming that Gauteng's PMTCT program had "saved 58 000 babies" explaining that this

figure was unlikely to be representative since it had been extrapolated from only 3% of infants who had returned at 12 months for HIV testing (57). To date, the national HIV transmission rate and hence the efficacy of the most important prevention strategy for HIV infection of children is unknown and will remain so until widespread infant diagnosis is implemented (58).

When this study was planned in 2000 the national antenatal clinic HIV prevalence rate was 24.5% putting almost a quarter of all babies born in the country at risk of HIV infection and in need of PMTCT follow up care; an HIV DNA PCR test cost approximately 10 times more than an HIV ELISA test and was unavailable in the public health care setting where the majority of HIV-exposed babies are seen. No antiretroviral drugs were available for treatment of HIV in the public sector.

Preliminary investigations leading to this study confirmed that a single HIV DNA PCR test at any age in conjunction with a clinical assessment was 100% sensitive and 98.5% specific in 101 children (59). In the same cohort, HIV ELISA readings at various ages were compared to the HIV status of the infant and a utility for these readings in diagnosing HIV earlier than 12 months of age was suggested (48).

Based on experience of PMTCT follow-up programs locally and in sub-Saharan Africa it became apparent that addressing the paediatric HIV epidemic hinged on diagnosing HIV infection as early in life as possible (55). Achieving this goal by defining an accurate, affordable diagnostic algorithm for low resource settings prompted this study.

## 2. RESEARCH AIMS

To advance diagnosis of HIV infection in infants:

- investigate *alternative diagnostic protocols* by assessing several HIV tests (both HIV antibody and viral detection assays) to establish accurate, cost-effective options for the diversity of poorly resourced settings where paediatric HIV infection is prevalent

- establish the *cost* of replacing the diagnostic protocol used in South Africa (at the time) with an alternative protocol that would provide a diagnosis early in infancy

- *disseminate* the results of this work to contribute towards practice guidelines, standard operating procedures and training materials to *advocate* for earlier diagnosis of infants in South Africa and other low resource settings

# 3. METHODS

All HIV infected women delivering at Coronation Women and Children's Hospital (CWCH) who could be contacted telephonically were eligible to enroll their infants at 6 weeks of age. For practical considerations, enrollment was limited to seven patients per week. The protocol was approved by the Ethics Committee of the University of the Witwatersrand (Protocol number M02-01-16) (Appendix 1). The signed informed consent (Appendix 2) and patient identifying data (Appendix 3) were filed separately from the clinical documents to maintain confidentiality. The study protocol was optimized according to the outcomes of a pilot study undertaken at the same facility in 2001.

The sample size was calculated using SAS software version 9 by estimating loss to follow-up (including deaths), vertical transmission and breastfeeding rates. A sample size of 300 was chosen in order to detect a difference of <1% in sensitivity or specificity of a 6-week HIV DNA PCR test assuming the sensitivity and specificity of the HIV DNA PCR test were between 90 and 99%.

Infants were managed according to national 'standard of care' guidelines (54) except that an earlier diagnosis was available at 4 months of age. Thus, all infants were followed to 12 months of age but their HIV infection status was established earlier according to CDC guidelines, which provided the 'gold standard' against which other HIV test results were assessed (Table 1). No antiretroviral therapy other than Nevirapine for PMTCT was available to these infants.

**Table 1. STUDY VISIT SCHEDULE**

| ASSESSMENT | 6 WEEKS | 3 MONTHS | 4 MONTHS | 7 MONTHS | 12 MONTHS |
|---|---|---|---|---|---|
| | | | | | |
| **History & clinical examination** | X | X | | X | X |
| **Laboratory tests** | | | X* | | |
| HIV DNA PCR (qualitative) | X (DBS) | X | | (X) | (X) |
| HIV RNA PCR (quantitative) | X | | | | |
| HIV ELISA (serum) | | X | | X | X ! |
| HIV ELISA (oral fluid) | | | | | X |
| **HIV status disclosure** | | | X | | |

(DBS) Dried blood spot for storage and subsequent analysis

* Further testing according to CDC guidelines if required (e.g. if two HIV PCR results are discrepant)

(X) Specimen to be separated, frozen and stored for later processing where necessary

! Children breastfed in the preceding 3 months to undergo further testing

Study visits were defined as follows:

| | | |
|---|---|---|
| 6 week visit | - | between 5 weeks and 8 weeks of age |
| 3 month visit | - | 2 weeks on either side of 3 months of age |
| 4 month visit | - | at least 2 weeks after the 3 month visit |
| 7 month visit | - | between 7 and 8 months of age |
| 12 month visit | - | between 11 and 12 months of age |

The protocol for sampling, preparation and storage of blood and oral fluid is detailed in Appendix 4. Clinical examination findings were standardized (Appendix 5) and clinical data sheets devised to document findings at each visit (Appendix 6). To ensure an unbiased clinical assessment, paediatricians and medical officers were blinded to the infants' HIV test results. At each clinical visit the infants' HIV infection status was determined according to the clinical findings. The infants' HIV infection status was documented as 'uninfected', 'infected' or 'unknown'.

