UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

MATERNAL SYPHILIS IN A RURAL AND URBAN COMMUNITY IN WESTERN CAPE, SOUTH AFRICA: A CROSS-SECTIONAL ANALYSIS

By

Maxine de Araujo

(DRJMAX001)

Minor dissertation

Submitted to the University of Cape Town in partial fulfillment of the requirements for the degree of MPhil Maternal and Child Health

FACULTY OF HEALTH SCIENCES

Department of Paediatrics and Child Health

UNIVERSITY OF CAPE TOWN

31 January 2025

Supervisors:

A/Prof Emma Kalk: Centre for Integrated Data and Epidemiological Research (CIDER), School of Public Health, University of Cape Town

Dr Stuart Maxwell Kroon: Division of Neonatal Medicine, Department of Paediatrics and Child Health, University of Cape Town

DECLARATION

I, Maxine de Araujo, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being or is to be submitted for another degree at this or any other university.

I empower the University of Cape Town to reproduce for the purpose of research either the whole or any portion of the contents of this dissertation in any manner whatsoever.

Signed by candidate

Signed: _____

Date: 31 January 2025_____

TABLE OF CONTENTS

List of Tables v
List of Figures v
List of Appendices v
Acknowledgments vi
List of Abbreviations vii
Glossary of Key Concepts ix
Abstract x

CHAPTER 1: INTRODUCTION
1. Introduction 1
2. Background 1
   2.1. Prevalence of Maternal and Congenital Syphilis 1
   2.2. Management of Maternal Syphilis 3
      2.2.1. Syphilis Screening 3
      2.2.2. South African Policies and Guidelines 4
3. Study Rationale 5
4. Purpose of the Study 5
   4.1. Research Questions 5
   4.2. Research Aim 5
   4.3. Research Objectives 6

CHAPTER 2: LITERATURE REVIEW
1. Introduction 7
2. Management of Maternal Syphilis 7
   2.1. Antenatal Screening 7
      2.1.1. Facilitators of POC Screening 8
      2.1.2. Obstacles of POC Screening 9
   2.2. Antenatal Treatment 9
   2.3. Partner Notification 10
3. Associations with Syphilis 11
   3.1. Clinical Factors 12
      3.1.1. Gravidity and Parity 12
      3.1.2. History of Obstetric Complications 12
      3.1.3. HIV 12
      3.1.4. Other STIs 13
   3.2. Sociodemographic and Behavioural Factors 13
      3.2.1. Maternal Age 13
      3.2.2. Level of Education 14
      3.2.3. Access to ANC Services 14
      3.2.4. Sexual Practices and Behaviours 15
      3.2.5. Substance Use 15
4. Conclusion 15

CHAPTER 3: METHODOLOGY
1. Introduction 17

2. Study Design 1
3. Study Population 17
3.1. Setting and Description of the Parent Study 17
3.2. Inclusion Criteria 18
3.3. Exclusion Criteria 19
3.4. Recruitment and Enrollment Procedures 19
3.5. Sample Size 19
4. Research Procedures 19
4.1. Data Collection Instruments 19
4.2. Data Collection Methods 20
4.3. Quality Control 21
4.4. Variables 21
4.5. Data Management and Analysis 24
4.6. Resources 24
5. Ethical Considerations 24
5.1. Risks and Benefits 24
5.2. HREC Approvals 25
5.3. Informed Consent Process 25
5.4. Privacy and Confidentiality 26
5.5. Reimbursement for Participation 26
5.6. Conflicts of Interest 26
5.7. Ethical Compliance 26

CHAPTER 4: RESULTS

1. Introduction 27
2. Sociodemographic Characteristics of Research Sites 27
2.1. Maternal Age 27
2.2. Socioeconomic Status 27
2.3. Maternal-Paternal Relationship 28
3. Clinical Characteristics at Research Sites 30
3.1. Obstetric History 30
3.2. HIV 32
4. Maternal Syphilis 33
4.1. POC Screening 33
4.2. Laboratory Testing 34
4.3. Factors Associated with Maternal Syphilis Status 36

CHAPTER 5: DISCUSSION

1. Introduction 38
2. Prevalence of Maternal Syphilis 38
3. Maternal Syphilis Screening Guideline Implementation 39
3.1. POC Screening 39
3.2. Laboratory Testing 40
4. Factors Associated with Maternal Syphilis Status 41
4.1. Sociodemographic Factors 41
4.2. Clinical Factors 43
5. Study Strengths 45

6. Study Limitations 45

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

1. Conclusion 47
2. Recommendations 47

REFERENCES 49

LIST OF TABLES

Table 1: Primary outcome variables 21
Table 2: Maternal syphilis status categorisation based on laboratory results 22
Table 3: Sociodemographic and clinical variables 23
Table 4: Description of sociodemographic characteristics by research site 29
Table 5: Description of participant obstetric history by research site 31
Table 6: Description of HIV-related characteristics by research site 33
Table 7: Maternal syphilis POC screening by research site 34
Table 8: Comparison of recorded POC screenings and TPHA laboratory tests by research site 34
Table 9: Maternal syphilis laboratory tests by research site 35
Table 10: Count of syphilis laboratory test results 35
Table 11: Proportion of participants categorised by maternal syphilis status based on laboratory results by research site 36
Table 12: Association of sociodemographic and clinical factors with maternal syphilis status 37

LIST OF FIGURES

Figure 1: Directed acyclic graph (DAG) of associations with syphilis in pregnancy 11

LIST OF APPENDICES

Appendix A: REDCap Data Collection Questionnaires 56
Appendix B: UCT HREC Approval 251/2024 80
Appendix C: SU CHERISH HREC Approval N20/08/084 83
Appendix D: UCT CHERISH HREC Approval 723/2021 85
Appendix E: WC Health Research Committee Approval WC_2021_09 93
Appendix F: Informed Consent Form Gugulethu Site 95
Appendix G: Informed Consent Form Breede Valley Site 104

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my supervisors, A/Prof Emma Kalk and Dr Max Kroon, for granting me the opportunity to work under their guidance and generously sharing their expertise. A profound thank you to the CHERISH study team for allowing me to build upon their project and their efforts in recruitment and data collection. A special acknowledgement goes to Heinrich Cupido, whose tireless efforts ensured we had the data necessary to complete this dissertation. Thank you to Elisma Schoeman and Ncumisa Msolo for their guidance.

This dissertation would not have been possible without my parents, who ensured I had a strong educational foundation, for which I am eternally thankful. I am deeply grateful to my partner, Kale-ab Tessera, who kept me motivated, shared his wisdom, and served as my ultimate sounding board. To my dear friend, Dr Mmamapudi Kubjane, thank you for being the catalyst for this project.

Finally, I extend my sincere gratitude to the Gugulethu and Breede Valley clinic staff and most importantly, the women who participated in this study.

LIST OF ABBREVIATIONS

Abbreviation	Full Text
ANC	Antenatal Care
ANCHSS	Antenatal HIV Sentinel Survey
ART	Antiretroviral Therapy
BPG	Benzathine Penicillin G
CDC	Centers for Disease Control and Prevention
CHAI	Clinton Health Access Initiative
CHERISH	Children HIV Exposed Uninfected – Research to Inform Survival and Health
CI	Confidence Interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
DAG	Directed Acyclic Graph
DHB	District Health Barometer
ECL	Electrochemiluminescence
EIA	Enzyme Immunoassay
FTA-ABS	Fluorescent Treponemal Antibody-Absorption Test
GA	Gestational Age
GHS	General Household Survey
HIV	Human Immunodeficiency Virus
HEU	HIV Exposed Uninfected
HUU	HIV Unexposed Uninfected
HREC	Health Research Ethics Committee
ID	Identification
IQR	Interquartile Range
KZN	KwaZulu Natal
LMICs	Low- and Middle-Income Countries
MCR	Maternity Case Record
MOU	Midwife Obstetric Unit
NDoH	National Department of Health
NHLS	National Health Laboratory Services
NICD	National Institute for Communicable Disease
NMC	Notifiable Medical Condition
PCR	Polymerase Chain Reaction
PHC	Primary Healthcare
PMTCT	Prevention of Mother to Child Transmission

POC	Point of Care
REDCap	Research Electronic Data Capture
RPR	Rapid Plasma Reagin
SA	South Africa
SES	Socioeconomic Status
SOP	Standard Operating Procedure
Stats SA	Statistics South Africa
STI	Sexually Transmitted Infection
SU	Stellenbosch University
TB	Tuberculosis
TOP	Termination of Pregnancy
TPHA	Treponema Pallidum Hemagglutination Assay
TPPA	Treponema Pallidum Particle Agglutination Assay
UCT	University of Cape Town
UNAIDS	United Nations Programme on HIV/AIDS
USA	United States of America
VDRL	Venereal Diseases Research Laboratory
VTP	Vertical Transmission Prevention
WC	Western Cape
WHO	World Health Organisation
WLHIV	Women living with HIV

GLOSSARY OF KEY CONCEPTS

Congenital syphilis: The World Health Organisation (WHO) case definition is as follows: "(i) a live birth or fetal death at >20 weeks of gestation or >500 g (including stillbirth) born to a woman with positive syphilis serology and without adequate syphilis treatment or (ii) a live birth, stillbirth or child <2 years of age born to a woman with positive syphilis serology or with unknown serostatus and with laboratory and/or radiographic and/or clinical evidence of syphilis infection (regardless of the timing or adequacy of maternal treatment)". [1]

Gestational age: The period measured in weeks used to describe how far along a pregnancy is. [2]

Gravidity: The number of pregnancies a woman has had, including the current pregnancy, regardless of pregnancy outcome. [2]

Maternal syphilis: Syphilis infection during pregnancy. [1]

Non-treponemal assay: Detects non-specific antibodies released in response to cell damage, indicating an active syphilis infection. [3] Includes the Rapid Plasma Reagin (RPR) test. [3]

Notifiable Medical Condition (NMC): A disease that poses considerable public health risk and is legally required to be reported to a surveillance authority by healthcare providers upon diagnosis. [4]

Parity: The number of times a woman has given birth (live or stillbirth) at a viable gestational age. [2]

Premature birth: A live birth that occurs before 37 completed weeks of gestation. [2]

Spontaneous abortion: An unexpected pregnancy loss of a fetus weighing less than 500g or occurring before 22 weeks of gestation. [2]

Stillbirth: A baby born with a birth weight above 500g and no signs of life. [2]

Treponemal assay: Detects antibodies to *Treponema pallidum* proteins, indicating a past or current syphilis infection. [3] Includes the Treponema Pallidum Hemagglutination Assay (TPHA) and most point of care syphilis tests. [3]

Vertical transmission: Mother-to-child transmission of an infection during pregnancy. [1]

ABSTRACT

Background: Syphilis affects 2 million pregnant women globally each year, with 63% of cases occurring in sub-Saharan Africa. Untreated infection during pregnancy can lead to vertical transmission, resulting in congenital syphilis in 50–90% of cases. Global rates exceed the World Health Organisation's target for congenital syphilis of 50 cases per 100,000 live births, with many countries experiencing a resurgence in the past decade. South Africa's (SA) maternal syphilis prevalence rate increased from 2.6% in 2019 to 3.1% in 2022. While syphilis screening is mandated for all pregnant women in SA, the complexities of screening algorithms and result interpretation, as well as resource constraints, remain a challenge. Further research is required to understand the resurgence of maternal and congenital syphilis, evaluate the effectiveness of existing interventions and inform future actions.

Aim: To describe maternal syphilis screening in terms of the implementation of the 2019 South African Prevention of Mother to Child Transmission (PMTCT) guidelines and prevalence in two communities, one urban and one rural, in the Western Cape (WC) province of South Africa.

Methodology: This cross-sectional study was a secondary analysis of the longitudinal parent study, Children Human Immunodeficiency Virus (HIV) Exposed Uninfected - Research to Inform Survival and Health (CHERISH). Our study analysed data collected between January 2022 and June 2024 from pregnant women (24- and 36-weeks gestation) living with and without HIV (in at least a 1:1 ratio), enrolled from two communities in the WC province of SA; Gugulethu (urban) and the Breede Valley (rural). Demographic and clinical data were collected from Maternity Case Records and face-to-face interviews and syphilis laboratory results were retrieved from the National Health Laboratory Services TrakCare database. Data analyses were performed by descriptive statistics using STATA version 12,1 and statistical significance was determined using Chi-square or Fisher Exact tests using a significance level of 0.05.

Results: Of 941 women, 600 were enrolled in Gugulethu and 341 in the Breede Valley. Most women were unemployed (67.3%), receiving government grants (57.2%), living in informal housing (57.2%), partnered with the father of the current pregnancy (83.5%), and attended their first antenatal care (ANC) visit in the second trimester (54.3%). Our study's HIV prevalence (57.6%) was affected by the enrollment of women with and without HIV in a ratio between 1:1 and 2:1.

Our study found maternal syphilis prevalences of 5.0% (95% CI 3.5%-6.4%) for active infection and 5.8% (95% CI 4.3%-7.3%) for past infection, with significantly higher rates in the Breede Valley than in Gugulethu (6.4% [95% CI 3.8%-9.1%] and 7.6% [95% CI 4.8%-10.5%] versus 4.2% [95% CI 2.6%-5.8%] and 4.8%[95% CI 3.1%-6.6%], respectively, $p = 0.00$). Overall, 87.8% of women had point of care (POC) syphilis screening results recorded, with significantly higher screening rates in the Breede Valley ($p = 0.00$). However, only 74.3% of women with POC screening results were screened at the first ANC visit. Although

73.6% of women underwent Treponema Pallidum Hemagglutination Assay (TPHA) laboratory testing, 18.9% with reactive POC results did not receive follow-up laboratory testing. All women with reactive or equivocal TPHA laboratory results underwent Rapid Plasma Reagin (RPR) testing to confirm active infection, although unnecessary RPR testing was conducted at both research sites.

Maternal syphilis status was significantly associated with socioeconomic status, relationship with the father of the pregnancy, parity, timing of ANC enrollment, HIV status, and pregnancy outcome. Prevalence rates of active (5.5%) and past (7.9%) syphilis were higher among women living with HIV than those without (4.3% and 3.0%, respectively). No significant associations were found with maternal age, gravidity, antiretroviral therapy in women living with HIV, prior obstetric complications, or hospitalisation during the current pregnancy.

Conclusion: Although national and facility-level maternal syphilis screening guidelines were not optimally implemented at either research site, the Breede Valley's practices aligned more closely with the 2019 PMTCT guidelines. Suboptimal implementation included low screening rates at the first ANC visit, poor documentation of POC results, inadequate confirmatory testing for reactive POC results, and the misuse of laboratory resources. Both sites showed active maternal syphilis prevalence rates higher than reported figures, with the Breede Valley more affected. Active syphilis was associated with delayed ANC enrollment, while both active and past infections were more prevalent in women living with HIV and associated with higher stillbirth rates. However, our study was not powered to quantify significant relationships, and potential confounders were not accounted for. Strengthening monitoring, addressing non-compliance, and improving staff training are critical for improving maternal syphilis screening and reducing rising congenital syphilis cases.

Key words: Maternal syphilis, congenital syphilis, syphilis screening, antenatal care, vertical transmission prevention.

CHAPTER 1: INTRODUCTION

1. Introduction

Syphilis, a sexually transmitted infection (STI) caused by the *Treponema pallidum* bacterium, typically occurs when bacteria penetrate the lining of the mouth or genitals through direct contact with a lesion. [5, 6] Globally, an estimated 2 million pregnant women have active syphilis each year. [7] Approximately, only 10% of these women are diagnosed and treated due to sub-optimal screening practices in many low- and middle-income countries (LMICs). [7, 8] Sub-Saharan Africa is responsible for 63% of the global maternal syphilis burden and the STI is associated with an estimated global cost of $309 million annually. [9, 10] Maternal syphilis is often asymptomatic. [9] Active infections may result in a usually painless lesion that spontaneously resolves after 4-6 weeks, while other symptoms, such as a macular rash, may go unnoticed. [3, 9] Once the infection becomes latent, all signs and symptoms usually resolve. [9]

Syphilis is entirely treatable, but if left untreated during pregnancy, it leads to vertical transmission in 50–90% of cases, either through the placenta or contact with lesions during birth. [5, 11] A Chinese study found that untreated maternal syphilis increases the risk of congenital syphilis 68-fold. [12] Infants with congenital syphilis may initially be asymptomatic but can develop signs and symptoms such as skin rash, desquamation, failure to thrive, hepatosplenomegaly, hepatitis, meningoencephalitis, seizures, cranial nerve palsies, pneumonitis, osteitis, anaemia, thrombocytopaenia, blindness, and hearing loss. [5, 13] This may result in long-term disability of the infant or death. [13] Furthermore, late manifestation of infection is possible, with signs and symptoms only becoming apparent after two years of age. [9]

Untreated maternal syphilis is also linked to adverse pregnancy and neonatal outcomes, occurring in an estimated 60% of cases and resulting in approximately 355,000 adverse outcomes annually. [13] These adverse outcomes include fetal death, stillbirth, premature birth, and low birth weight, and are thought to be caused by syphilis-mediated inflammatory markers and cytokines. [5, 13, 14] Maternal syphilis is the second most common infectious cause of stillbirth, with studies in sub-Saharan Africa suggesting it may be associated with 25–50% of cases. [6, 13] A retrospective study in Botswana found that the rate of stillbirth increased in women co-infected with syphilis and Human Immunodeficiency Virus (HIV). [13] The impact of this co-infection is a crucial consideration in South Africa (SA), which has a high antenatal HIV prevalence of approximately 28%. [15] Given the consequences of untreated maternal syphilis, it is crucial to monitor infection prevalence and assess the effectiveness of local interventions.

2. Background

2.1. Prevalence of Maternal and Congenital Syphilis

A study conducted by Korenromp *et al.* estimated global maternal syphilis prevalence at 0.7% in 2012 and 0.69% in 2016. [16] However, this stable trend has shifted in recent years. The 2007 World Health

Organisation (WHO) Global Plan for the Elimination of Congenital Syphilis set a target of 50 cases per 100,000 live births, but in 2020, the global rate was an extraordinary 425 cases per 100,000 live births. [13]

Korenromp *et al.* estimated that in 2016, maternal syphilis prevalence in Africa was more than twice the global rate, at 1.5%.[16] A 2018 meta-analysis found a pooled maternal syphilis prevalence of 2.9% in sub-Saharan Africa, equating to 1 million pregnancies annually, with a prevalence of 2.5% in Southern Africa. [7] Despite most cases occurring in LMICs, a recent resurgence of congenital syphilis has been documented in high-income countries, such as Australia and the United Kingdom. [13] Anecdotally, the New York Times cited a report from the Centers for Disease Control and Prevention (CDC) stating that congenital syphilis cases in the United States of America (USA) increased 11-fold between 2012 and 2022. [17] Similar patterns are expected to have occurred in LMICs, but prevalence rates are often under-reported due to underdeveloped surveillance and information systems. [13]

In SA, the 2022 Antenatal HIV Sentinel Survey (ANCHSS) reported a national maternal syphilis prevalence of 3.1%.[15] This indicates a 19% increase from the 2019 national prevalence of 2.6% and a 55% rise from the 2015 prevalence of 2.0%.[15] KwaZulu-Natal (KZN) had the highest provincial prevalence at 4.4%, while Limpopo had the lowest at 1.4%.[15] The Western Cape (WC) reported a prevalence of 3.9%, with district rates ranging from 2.6% to 5.3%, marking an increase from 2.2% in 2019 and 1.7% in 2015. [15] Of the women who tested positive nationally, 85% received treatment with at least one dose (2.4 million units) of benzathine penicillin G (BPG). [15] Intramuscular BPG is the only recommended treatment for maternal syphilis, with 98% efficacy in preventing congenital syphilis. [18] However, recurrent global BPG stock shortages since 2015 have raised concern. [8]

Maternal syphilis prevalence has been studied by several researchers in the KZN province, with varying results. A 2020 clinical audit at a regional hospital found a prevalence of 1.1%, while a 2021 retrospective study at a primary healthcare (PHC) clinic reported a prevalence of 3.8%.[19, 20] A 2023 cross-sectional study observed a prevalence of 5.2% among pregnant women living with HIV (WLHIV). [21] Although recent study data from the WC province are limited, a 2019 study reported a maternal syphilis prevalence of 2.2% at a Cape Town antenatal care (ANC) clinic. [22] A 2015 study estimated 1,771 annual adverse pregnancy outcomes in SA due to undetected maternal syphilis, however, this figure has likely risen given the increasing prevalence rates. [23]

In SA, congenital syphilis is a notifiable medical condition (NMC), requiring reporting within seven days to the National Institute for Communicable Disease (NICD). [5] In the event of a diagnosis, healthcare providers are required to submit a paper-based notification form or complete an electronic form on the NMC surveillance platform. [5] In agreement with the ANCHSS, the NICD has reported a sustained increase in congenital syphilis notifications and Rapid Plasma Reagin (RPR)-reactive infants since the initiation of

surveillance. [24] According to their latest surveillance report, there were 1,370 notifications of congenital syphilis cases and 18,518 RPR-reactive results among children under two years from July 2017 to June 2022. [24]

2.2. Management of Maternal Syphilis

Eliminating the vertical transmission of syphilis has been a WHO priority since the publication of the Global Plan for the Elimination of Congenital Syphilis in 2007. [5] In 2014, the WHO set targets to achieve this goal, which includes a congenital syphilis case rate of less than 50 cases per 100,000 live births, ANC coverage of at least 95%, 95% of pregnant women attending ANC being tested for syphilis, and 95% of syphilis seropositive pregnant women receiving treatment. [13, 25] As of December 2024, only 18 countries have met these impact and process criteria. [26]

2.2.1. Syphilis Screening

Serological screening for syphilis can be conducted using two methods, namely treponemal and non-treponemal assays, with the sensitivity of each depending on the stage of the disease. [19] Treponemal tests, such as the Treponema Pallidum Hemagglutination Assay (TPHA), detect antibodies to *Treponema pallidum* proteins and will remain reactive lifelong, regardless of treatment. [3] Although treponemal testing cannot distinguish between past and current infections, it is more sensitive than non-treponemal methods and is used in most rapid syphilis tests. [27] Rapid tests can be conducted at the point of care (POC) and allow for immediate treatment, reducing the risk of losing seropositive women to follow-up. [3] Other types of treponemal tests include electrochemiluminescence (ECL), enzyme immunoassay (EIA), *Treponema pallidum* particle agglutination assay (TPPA), and fluorescent treponemal antibody-absorption test (FTA-ABS). [28] Non-treponemal tests, such as Rapid Plasma Reagin (RPR) and Venereal Diseases Research Laboratory (VDRL), detect antibodies released in response to cell damage and therefore, are non-specific. [3] Since non-treponemal biomarkers vary based on disease progression, they are used to confirm an active infection and monitor treatment efficacy. [3] A RPR titer equal to or above 1:8 indicates an active infection and 95% of RPR tests will be non-reactive after two years if full treatment is provided. [3]

Syphilis serological testing is conducted using a traditional or reverse sequence algorithm. [9] The traditional algorithm involves an initial non-treponemal test followed by a treponemal test to confirm the presence of syphilis-specific antibodies. [9] The reverse sequence algorithm involves an initial treponemal test, often a rapid test conducted at POC, followed by a non-treponemal test to determine whether the infection is active. [9] Countries publish guidelines with varying algorithms based on resource availability and prevalence, but the reverse sequence algorithm is increasing in popularity. [9, 27]

Additionally, when lesions are present, syphilis can be diagnosed using direct detection methods including dark field microscopy, the direct fluorescent antibody test (DFA-TP), and nucleic acid amplification techniques such as polymerase chain reaction (PCR). [28] These diagnostic methods are not effective in asymptomatic syphilis cases and, therefore, are not recommended for routine antennal screening. [28]

2.2.2. South African Policies and Guidelines

In 2017, the SA National Department of Health (NDoH) released the National Strategy for Sexually Transmitted Infections 2017–2022. [29] In the same year, the NICD was tasked with collating NMC data, which had previously been conducted by the NDoH. [5] However, syphilis in pregnant women and women of reproductive age are not NMCs in SA, as they are in countries such as Brazil and Japan. [15] This level of surveillance would serve as the ultimate early warning system for rising congenital syphilis rates, enabling early intervention. [5]

In 2019, SA adopted the WHO case definition of congenital syphilis and released the Guidelines for the Prevention of Mother-to-Child Transmission (PMTCT) of Communicable Infections. [3] This guideline mandates that all pregnant women are tested for syphilis at their first ANC visit using either an RPR or TPHA test, with reactive results confirmed by the alternative test. [3] Women confirmed to have an active infection must be treated with intramuscular BPG once weekly for three weeks. [3] If the initial test is conducted before 20 weeks of gestation with a non-reactive result, a repeat test is to be conducted at 32-34 weeks. [3] The guideline also encourages partner testing. [3] In 2022, the Southern African HIV Clinicians Society released a guideline for the management of STIs, which is in alignment with the 2019 PMTCT guidelines. [30]

More recently, in August 2023, an updated vertical transmission prevention (VTP) guideline, titled "Guideline for Vertical Transmission Prevention of Communicable Infections" was released, introducing significant changes to maternal syphilis screening. [31] This guideline mandates the screening of all women at their first ANC visit, with re-testing at each subsequent visit (approximately every four weeks) and at delivery if the initial result is non-reactive, aligning with HIV screening guidelines. [31] It also requires maternal syphilis testing in cases of intrauterine death or spontaneous abortion. [31] The guideline follows a reverse sequence screening algorithm, starting with a rapid POC TPHA test, followed by a confirmatory RPR laboratory test if the POC result is reactive. [31] Regarding treatment, the guideline requires an immediate BPG dose in the case of a reactive POC test, followed by two additional doses if laboratory results confirm an active infection. [31] The guideline also encourages the use of dual HIV-syphilis rapid tests where applicable and the tracing and testing of sexual partners. [31] Facility-level training on this guideline was still in process nationwide at the time of conducting this study. In April 2024, the Southern African HIV Clinicians Society released a

guideline for the clinical management of syphilis, highlighting the complexities of diagnosis and treatment and briefly addressing the national resurgence of maternal and congenital syphilis. [27]

3. Study Rationale

Despite syphilis screening and treatment being relatively inexpensive and effective, there has been a notable increase in maternal and congenital syphilis cases in recent years, both globally and in SA. [13] SA is a country already burdened with HIV, tuberculosis (TB), and non-communicable diseases, as is true for many other LMICs. A systematic review of LMICs found that both healthcare providers and pregnant women commonly viewed syphilis during pregnancy as less of a concern than HIV, highlighting a lack of awareness about its devastating effects. [32]

Maternal syphilis is completely treatable, and therefore, congenital syphilis is completely preventable. The release of the 2023 VTP guidelines and the inclusion of significant updates to maternal syphilis screening suggests that the SA NDoH has recognised the seriousness of the local resurgence in congenital syphilis. It is essential for healthcare providers caring for pregnant women to understand the gravity of the situation and the importance of complying with screening, treatment and partner-notification guidelines. Research is required to understand the resurgence of maternal and congenital syphilis in SA, identify the likely social and biological driving factors that are at play, evaluate the effectiveness of existing interventions, and inform future actions.

4. Purpose of the Study

4.1. Research Questions

This study addressed the following questions:

1. Did the ANC facilities serving two communities in the Western Cape province of South Africa, Gugulethu in Cape Town (urban) and the Breede Valley in the Cape Winelands (rural), comply with the 2019 South African PMTCT screening guidelines for maternal syphilis?
2. What were the prevalence rates of active and past syphilis among ANC attendees in two Western Cape communities, Gugulethu and the Breede Valley?
3. Which clinical and sociodemographic factors were associated with maternal syphilis in Gugulethu and the Breede Valley?

4.2. Research Aim

This study aimed to describe maternal syphilis screening in terms of the implementation of the 2019 South African PMTCT guidelines and syphilis prevalence in two communities, one urban and one rural, in the Western Cape province of South Africa.

4.3. Research Objectives

The objectives of this study were as follows:

1. Determine the proportion of pregnant women in two study populations who received routine antenatal syphilis screening and describe adherence to the 2019 South African PMTCT screening guidelines by research site.
2. Determine the proportion of pregnant women who tested positive for an active and past syphilis infection and describe any associations with relevant clinical and sociodemographic variables.

CHAPTER 2: LITERATURE REVIEW

1. Introduction

This chapter reviews relevant literature on maternal and congenital syphilis with the intention of contextualising and justifying our study. A total of 38 journal articles were sourced from four health sciences databases: PubMed, Scopus, CINAHL, and Africa-Wide Information. The following search terms were used: "maternal syphilis", "syphilis in pregnancy", "antenatal syphilis", and "treponemal infection". The following terms were used with Boolean operators: "risks", "risk factors", "predicators", "management", "Africa" and "South Africa". Literature was only included if available in English and published in the preceding ten years (2014-2024). Seminal literature was included where appropriate. The WHO, CDC, Statistics South Africa (Stats SA), NICD, NDoH and Health Systems Trust websites were used to source a total of ten relevant guidelines, reports, and policy documents.

Syphilis, a preventable and treatable STI, was historically most prevalent among men who have sex with men. [33] However, its prevalence has risen drastically in other groups, including pregnant women, creating further public health concerns. [33] This increase in prevalence is likely multifactorial, including factors such as sexual violence, poor compliance with safe sex practices, limited ANC access, inadequate screening resources, global BPG shortages, insufficient healthcare provider training, non-adherence to national guidelines, and ambiguous screening and treatment algorithms. [33] Furthermore, Stafford *et al.* suggest that the prevalence of STIs, including syphilis, saw an increase in 2020-2021 as efforts shifted to managing the COVID-19 pandemic. [9]

2. Management of Maternal Syphilis

Most sub-Saharan African countries have included antenatal syphilis screening in standard ANC in alignment with WHO recommendations, although the methods and coverage vary greatly. [34] In 2016, the estimated rate of maternal syphilis screening in Africa was low at 47%, with a treatment rate of 76% with at least one BPG dose. [16] According to the 2022/23 District Health Barometer (DHB), SA's ANC coverage rate (at least one visit) is 76.4%.[35] The 2022 ANCHSS reported that 97.5% of women were screened for syphilis at these ANC visits, up from 96.4% in 2019. [15]

2.1. Antenatal Screening

Antenatal syphilis screening ensures early detection and treatment, which is imperative in preventing congenital syphilis and has the potential to reduce global perinatal deaths by 50%.[36] A SA study reviewed notified congenital syphilis cases and found that 87% of mothers were screened for syphilis antenatally, with only 65% screened at their first ANC visit and only 40% screened more than 28 days before delivery. [37] The 2019 ANCHSS found syphilis screening coverage was 90% at the first ANC visit, and 99% at follow-up visits. [38] These figures are concerning as they indicate that women are not always screened at their first ANC visit, as required by the PMTCT guidelines. A Chinese study showed that delaying treatment for maternal syphilis increased the risk of congenital syphilis by 125% per week. [39] Furthermore,

infants whose mothers received treatment within four weeks of delivery were 17 times more likely to have congenital syphilis than those treated at least four weeks before giving birth. [39] This increased risk over time is hypothesised to result from increased creatinine clearance and decreased protein concentration in late pregnancy, leading to reduced BPG efficacy. [12]

In the WC, antenatal syphilis screening is conducted using serological tests, namely treponemal TPHA and non-treponemal RPR assays. The TPHA assay detects syphilis-specific antibodies and will remain reactive lifelong, regardless of treatment. [3] The RPR assay detects non-specific antibodies released during cell damage and is used to confirm an active infection and monitor treatment efficacy. [3] RPR screening in PHC is limited by the need for specialised laboratory staff, costly equipment, and access to electricity. [36, 40]

Rapid treponemal POC tests have proven highly effective due to their cost-effectiveness, accuracy, practicality, minimal training requirements, and the ability to initiate treatment immediately without waiting for laboratory test results. [34] Therefore, the WHO recommends using rapid treponemal tests for ANC screening, resulting in more frequent use of the reverse sequence algorithm. [28] In KZN, a time series analysis observed a significant reduction in the increase of maternal mortality rate following the provincial implementation of antenatal syphilis POC screening. [41]

2.1.1. Facilitators of POC Screening

Due to a high national HIV prevalence, SA has well-established and relatively well-funded HIV VTP policies and interventions, including antenatal HIV screening. [42] This provides an ideal opportunity for integrating syphilis screening into standard ANC. This integration maximises limited resources, avoids duplication, provides concurrent sexual health education, and uses transferable testing and counselling skills. [40] Furthermore, no change in facility organisation and patient flow is required. [40] This synergy is evident in a Ghanaian study which found that women screened for HIV were more likely to be screened for syphilis. [34]

Numerous qualitative and quantitative studies in sub-Saharan Africa have examined the integration of syphilis POC screening into existing ANC procedures. These studies show that PHC staff found integration easy, and pregnant patients accepted screening due to their familiarity with HIV screening. [40, 43, 44] Furthermore, they found that the decentralisation of syphilis screening to PHC facilities improved accessibility, ANC waiting room education increased patient willingness for screening, and national guidelines encouraged provider compliance. [43, 44]

The 2023 updated SA VTP guidelines recommend using dual HIV-syphilis rapid tests to further improve efficiency. [31] A Ugandan study observed an increase in antenatal syphilis screening coverage from 48% to 94% using these dual rapid tests. [42] However, the use of such tests in HIV-prevalent

countries raises concerns; it is crucial to clarify that, while dual tests should not be used for WLHIV, antenatal syphilis screening must still be performed. [38]

2.1.2. Obstacles of POC Screening

Numerous quantitative and qualitative studies in sub-Saharan Africa have explored the challenges of effective antenatal syphilis screening. [32] A major obstacle to POC screening is that most rapid tests are treponemal and cannot differentiate between active and past infection. [3] Using these tests in isolation can result in over-treatment, which is resource intensive. Consequently, in SA, the 2023 VTP guidelines require a confirmatory RPR laboratory test before administering the second and third doses of BPG. [36]

Studies highlight several structural and operational issues within the health system, including poor PHC accessibility, facility electricity cuts, staff shortages, shortages of screening kits and consumables, receipt of short-dated screening kits, and lack of referral to other facilities during shortages. [32, 40, 43, 44] A study in Ghana observed that women who were not screened for syphilis were more likely to have attended a private ANC facility. [34] Although only 15.7% of SA's population uses private healthcare, private ANC providers must still be mandated to comply with the national ANC guidelines. [45]

Literature has also explored healthcare provider uptake of POC antenatal syphilis screening, reporting low staff confidence and feelings of work overload. [40, 43] Low confidence stems from a lack of test kit quality assurance and poor staff training procedures. [32, 43] Complaints regarding training involve off-site training attended by a single staff member who is responsible for reporting back, high turnover leading to untrained staff, and lack of refresher trainings. [32, 40, 43, 44] Staff reluctance to follow guidelines and low motivation tend to arise from staff feeling overwhelmed by documentation requirements, time-constrained with lengthening ANC visits and finding difficulty in keeping up with changing guidelines. [40, 43, 44] While a study in Burkina Faso found that syphilis screening only added an average of three minutes to ANC consultations, PHC staff are still required to document programme data. [40] To manage workload, it is recommended that facilities maintain a single register incorporating all antenatal programme data. [43]

As with many STIs, studies have shown that pregnant women can also be reluctant to undergo screening. [32, 44] Women report fearing antenatal syphilis screening due to concerns about positive results and their consequences, such as stigma and relationship breakdown. [32, 44]