HIV tests detailed in Table 1 and Appendix 4 were performed prospectively on blood:

HIV DNA PCR: Roche Amplicor HIV-1 DNA version 1.5 assay
HIV RNA PCR: Roche Amplicor Monitor version 1.5 assay
(Roche Diagnostic Systems Inc, Branchburg, NJ)
HIV ELISA: HIV-1/HIV-2 III Plus (IMx System)
(Abbott Diagnostics Division, Wiesbaden, Germany)

and oral fluid:

OraSure collection device (Orasure Technologies, Inc., Bethlehem, PA) and the Vironostika Microelisa system
(Organon Teknika Corporation, Durham, NC)
Oraquick Rapid HIV-1/2 Antibody test
(Orasure Technologies, Inc., Bethlehem, PA).

HIV DNA PCR on stored dried blood spots and the Ultrasensitive p24 Antigen assay (HIV-1 p24 Ag Ultra kit, PerkinElmer Life Sciences, Turku, Finland) on stored plasma were performed later once the methodology of these assays had been optimized.

The costing substudy datasheet (Appendix 7) was designed and tested for completion on a convenience sample of 30 patients at each of their five visits. For practical reasons two new patients were randomly enrolled per week.

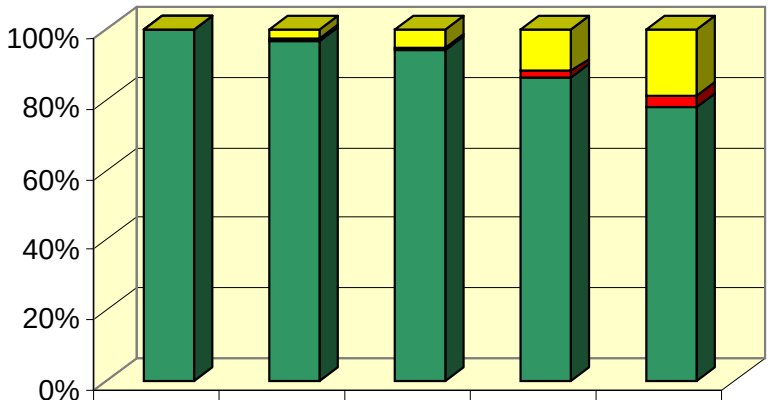
# 4. RESULTS

The outcomes are presented as six publications and unpublished work. Reference is made to additional publications emanating from the study. Inconsistencies are outlined in Appendix 8.

Infants were enrolled between January and October 2002 and their follow up completed by September 2003. The infant's 3-month HIV ELISA test served as a check of the mother's positive HIV status since her HIV test results were not always available due to incomplete antenatal clinic records. To achieve the sample size of 300, 302 infants were enrolled. One infant was excluded on the basis of a negative 3-month HIV ELISA result and although the mother's positive HIV status was subsequently confirmed there was no definitive evidence of HIV exposure at birth. A second infant was initially excluded because no 6-week HIV PCR results were available until the dried blood spot HIV DNA PCR tests were processed after the study was completed. Of the 300 infants, 151 were female and 149 were male.

Figure 3 illustrates the study follow-up rates and provides an update to Figure 1A in Publication 1 when study visits were incomplete. The intervals at which 10 (38.5%) of the 26 HIV-infected children died are shown. This concurs with a later study demonstrating that 35% of HIV-infected infants in sub-Saharan Africa die within the first year of life (60). No HIV-uninfected infants were known to have died during the study period. By 12-months of age 57 (19%) patients had defaulted from the study.

**Figure 3. Study visit attendance**




| | 6wks | 3mo | 4mo | 7mo | 12mo |
|---|---|---|---|---|---|
| Visits missed | 0 | 7 | 15 | 36 | 57 |
| Deaths | 0 | 2 | 3 | 5 | 10 |
| Visits attended | 301 | 292 | 283 | 260 | 234 |

**Determination of the HIV infection status of 301 infants** (Publication 2)

No discordant qualitative or quantitative HIV PCR test result was recorded for any infant irrespective of their age.

All 26 infants diagnosed as HIV-infected fulfilled the CDC criteria (3). At least two positive HIV PCR test results at 6-weeks and 3-months of age were recorded for 24 infants. Two infants died prior to the 3-month visit but were clinically symptomatic and tested HIV DNA and RNA PCR positive at 6-weeks of age. A third HIV DNA PCR test was prospectively performed in six (23%) infected infants at 4-months of age because they appeared clinically unaffected, evidence of the lack of sensitivity of a clinical examination early in infancy.

Of the 275 infants diagnosed as uninfected, 30 (10%) did not fulfill CDC criteria chiefly because they were lost to follow up. Seven infants defaulted before the 3-month visit but were considered uninfected at 6-weeks of age on the basis of being clinically asymptomatic and having three negative HIV PCR results (viz.

liquid blood HIV DNA and RNA PCR and DBS HIV DNA PCR (Table 1)). The remaining 23 infants all had at least two negative HIV PCR results at 6-weeks and 3-months of age.

The balance of 245 infants diagnosed as uninfected fulfilled CDC criteria in that they all had two negative HIV PCR results at 6-weeks and 3-months of age, and either seroreverted by 12-months of age (n=124) or had an additional negative HIV PCR result obtained retrospectively on stored sample from their 12- or 7-month visit (n=121).

**Clinical diagnosis of HIV infection in infancy**

Table 2 illustrates the outcome of the prospective clinical assessments performed by the study doctors. Despite being performed by experienced doctors, a clinical examination alone at 6-weeks of age identified a little over half of HIV-infected infants although sensitivity did improve dramatically with age.
One year after commencement of this study, a clinical algorithm to identify children with suspected HIV infection for HIV testing was incorporated into the South African Integrated Management of Childhood Illness (IMCI) guidelines (38, 61). When the clinical findings of this study were retrospectively analysed according to the IMCI algorithm, significantly less HIV-infected children were identified as evidenced by lower sensitivities in all age groups under 1 year (38, 62) (Table 2). By 12 months of age the IMCI algorithm identified only half of all HIV-infected infants but even more worrying was its failure to detect 50% of HIV-infected study infants who died before 12-months of age (62). Clinical examination, although useful when used in conjunction with HIV testing, fails to identify infants at risk of HIV-related deaths.

| Table 2. | | | | | | | PERFORMANCE OF CLINICAL ASSESSMENTS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | n | True HIV status | | Clinical assessments by study doctors | | | Study doctors' assessments (according to Appendix 5) | | | | IMCI clinical algorithm | | | |
| | | N | P | N | P | Unknown [% of total] | SN % | SP % | PPV % | NPV % | SN % | SP % | PPV % | NPV % |
| **6 weeks** | 301 | 275 | 26 | 210 | 33 | 58 [19] | 56** | 90** | 30 | 96 | 17 | 97 | 30 | 94 |
| **3 months** | 290 | 267 | 23 | 224 | 21 | 45 [16] | 67** | 96* | 57 | 97* | 11 | 100 | 100 | 93 |
| **7 months** | 258 | 238 | 20 | 223 | 18 | 17 [7] | 94** | 99.5 | 89 | 99.5* | 47 | 99.6 | 89 | 96 |
| **12 months** | 234 | 219 | 15 | 208 | 15 | 11 [5] | 93** | 99.5 | 87 | 99.5* | 50 | 99.5 | 88 | 97 |

The accuracy of the "**Study doctors' assessments**" is over-estimated since infants of "**unknown status**" were excluded.

The accuracy of the "**IMCI clinical algorithm**" was determined retrospectively.

N= negative; P= positive; SN= sensitivity; SP= specificity; PPV= positive predictive value; NPV= negative predictive value

* statistically significantly different $p < 0.05$ ($\chi^2$ test) ; ** statistically significantly different $p < 0.005$ ($\chi^2$ test)

**Improving the clinical utility of HIV antibody assays in infants**

The pattern of HIV antibody changes over time (Figure 4) was similar to that previously documented for vertically exposed South African infants where the divergent pattern of HIV antibodies in infected and uninfected infants emerged at about 6 months of age (43).