2.2. Antenatal Treatment

A SA study reviewing notified congenital syphilis cases found that among the screened mothers, only 72% were recorded to have received treatment with BPG. [37] The WHO, CDC and SA NDoH recommend intramuscular BPG as the only treatment for maternal syphilis due to its safety and 98% effectiveness in

preventing congenital syphilis. [12, 18] Other typical syphilis treatments, such as azithromycin, doxycycline and erythromycin, are contraindicated in pregnancy or cannot cross the placental barrier to treat the fetus. [8] The WHO recommends at least one dose (2.4 million units) of BPG for early syphilis and the same dose weekly for three consecutive weeks for late or unknown-stage infection. [28] However, due to challenges in determining the disease stage, there can be a tendency to overtreat. [27] Notably, a Chinese study observed that women with a history of treated syphilis had a reduced risk for infants with congenital syphilis, suggesting that treatment may also protect future pregnancies. [39]

However, relying solely on BPG to treat maternal syphilis presents significant challenges. Firstly, despite a low anaphylactic reaction rate, qualitative studies have reported that ANC staff fear administering BPG. [8, 44] These fears, coupled with misconceptions that BPG is an obsolete drug, may lead to the use of ineffective treatment alternatives or unnecessary referrals to tertiary facilities resulting in treatment delays. [8] In the case of a known penicillin allergy, the latest local VTP guidelines recommend hospital referral for penicillin desensitisation and treatment under close observation. [31]

Secondly, global stock shortages of BPG have arisen, and in 2016, the 69th World Health Assembly recognised it as an essential drug with a high risk for stock-outs. [8] A WHO and Clinton Health Access Initiative (CHAI) study found that 41% of the 95 assessed countries experienced BPG stock shortages between 2014 and 2016, including SA. [8] To manage this stock-out, SA prioritised BPG stock for pregnant women with syphilis and requested emergency authorisation for the use of unregistered products. [38] These global BPG shortages are multifactorial, but a major contributor is production cessation by many manufacturers due to high manufacturing costs and low selling prices. [8] Other factors include long production lead times, inflexible production cycles, poor quality leading to product recalls, large minimum purchasing quantities, inaccurate buyer forecasting, lack of funding to purchase buffer stock, and rigid tender systems. [8] Other effective and safe treatment options are necessary, and an ongoing study is assessing high-dose amoxicillin and other alternatives, but these are not yet recommended. [9]

2.3. Partner Notification

Although our study did not investigate partner tracing and testing, it is crucial to recognise that managing syphilis in pregnancy cannot occur in isolation. Given the heightened risks of syphilis contracted during pregnancy, partner notification, testing, treatment, education, and monitoring are essential. [46] A Chinese study found that the risk of congenital syphilis increased 12-fold if the mother was in the early infection stage at diagnosis and three-fold if both parents were infected or if paternal syphilis status was unknown. [39]

While untreated sexual partners have the potential to compromise other efforts to reduce congenital syphilis rates, partner notification is no easy feat. [46] Many complex factors must be considered, including

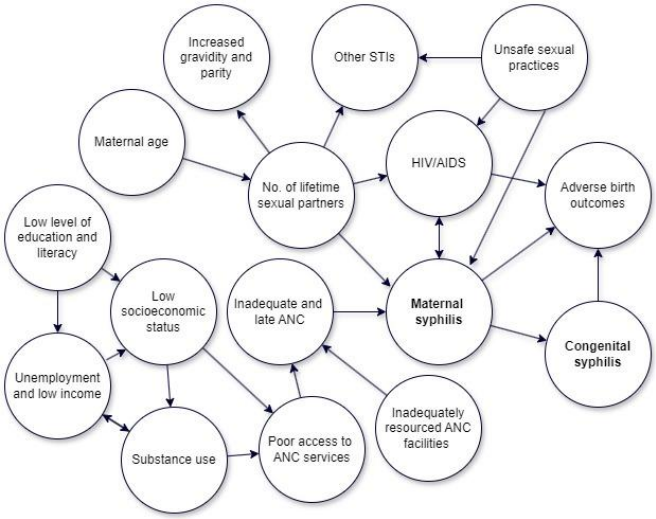
culture, possible infidelity, domestic violence risk, and potential relationship breakdown leading to the possible loss of emotional and financial support. [46] Qualitative studies have found that ANC staff often feel unequipped to manage partner notification, are unaware of relevant protocols, and frequently handle it by advising pregnant women to inform their partners. [46] Furthermore, qualitative studies have found that pregnant women report discomfort and fear in discussing their diagnosis with their partner, difficulty in correctly relaying information and feeling coerced by healthcare providers to notify their partner. [32, 46, 47] Studies suggest that pregnant women should not be responsible for notifying their partners and a study in Burkina Faso, where patriarchal relationships are common, observed no partner clinic attendance when using patient-driven notification. [40, 47]

In SA, the National Strategy for STIs 2017–2022 reported that current partner notification processes which mainly rely on patient-driven notification, have shown success in only 12% of cases. [29] The 2019 PMTCT guidelines briefly mention the importance of partner treatment, requiring the provision of a notification slip for each sexual partner, placing the onus on pregnant women. [3] The updated 2023 VTP guidelines include more detail by including partner tracing and testing in the screening algorithm and outlining partner testing methods. [31] Although the 2023 VTP guidelines include a section promoting paternal ANC involvement and counselling, it lacks specific guidance on managing the complexities of partner notification, while considering women's social circumstances and the high rates of gender-based violence in SA. [31]

3. Associations with Syphilis

Maternal syphilis screening protocols vary worldwide, with some guidelines, such as those published by the CDC, recommending repeat testing for high-risk individuals. [48] Despite prevalence rates being unevenly distributed, there are no standardised criteria for classifying women as high-risk. The literature suggests that the risk of syphilis in pregnancy is significantly associated with specific clinical, sociodemographic, and behavioural factors (Figure 1).

Figure 1: Directed acyclic graph (DAG) of associations with syphilis in pregnancy



3.1. Clinical Factors

3.1.1. Gravidity and Parity

A Tanzanian study found a significant association between increasing gravidity and syphilis infection. [49] This finding was confirmed by the 2019 ANCHSS, which indicated that women with gravidity of 2 or more were 1.5 times more likely to have maternal syphilis. [38] A retrospective study at a PHC clinic in KZN observed that the risk of syphilis infection significantly increased with higher parity. [20] The increased risk of maternal syphilis with higher gravidity and parity is likely due to a higher maternal age, and the literature suggests that syphilis risk increases with age. [20, 38, 50]

3.1.2. History of Obstetric Complications

The literature presents conflicting results on the relationship between maternal syphilis and a history of spontaneous abortion. A Brazilian study observed a four-fold increased risk in women with a history of spontaneous abortion, while a Ugandan study observed a 79% reduced risk. [51, 52] The Ugandan study hypothesised that the reduced risk may be attributed to more caution and ANC attendance in subsequent pregnancies following an adverse pregnancy outcome. [51]

An Ethiopian study observed no significant association between maternal syphilis and a history of pregnancy termination. [53] Studies in the Americas have observed a significantly higher rate of maternal syphilis in women with a history of premature birth, cesarean delivery, and neonatal death, which may be indicative of previous syphilis infection. [14, 52] A USA study observed that pregnant women infected with syphilis were significantly more likely to have a history of pregnancy-related hypertensive disorders but no significant relationship with a history of gestational diabetes. [14]

3.1.3. HIV

SA has the highest global burden of HIV, with approximately 8.45 million infected people, and adolescent girls and young women are disproportionately affected. [54] The 2023 Global Update by the United Nations Programme on HIV/AIDS (UNAIDS) reported that SA diagnosed 43,300 new HIV cases among females aged 15-24 in 2022, compared to 12,200 new cases among males in the same age group. [55] Syphilis is the most common co-infection in pregnant WLHIV, and the literature documents a notable relationship. [56, 57] A Rwandan study observed that individuals with HIV were six-times more likely to have syphilis, while a Ugandan study found a four-fold increase in syphilis in pregnant WLHIV. [50, 51] A KZN regional hospital study found that pregnant WLHIV were twice as likely to be diagnosed with syphilis, while research at a PHC clinic found that women living without HIV were 56% less likely to be diagnosed. [19, 20] The 2019 ANCHSS found that HIV status was independently and significantly associated with a two-fold increase in maternal syphilis risk, regardless of antiretroviral therapy (ART) use. [38]

HIV appears to accelerate the natural progression of syphilis, commonly resulting in atypical clinical syphilis presentations and repeat syphilitic episodes. [38, 56] Furthermore, HIV infection and ART may affect the accuracy of syphilis serology, potentially resulting in undetected infection and may even be associated with syphilis treatment failure. [19, 56] Importantly, an Ethiopian study found that maternal ART use can be protective against congenital syphilis, with infants born to women with untreated HIV being 2.6 times more at risk. [58]

HIV-syphilis co-infection is also associated with higher HIV viral loads and lower CD4 T-lymphocyte counts, suggesting that syphilis worsens HIV outcomes. [56] Furthermore, the literature shows that co-infection increases HIV transmission risk by two to five times, including vertical transmission, even with ART use. [42, 50, 59] A Brazilian study observed that newborns of women with co-infection were 4.5 times more likely to contract HIV, even after accounting for confounders. [57] This increased risk likely involves syphilis-mediated increases in maternal viral load and the compromised integrity of the maternal-fetal barriers. [59] Therefore, although SA has made great strides in reducing vertical HIV transmission, rising rates of maternal syphilis may threaten this progress.

3.1.4. Other STIs

Since STIs share modes of transmission and behavioural risk profiles, studies have documented high rates of concurrent STI infections among pregnant women with syphilis. [6, 14, 60] A Brazilian study concluded that women with a previous STI diagnosis are almost ten times more likely to be diagnosed with maternal syphilis. [60] A study in the USA found that women with maternal syphilis were seven times more likely to have concurrent gonorrhoea and four times more likely to have concurrent chlamydia. [14] A Nigerian study found that 11.9% of women with maternal syphilis were co-infected with hepatitis B or C. [6] In contrast, an Ethiopian study found no cases of syphilis-hepatitis B co-infection in pregnant women. [53]

3.2. Sociodemographic and Behavioural Factors

3.2.1. Maternal Age

A Rwandan study observed that individuals 25-49 years old were twice as likely to have syphilis than those aged 15-24. [50] A study at a KZN PHC clinic found that pregnant women aged 35 and older were significantly more likely to have active syphilis, while the 2019 ANCHSS indicated that the likelihood of maternal syphilis increased with age up to 34 years. [20, 38] Although not statistically significant, an Ethiopian study observed that children born to women over 36 years old were 11 times more likely to contract congenital syphilis compared to those born to girls under 15 years old. [58] On the contrary, a Ugandan study found that HIV-syphilis co-infected pregnant women were significantly more likely to be younger, and a Peruvian study observed that pregnant women with active syphilis were significantly younger than those without. [11, 36]

The observation that numerous studies in sub-Saharan Africa associate syphilis with increased age is thought-provoking, as most other STIs are associated with a younger maternal age. [14] Since many studies do not differentiate between active and past syphilis infection, Gulersen *et al.* hypothesise that this association may be due to older individuals being more likely to have had past infections resulting in persistent syphilis antibodies and lifelong seropositive treponemal results. [14] Furthermore, increased maternal age typically correlates with more years of sexual activity and therefore, increased likelihood of STI exposure. [14]

3.2.2. Level of Education

A USA study reported that syphilis-infected pregnant women were likely to have attained a lower education level, with 29% of women with syphilis not having completed high school, compared to 13% of women without syphilis. [14] Brazilian studies found that lower maternal education increased the risk of both maternal and congenital syphilis. [60, 61] Illiterate women or those who had not completed primary school were twice as likely to have maternal syphilis, while ten or more years of maternal schooling were protective against congenital syphilis. [60, 61]

Data from the 2019 ANCHSS indicated that education level was independently and significantly associated with maternal syphilis in SA. [38] Women with only primary schooling were 1.6 times more likely to have maternal syphilis, and those with secondary schooling were 1.3 times more likely compared to those with tertiary education. [38] Conversely, a study at a KZN tertiary hospital observed no significant association between education level and syphilis infection in pregnant WLHIV. [21]

3.2.3. Access to ANC Services

A Rwandan study observed no significant difference in syphilis prevalence between rural and urban areas, while a Tanzanian study found that women attending rural or semi-urban ANC clinics were twice as likely to have maternal syphilis, which may be due to poorer ANC services or improved syphilis screening at these sites. [49, 50] Conversely, the 2019 ANCHSS reported that women attending urban ANC clinics were 1.3 times more likely to be infected than those in rural areas. [38]

Studies in the USA found that maternal syphilis risk increased four-fold with no ANC and doubled if ANC was initiated in the second or third trimester as opposed to in the first trimester. [12, 14] Furthermore, infants born to women with no ANC were six times more likely to contract congenital syphilis. [12] A Brazilian study observed that women who attended 0 to 3 ANC visits were three times more likely to have maternal syphilis compared to those with seven or more visits, with the risk decreasing as the number of ANC visits increased. [60]

Although syphilis treatment at any point in pregnancy will treat the mother, it may not effectively treat the fetus in late pregnancy. [12] A systematic review found a significant association between late or

incomplete maternal syphilis treatment and congenital syphilis, supported by a meta-analysis that concluded treatment is most efficient when administered in the first two trimesters of pregnancy. [10, 12] Chinese studies also emphasise the importance of early treatment, indicating that the risk of congenital syphilis increased eight-fold if maternal syphilis was treated after 28 weeks of gestation and 25-fold if treated after 36 weeks. [12]

3.2.4. Sexual Practices and Behaviours

A Brazilian study observed that pregnant women with three or more partners in the past twelve months had a three-fold risk of maternal syphilis, while an Ethiopian study observed a 21-fold risk increase in those with multiple sexual partners in the previous six months. [53, 60] On the other hand, a KZN study observed no significant association between the lifetime number of sexual partners and maternal syphilis in WLHIV. [21] The 2019 ANCHSS reported that pregnant women were significantly less likely to have syphilis when married to the fathering individual, regardless of living situation, and most likely to have syphilis if they had no relationship with the father. [38]

A Chinese study reported that 57% of women with active maternal syphilis admitted to not using condoms in the past six months, which may be influenced by the perception that condom use signifies mistrust or infidelity. [39, 60] A systematic review concluded no significant association between congenital syphilis and maternal sex work, and an Ethiopian study found no association between maternal syphilis and a history of sexual abuse. [12, 53]

3.2.5. Substance Use

The association between illicit substance use and syphilis in pregnancy is well-documented. [14, 33, 52, 60] Stafford *et al.* reported that among women with maternal syphilis at a USA hospital, 15% used cannabis, 5% used cocaine, 1.4% used benzodiazepines and 1.4% used opiates during pregnancy. [33] Another USA study found that pregnant women with syphilis were twice as likely to use tobacco. [14] Studies in Brazil found that women reporting illicit drug use during their current pregnancy were 13 times more likely to have syphilis, while those with a partner using illicit drugs were twice as likely. [52, 60] This association is thought to result from drug use leading to high-risk sexual behaviours and reduced use of ANC services. [33, 39, 52]

4. Conclusion

SA has developed detailed guidelines for screening and treating maternal syphilis to reduce vertical transmission of the infection. Despite these efforts, numerous obstacles to optimal screening, treatment and partner notification are likely contributing to the rising rates of maternal and congenital syphilis. The literature demonstrates clear associations between syphilis and various clinical factors, most notably HIV. HIV increases the risk of contracting syphilis, and co-infection increases the risk of HIV vertical transmission.

Additionally, older women, with increased gravidity and parity, are shown to be at greater risk for maternal syphilis. The literature suggests that this risk also rises with worsening socioeconomic status (SES) and related behaviours, such as poor ANC attendance and illicit substance use. This indicates that maternal syphilis is influenced by social health determinants and inequities, highlighting the importance of a comprehensive approach.

CHAPTER 3: METHODOLOGY

1. Introduction

This chapter discusses the methodology of our cross-sectional study including a detailed description of the study design, study populations from Gugulethu and the Breede Valley, research procedures, data analysis and management, and ethical considerations.

2. Study Design

This cross-sectional study was a secondary analysis of data collected at enrollment from pregnant women participating in the Children HIV Exposed Uninfected – Research to Inform Survival and Health (CHERISH) study, which began in January 2022. [62] The CHERISH study aimed to prospectively compare the growth, neurodevelopment, hospitalisation, and survival of children who were HIV-exposed uninfected (HEU) with those who were HIV-unexposed uninfected (HUU). [62] Pregnant women living with and without HIV were enrolled antenatally, and their live-born children were followed up. The CHERISH study will be referred to as the parent study for the remainder of this dissertation.

Antenatal syphilis data were collected as part of the parent study, with these data being the focus of the secondary analysis. The cross-sectional design of our study allowed for a detailed description of maternal syphilis in the two study communities. This involved the comparison of antenatal syphilis screening practices to the 2019 PMTCT guidelines, and the determination of the prevalence rate of active and past syphilis. Only data collected during the parent study's enrollment visit were included in our secondary analysis.

3. Study Population

3.1. Setting and Description of the Parent Study

The data used in our study were collected as part of the parent study conducted in the WC province of SA. The WC is one of nine SA provinces with a population of 7.2 million, per the 2022 mid-year estimates. [45] The results of the 2023 General Household Survey (GHS) indicate that of the households in the WC, 19% live in an informal dwelling, 99% have access to piped or tap water, 95% have a flush toilet, 94% have electricity, 39% receive a government grant, and 18% report food inadequacy. [45] Furthermore, only 55% of people in the province aged 20 years and older have a minimum education level of grade 12. [45]

In the WC, 74% of the population relies on the public health system, including approximately 110,000 pregnant women resulting in 90,000 deliveries annually. [45] According to the 2022/23 DHB, the WC has an ANC coverage rate (at least one visit) of 78.6%, the lowest provincial in-facility maternal mortality rate at 62 per 100,000 live births, and the lowest provincial in-facility neonatal mortality rate at 7.1 per 1,000 live births. [35] Furthermore, the WC has the lowest number of healthcare professionals (including medical practitioners, professional nurses and pharmacists) per 100,000 uninsured population at 159, disproportionally affected by a low ratio of professional nurses per 100,000 uninsured population. [35] In the

WC, antenatal HIV prevalence was 16% in 2022, and the vertical HIV transmission rate was 2-3% at 18 months of age. [15]

The parent study enrolled cohorts in two communities in the WC, Gugulethu and the Breede Valley, with the aim to prospectively study children who were HEU and HUU. Gugulethu is an urban community located in the Cape Town Metropolitan district. According to the 2022/23 DHB, this district has an ANC coverage rate of 74.5%, an in-facility maternal mortality rate of 75.1 per 100,000 live births, and an in-facility neonatal mortality rate of 7.2 per 1,000 live births. [35] Furthermore, the district has a ratio of medical practitioners per 100,000 uninsured population above the national average but one of the lowest ratios of professional nurses per 100,000 uninsured population, at 46.1 and 104.5, respectively. [35] The overall district has an antenatal HIV prevalence of 20%, while Gugulethu's prevalence is 29%.[15] ANC and primary childbirth services are provided by the Gugulethu Midwife Obstetric Unit (MOU) to approximately 5,500 pregnant women annually. [62] Mowbray Maternity Hospital provides secondary care, while Groote Schuur Hospital offers tertiary care. [62]

The Breede Valley is a rural farming community near the town of Worcester, roughly 110 km from Cape Town, located in the Cape Winelands district. According to the 2022/23 DHB, this district has an ANC coverage rate of 82.9%, an in-facility maternal mortality rate of 44.2 per 100,000 live births, and an in-facility neonatal mortality rate of 6.4 per 1,000 live births. [35] Furthermore, the district has a ratio of medical practitioners per 100,000 uninsured population near the national average but one of the lowest ratios of professional nurses per 100,000 uninsured population, at 36 and 82.3, respectively. [35] The overall district and the Breede Valley both have an antenatal HIV prevalence of 15%.[15] In the Breede Valley, primary ANC is provided by Worcester MOU and six smaller PHC clinics serving approximately 3,700 pregnant women annually. [62] Worcester MOU also provides primary childbirth services, while secondary and tertiary care are provided by Worcester and Tygerberg Hospitals, respectively. [62]

3.2. Inclusion Criteria

Our secondary analysis included the enrollment data for all participants of the parent study from commencement in January 2022 to 12 June 2024. The following inclusion criteria were applied to the parent study:

- Pregnant women who presented at Gugulethu and Breede Valley ANC clinics. [62]
- Women between 24- and 36 weeks of gestation at enrollment estimated by last menstrual period or routine obstetric ultrasounds. [62] Although women in these communities often present for their first ANC visit at 18−20 weeks of gestation, they were not enrolled at this point to avoid including pregnancies with early losses. [62]
- Pregnant girls under 18 years were only included if accompanied by a parent or legal guardian who consented, and the pregnant adolescent assented to participation. [62]

3.3. Exclusion Criteria

The secondary analysis included all enrollment data without any exclusion criteria. The following exclusion criteria applied to the parent study:

- Women with unknown HIV status who declined routine antenatal HIV testing. [62] However, antenatal HIV-testing uptake is high in the Gugulethu and Breede Valley communities. [62]

3.4. Recruitment and Enrollment Procedures

Under the parent study, enrollment of women at the Gugulethu and Breede Valley ANC clinics began in January 2022. Research staff responsible for recruitment were trained on inclusion and exclusion criteria, with the database used to collect data prompting checks to ensure compliance. Research staff approached pregnant women of 24−36 weeks of gestation in the antenatal clinic waiting areas during routine ANC visits. [62] Interested potential participants were taken to a private room at the research site for further discussions. These included ensuring inclusion criteria were met, confirming residence in the catchment area, reviewing maternal HIV status, and obtaining informed consent. [62] Research staff were supported by a senior research nurse or study coordinator at each site, and due to previous maternal health studies, the research teams had long-standing working relationships with facility staff. [62]

3.5. Sample Size

All pregnant women enrolled in the parent study at the two research sites, Gugulethu and the Breede Valley, between January 2022 and 12 June 2024 were included in the secondary analysis with no sampling. [62] A total of 600 participants were recruited from the Gugulethu site and 341 from the Breede Valley site. The sample size was determined by the parent study, which was powered to detect differences in mortality, hospitalisation, and neurodevelopment between HEU and HUU children. Our study was descriptive and not powered to quantify statistically significant relationships.

Given that maternal HIV status was the primary exposure variable of the parent study and due to the heterogeneity of HIV treatment, the parent study aimed to enroll women with and without HIV in a 2:1 ratio, with no less than a 1:1 ratio. [62] To account for seasonal influences on child morbidity and mortality, which applied to the parent study, they aimed to enroll women with and without HIV at an equivalent pace throughout the study. [62]

4. Research Procedures

4.1. Data Collection Instruments

Our study exclusively used data collected at enrollment in the parent study. Data were collected by research assistants through record reviews and face-to-face interviews conducted using a standardised script. The data were directly entered into an access-controlled Research Electronic Data Capture (REDCap) database using portable tablets (Appendix A). [63, 64] REDCap is a secure, web-based software

designed for validated data capture and auditing, and allows for data exports to popular statistical packages and data integration. [63, 64] The parent study did not involve paper-based data collection. [62]

During the data collection period, syphilis POC screening in the WC was conducted using the Avacare™ One Step Rapid Test, a treponemal test obtained from the WC Central Medical Depot. At the Gugulethu MOU, protocol required all pregnant women to undergo POC screening, allowing for immediate treatment if reactive, and a TPHA laboratory test performed by the National Health Laboratory Services (NHLS). If the TPHA laboratory result was reactive, a confirmatory RPR test was to be performed on the same blood sample. In the Cape Winelands district, where the Breede Valley is located, protocol required all pregnant women to undergo a POC test at their first ANC visit. For reactive POC results, a blood sample was to be obtained and both TPHA and RPR laboratory tests were to be performed by the NHLS.

4.2. Data Collection Methods

At both research sites, standard care at the first ANC visit included a rapid POC syphilis test for all women and HIV testing for women not already known to be living with HIV. This screening was conducted by the clinic staff, who were required to initiate treatment with BPG immediately if the POC syphilis test was reactive. At the Gugulethu site, blood samples for syphilis testing were sent to the local NHLS facility for all pregnant women, regardless of the POC result. In contrast, in the Breede Valley, laboratory testing was only required for women with reactive POC results.

Research staff collected routine data on first ANC visit dates, gestational age (GA), obstetric history, syphilis POC screening, and HIV from the Maternity Case Record (MCR). The MCR is a national, standardised, client-held document used across healthcare facilities to record care for pregnant women and their newborn infants, promoting continuity and quality of perinatal care. The obstetric history collected from the MCR included gravidity, parity, history of termination of pregnancy (TOP), history of adverse pregnancy outcomes and history of pregnancy complications. HIV-related data collected from the MCR included HIV status, CD4 counts and viral loads. HIV-related laboratory test results were only included in the secondary analysis if conducted within a maximum of 12 months before conception of the current pregnancy. Neither the parent study nor this secondary analysis involved any study-specific diagnostic procedures or the storage of biological samples. [62] Laboratory syphilis results were retrieved from the NHLS-TrakCare database by research staff.

Research staff conducted face-to-face interviews in one of the major local languages (isiXhosa, Afrikaans, or English) as chosen by the participant. [62] These interviews collected data on HIV status and diagnoses, ART use, TB diagnoses, hospitalisations during pregnancy, demographics and socioeconomics.

4.3. Quality Control

In accordance with the parent study protocol, data were entered into REDCap, which allowed for input validations throughout data collection. Permissions within REDCap were managed by the parent study's data coordinator, permitting only the data coordinators and principal investigators to edit data after entry. [62] Research staff conducting interviews were only granted permission for data entry and could not modify data. [62] Furthermore, these staff were provided with and trained on a standardised interview script to ensure consistency (Appendix A).

The parent study did not involve paper-based data collection, reducing the risk of transcription errors. The parent study coordinator was responsible for ensuring that standard operating procedures (SOPs) relating to data collection were implemented and applied consistently. [62]

4.4. Variables

The primary outcome variables of our study were the implementation of antenatal syphilis screening guidelines and maternal syphilis status, as outlined in Table 1. Study objective 1 was fulfilled by determining the proportions of women who underwent syphilis screening via rapid POC and laboratory tests. Objective 2 was partially met by assessing the maternal syphilis infection status, as determined by the TPHA and RPR laboratory test results. The NHLS reports the RPR reactivity based on the qualitative test and the RPR titer on the semi-quantitative test. Research assistants recorded RPR results as reported in the NHLS-TrakCare database.

Table 1: Primary outcome variables

Variable	Description
POC test result available for current pregnancy	Categorical, nominal (Yes/No)
POC test conducted at first ANC visit	Categorical, nominal (Yes/No)
POC test result	Categorical, nominal (Reactive/Non-reactive)
TPHA laboratory test available for current pregnancy	Categorical, nominal (Yes/No)
TPHA laboratory test result	Categorical, nominal (Reactive/Non-reactive/Equivocal/Rejected)
RPR laboratory test available for current pregnancy	Categorical, nominal (Yes/No)
RPR laboratory result	Categorical, nominal (Reactive/Non-reactive)
RPR titer[a]	Categorical, nominal (< 1:8/≥ 1:8)
Maternal syphilis status based on laboratory tests	Categorical, nominal (Active/Past/No infection/Unknown)

a = Variable collected as numerical and converted into categorical.

Maternal syphilis status was categorised based on TPHA and RPR laboratory test results collected from the NHLS-TrakCare database, as detailed in Table 2. POC test results were not considered in this categorisation.

Table 2: Maternal syphilis status categorisation based on laboratory results

		TPHA Result				
		Reactive	**Non-reactive**	**Equivocal**	**Rejected**	**No test**
RPR Result	**Reactive**	Active infection	No infection	N/A	N/A	N/A
	Non-reactive	Past infection	No infection	Unknown	N/A	Unknown
	No test	N/A	No infection	N/A	Unknown	Unknown

NHLS = National Health Laboratory Services; TPHA = Treponema Pallidum Hemagglutination Assay; RPR = Rapid Plasma Reagin

The sociodemographic and clinical data that were requested from the parent study are outlined in Table 3. While this study analysed data collected at enrollment, pregnancy outcomes (i.e. spontaneous abortion/stillbirth/live birth) were obtained from the parent study, where available. Pregnancy outcomes were collected from MCRs and telephonic interviews with participants. For participants enrolled towards the end of the data collection period, pregnancy outcome data were unavailable as they had not given birth at the time of commencing our study. The inclusion of this variable does not affect the study design, as the data were incomplete, it is beyond the study objectives, and the study was not designed as a cohort study.

Table 3: Sociodemographic and clinical variables

Variable	Description
Sociodemographic Variables	
Research site	Categorical, nominal (Gugulethu/Breede Valley)
Maternal age[a]	Categorical, ordinal (≤20, 21-30, 31-40, >40 years)
Home language	Categorical, nominal
Level of education	Categorical, nominal (None/Primary/Secondary/Tertiary/ Unknown)
Employment status	Categorical, nominal (Employed/Unemployed/Full-time student with no income)
Monthly income	Categorical, ordinal (ZAR0/<1,000/1,001-5,000/ 5,001-10,000/10,001-15,000/>15,000)
Receipt of government grant	Categorical, nominal (Yes/No)
In the past week, ≥1 day without a meal	Categorical, nominal (Yes/No)
No. of people in household[a]	Categorical, ordinal (1-4/5-8/9-12/>12 people)
Housing type	Categorical, nominal (Formal/Informal)
Socioeconomic status[b]	Categorical, nominal (Low/Middle/High)
Relationship with father of pregnancy	Categorical, nominal (Coupled and cohabiting/Coupled and not cohabiting/Not coupled/Uncertain)
Clinical Variables	
GA at enrollment	Numerical, continuous
Trimester at first ANC visit[a,c]	Categorical, nominal (First/Second/Third/Unknown)
Gravidity[a]	Categorical, nominal (Primi/Multi/Grand multigravida)
Parity[a]	Categorical, nominal (Nulli/Primi/Multi/Grand multiparous)
History of adverse pregnancy outcomes (i.e., pregnancy-related hypertension, spontaneous abortion, TOP, premature birth, stillbirth, neonatal death, infant death)	Categorical, nominal (Yes/No/Unknown)
TB diagnosis in current pregnancy	Categorical, nominal (Yes/No)
Hospital admission related to current pregnancy before enrollment	Categorical, nominal (Yes/No/Unknown)
Pregnancy outcome (where available)	Categorical, nominal (Spontaneous abortion/Stillbirth/Live birth)
Living with HIV	Categorical, nominal (Yes/No)
Diagnosed with HIV in current pregnancy[a]	Categorical, nominal (Yes/No)
HIV viral load available	Categorical, nominal (Yes/No)
HIV virally suppressed[a]	Categorical, nominal (Yes/No)
CD4 count available	Categorical, nominal (Yes/No)
CD4 count[a]	Categorical, nominal (<200/201- 350/351-500/>500 cell/mm^3)
ART status at enrollment	Categorical, nominal (Treated/Untreated/Unknown)
Timing of ART initiation[a]	Categorical, nominal (Before/During current pregnancy/ Unknown)
No. days ART was missed in last 30 days	Categorical, ordinal (0/1-10/11-20/21-30 days/Unknown)
Sexual partner/s aware of HIV status	Categorical, nominal (Yes/No/Unknown)

ANC = Antenatal Care; TOP = Termination of Pregnancy; TB = Tuberculosis; HIV = Human Immunodeficiency Virus; ART = Antiretroviral Therapy; GA = Gestational Age

a = Variable collected as numerical and converted into categorical; *b* = Variable created through an asset score based on other variables, including asset counts; *c* = First (≤12 weeks GA), second (13-27 weeks GA), and third (≥28 weeks GA) trimesters

4.5. Data Management and Analysis

In the parent study, data were collected and managed using REDCap, hosted on a secure, firewall-protected university server. [62] Datasets were securely backed up on a university OneDrive account every three months. [62] The parent study protocol states that data will be kept for 20 years, and its secure electronic storage is ensured by the principal investigators. [62]

Following the Human Research Ethics Committee (HREC) approval of our study, a dataset was requested from the parent study's data coordinator. Data, which had been deidentified, were issued under unique alphanumeric participant identifications (IDs) and sent via secure email transfer. Data were stored on the investigator's password-protected laptop and backed up on a secure university OneDrive account.

The data were cleaned, and analyses were conducted using STATA version 12.1 software by descriptive statistics. Our study included only quantitative data, comprising both categorical and numerical variables (Tables 1, 3). The distribution of numerical variables was determined, and appropriate measures of central tendency were used. i.e. mean for normally distributed continuous data and median for skewed data. Data dispersions were analysed by calculating standard deviation and interquartile range (IQR) in normally distributed and skewed data, respectively. Numerical variables were converted into categorical variables where applicable.

Sociodemographic, clinical and syphilis-related variables were compared by research site using bivariate analysis. Participants were categorised by maternal syphilis status based on laboratory results, (Table 2), and prevalence rates of active and past maternal syphilis were calculated. Subsequently, relevant sociodemographic and clinical variables were analysed by maternal syphilis status using bivariate analysis. Statistical significance was determined using Chi-square or Fisher Exact tests using a significance level of 0.05. Data were presented using frequency tables and bar charts.

4.6. Resources

This study did not require specific equipment or funding. Data collection was conducted under the parent study, which had already secured the necessary resources.

5. Ethical Considerations

5.1. Risks and Benefits

To ensure the communities' needs were met, the parent study team engaged with a Community Advisory Board, community representatives in Gugulethu, researchers in the Breede Valley, and local antenatal group leaders before commencing the study. [62] Furthermore, two focus groups were conducted with 20 of the first enrolled participants to obtain their feedback. [62] The greatest risk for participants in the parent study was a breach of confidentiality, as research staff had access to sensitive information, including HIV

and syphilis results. [62] The attempts to minimise this risk are described in section 5.4 (Privacy and Confidentiality).

The parent study considered the risk of STI-associated stigma deeming it minor, given that women with and without HIV and syphilis were enrolled, and the research sites were not specifically associated with STI care. [62] Since non-routine information was gathered during face-to-face interviews, participants could have experienced psychological distress. [62] To minimise this, parent study staff received training in handling sensitive situations and providing appropriate referrals when necessary. [62] The parent study, and therefore our study, were unlikely to cause any physical, social, legal, or economic harm to participants. [62]

Neither the parent study nor our study provided immediate direct benefits to participants. However, research indicates a notable increase in maternal and congenital syphilis, highlighting the importance of understanding this issue within the local context to improve the health of SA women and children. Both studies explored maternal and child health issues through purely observational methodology, with the potential to inform evidence-based policies and interventions. While women without syphilis were unlikely to benefit, their inclusion was necessary to determine maternal syphilis prevalence rates.

Overall, our study offers insight into maternal and congenital syphilis in the WC and aligns with the 2030 Sustainable Development Goals and the SA government's effort to optimise maternal and child survival and wellbeing.

5.2. HREC Approvals

Our study was approved by the HREC of the University of Cape Town (UCT) (251/2024 – Appendix B). The dynamic cohorts of the parent study were approved by the HREC of Stellenbosch University (SU) for the Breede Valley site (N20/08/084 - Appendix C) and by the HREC of UCT for the Gugulethu site (723/2021 – Appendix D). The WC Government Health Research Committee approved access to participants at the Gugulethu and Breede Valley antenatal clinics (WC_2021_09_007 – Appendix E).