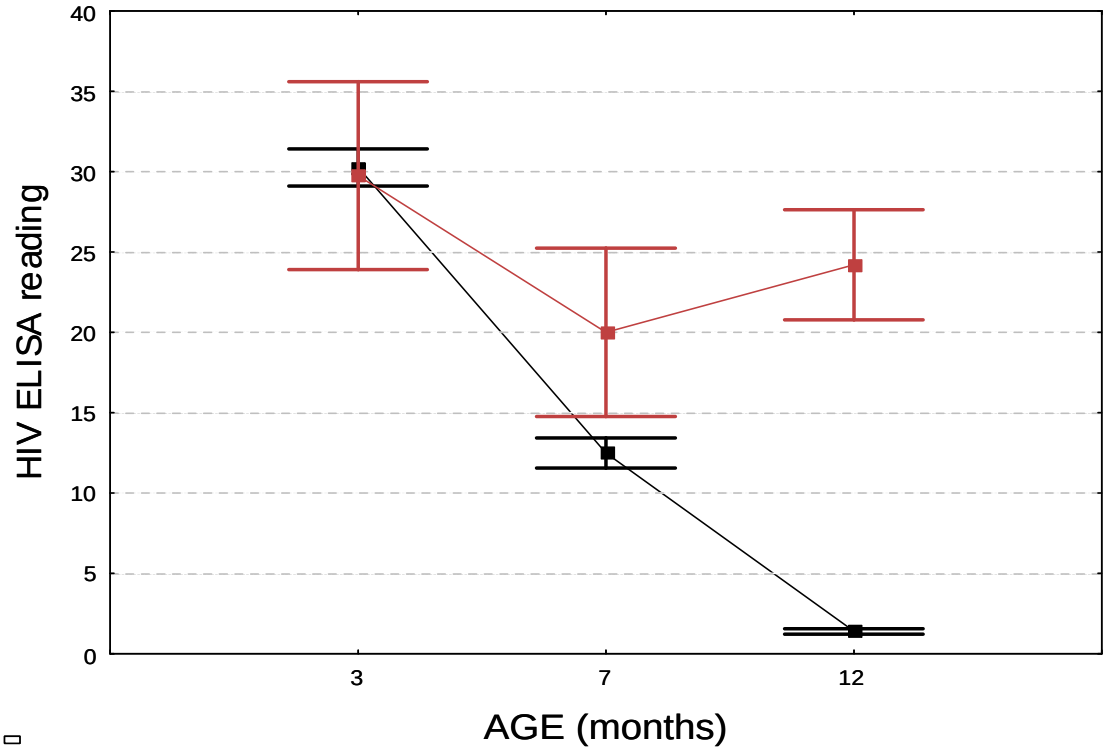



**Figure 4.** Serial HIV ELISA readings from 301 HIV-exposed infants.

The graph depicts the mean ± 95% confidence intervals of serial ELISA readings for **26 HIV-infected** and **275 HIV-uninfected** infants at median ages of 3.0, 7.5 and 12.1 months respectively. Results are available for 15 infected and 212 uninfected infants at all three time points. At 7-months of age outliers (not depicted here) prevent effective discrimination of HIV status on the basis of ELISA readings but by 12-months of age there is much less overlap.

ELISA tests deliver a qualitative result by measuring specific HIV antibodies and using a cutoff reading, statistically set from clinical populations, above which the test is positive and below which the test is negative. The manufacturer's recommended cutoff value for the ELISA reading is 1 therefore readings greater than 1 are positive (Figure 5). At 12-months of age, 122 of the 219 HIV-uninfected children (specificity = 56%) had negative HIV ELISA results (Figure 5). If the same test results are interpreted using a higher cutoff reading of 5, 208 of the 219 uninfected children (specificity = 95%) can be correctly identified as being HIV-uninfected.

HIV-infected children had higher ELISA readings and were clinically symptomatic in comparison to HIV-uninfected children. Reduced antibody production during end stage acquired immunodeficiency syndrome may result in low HIV ELISA readings in an infected child however a clinical assessment would readily identify such a child (47). Since virtually all 12-month old HIV-infected children display signs and symptoms of HIV infection, the sensitivity of the HIV ELISA test is further safeguarded (62).

All infants under 18 months of age with positive HIV ELISA results require repeat testing to distinguish between HIV-infection and HIV-exposure. Of the 234 children tested only 26 (11%) instead of 112 (48%), using a cutoff value of 5 and 1 respectively, would need to return for repeat testing after 12-months of age provided they were not being breastfed. Therefore increasing the ELISA reading cutoff from 1 to 5 at 12-months of age increased the specificity of the HIV ELISA test by 39% whilst maintaining the sensitivity at 100%.

Further investigation is warranted to explore the earliest age at which HIV infection can be excluded in HIV-exposed children using antibody detection assays. In particular, rapid HIV tests on blood and oral fluid (Publication 5) may be effective as early as 6-months of age.

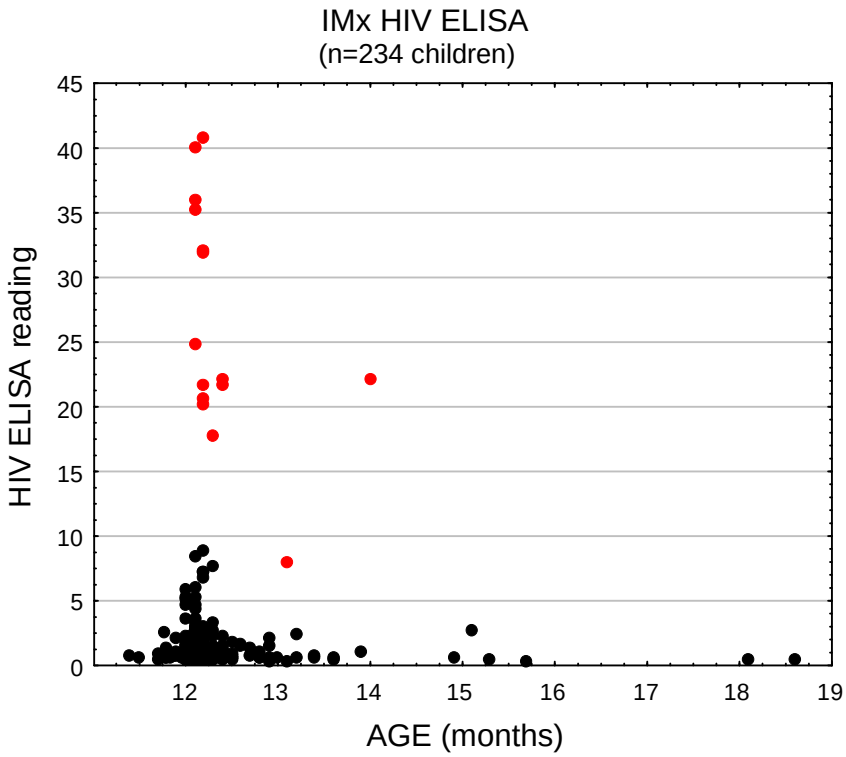



**Figure 5.** ELISA readings at a median of 12.1 months of age in 15 HIV-infected ● and 219 HIV-uninfected ● children

The 13-month old HIV-infected child with a reading of 8 had persistent, multiple clinical features consistent with HIV-infection since 7-months of age. Conversely, HIV-uninfected children with readings between 5 and 10 were clinically asymptomatic.