5.3. Informed Consent Process

Upon enrollment in the parent study, all participants underwent an informed consent process conducted by research staff in their preferred language. [62] This process was held in a private setting, where research staff verbally explained the informed consent form, provided participants time to study it, and encouraged them to ask questions. To ensure understanding, participants were asked questions about the study. [62] Research staff did not provide healthcare to participants, ensuring to distinguish between standard care and research activities. Participants signed an informed consent document (Appendices F and G), which included consent to access their linked electronic data.

5.4. Privacy and Confidentiality

In the parent study, participant names were restricted to informed consent forms, tracing information, and the participant ID key, which were accessible only to the principal investigators and study coordinator. [62] This information was stored separately from other study documents in locked cabinets within access-controlled rooms at each site. [62] Upon enrollment, participants were assigned unique alphanumeric participant IDs, which were used for all other study documentation and the REDCap database. [62] Access to REDCap was managed by the parent study's data coordinator, ensuring that only data coordinators and principal investigators were authorised to extract datasets. [62] Furthermore, the REDCap database was housed on a firewall-protected server, requiring dual authentication with Google Authenticator and a university login. [62] Parent study staff were required to sign confidentiality agreements and undergo training on a confidentiality SOP and human subjects' protection. [62]

The parent study data coordinator shared the deidentified data for our study via a secure email transfer, requiring a password to download the file and a second password to open the document. These passwords were sent in separate communications. The data was stored on a password-protected laptop and backed up on a secure UCT student OneDrive account. No identifiable data or documentation, including dates of birth, were accessed during this study. The privacy procedures of the parent study and this secondary analysis complied with SA's Protection of Personal Information Act 4 of 2013.

5.5. Reimbursement for Participation

Participants were not reimbursed at the time of enrollment in the parent study. However, the parent study provided ZAR30 for telephonic interviews and ZAR300 for in-person follow-up visits after enrollment. Data collected during these follow-up contacts were not utilised in our study. [62] Therefore, there were no reimbursements for this secondary analysis.

5.6. Conflicts of Interest

There are no conflicts of interest to declare for this study.

5.7. Ethical Compliance

This study required the inclusion of pregnant women to fulfil its objectives. Neither the parent study nor this study involved medical or behavioural interventions, and the participants' pregnancies continued to be managed in accordance with provincial care guidelines. [62] Pregnant girls under 18 years were included in the study only if they provided assent and their parent or legal guardian provided consent. [62]

The parent study and our study comply with the latest versions of the Declaration of Helsinki, the Guidelines for Good Clinical Practice in the Conduct of Clinical Trials in Human Participants in South Africa, and the Department of Health: Principles Structures and Processes of Ethics in Health Research.

CHAPTER 4: RESULTS

1. Introduction

The following chapter presents the study's findings and details the outcomes of the analyses. This includes sociodemographic and clinical characteristics of both research sites, maternal syphilis screening practices, maternal syphilis prevalence and factors associated with maternal syphilis. Data analyses were conducted using STATA version 12.1, and statistical significance testing was performed using the Chi-square or Fisher Exact tests with a significance level of 0.05.

2. Sociodemographic Characteristics of Research Sites

A total of 941 participants were included in our study, with 63.8% from Gugulethu and 36.2% from the Breede Valley. The parent study began enrollment in 2022, with 37.4% enrolled that year, 50.3% in 2023 and 12.3% in 2024 by 12 June. Of the total participants, 77.5% were enrolled under the 2019 PMTCT guidelines, while the remaining were enrolled following the release of the updated 2023 VTP guidelines. Table 4 summarises the sociodemographic characteristics of the study participants at each research site.

2.1. Maternal Age

The median maternal age was 29.1 years, with an interquartile range (IQR) of 24.6 to 34.3 years. Most women were between 21 and 40 years old (87.0%), with no significant difference between the research sites (Table 4).

2.2. Socioeconomic Status

Most participants (81.7%) had a secondary level (grades 8-12) of education, with a significant difference between the sites (p = 0.00). A higher proportion of participants in Gugulethu had tertiary education (17.7%), while the Breede Valley had a higher proportion with only primary or no formal education (6.5%) (Table 4).

The majority of women (67.3%) included in our study were unemployed. Although the unemployment rate was high at both sites, a significant difference was noted (p = 0.03) due to Gugulethu having a higher proportion of full-time students without an income (5.7%) (Table 4). Among those employed, most (82.8%) earned a low monthly income of between ZAR1,001 and ZAR5,000, consistent at both sites (Table 4). Most women (57.2%) reported receiving a social grant from the SA government at the time of enrollment, though this was more common at Gugulethu (p = 0.00, Table 4). Overall, 30.8% of women had no income, including no social grant. Regarding food security, 14.7% of women reported going without a meal at least one day in the past week, with a median of 2 days (IQR: 2-3 days). This was at a higher proportion in Gugulethu (18.0%) (p = 0.00, Table 4).

Most women lived in households of 1-4 people (61.6%), with a significant difference between sites, indicating that overcrowding was more common in Gugulethu (p = 0.00, Table 4). The majority lived in

informal housing (57.2%), with no difference between sites (p = 0.34, Table 4). Almost all participants had access to an onsite toilet (97.5%), running water (98.6%), and electricity (90.5%). Additionally, 15.0% had a vehicle, 77.7% had a refrigerator, and 74.4% had a television.

Data on education, employment, income, food security, housing, and access to amenities were used to calculate an asset score for each participant, which classified them as low, middle or high socioeconomic status (SES). Most women (61.6%) were classified as middle SES, but a significant difference between sites was observed (p = 0.00, Table 4). The Breede Valley showed greater SES diversity, with higher proportions of low (27.6%) and high (18.2%) status women (Table 4).

2.3. Maternal-Paternal Relationship

Participants were questioned about their relationship status with the father of their current pregnancy. Most participants were in a relationship with the father (83.5%); 40.8% were in a relationship and cohabiting, and 42.7% were in a relationship but not cohabiting. A significant difference between sites was noted, as higher proportions of Gugulethu women were in a relationship but not cohabiting (47.3%) and not in a relationship (17.0%) (p = 0.00, Table 4). Most women reported receiving financial (76.8%) and emotional (80.3%) support from the father.

Table 4: Description of sociodemographic characteristics by research site

n (%)	Total (n = 941)	Gugulethu (n = 600)	Breede Valley (n = 341)	*p*-value (<0.05)
Maternal age (*years*)				0.30[a]
≤20	74 (7.9%)	50 (8.3%)	24 (7.0%)	-
21-30	460 (48.9%)	292 (48.7%)	168 (49.3%)	-
31-40	359 (38.1%)	222 (37.0%)	137 (40.2%)	-
> 40	48 (5.1%)	36 (6.0%)	12 (3.5%)	-
Home language				0.00 [b]
isiXhosa	822 (87.4%)	584 (97.5%)	238 (69.8%)	-
Afrikaans	64 (6.8%)	2 (0.3%)	62 (18.2%)	-
English	17 (1.8%)	4 (0.7%)	13 (3.8%)	-
Sesotho	15 (1.6%)	0 (0.0%)	15 (4.4%)	-
Shona	15 (1.6%)	2 (0.3%)	13 (3.8%)	-
isiZulu	5 (0.5%)	5 (0.8%)	0 (0.0%)	-
Unknown	3 (0.3%)	3 (0.5%)	0 (0.0%)	-
Education Level				0.00 [b]
None	1 (0.1%)	0 (0.0%)	1 (0.3%)	-
Primary (grades 1-7)	36 (3.8%)	15 (2.5%)	21 (6.2%)	-
Secondary (grades 8-12)	769 (81.7%)	478 (79.7%)	291 (85.3%)	-
Tertiary	134 (14.3%)	106 (17.7%)	28 (8.2%)	-
Unknown	1 (0.1%)	1 (0.2%)	0 (0.0%)	-
Employment status				0.03 [a]
Employed	267 (28.4%)	168 (28.0%)	99 (29.0%)	-
Unemployed	633 (67.3%)	398 (66.3%)	235 (68.9%)	-
Full-time student with no income	41 (4.3%)	34 (5.7%)	7 (2.1%)	-
Monthly income				0.78 [b]
ZAR 0	674 (71.6%)	432 (72.0%)	242 (70.9%)	-
< ZAR 1,000	15 (1.6%)	8 (1.3%)	7 (2.1%)	-
ZAR 1,001 – 5,000	221 (23.5%)	142 (23.7%)	79 (23.2%)	-
ZAR 5,001 – 10,000	30 (3.2%)	17 (2.8%)	13 (3.8%)	-
ZAR 10,001 – 15,000	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
>ZAR 15,000	1 (0.1%)	1 (0.2%)	0 (0.0%)	-
Government grant				0.00 [a]
Yes	538 (57.2%)	374 (62.3%)	164 (48.1%)	-
No	403 (42.8%)	226 (37.7%)	177 (51.9%)	-
In the past week, ≥1 day without a meal				0.00 [a]
Yes	138 (14.7%)	108 (18.0%)	30 (8.8%)	-
No	803 (85.3%)	492 (82.0%)	311 (91.2%)	-
No. of people in the household				0.00 [b]
1-4	579 (61.6%)	355 (59.2%)	224 (65.7%)	-
5-8	291 (30.9%)	189 (31.5%)	102 (29.9%)	-
9-12	55 (5.8%)	40 (6.7%)	15 (4.4%)	-
>12	16 (1.7%)	16 (2.6%)	0 (0.0%)	-
Housing type				0.34 [a]
Formal	403 (42.8%)	264 (44.0%)	139 (40.8%)	-
Informal	538 (57.2%)	336 (56.0%)	202 (59.2%)	-
SES				0.00 [a]
Low	217 (23.1%)	123 (20.5%)	94 (27.6%)	-
Medium	580 (61.6%)	395 (65.8%)	185 (54.2%)	-
High	144 (15.3%)	82 (13.7%)	62 (18.2%)	-
Relationship with father of pregnancy				0.00[b]
Coupled and cohabiting	384 (40.8%)	210 (35.0%)	174 (51.0%)	-
Coupled and not cohabiting	402 (42.7%)	284 (47.3%)	118 (34.6%)	-
Not coupled	149 (15.8%)	102 (17.0%)	47 (13.8%)	-
Mother uncertain of relationship status	6 (0.6%)	4 (0.7%)	2 (0.6%)	-

SES = Socioeconomic Status

a = Chi-square test, *b* = Fisher's Exact test

3. Clinical Characteristics at Research Sites

3.1. Obstetric History

The GA at enrollment ranged from 24- to 36 weeks, as specified by the inclusion criteria. The overall median GA at enrollment was 29.0 weeks (IQR: 25.6–32.3 weeks); 28.6 weeks (IQR: 25.3-32.4 weeks) at Gugulethu, and 29.1 weeks (IQR: 26.2-32.2 weeks) at the Breede Valley.

The first ANC visit date was recorded, allowing for determining the pregnancy trimester when participants first accessed ANC. Most women (54.3%) attended their first ANC visit in the second trimester, with a significant difference between sites. The Breede Valley had a higher proportion of women attending in the first trimester (37.2%), and Gugulethu had a higher proportion only attending in the third trimester (17.7%) ($p = 0.00$, Table 5).

Gravidity among participants ranged from 1 to 8, with a median of 2. Most women were multigravida (70.7%), consistent at each site ($p = 0.83$, Table 5). Parity ranged from 0 to 6, with a median of 1, and most women were multiparous (39.9%), with no significant difference between sites ($p = 0.53$, Table 5). Overall, 26.4% of women reported at least one obstetric complication in previous pregnancies: 14.7% spontaneous abortion, 5.3% termination of pregnancy (TOP), 3.1% premature birth, 2.6% stillbirth, and 1.6% pregnancy-related hypertension. The only past obstetric complication to differ significantly by site was TOP, partly due to a higher proportion of women from the Breede Valley with no recorded data (25.5%) ($p = 0.00$, Table 5). The historical death of a neonate and an infant was reported by 1.7% and 1.8% of women, respectively, with no significant difference by site ($p = 0.75$, $p = 0.32$, Table 5).

During their current pregnancy and prior to study enrollment, 0.5% of women were diagnosed with TB, and 2.7% had been hospitalised for pregnancy-related reasons. A significant difference between sites was noted for pregnancy-related hospitalisation, as the Breede Valley had a higher proportion of admissions (5.0%) ($p = 0.00$, Table 5).

At the time of data retrieval from the parent study, pregnancy outcome information was available for 84.2% of participants. Most of these births (99.5%) were live births, and no significant difference by site was detected ($p = 0.09$, Table 5). However, the spontaneous abortion and two of the three stillbirths occurred in the Breede Valley.

Table 5: Description of participant obstetric history by research site

n (%)	Total (n = 941)	Gugulethu (n = 600)	Breede Valley (n = 341)	*p*-value (<0.05)
Trimester at first ANC visit				0.00[a]
First (≤12 weeks)	210 (22.3%)	83 (13.8%)	127 (37.2%)	-
Second (13-27 weeks)	511 (54.3%)	350 (58.3%)	161 (47.2%)	-
Third (≥28 weeks)	122 (13.0%)	106 (17.7%)	16 (4.7%)	-
Unknown	98 (10.4%)	61 (10.2%)	37 (10.9%)	-
Gravidity				0.83[a]
Primigravida (1)	203 (21.5%)	127 (21.1%)	76 (22.3%)	-
Multigravida (2-4)	665 (70.7%)	428 (71.4%)	237 (69.5%)	-
Grand multigravida (≥5)	73 (7.8%)	45 (7.5%)	28 (8.2%)	-
Parity				0.53[b]
Nulliparous (0)	233 (24.8%)	146 (24.3%)	87 (25.5%)	-
Primiparous (1)	322 (34.2%)	198 (33.0%)	124 (36.4%)	-
Multiparous (2-4)	376 (39.9%)	250 (41.7%)	126 (36.9%)	-
Grand multiparous (≥5)	10 (1.1%)	6 (1.0%)	4 (1.2%)	-
History of spontaneous abortion				0.44[a]
Yes	138 (14.7%)	84 (14.0%)	54 (15.8%)	-
No	803 (85.3%)	516 (86.0%)	287 (84.2%)	-
History of TOP				0.00[a]
Yes	50 (5.3%)	25 (4.2%)	25 (7.3%)	-
No	792 (84.2%)	563 (93.8%)	229 (67.2%)	-
Unknown	99 (10.5%)	12 (2.0%)	87 (25.5%)	-
History of premature birth (<37 weeks)				0.90[b]
Yes	29 (3.1%)	18 (3.0%)	11 (3.2%)	-
No	911 (96.8%)	581 (96.8%)	330 (96.8%)	-
Unknown	1 (0.1%)	1 (0.2%)	0 (0.0%)	-
History of stillbirth				0.25[a]
Yes	24 (2.5%)	18 (3.0%)	6 (1.8%)	-
No	917 (97.5%)	582 (97.0%)	335 (98.2%)	-
History of pregnancy-related hypertension				0.54[b]
Yes	15 (1.6%)	10 (1.7%)	5 (1.5%)	-
No	925 (98.3%)	590 (98.3%)	335 (98.2%)	-
Unknown	1 (0.1%)	0 (0.0%)	1 (0.3%)	-
History of neonatal death (0-28 days)				0.75[b]
Yes	16 (1.7%)	9 (1.5%)	7 (2.1%)	-
No	924 (98.2%)	590 (98.3%)	334 (97.9%)	-
Unknown	1 (0.1%)	1 (0.2%)	0 (0.0%)	-
History of infant death (29-365 days)				0.32[b]
Yes	17 (1.8%)	13 (2.2%)	4 (1.2%)	-
No	924 (98.2%)	587 (97.8%)	337 (98.8%)	-
TB in current pregnancy before enrollment				0.36[b]
Yes	5 (0.5%)	2 (0.3%)	3 (0.9%)	-
No	936 (99.5%)	598 (99.7%)	338 (99.1%)	-
Hospital admission related to current pregnancy before enrollment				0.00[b]
Yes	25 (2.7%)	8 (1.3%)	17 (5.0%)	-
No	914 (97.1%)	592 (98.7%)	322 (94.4%)	-
Unknown	2 (0.2%)	0 (0.0%)	2 (0.6%)	-
Pregnancy outcome (n = 792, 84.2%)				0.09[b]
Live birth	788 (99.5%)	546 (99.8%)	242 (98.8%)	-
Stillbirth	3 (0.4%)	1 (0.2%)	2 (0.8%)	-
Spontaneous abortion	1 (0.1%)	0 (0.0%)	1 (0.4%)	-

ANC = Antenatal Care; TOP = Termination of Pregnancy; TB = Tuberculosis *a* = Chi-square test, *b* = Fisher's Exact test

3.2. HIV

A total of 542 participants were WLHIV at the time of enrollment, resulting in an overall HIV study prevalence of 57.6%, with no difference by site (p = 0.74, Table 6). Our study's HIV prevalence figures are not representative of the true HIV prevalence in these communities, as the parent study aimed to enroll women with and without HIV in a ratio of 2:1 and no less than 1:1. Of the WLHIV, 52.0% were diagnosed during any pregnancy, and 19.6% during the current pregnancy. The Breede Valley had a significantly higher proportion of women diagnosed during the current pregnancy (25.8%) (p = 0.01, Table 6).