## 4.1 INTRODUCTION TO PUBLICATION 1

*Sherman GG, Jones SA, Coovadia AH, Urban MF, Bolton KD.*

***PMTCT from research to reality – results from a routine service.***

*S Afr Med J 2004;94(4):289-292*

ISI 2004 journal impact factor: 1.107

Permission to reproduce: Appendix 9

The study provided the first opportunity to assess the HIV transmission rate of the CWCH PMTCT program. Additionally the efficacy of Nevirapine administration, infant feeding practices and infant follow-up rates for the program could be evaluated. The paper sets the scene of how routine PMTCT care is practiced in South Africa and illustrates the importance of earlier infant diagnosis for monitoring the efficacy of PMTCT programs and identifying HIV-infected children for care. It suggests that the 'low resource setting' infant diagnostic protocol must be revised to achieve an earlier, accurate and affordable HIV diagnosis.

At the time of this publication, a single 6-week HIV DNA PCR test result appeared highly accurate in determining the HIV infection status of exposed, non-breastfed infants. This was borne out in the final analysis of 26 HIV-infected and 274 HIV-uninfected 6-week old infants demonstrating that a single HIV DNA PCR test performed on liquid blood yielded a sensitivity of 100%, specificity of 99.6% (99.0-100.3%) due to one false positive result, positive predictive value of 96.3% (94.2-98.4%) and negative predictive value of 100% where the bracketed values indicate 95% confidence intervals. In Publication 2 when the cohort was combined with two others, a single 6-week HIV DNA PCR test was shown to be 98.8% sensitive and 99.4% specific in 627 infants.

Quantitative HIV RNA PCR results on liquid blood were available at 6-weeks of age for 24 infected and 257 uninfected children. Results concurred with the final HIV infection status of every child except for one false positive result of 109 copies per milliliter. Because of the very low viral copy number and previous descriptions of low false positive results in these instances (63, 64), this was presumed to be a false positive at the time the result was received. The sensitivity of 100% and specificity of 99.6% of this viral load assay equals the performance of the HIV DNA PCR assay but since it did not add any accuracy and cost more at the time, it was not pursued further.

## 4.2 INTRODUCTION TO PUBLICATION 2

*Sherman GG, Cooper PA, Coovadia AH, Puren AJ, Jones SA, Mokhachane M, Bolton KD.*

**Polymerase Chain Reaction for Diagnosis of Human Immunodeficiency Virus Infection in Infancy in Low Resource Settings**.

*Pediatr Infect Dis J 2005;24(11):993-997.*

ISI 2004 journal impact factor: 2.735

Permission to reproduce: Appendix 10

Although a single HIV DNA PCR version 1.5 assay at 6 weeks of age in the setting of this study performed extremely well, further validation of this approach was sought by increasing the sample size and broadening the clinical and laboratory conditions. The study cohort was combined with two other clinical cohorts where the HIV infection status of children had been established and 6-week HIV DNA PCR results, performed in a different molecular laboratory, were available for assessment.

## 4.3 INTRODUCTION TO PUBLICATION 3

*Sherman GG, Stevens G, Jones SA, Horsfield P, Stevens WS.*

***Dried blood spots improve access to HIV diagnosis and care for infants in low-resource settings.***

*J Acquir Immune Defic Syndr 2005;38(5):615-617*

ISI 2004 journal impact factor: 4.1

Permission to reproduce: Appendix 10

Infants' access to a diagnosis is dramatically enhanced when HIV laboratory tests can be performed on DBS. Most healthcare workers in low resource settings would not have the skills required to venesect 6-week old babies foiling implementation of widespread early HIV PCR testing. In contrast, blood sampling by heelprick is widely practiced and requires less training.

This paper describes how the HIV DNA PCR test method was modified to analyse DBS instead of liquid blood and demonstrates that the modified assay maintains its accuracy on DBS collected from 6-week old infants. Poorly resourced settings with high throughput routine laboratories will not have an abundance of skilled personnel to troubleshoot therefore strategies to minimize laboratory error are important. Examples of such strategies include using standardized, commercially available test kits and simplifying test methods as far as possible. The unique extraction procedure described in this paper means that the method for performing HIV DNA PCR tests is highly similar whether on DBS or liquid blood.

Whatman filter paper no. 1 (Whatman Inc., Maidstone, Kent, UK) (Figure 6) was used because it is ubiquitous in laboratories and less expensive than the FDA-approved Schleicher and Schuell filter paper (S&S 903, Whatman Inc., Sanford, Maine, USA) (Figure 7) however, it is less absorbent resulting in blood spillage and thereby an increased biohazard and cross contamination risk in the field. S&S filter paper is more standardized than the Whatman filter paper with

demarcated circles for blood collection and dedicated space for labeling the sample. The consensus opinion amongst South African policy makers was to adopt S&S 903 paper for DBS HIV DNA PCR testing.