In SA, the 2019 PMTCT and 2023 VTP guidelines recommend regular viral load monitoring throughout pregnancy for WLHIV, while CD4 testing is only required at ART initiation and one year thereafter. [3, 31] The most recent HIV-related laboratory test results were deemed relevant if conducted within a maximum of 12 months before conception of the current pregnancy. Viral load results indicated that 76.0% of women with relevant results were virally suppressed, with no significant difference between sites (p = 0.99, Table 6). Relevant CD4 count results were available for 17.7% of WLHIV, with the majority (62.5%) of results below 500 cells/mm^3. However, a significantly higher proportion of women at Gugulethu had a CD4 count above 500 cells/mm^3 (42.4%) (p = 0.02, Table 6). Although CD4 testing at both research sites did not fully align with the requirements of national guidelines, over-testing was more common in Gugulethu. [3, 31]

Overall, 95.9% of WLHIV reported taking ART at the time of enrollment, and 22.9% had initiated or reinitiated ART during the current pregnancy. Gugulethu had significantly more untreated WLHIV (5.4%, p = 0.00), but significantly more women who were receiving treatment at the time of conceiving the current pregnancy (80.7%) (p = 0.01, Table 6). Most women (72.7%) reported taking treatment daily over the past 30 days, with the Breede Valley reporting higher adherence (p = 0.00, Table 6).

Table 6: Description of HIV-related characteristics by research site

n (%)	Total (n = 542)	Gugulethu (n = 348)	Breede Valley (n = 194)	*p*-value (<0.05)
Living with HIV (n = 941)				0.74[a]
Yes	542 (57.6%)	348 (58.0%)	194 (56.9%)	-
No	399 (42.4%)	252 (42.0%)	147 (43.1%)	-
Diagnosed with HIV in current pregnancy				0.01[a]
Yes	106 (19.6%)	56 (16.1%)	50 (25.8%)	-
No	436 (80.4%)	292 (83.9%)	144 (74.2%)	-
Viral load available				0.00[a]
Yes	175 (32.3%)	96 (27.6%)	79 (40.7%)	0.99[a]
Virally suppressed (≤50 copies/ml)	133 (76.0%)	73 (76.0%)	60 (76.0%)	-
Not virally suppressed (>50 copies/ml)	42 (24.0%)	23 (24.0%)	19 (24.0%)	-
No	367 (67.7%)	252 (72.4%)	115 (59.3%)	-
CD4 count available				0.31[a]
Yes	96 (17.7%)	66 (19.0%)	30 (15.5%)	0.02[b]
>500 cells/mm^3	36 (37.5%)	28 (42.4%)	8 (26.7%)	-
351-500 cells/mm^3	23 (24.0%)	17 (25.8%)	6 (20.0%)	-
201-350 cells/mm^3	22 (22.9%)	9 (13.6%)	13 (43.3%)	-
<200 cells/mm^3	15 (15.6%)	12 (18.2%)	3 (10.0%)	-
No	446 (82.3%)	282 (81.0%)	164 (84.5%)	-
ART status at enrollment				0.00[b]
Treated	520 (95.9%)	326 (93.7%)	194 (100.0%)	0.01[b]
Re/initiated during current pregnancy	119 (22.9%)	62 (19.0%)	57 (29.4%)	
Initiated before current pregnancy	399 (76.7%)	263 (80.7%)	136 (70.1%)	
Unknown	2 (0.4%)	1 (0.3%)	1 (0.5%)	
Untreated	19 (3.5%)	19 (5.4%)	0 (0.0%)	-
Unknown	3 (0.6%)	3 (0.9%)	0 (0.0%)	-
No. days ART was missed in last 30 days				0.00[b]
0	394 (72.7%)	225 (64.7%)	169 (87.1%)	-
1-10	109 (20.1%)	87 (25.0%)	22 (11.4%)	-
11-20	3 (0.6%)	2 (0.6%)	1 (0.5%)	-
21-30	31 (5.7%)	29 (8.3%)	2 (1.0%)	-
Unknown	5 (0.9%)	5 (1.4%)	0 (0.0%)	-
Sexual partner/s aware of HIV status				0.50[b]
Yes	353 (65.1%)	223 (64.1%)	130 (67.0%)	-
No	186 (34.3%)	122 (35.0%)	64 (33.0%)	-
Unknown	3 (0.6%)	3 (0.9%)	0 (0.0%)	-

HIV = Human Immunodeficiency Virus; ART = Antiretroviral Therapy
a = Chi-square test, *b* = Fisher's Exact test

4. Maternal Syphilis

4.1. POC Screening

Among all participants, 87.8% had maternal syphilis POC screening results recorded in their MCRs for the current pregnancy, with a higher proportion in the Breede Valley (97.9%) (p = 0.00, Table 7). The remaining participants included 5.5% with recorded screening dates but no documented results, and 6.6% whose MCRs were unavailable. One woman in the Breede Valley, had neither a recorded screening date nor result, indicating she may not have been screened. Of those with recorded screening results, 70.0% were screened at their first ANC visit, as required by the 2019 PMTCT guidelines, with no significant difference between sites (p = 0.08, Table 7).

Of the screened women (n = 826), 127 tests were reactive, resulting in a syphilis prevalence of 15.4% based on POC screening results alone, with no significant difference between sites (15.4% at Gugulethu and 15.3% at Breede Valley) (p = 0.95, Table 7). Among those with reactive POC results, 81.1% had corresponding TPHA laboratory results available on the NHLS-TrakCare database, in accordance with the 2019 PMTCT guidelines. Gugulethu was significantly more compliant with this aspect of the guideline since 86.8% of reactive women had an available TPHA laboratory result (p = 0.04, Table 7).

Table 7: Maternal syphilis POC screening by research site

n (%)	**Total (n = 941)**	**Gugulethu (n = 600)**	**Breede Valley (n = 341)**	***p*-value (<0.05)**
POC Screening Result Available				0.00[a]
Yes	826 (87.8%)	492 (82.0%)	334 (97.9%)	0.08[a]
At the first ANC visit	614 (74.3%)	379 (77.0%)	235 (70.4%)	-
At follow-up ANC visit	212 (25.7%)	113 (23.0%)	99 (29.6%)	-
No	115 (12.2%)	108 (18.0%)	7 (2.1%)	-
POC Screening Results (n = 826)				0.95[a]
Reactive	127 (15.4%)	76 (15.4%)	51 (15.3%)	0.04[a]
Laboratory results available	103 (81.1%)	66 (86.8%)	37 (72.5%)	-
No laboratory results available	24 (18.9%)	10 (13.2%)	14 (27.5%)	-
Non-reactive	699 (84.6%)	416 (84.6%)	283 (84.7%)	-

ANC = Antenatal Care; POC = Point of Care
a = Chi-square test

As detailed in Chapter 2, the two research sites had distinct protocols for maternal syphilis screening. At Gugulethu, it is protocol that all pregnant women receive a POC rapid test and TPHA laboratory test. In the Breede Valley, it is protocol that all pregnant women receive a POC rapid test, with TPHA laboratory testing conducted only if the POC test is reactive. Table 8 summarises adherence to these protocols at each research site by comparing the conducted POC rapid screenings and TPHA laboratory tests. Gugulethu adhered to its protocol of conducting both tests in most cases (80.4%). In 10.7% of cases, women were screened at POC with rapid tests but did not undergo TPHA laboratory testing, which remains compliant with the national guidelines, as the POC tests were non-reactive.

Table 8: Comparison of recorded POC screenings and TPHA laboratory tests by research site

n (%)		**Gugulethu (n = 540)**		**Breede Valley (n = 339)**	
		TPHA Laboratory Testing		**TPHA Laboratory Testing**	
		Yes	No	Yes	No
POC Screening	Yes	434 (80.4%)	58 (10.7%)	157 (46.3%)	177 (52.2%)
	No	42 (7.8%)	6 (1.1%)	4 (1.2%)	1 (0.3%)

TPHA = Treponema Pallidum Hemagglutination Assay; POC = Point of Care

4.2. Laboratory Testing

Of all participants, 73.6% had a TPHA laboratory result available for the current pregnancy in the NHLS-TrakCare database, while 15.5% had an RPR laboratory result. A significantly higher proportion of

Gugulethu women had TPHA results (88.3%) (p = 0.00, Table 9), with no significant difference between sites for RPR results (p = 0.84, Table 9). Of the TPHA results, 14.7% were reactive, with a significantly higher proportion of reactive results in the Breede Valley, which is expected as it is protocol to only conduct laboratory testing in the case of a reactive POC test (29.5%) (p = 0.00, Table 9). For the RPR results, 32.9% of those tested were reactive, with no significant difference between sites (p = 0.12, Table 9). The majority (52.1%) of RPR titer results were equal to or above 1:8, with a higher proportion of these coming from the Gugulethu site (65.4%) (p = 0.05, Table 9).

Table 9: Maternal syphilis laboratory tests by research site

n (%)	**Total (n = 941)**	**Gugulethu (n = 600)**	**Breede Valley (n = 341)**	***p*-value (<0.05)**
TPHA Laboratory Test				0.00[a]
Yes	693 (73.6%)	530 (88.3%)	163 (47.8%)	0.00[b]
Reactive	102 (14.7%)	54 (10.2%)	48 (29.5%)	-
Non-reactive	575 (83.0%)	464 (87.5%)	111 (68.1%)	-
Equivocal	15 (2.2%)	11 (2.1%)	4 (2.4%)	-
Rejected	1 (0.1%)	1 (0.2%)	0 (0.0%)	-
No	248 (26.4%)	70 (11.7%)	178 (52.2%)	-
RPR Laboratory Test				0.84[a]
Yes	146 (15.5%)	92 (15.3%)	54 (15.8%)	0.12[a]
Reactive	48 (32.9%)	26 (28.3%)	22 (40. 7%)	-
Non-reactive	98 (67.1%)	66 (71.7%)	32 (59.3%)	-
No	795 (84.5%)	508 (84.7%)	287 (84.2%)	-
RPR Titer (n = 48)				0.05[a]
< 1:8	23 (47.9%)	9 (34.6%)	14 (63.6%)	-
≥ 1:8	25 (52.1%)	17 (65.4%)	8 (36.4%)	-

TPHA = Treponema Pallidum Hemagglutination Assay; RPR = Rapid Plasma Reagin
a = Chi-square test, *b* = Fisher's Exact test

Participants were categorised according to their infection status based on laboratory results, using the categorisation system detailed in Table 2 of Chapter 2. The categories include active syphilis infection (positive TPHA, positive RPR), past syphilis infection (positive TPHA, negative RPR), no syphilis infection (negative TPHA, negative RPR), and unknown. The categorisation process is detailed in Table 10, and the results are presented in Table 11.

Table 10: Count of syphilis laboratory test results

Total (n = 941)		**TPHA Result**				
		Reactive	**Non-reactive**	**Equivocal**	**Rejected**	**No test**
RPR Result	**Reactive**	47	1	0	0	0
	Non-reactive	55	26	15	0	2
	No test	0	548	0	1	246

TPHA = Treponema Pallidum Hemagglutination Assay; RPR = Rapid Plasma Reagin

The overall prevalence in this study was 5.0% (95% CI 3.5%-6.4%) for active maternal syphilis and 5.8% (95% CI 4.3%-7.3%) for past maternal syphilis, based solely on laboratory results (Table 11). A significant

difference was observed between sites, with the rural Breede Valley showing higher prevalence rates for both active (6.4%, 95% CI 3.8%-9.1%) and past (7.6%, 95% CI 4.8%-10.5%) maternal syphilis compared to Gugulethu (4.2% [95% CI 2.6%-5.8%] and 4.8% [95% CI 3.1%-56.6%], respectively) (p = 0.00, Table 11). Among women with unknown maternal syphilis status based on laboratory results, including those with equivocal TPHA results, most (83.0%) had non-reactive POC test results, requiring no further testing per the 2019 PMTCT guidelines.[3] However, 11.0% had reactive POC test results, with a significantly higher proportion at Gugulethu (p = 0.00, Table 11).

Table 11: Proportion of participants categorised by maternal syphilis status based on laboratory results by research site

n (%; 95% CI)	**Total (n = 941)**	**Gugulethu (n = 600)**	**Breede Valley (n = 341)**	***p*-value (<0.05)**
Maternal syphilis status				0.00[a]
Active infection	47 (5.0%; 3.5-6.4)	25 (4.2%; 2.6-5.8)	22 (6.4%; 3.8-9.1)	-
Past infection	55 (5.8%; 4.3-7.3)	29 (4.8%; 3.1-6.6)	26 (7.6%; 4.8-10.5)	-
No infection	575 (61.1%; 57.9-64.2)	464 (77.3%; 74.0-80.7)	111 (32.6%; 27.6-37.5)	-
Unknown	264 (28.1%; 25.2-30.9)	82 (13.7%; 10.9-16.4)	182 (53.4%; 48.1-58.7)	0.00[b]
Reactive POC test	29 (11.0%)	13 (15.8%)	16 (8.8%)	-
Non-reactive POC test	219 (83.0%)	54 (65.9%)	165 (90.7%)	-
Unknown/no POC result	16 (6.0%)	15 (18.3%)	1 (0.5%)	-

CI = Confidence Interval
a = Chi-square test, *b* = Fisher's Exact test

4.3. Factors Associated with Maternal Syphilis Status

Maternal syphilis status demonstrated a significant association with several factors, including SES (p = 0.00), parity (p = 0.00), relationship with the father of the pregnancy (p = 0.01), timing of the first ANC visit (p = 0.00), HIV status (p = 0.00), and the pregnancy outcome (p = 0.00) (Table 12).

A significantly higher proportion of women with active maternal syphilis were nulliparous (36.2%) and belonged to a higher SES class (25.5%) (Table 12). In contrast, a significantly lower proportion of these women were coupled and co-habiting with the father of the current pregnancy (21.3%) and had initiated ANC in the first trimester (14.9%) (Table 12). A significantly higher proportion of women with past syphilis were WLHIV (78.2%) (Table 12). The prevalence of both active (5.5%) and past (7.9%) syphilis was higher among WLHIV compared to those without HIV (4.3% and 3.0%, respectively). Furthermore, both active and past syphilis infections were associated with a significantly higher incidence of stillbirth where pregnancy outcomes were available, at 6.1% and 2.3%, respectively (Table 12).

Maternal syphilis did not show a significant association with maternal age (p = 0.07), gravidity (p = 0.41), history of obstetric complications (p = 0.22), hospitalisation during the current pregnancy (p = 0.65), and ART treatment (p = 0.45) (Table 12).

Table 12: Association of sociodemographic and clinical factors with maternal syphilis status

n (%)	Total (n = 941)	Active syphilis (n = 47)	Past syphilis (n = 55)	No infection (n = 575)	Unknown (n = 264)	*p*-value (<0.05)
Sociodemographic Factors						
Maternal age (*years*)						0.07[a]
≤20	74 (7.9%)	7 (14.9%)	3 (5.5%)	43 (7.5%)	21 (7.9%)	-
21-30	460 (48.9%)	21 (44.7%)	21 (38.2%)	275 (47.8%)	143 (54.2%)	-
31-40	359 (38.1%)	17 (36.2%)	24 (43.6%)	227 (39.5%)	91 (34.5%)	-
> 40	48 (5.1%)	2 (4.2%)	7 (12.7%)	30 (5.2%)	9 (3.4%)	-
SES						0.00 [a]
Low	217 (23.1%)	14 (29.8%)	19 (34.5%)	132 (22.9%)	52 (19.7%)	-
Medium	580 (61.6%)	21 (44.7%)	30 (54.6%)	370 (64.4%)	159 (60.2%)	-
High	144 (15.3%)	12 (25.5%)	6 (10.9%)	73 (12.7%)	53 (20.1%)	-
Relationship with father of pregnancy						0.01[a]
Coupled and cohabiting	384 (40.8%)	10 (21.3%)	25 (45.4%)	224 (38.9%)	125 (47.3%)	-
Coupled and not cohabiting	402 (42.7%)	24 (51.1%)	21 (38.2%)	261 (45.4%)	96 (36.4%)	-
Not coupled	149 (15.8%)	13 (27.6%)	9 (16.4%)	88 (15.3%)	39 (14.8%)	-
Mother uncertain of status	6 (0.6%)	0 (0.0%)	0 (0.0%)	2 (0.4%)	4 (1.5%)	-
Clinical Factors						
Trimester at first ANC visit						0.00 [a]
First (≤12 weeks)	210 (22.3%)	7 (14.9%)	13 (23.6%)	98 (17.0%)	92 (34.9%)	-
Second (13-27 weeks)	511 (54.3%)	35 (74.5%)	26 (47.3%)	323 (56.2%)	127 (48.1%)	-
Third (≥28 weeks)	122 (13.0%)	3 (6.4%)	9 (16.4%)	88 (15.3%)	22 (8.3%)	-
Unknown	98 (10.4%)	2 (4.2%)	7 (12.7%)	66 (11.5%)	23 (8.7%)	-
Gravidity						0.41[a]
Primigravida (1)	203 (21.5%)	13 (27.7%)	6 (10.9%)	120 (20.9%)	64 (24.2%)	-
Multigravida (2-4)	665 (70.7%)	31 (65.9%)	44 (80.0%)	409 (71.1%)	181 (68.6%)	-
Grand multigravida (≥5)	73 (7.8%)	3 (6.4%)	5 (9.1%)	46 (8.0%)	19 (7.2%)	-
Parity						0.00[a]
Nulliparous (0)	233 (24.8%)	17 (36.2%)	8 (14.5%)	129 (22.4%)	79 (29.9%)	-
Primiparous (1)	322 (34.2%)	16 (34.0%)	16 (29.1%)	193 (33.6%)	97 (36.7%)	-
Multiparous (2-4)	376 (39.9%)	12 (25.5%)	31 (56.4%)	247 (43.0%)	86 (32.6%)	-
Grand multiparous (≥5)	10 (1.1%)	2 (4.3%)	0 (0.0%)	6 (1.0%)	2 (0.8%)	-
History of obstetric complications						0.22 [a]
Yes	239 (25.4%)	15 (31.9%)	17 (30.9%)	133 (23.1%)	74 (28.0%)	-
No	702 (74.6%)	32 (68.1%)	38 (69.1%)	442 (76.9%)	190 (72.0%)	-
Hospital admission related to current pregnancy before enrollment						0.65[a]
Yes	25 (2.7%)	3 (6.4%)	2 (3.6%)	12 (2.1%)	8 (3.0%)	-
No	914 (97.1%)	44 (93.6%)	53 (96.4%)	562 (97.7%)	255 (96.6%)	-
Unknown	2 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	1 (0.4%)	-
Living with HIV						0.00[a]
Yes	542 (57.6%)	30 (63.8%)	43 (78.2%)	363 (63.1%)	106 (40.2%)	-
No	399 (42.4%)	17 (36.2%)	12 (21.8%)	212 (36.9%)	158 (59.8%)	-
ART status at enrollment (n = 542)						0.45[a]
Treated	520 (95.9%)	29 (96.7%)	43 (100%)	344 (94.8%)	104 (98.2%)	-
Untreated	19 (3.5%)	1 (3.3%)	0 (0.0%)	17 (4.7%)	1 (0.9%)	-
Unknown	3 (0.6%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	1 (0.9%)	-
Pregnancy outcome (n = 792)						0.00[a]
Live birth	788 (99.5%)	31 (93.9%)	43 (97.7%)	507 (99.8%)	207 (100%)	-
Stillbirth	3 (0.4%)	2 (6.1%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	-
Spontaneous abortion	1 (0.1%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	-

SES = Socioeconomic Status*;* ANC = Antenatal Care; HIV = Human Immunodeficiency Virus; ART = Antiretroviral Therapy
a = Chi-square test

CHAPTER 5: DISCUSSION

1. Introduction

Our study described maternal syphilis screening and results in an urban and rural community in the WC province of SA. The following chapter discusses the study findings in relation to the study objectives. This includes a comparison of the observed maternal syphilis prevalence rates, which were notably higher, with national and provincial reports, and a detailed discussion of how screening practices were often suboptimal at both sites compared to facility protocols and the 2019 PMTCT guidelines. This chapter also addresses the study's strengths and limitations.