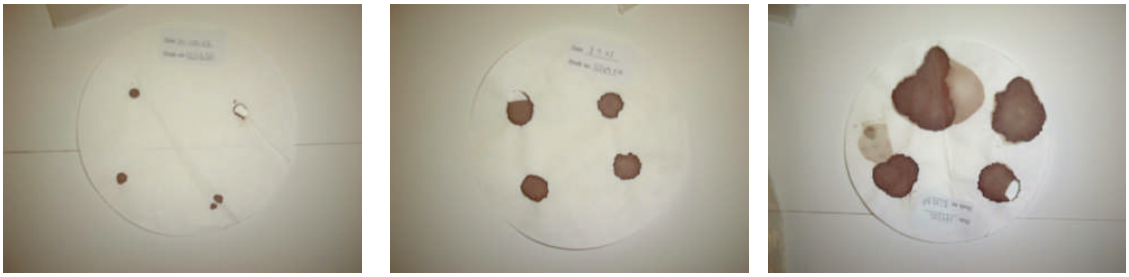


**Figure 6.** DBS from three 6-week old infants collected on Whatman no. 1 filter paper

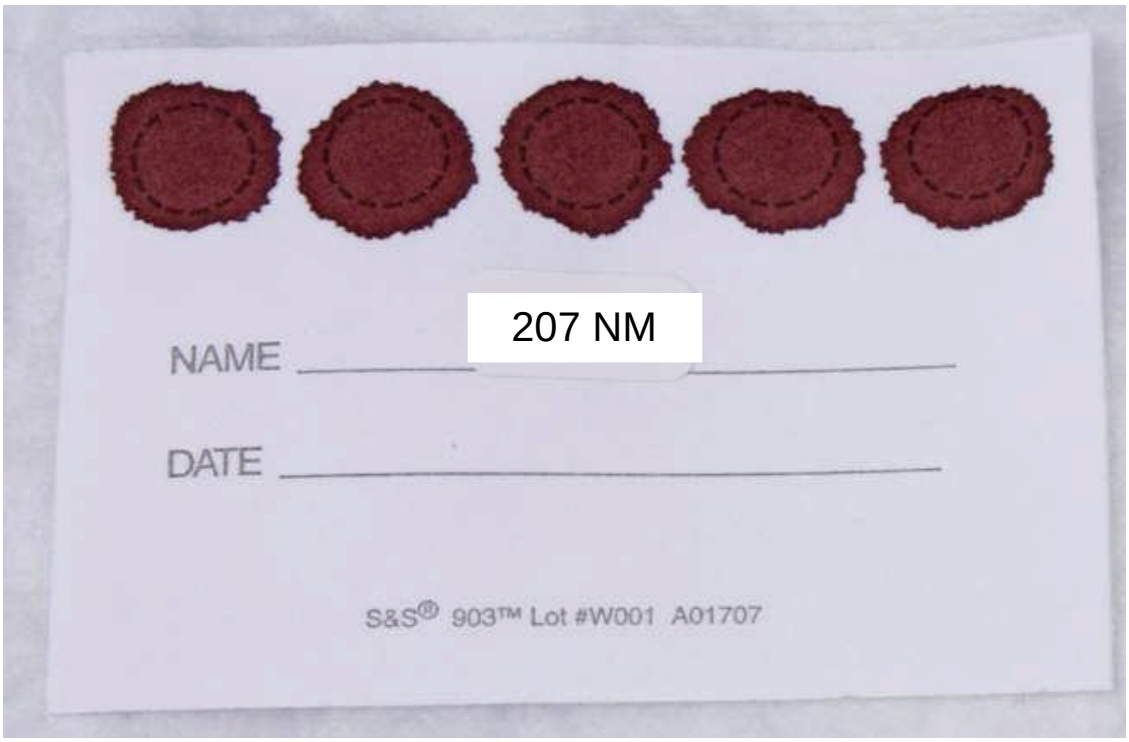



**Figure 7.** S&S 903 filter paper (Guthrie card) for DBS collection

## 4.4 INTRODUCTION TO PUBLICATION 4

*Sherman GG, Stevens G, Stevens WS.*

**Affordable diagnosis of human immunodeficiency virus infection in infants by p24 antigen detection**.

*Pediatr Infect Dis J 2004;23(2):173-176.*

ISI 2004 journal impact factor: 2.735

Permission to reproduce: Appendix 10

The performance of the Ultrasensitive p24 Antigen ELISA using Schupbach's lysis buffer (65) was assessed at predominantly 6 weeks and 3 months of age on stored plasma. This viral detection assay provides a third option for early diagnosis of HIV in infants to add to HIV DNA and RNA PCR. At 6-weeks of age it demonstrated equal specificity and only slightly less sensitivity at 96% (resulting from a single false negative result). The assay is well suited to settings where skills and equipment to perform PCR are limited. Disadvantages currently associated with the assay, but that can be circumvented, are that the commercially available kit is supplied with an inferior lysis buffer, pricing is inflated and accessibility will remain limited until DBS can be used which requires validation of the DBS methodology by more laboratories (66). The assay does yield accurate results on dried plasma spots (67) however DBS collection is easier in the field and would therefore be the preferred sample.

## 4.5 INTRODUCTION TO PUBLICATION 5

*Sherman GG and Jones SA.*

**Oral Fluid Human Immunodeficiency Virus Tests. Improved Access to Diagnosis for Infants in Poorly Resourced Prevention of Mother to Child Transmission Programs.** *Pediatr Infect Dis J 2005;24(3):253-256.*

ISI 2004 journal impact factor: 2.735

Permission to reproduce: Appendix 10

Viral detection assays are currently unavailable in the majority of low resource settings and for the time being HIV antibody detection assays have to suffice. Since it is less than ideal to diagnose HIV late in infancy, the efficacy of this group of tests has to be maximised.
Manipulating HIV antibody detection assays to provide the earliest, most accurate diagnosis possible was investigated by assessing two oral fluid HIV antibody tests in this paper.

Oral fluid HIV tests are not FDA-approved for children under the age of 13 years. The use of oral fluid instead of blood increases the accessibility and acceptability of HIV testing since less skill is required to obtain the sample and sampling is less painful. Oral fluid has a substantially lower total antibody concentration in comparison to serum providing a naturally 'dilute' sample which would be expected to reduce sensitivity of antibody detection, ideal for the setting of reducing false positive results due to waning maternal HIV antibodies during seroreversion. This paper demonstrates how effective oral fluid is in reducing the need for repeat testing at 12 months of age and outlines issues that require further research. The sample size of 321 children was comprised predominantly of study patients in addition to infants presenting to the CWCH PMTCT clinic for a diagnosis of HIV infection status. Informed consent was obtained for all infants.

## 4.6 INTRODUCTION TO PUBLICATION 6

*Sherman GG, Matsebula TC, Jones SA.*

***Is early HIV testing of infants in poorly-resourced PMTCT programs unaffordable?***

*Trop Med Int Health 2005;10(11):1108-1113*

ISI 2004 journal impact factor: 1.969

Permission to reproduce: Appendix 11

The outcome of the cost analysis substudy is detailed here. The main study was concerned with assessing various age-appropriate HIV tests to provide evidence for the accuracy of a new diagnostic algorithm. Examining the financial implications of implementing this new algorithm is crucial in advocating for a change in the HIV diagnosis policy. This paper measured the average cost of ascertaining an infant's HIV infection status for two diagnostic algorithms and illustrates that using a cheaper HIV test late in infancy is costlier to society than earlier testing with a more expensive HIV PCR test.

## 4.7 PUBLICATIONS 1 - 6

### 4.7.1. PUBLICATION 1

### 4.7.2. PUBLICATION 2

### 4.7.3. PUBLICATION 3

### 4.7.4. PUBLICATION 4

### 4.7.5. PUBLICATION 5

### 4.7.6. PUBLICATION 6

# 5. CONCLUSIONS

The strength of the longitudinal study design was the ability to assess the accuracy of each HIV test in relation to the clinical HIV infection status of the infant established over 12 months according to a 'gold standard' (3). Furthermore, the HIV tests were performed at specific, tightly controlled ages rather than the more commonly encountered range of ages. This has the advantage of being able to inform practical diagnostic guidelines that take cognisance of the healthcare visits infants are committed to such as immunization schedules at 6 weeks and 14 weeks (3 months) of age.
The longitudinal design and low prevalence of breastfeeding were vital in demonstrating that *all* HIV infection transmitted in-utero and intrapartum was detected by HIV DNA PCR at 6 weeks of age contrary to earlier concerns (3, 50-52).

The originality of the study is manifested by many firsts. The first

- assessment of the efficacy of the CWCH PMTCT program (Publication 1)
- demonstration of the accuracy of a single 6-week HIV DNA PCR result in determining the HIV infection status of non-breastfed infants (Publication 2)
- modification of the extraction method of the HIV DNA PCR assay for DBS testing in high throughput laboratories ushering it into the realm of routine diagnostics and the demonstration that it performed as well on DBS as on liquid blood in 6-week old infants (Publication 3)
- assessment of the ultrasensitive p24 Ag assay for infant diagnosis in subtype C virus (Publication 4)
- evaluation of two oral fluid HIV tests in children and the proposal of a unique clinical setting in which oral fluid testing may be superior to blood for excluding HIV infection (Publication 5)
- measurement of the cost of establishing an infant's HIV infection status (Publication 6)

- illustration of how modifying serum HIV ELISA cutoff readings in 12-month old infants can accelerate a definitive diagnosis (unpublished data).

In the ideal PMTCT setting assuming Nevirapine administration (68) and exclusive formula feeding, the Health Systems Trust estimated that the HIV transmission rate in South Africa could decrease from 35% to 13% at 6 months of age (56). The CWCH PMTCT program demonstrated that the transmission rate could be reduced below 9% at 3 months of age (Publication 1) which has been confirmed by ongoing monitoring of the program. In 2003 after the FDA raised questions regarding the HIVNET012 study and the Medicines Control Council reviewed the use of Nevirapine for PMTCT, data from this study were used to successfully support continued use of Nevirapine in national PMTCT programs (Appendix 12).