2. Prevalence of Maternal Syphilis

Our study found a maternal syphilis prevalence (active or past) of 15.4% based solely on POC screening: 15.4% in Gugulethu and 15.3% in the Breede Valley (p = 0.95, Table 7). When based on reactive TPHA laboratory results, the overall prevalence decreased to 10.8%, with 9.0% in Gugulethu and 14.0% in the Breede Valley (p = 0.00, Table 11). Notably, the prevalence rates observed with laboratory results were lower than those of POC results. This suggests that the POC tests yielded false positives, indicating a lower specificity compared to laboratory tests. [65] Furthermore, the high prevalence of HIV and ART use in our study may have affected the accuracy of rapid syphilis serology. [56]

Since rapid POC tests cannot distinguish between active and past infection, participants were categorised by infection status based on TPHA and RPR laboratory results (Table 2). This resulted in a study prevalence of 5.0% (95% CI 3.5%-6.4%) for active infection and 5.8% (95% CI 4.3%-7.3%) for past infection, highlighting the importance of laboratory confirmation in preventing overtreatment. The rural area of the Breede Valley had significantly higher rates of both active (6.4%, 95% CI 3.8%-9.1%) and past (7.6%, 95% CI 4.8%-10.5%) syphilis compared to Gugulethu (4.2% [95% CI 2.6%-5.8%] and 4.8% [95% CI 3.1%-6.6%], respectively) (p = 0.00, Table 11). Similarly, a Tanzanian study found that women attending rural or semi-urban ANC clinics were twice as likely to be diagnosed with syphilis than those in urban areas. [49] However, in contrast, the 2019 ANCHSS reported a 1.3 times higher risk in urban ANC clinics, while a Rwandan study found no significant difference. [38, 50]

In 2016, Korenromp *et al.* estimated active maternal syphilis prevalence at 0.69% globally and 1.5% in Africa.[22] However, numerous reports, including those from the CDC and South African NICD, draw attention to a national and global resurgence of maternal and congenital syphilis over recent years. [13, 15, 17, 24] Given that the elimination of vertical syphilis transmission has been a WHO priority since 2007, and maternal syphilis is entirely preventable and treatable, this is a cause for concern. [5] Our study showed even higher prevalence rates of active maternal syphilis than comparable reports, possibly due to the purposeful sampling of women living with and without HIV in a ratio of no less than 1:1. The 2022 ANCHSS reported a national prevalence of 3.1%, and 3.9% in the WC, while a 2019 study conducted at a Cape Town ANC clinic found a prevalence of

2.2%.[12 22] These local prevalence rates were based solely on MCR reviews suggesting they may have been underestimated if results were not documented, whereas our study also incorporated laboratory results from the NHLS TrakCare database. Additionally, the WC province, like much of SA, faces significant socioeconomic inequity, with higher disease prevalence in poverty-stricken areas such as Gugulethu and the Breede Valley.

In summary, although national and provincial active maternal syphilis prevalence rates have reportedly increased, our study's prevalence of 5.0% (95% CI 3.5%-6.4%) exceeds these estimates, with significantly higher infection rates in the rural Breede Valley community.

3. Maternal Syphilis Screening Guideline Implementation

The two research sites had distinct protocols for maternal syphilis screening. In Gugulethu, all pregnant women were required to receive a POC rapid test and TPHA laboratory test. In contrast, in the Breede Valley, the protocol required all pregnant women to receive a POC rapid test, with a blood sample sent for laboratory testing only if the POC test was reactive. At both sites, if the laboratory-performed TPHA was positive, an RPR test was performed. One of our research questions was whether the research sites adhered to their respective screening protocols and the national 2019 PMTCT guidelines.

Gugulethu's screening protocol did not align with the 2019 PMTCT and 2023 VTP guidelines, which only recommend confirmatory laboratory tests for reactive POC results. [3, 31] While applying Gugulethu's protocol nationally would increase maternal syphilis case detection and help identify false-negatives POC test results, it would come at an increased cost. The national guidelines, along with the WHO guidelines, limit laboratory testing due to its high resource demands, while rapid POC tests are cost-effective, accurate, practical, and enable immediate treatment initiation. [28, 34] By not aligning with these guidelines, Gugulethu's protocol is likely to lead to unnecessary resource use in a resource-constrained setting.

3.1. POC Screening

POC syphilis screening is essential as it allows for immediate treatment of reactive women with a single dose of BPG while awaiting confirmation of active syphilis through laboratory testing. Overall, 87.8% of women had recorded results for POC screening, falling below the 99.0% provincial screening coverage reported in the 2022 ANCHSS. [15]

Significantly more women from the Breede Valley (97.9%) had recorded POC screening results compared to Gugulethu (82.0%) (p = 0.00, Table 7), perhaps because laboratory testing was required for all women in Gugulethu. Among those without available results, 44.4% in Gugulethu and 57.1% in the Breede Valley had POC syphilis screening dates, but no results were documented in their MCRs. This suggests that while POC screening is being conducted, results may not always be recorded by nursing staff. This was also noted in the 2019 ANCHSS, where nearly 20% of screened women had pending or unrecorded results. [38]

A Ghanaian study identified poor documentation of maternal syphilis test results and treatment, which facility staff attributed to increasing documentation requirements due to multiple programme registers. [43] This may apply in this study, particularly in Gugulethu.

Despite national guidelines and facility protocols mandating syphilis screening for all pregnant women at their first ANC visit, only 74.3% of those with POC screening results were screened at this visit, with no significant difference between sites ($p = 0.08$, Table 7). This is substantially lower than the 90% screening rate at the first ANC visit reported in the 2019 ANCHSS but higher than the 65% shown in a study of NICD-notified congenital syphilis cases. [37, 38] This finding is concerning as a quarter of women were only screened at follow-up visits, potentially prolonging fetal exposure to syphilis and increasing the risk of adverse outcomes. A Chinese study found that congenital syphilis risk increased eight-fold with maternal treatment after 28 weeks of gestation and 25-fold after 36 weeks, while another reported a 125% increased risk with each week of treatment delay. [12, 39] Stockouts of rapid POC tests, noted from 31 July to 16 August 2023 and 10 April to 4 June 2024, may have contributed to the screening delays at both sites. [66]

Among women with reactive POC test results, 18.9% did not receive follow-up TPHA laboratory testing, as required by the national guidelines. This was significantly more common in the Breede Valley (27.5%) compared to in Gugulethu (13.2%) ($p = 0.04$, Table 7), which is likely due to Gugulethu's protocol mandating TPHA laboratory testing for all pregnant women. The lack of laboratory confirmation in nearly one-fifth of cases is concerning as rapid POC tests have shown to have lower sensitivity (67–97% on whole blood) than TPHA laboratory tests (85–100%), potentially resulting in more frequent false negatives. [65, 67] Furthermore, POC tests cannot distinguish between active and past infection which may result in overtreatment, a genuine concern given reductions in the production of BPG, and the subsequent stockouts experienced globally and in SA. [8, 12]

In summary, this study revealed suboptimal implementation of POC screening guidelines at both sites, including low screening rates at the first ANC visit, insufficient confirmatory testing for reactive POC results, and inadequate documentation of screening results.

3.2. Laboratory Testing

Overall, 73.6% of women underwent TPHA laboratory testing, with a significantly higher testing rate in Gugulethu (88.3%), as per the facility protocol ($p = 0.00$, Table 9). Of those with TPHA laboratory test results, 14.7% were reactive, indicating detection of past or active syphilis, with a significantly higher proportion in the Breede Valley (29.5%) ($p = 0.00$, Table 9). This is expected, as, unlike in Gugulethu, the facility protocol only requires laboratory testing in the case of reactive POC test results. One participant had a rejected TPHA result, and 15 had equivocal results, five of whom had reactive POC test results. According to the NHLS-TrakCare database, no new laboratory samples were sent at the time of data

collection. This highlights the importance of clinic staff promptly retrieving laboratory results and acting on rejected, equivocal or positive results, as well as the need for facility managers to implement efficient monitoring strategies.

Gugulethu adhered to its protocol of conducting both POC and TPHA laboratory tests in most cases (80.4%) (Table 8). TPHA laboratory tests were not conducted in 70 (11.7%) women in Gugulethu, two of whom had RPR test results, indicating that blood was drawn and sent to the laboratory. In the Breede Valley, 157 TPHA laboratory tests were conducted despite only 51 reactive POC tests. This indicates non-compliance with their protocol and the unnecessary use of laboratory resources.

All women with reactive or equivocal TPHA laboratory results were RPR tested to confirm active or past infection. However, in Gugulethu, 25 women (4.7%) with non-reactive TPHA results were also RPR tested, as were two women (1.2%) in the Breede Valley. This indicates deviation from the national guidelines, which resulted in unnecessary use of valuable laboratory resources. It is important to note that distinguishing active from past infections to avoid overtreatment is only effective when results are followed up and treatment is discontinued for non-reactive RPR results. Gugulethu showed nearly twice as many RPR titers of 1:8 or higher, which is typically indicative of early infection and substantially increases the risk of vertical transmission of syphilis ($p = 0.05$, Table 9). [39]

In summary, this study showed inadequate implementation of maternal syphilis laboratory testing guidelines at both sites, particularly in failing to perform the appropriate tests on the correct women. This may lead to the misuse of resources and inadequate detection of maternal syphilis.

4. Factors Associated with Maternal Syphilis Status

4.1. Sociodemographic Factors

Maternal syphilis status showed a significant association with SES ($p = 0.00$, Table 12). Women with active maternal syphilis were evenly distributed across the three SES classes, likely due to the low numbers of infected women and the method of SES classification using an asset score. Furthermore, confounding factors were not considered. SES classification showed that over a quarter of women in the Breede Valley, where syphilis prevalence was higher, were classified as low SES ($p = 0.00$, Table 4).

Research supports a strong correlation between education level, a key determinant of SES, and the risk of maternal syphilis. The 2019 ANCHSS found that women with only primary schooling had a 1.6 times higher risk of maternal syphilis, and those with secondary schooling had a 1.3 times higher risk compared to those with tertiary education. [38] Similarly, Brazilian studies found that illiterate women or those with low education levels were twice as likely to have maternal syphilis. [60, 61] The 2023 GHS reported that 55% of WC adults had at least a Grade 12 education. [45] However, only 35.8% of women in Gugulethu and 21.4% in the Breede Valley reached this level of education, indicating lower educational attainment

compared to surrounding areas. Women in Gugulethu had significantly higher education levels than those in the Breede Valley (p = 0.00, Table 4). In addition to directly increasing the risk of maternal syphilis, lower education levels are linked to delayed or no ANC attendance and reduced likelihood of antenatal syphilis screening. [34, 68, 69]

Our study found that unemployment rates in both communities were considerably higher than the national estimate for women of 35.8%, with 66.3% in Gugulethu and 68.9% in the Breede Valley (p = 0.03, Table 4). [70] Both sites also had higher proportions of women receiving social grants than the 39.0% reported in the 2023 GHS, with significantly more in Gugulethu (62.3%) (p = 0.00, Table 4). [45] This difference by site may be attributed to better access to social care resources in the urban setting. Furthermore, both communities had double the proportion of women living in informal housing compared to the 2023 GHS report of 19% of WC households, with overcrowding significantly more prevalent among urban women in Gugulethu (p = 0.00, Table 4). [45] These figures highlight the inequities within the South African population.

There was also a significant association between maternal syphilis status and the relationship with the father of the current pregnancy (p = 0.01, Table 12), although adjustments were not made for potential confounders. A smaller proportion of women with active maternal syphilis were in a cohabiting relationship with the father, consistent with the 2019 ANCHSS report, which found women less likely to have syphilis if married to the father and more likely if they had no relationship. [38] Although most women in both communities remained in a relationship with the father of the pregnancy, this was significantly less common in Gugulethu (p = 0.00, Table 4). This variable highlights the potential syphilis exposure risks associated with non-monogamy, which should be considered for effective partner notification. Uncoupled women may be more likely to have multiple sexual partners during pregnancy and be at increased risk for seroconversion or reinfection. [22, 38]

Maternal syphilis status was not significantly associated with maternal age (p = 0.07), in contrast with several studies. A KZN PHC clinic study found pregnant women aged 35 and older to be significantly more likely to have active maternal syphilis, while the 2019 ANCHSS indicated that prevalence increased with age up to 34 years. [20, 38] A Rwandan study observed that individuals 25-49 years old were twice as likely to have syphilis than those younger than 25 years, while a US retrospective study showed an increasing odds ratio with age. [14, 50] The lack of association observed in our study may be due to the lack of adjustment for confounding factors, as was performed in the ANCHSS and two of the comparative studies. [14, 20, 38]

In summary, maternal syphilis status was significantly associated with SES and the relationship with the father of the pregnancy but not significantly associated with maternal age. Our study also highlights

considerable economic challenges among women attending ANC in Gugulethu and the Breede Valley, including high unemployment, low education levels, and poor housing.

4.2. Clinical Factors

Maternal syphilis status was significantly associated with parity ($p = 0.00$) but not gravidity ($p = 0.41$, Table 12), which is in contrast with previous reports. Research, including the 2019 ANCHSS, shows an association between maternal syphilis and increasing gravidity and parity, likely due to an increase in maternal age. [20, 38, 49] The lack of association observed in our study may be due to the lack of adjustment for confounding factors, as was performed in the ANCHSS and the comparative studies. [20, 38, 49] Our study found that a higher proportion of women with active syphilis were nulliparous, which may be attributed to the non-randomised nature of participant enrollment and the absence of a statistically significant association between maternal age and syphilis.

Syphilis status was also significantly associated with the timing of the first ANC visit, with fewer women with active maternal syphilis initiating ANC in the first trimester ($p = 0.00$, Table 12). This is supported by research showing that maternal syphilis risk increases four-fold with no ANC and doubles if ANC is initiated in the second or third trimesters. [12, 14] Despite living in a rural community, women in the Breede Valley were significantly more likely to enroll in ANC during the first trimester, while 17.7% of Gugulethu women only enrolled at 28 weeks or later ($p = 0.00$, Table 5). This finding contrasts with research showing lower ANC utilisation in rural areas and that rural women are more likely to enroll after 20 weeks of gestation. [68, 69] Later ANC enrollment observed in Gugulethu may be attributed to women initially booking their visits at a basic ANC clinic before being referred to the Gugulethu MOU. This could also reflect higher patient loads at urban clinics, where women report waiting for ANC appointments despite early presentation. [69] Other common barriers to early ANC enrollment include limited transport access, financial constraints, childcare responsibilities, distance to clinics, and long waiting times. [44, 69]

Our study found a significant association between maternal syphilis status and HIV status ($p = 0.00$) but no association with ART treatment among WLHIV ($p = 0.45$, Table 12). However, these results may be influenced by the purposeful sampling of women living with and without HIV in a minimum ration of 1:1, as well as the lack of adjustment for potential confounding factors. Prevalence rates of both active (5.5%) and past (7.9%) syphilis were higher among WLHIV than those without HIV (4.3% and 3.0%, respectively). This observed relationship between HIV and maternal syphilis is consistent with the literature. The 2019 ANCHSS found a significant and independent association with a two-fold increase in maternal syphilis risk in WLHIV, which was confirmed by a KZN hospital study. [19, 38] Studies in Rwanda and Uganda reported a six-fold and four-fold increase, respectively, in maternal syphilis among WLHIV. [50, 51] Importantly, this co-infection increases HIV vertical transmission risk by two to five times, as well as the risk of congenital syphilis. [42, 50, 57, 59]

The study HIV prevalence rates were comparable between the research sites, with 58.0% in Gugulethu and 56.9% in the Breede Valley owing to the study design. According to the 2022 ANCHSS, the antenatal HIV prevalence was reported at 20.0% in the Cape Town Metropolitan district (which includes Gugulethu) and 15.0% in the Cape Winelands district (which includes the Breede Valley). [15] A retrospective study conducted in Gugulethu from 2010-2013 reported an antenatal HIV prevalence of 30%.[71] Since the parent study enrolled women living with and without HIV in a ratio no less than 1:1, the true antenatal HIV prevalence rates in these communities are likely closer to these estimates. One-third of WLHIV had not notified their sexual partner(s) of their diagnoses, suggesting they may be reluctant to do so if screened positive for maternal syphilis. Partner notification is an essential aspect of HIV and syphilis management, but a Ugandan study found that only 58% of pregnant women diagnosed with syphilis consented to partner notification. [36]

There was a significant difference in the timing of HIV diagnosis between sites, with a quarter of WLHIV in the Breede Valley being diagnosed in their current pregnancy (p = 0.01, Table 6). This finding may indicate gaps in HIV interventions within the general population of the Breede Valley, resulting in women being first diagnosed during ANC. Urban PHC facilities are generally more accessible, better staffed and better resourced, potentially resulting in more HIV diagnoses outside of ANC in Gugulethu. [68]Alternatively, it may suggest more effective antenatal HIV screening in the Breede Valley. Furthermore, the Breede Valley had a significantly higher proportion of treated women (p = 0.00) and higher ART adherence at enrollment (p = 0.00, Table 6). However, since more Breede Valley WLHIV had initiated treatment during their current pregnancy, fewer were on treatment at the time of conception. Although viral suppression rates were comparable at both sites, significantly more women in the Breede Valley had recent viral load results (40.7%), suggesting better implementation of the PMTCT guidelines (p = 0.00, Table 6).