The most outstanding achievement of this study was the adoption by the South African National Department of Health in April 2004 of a new diagnostic algorithm based on a single 6-week HIV DNA PCR test (Appendix 13) (69). Implementation of the policy is evident by the more than 10 fold increase in number of HIV DNA PCR tests being performed nationally and the National Health Laboratory Services' strategic plan to increase the number of laboratories capable of performing HIV DNA PCR testing from three to 11 by the end of 2006 (personal communication Dr T. Marshall).

The global commitment to providing antiretroviral treatment in low resource settings cannot be achieved without a diagnosis of HIV infection. Hence this work has attracted enormous interest from the international community culminating in multiple invitations to present at Paediatric HIV Care Consultation meetings hosted by CDC, WHO, UNICEF and ANECCA. It is widely quoted in the draft WHO guidelines "Antiretroviral treatment of HIV infection in infants and children in resource-limited settings, towards universal access: Recommendations for a public health approach" due for release in 2006 (http://www.who.int/en/).

Opportunities that have arisen from this work include an invitation to chair the HIV Collaborative Forum's (based in Washington D.C) recently launched 'Pediatric HIV Diagnosis and Monitoring Working Group' and chairing the 'Early identification Task Team' established by the South African Concerned Child Healthcare workers and the National Department of Health to improve the quality of HIV care to children in this country.

Additional contributions include modification of the ultrasensitive p24 Ag assay method for use on DBS to improve accessibility of the assay in low resource settings (66), exploration of socio-economic reasons for the high loss to follow-up rates at the CWCH PMTCT program (70), establishing decay patterns of the K103N mutation after single-dose Nevirapine for PMTCT to inform treatment decisions (71), insights into the psychosocial consequences of an early diagnosis of infants (72) and disclosure issues in the context of vertical transmission (73).

Before DBS testing was adopted, further investigation was requested to demonstrate that the HIV DNA PCR assay performed as well on DBS made from heelpricks (capillary blood) as it did on DBS prepared from formal venesection (venous blood). The latter was used in this study. Informed consent to perform formal venesection as the 'gold standard' sample and a heelprick onto S&S 903 filter paper on the same child at a single visit was obtained. The sensitivity of the heelprick sample was 98.3% due to a single false negative result and the specificity was 98.6%. Although DBS are ideal for the clinical setting, in the laboratory they are more laborious to process than liquid blood and investigation of automated punches to overcome this is underway.

At commencement of this study in 2002, early infant diagnosis of HIV was a new phenomenon in the public health care setting. According to the Child Care Act of 1983 informed consent had to be obtained from a parent or legal guardian. The Act failed to recognize the reality of the situation in South Africa which was the existence of substantial numbers of orphaned children who had neither parents

nor legal guardians. In order to safeguard healthcare workers on this study and in other situations dealing with HIV testing of children the Wits AIDS Law Project on behalf of the Wits Paediatric HIV Working Group, obtained a High Court Order on 5 December 2003 to allow informed consent to be taken from the child's primary caregiver provided that an HIV test was in the best interest of the child (Appendix 14) (74). This set a precedent that has been incorporated into the new Children's Bill (75).

Following the global commitment to treating HIV infection in poorly resourced settings, the need for diagnosing infants early has become evident and renewed efforts in this field are manifested by investigation of DBS testing (67, 76-78), real time PCR (77, 79), the ultrasensitive p24 Ag assay (80) and near patient testing (81). Quantitative HIV RNA tests are required in low resource settings for initiating and monitoring antiretroviral therapy. To reduce the panel of laboratory tests necessary to manage the epidemic, these assays may emerge as the logical choice for infant diagnosis but will require further validation (64, 79, 82, 83).

The results of this study have allowed additional funding to be secured for further research and to support scale-up of infant diagnosis in South Africa.
Ongoing studies stemming from this work comprise

- assessment of other viral detection assays on DBS including use of real time PCR and automated laboratory systems to increase capacity
- validation of the DBS Ultrasensitive p24 Ag method for 6-week old infants
- further evaluation of HIV antibody detection assays including 4$^{th}$ generation and oral fluid HIV ELISA assays and rapid HIV tests.

A mobile 'infant diagnostic team' comprising a doctor and primary health care nurse has been trained and will perform outreach work to initiate sustainable infant diagnostic services in collaboration with the Gauteng Department of Health's HAST (HIV/AIDS and Sexually Transmitted diseases) Directorate.

Funding to increase the capacity of the Johannesburg Hospital's molecular laboratory has been secured to launch DBS HIV DNA PCR testing and provide laboratory training and support. Clinical and laboratory standard operating procedures for infant diagnosis of HIV are being collated for widespread dissemination nationally and internationally. Appendix 15 contains an example of one of the clinical standard operating procedures developed from this study for use in training to ensure good clinical practice.

Local and international clinical and laboratory collaborative networks as a result of this study continue to expand and are essential to achieving rapid, global scale-up of HIV diagnosis in infants. The world that once believed that HIV could not be treated in low resource settings has witnessed heartening results with antiretroviral therapy. Now it seems possible that HIV diagnosis in infancy may also be attainable….