Maternal syphilis status was also significantly associated with poor pregnancy outcomes (p = 0.00, Table 12); however, our study was not powered to demonstrate this, the number of poor outcomes was small, and no adjustments were made for potential confounders. Both women with active and past syphilis infections had higher rates of stillbirths (6.1% and 2.3%, respectively), which is a well-documented adverse outcome of maternal syphilis. [5, 13, 14] Syphilis is the second most common infectious cause of stillbirth, with studies in sub-Saharan Africa suggesting it may be associated with 25–50% of cases. [6, 13] Korenromp *et al.* estimated 143,000 early fetal deaths and stillbirths globally from maternal syphilis in 2016, while 6% of congenital syphilis cases reported to the CDC in 2022 were stillbirths. [16, 72] Adverse pregnancy outcomes (stillbirth and spontaneous abortion) were six times higher in the Breede Valley than in Gugulethu (p = 0.09, Table 5), consistent with studies showing that rural women are more likely to experience adverse pregnancy outcomes. [73]

There was no significant association between maternal syphilis status and a history of obstetric complications ($p = 0.22$) or hospitalisation during the current pregnancy but before enrollment ($p = 0.65$, Table 12). Studies in the Americas have observed higher maternal syphilis rates in women with a history of spontaneous abortion, premature birth, and neonatal death, possibly indicative of past infection. [14, 52] Women in the Breede Valley experienced more previous spontaneous abortions, TOPs, premature births, and neonatal deaths, while Gugulethu experienced more previous stillbirths and infant deaths, with no significant difference between sites except for previous TOPs. Additionally, significantly more women from the Breede Valley had required hospitalisation during their current pregnancy ($p = 0.00$, Table 5).

In summary, maternal syphilis was associated with later ANC enrolment, HIV co-infection and an increased rate of stillbirth. Women in the Breede Valley had significantly earlier ANC enrollment but more hospitalisation and adverse outcomes related to the current pregnancy.

5. Study Strengths

Our study is timely and relevant considering the notable increases in maternal and congenital syphilis globally, including in SA, as reported by the NICD and ANCHSS. [15, 24, 38] The reasons for this resurgence are unclear and thought to be multifactorial. Studies such as this offer insight into maternal syphilis screening practices and assist in identifying gaps which may be contributing to the increasing prevalence rates. Furthermore, such research contributes to the 2030 Sustainable Development Goals and the SA government's effort to optimise maternal and child survival and well-being.

Besides insight into screening practices using a large sample, our study compared such practices in two geographical locations, one rural and the other urban. Both sites are resource-restricted and should adhere to the same national guidelines, making them comparable and generalisable to similar South African contexts. Furthermore, the study design allowed the comparison of the syphilis rapid POC and laboratory test results in the same women, and since data were collected over two and a half years, a long-term view of screening practices was presented.

6. Study Limitations

This study used data from the parent study, which restricted the recruitment and data collection methods and limited the available variables, including over-recruiting WLHIV. Furthermore, the sample size was determined by the parent study, which was powered to detect differences in mortality, hospitalisation, and neurodevelopment between HEU and HUU children. As a descriptive study, our study was not powered to quantify significant relationships, and we cannot account for unmeasured confounders.

The parent study began recruiting participants in January 2022, and this study included participants enrolled up until 12 June 2024. Since the latest national VTP guidelines, which included updated syphilis testing guidelines, were only released in August 2023, most data in this study preceded their implementation.

Consequently, there were insufficient participants enrolled after their implementation to allow for a meaningful comparison between the two guidelines.

The reported prevalence of maternal syphilis may be underestimated for several reasons. This secondary analysis relied on data collected at the enrollment visit, focusing solely on initial maternal syphilis screening and not considering follow-up screenings or seroconversion later in pregnancy. Additionally, by only including women attending ANC facilities, the study excludes those who did not attend ANC and may be at higher risk. [12, 14, 60] Finally, the denominator used in the prevalence calculation comprised all women, including those without documented POC test results who were assumed to be uninfected, which may have reduced the estimated prevalence.

The analysis of partner notification, which is essential for preventing reinfection, protecting future pregnancies, and reducing transmission, was beyond the scope of our study. Furthermore, data on maternal syphilis treatment were unavailable, and pregnancy outcome data were incomplete and did not include information on premature birth or congenital syphilis.

At the time of data collection, the two research sites, Gugulethu and the Breede Valley, followed different maternal syphilis screening protocols. Gugulethu's protocol involved conducting a treponemal POC test and a TPHA laboratory test for all pregnant women, whereas the Breede Valley only performed a laboratory test if the POC result was reactive. This difference in screening protocols likely influenced the comparability of the sites. Regardless of this difference, all available data for maternal syphilis screening were included.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

1. Conclusion

Our study revealed that national and facility-level maternal syphilis screening guidelines were not optimally implemented at both research sites. In the Breede Valley, the rate of recorded POC screening was comparable to the 2022 ANCHSS national screening coverage rate. However, Gugulethu had a significantly lower rate, raising concerns about poor documentation and potential treatment delays. Both research sites showed suboptimal screening rates at the first ANC visit, indicating delayed case detection. The findings also showed insufficient confirmatory testing for reactive POC results and laboratory testing out of protocol. These deviations from screening guidelines may contribute to rising congenital syphilis rates and indicate inefficient resource use.

Our study highlights a noteworthy concern regarding maternal syphilis, finding a 5.0% % (95% CI 3.5%-6.4%) prevalence rate for active infection, with a significantly higher rate in the rural community of the Breede Valley. The prevalence rates observed in this study exceeded the already rising national and provincial rates reported by the 2022 ANCHSS.

Maternal syphilis status was significantly associated with SES, relationship with the father of the pregnancy, parity, timing of ANC enrollment, HIV status, and pregnancy outcomes. Women with active syphilis enrolled in ANC later, while both active and past infections were more prevalent in WLHIV and associated with higher rates of stillbirth. No significant associations were found with maternal age, gravidity, ART treatment in WHLIV, prior obstetric complications, or hospitalisation during the current pregnancy.

In conclusion, while both research sites faced challenges related to maternal syphilis screening, the Breede Valley's practices aligned more closely with the national guidelines. Both sites showed active maternal syphilis prevalence rates higher than reported figures, with the Breede Valley more affected. Strengthening monitoring processes, addressing non-compliance, and improving staff training are crucial for improving maternal syphilis screening to reduce rising congenital syphilis cases.

2. Recommendations

Based on the findings of our study, it is recommended that all ANC facilities in SA align their maternal syphilis screening protocols with the August 2023 VTP guidelines set by the NDoH. Standardisation across facilities will allow for centralised staff training and better clarity among staff, as well as will improve regional and national monitoring. It is essential to implement consistent monitoring of staff training, protocol compliance, effective record-keeping, and any failures to retrieve laboratory results and act accordingly. Furthermore, the high prevalence rates observed in this study highlight the urgent need for PHC facilities in the WC to invest in comprehensive primary syphilis prevention strategies.

Further research should be conducted to evaluate the effectiveness of the updated August 2023 guidelines in improving maternal and neonatal outcomes through better case detection and reducing maternal and congenital syphilis prevalence rates. [14] Specifically, it is important to determine whether ANC providers can successfully implement the requirement for syphilis screening at every ANC visit and link women to treatment. [31] Since POC test results are not captured electronically, additional data collection will be required to assess this implementation. Measures to avoid test kit stockouts with a more robust supply chain are also critical.

Syphilis screening and diagnosis can be complex, involving various testing methods and algorithms. Future studies on syphilis prevalence should specify the screening algorithm used, whether seroconversion was considered, and how prevalence rates are determined (i.e. POC, TPHA, RPR or a combination of results). Furthermore, partner notification plays a critical role in syphilis management, and future studies should evaluate the effectiveness of current processes, particularly in SA.

REFERENCES

1. WHO. Global guidance on criteria and processes for validation: Elimination of mother-to-child transmission of HIV, syphilis and hepatitis B virus. Geneva, Switzerland: World Health Organization (WHO); 2021 [cited 2024 Jan 6]. Available from: https://iris.who.int/bitstream/handle/10665/349550/9789240039360-eng.pdf?sequence=1.
2. Pattinson RC, Buchmann EJ. Basic antenatal care plus handbook. Pretoria, South Africa: South African National Department of Health (SA NDoH); 2017 [cited 2025 Jan 5]. 2:[Available from: https://www.health.gov.za/wp-content/uploads/2023/05/BANC-Plus-Handbook-Feb-2017-with-NDoH-logo.pdf.
3. NDoH. Guideline for the prevention of mother-to-child transmission of communicable infections. Pretoria, South Africa: South African National Department of Health (NDoH); 2019 Nov [cited 2024 Jan 6]. Available from: https://www.nicd.ac.za/wp-content/uploads/2019/11/Guidelines-for-the-Prevention-of-Transmission-of-Communicable-Diseases-from-mother-to-child_28-October.pdf.
4. CDC. Notifiable disease. Atlanta, USA: Centers for Disease Control and Prevention (CDC); 2024 Jul [cited 2025 Jan 5]. Available from: https://www.cdc.gov/nchs/hus/sources-definitions/notifiable-disease.htm.
5. Morifi M, Malevu N, Odayan S, McCarthy K, Kufa T. Congenital syphilis case surveillance in South Africa 2017–19: Experience, challenges and opportunities. Journal of Tropical Pediatrics. 2021;67(4):1-11.
6. Umoke M, Sage P, Bjoernsen T, Umoke PCI, Ezeugworie C, Ejiofor D, *et al*. Co-infection and risk factors associated with STIs among pregnant women in rural health facilities in Nigeria: A retrospective stuSdy. The Journal of Health Care Organization, Provision, and Financing. 2021;58:1-9.
7. Hussen S, Tadesse BT. Prevalence of syphilis among pregnant women in sub-Saharan Africa: A systematic review and meta-analysis. BioMed Research International. 2019(1):4562385.
8. Nurse-Findlay S, Taylor MM, Savage M, Mello MB, Saliyou S, Lavayen M, *et al*. Shortages of benzathine penicillin for prevention of mother-to-child transmission of syphilis: An evaluation from multi-country surveys and stakeholder interviews. PLoS Medicine. 2017;14(12):e1002473.
9. Stafford IA, Workowski KA, Bachmann LH. Syphilis complicating pregnancy and congenital syphilis. New England Journal of Medicine. 2024;390(3):242-53.
10. Perez F, Mayaud P. One step in the right direction: Improving syphilis screening and treatment in pregnant women in Africa. The Lancet Global Health. 2019;7(5):550-1.
11. Carcamo CP, Velasquez C, Rocha SC, Centurion-Lara A, Lopez-Torres L, Parveen N. Sociodemographic and clinical characteristics associated with maternal and congenital syphilis: A prospective study in Peru. International Journal of Infectious Diseases. 2024;143:107041.
12. Pascoal LB, Carellos EVM, Tarabai BHM, Vieira CC, Rezende LG, Salgado BSF, *et al*. Maternal and perinatal risk factors associated with congenital syphilis. Tropical Medicine & International Health. 2023;28(6):442-53.

13. Moseley P, Bamford A, Eisen S, Lyall H, Kingston M, Thorne C, *et al*. Resurgence of congenital syphilis: New strategies against an old foe. The Lancet Infectious Diseases. 2023;24:24-35.
14. Gulersen M, Lenchner E, Eliner Y, Grunebaum A, Johnson L, Chervenak FA, *et al*. Risk factors and adverse outcomes associated with syphilis infection during pregnancy. American Journal of Obstetrics & Gynecology MFM. 2023;5(6):100957.
15. Kufa-Chakezha T, Shangase N, Lombard C, Manda S, Puren A. The 2022 National Antenatal HIV Sentinel Survey (ANCHSS) key findings. Johannesburg, South Africa: National Institute for Communicable Diseases (NICD); 2022 [cited 2024 Jan 6]. Available from: https://www.nicd.ac.za/wp-content/uploads/2024/01/Antenatal-survey-2022-report_National_Provincial_12Jul2023_Clean_01.pdf
16. Korenromp EL, Rowley J, Alonso M, Mello MB, Wijesooriya NS, Mahiané SG, *et al*. Global burden of maternal and congenital syphilis and associated adverse birth outcomes: Estimates for 2016 and progress since 2012. PLoS ONE. 2019;14(2):e0211720.
17. Mandavilli A. Infants are born with syphilis in growing numbers, a sign of a wider epidemic. New York, USA: The New York Times; 2023 Nov 7 [cited 2024 Jan 6]. Available from: https://www.nytimes.com/2023/11/07/health/syphilis-babies.html.
18. Eppes CS, Stafford I, Rac M. Syphilis in pregnancy: An ongoing public health threat. American Journal of Obstetrics and Gynecology. 2022;227(6):822-38.
19. Onyangunga OA, Naicker T, Moodley J. A clinical audit of maternal syphilis in a regional hospital in KwaZulu-Natal, South Africa. Southern African Journal of Infectious Diseases. 2020;35(1):1-5.
20. Hoque M, Hoque ME, Van Hal G, Buckus S. Prevalence, incidence and seroconversion of HIV and syphilis infections among pregnant women of South Africa. Southern African Journal of Infectious Diseases. 2021;36(1).
21. Mabaso N, Ngobese B, Hassan WM, Abbai N. Prevalence of syphilis in pregnant women living with Human Immunodeficiency Virus (HIV) from South Africa using a molecular-based assay. International Journal of STD & AIDS. 2023;34(9):624-32.
22. Joseph Davey DL, Nyemba DC, Gomba Y, Bekker L-G, Taleghani S, DiTullio DJ, *et al*. Prevalence and correlates of sexually transmitted infections in pregnancy in HIV-infected and -uninfected women in Cape Town, South Africa. PLoS ONE. 2019;14(7):e0218349.
23. Kuznik A, Habib AG, Manabe YC, Lamorde M. Estimating the public health burden associated with adverse pregnancy outcomes resulting from syphilis infection across 43 countries in sub-Saharan Africa. Sexually Transmitted Diseases. 2015;42(7):369-75.
24. Kufa-Chakezha T. Update on congenital syphilis surveillance in South Africa. Johannesburg, South Africa: National Institute for Communicable Diseases (NICD); 2022 Sept [cited 2024 Jul 26]. Available from: https://www.nicd.ac.za/wp-content/uploads/2022/09/Update-on-congenital-syphilis-surveillance-in-South-Africa.pdf.

25. WHO. Global guidance on criteria and processes for validation: Elimination of mother-to-child transmission of HIV and syphilis. Geneva, Switzerland: World Health Organization (WHO); 2017 [cited 2024 Jan 6]. 2: Available from: https://iris.who.int/bitstream/handle/10665/259517/9789241513272-eng.pdf?sequence=1.

26. WHO. Validation of elimination of mother-to-child transmission of HIV, syphilis and hepatitis B. Geneva, Switzerland: World Health Organization (WHO); 2024 [cited 2024 Jul 26]. Available from: https://www.who.int/initiatives/triple-elimination-initiative-of-mother-to-child-transmission-of-hiv-syphilis-and-hepatitis-b/validation.

27. Peters RP, Nel JS, Sadiq E, Kufa T, Smit DP, Sorour G, *et al*. Southern African HIV Clinicians Society guideline for the clinical management of syphilis. Southern African Journal of HIV Medicine. 2024;25(1):1577.

28. WHO. WHO guidelines for the treatment of Treponema pallidum (syphilis). Geneva, Switzerland: World Health Organization (WHO); 2016 [cited 2024 Mar 22]. Available from: https://iris.who.int/bitstream/handle/10665/249572/9789241549806-eng.pdf?sequence=1.

29. NDoH. National strategy for sexually transmitted infections 2017–2022. Pretoria, South Africa: South African National Department of Health (NDoH); 2017 May [cited 2024 Jan 6]. Available from: https://platform.who.int/docs/default-source/mca-documents/policy-documents/plan-strategy/ZAF-RH-43-01-PLAN-STRATEGY-2017-eng-National-STI-Strategy.pdf

30. Peters RP, Garrett N, Chandiwana N, Kularatne R, Brink AJ, Cohen K, *et al*. Southern African HIV Clinicians Society 2022 guideline for the management of sexually transmitted infections: Moving towards best practice. Southern African Journal of HIV Medicine. 2022;23(1):1450.

31. NDoH. Guideline for vertical transmission prevention of communicable infections. Pretoria, South Africa: South African National Department of Health (NDoH); 2023 Aug [cited 2024 Jan 6]. Available from: https://knowledgehub.health.gov.za/system/files/elibdownloads/2023-09/2023%20Vertical%20Transmission%20Prevention%20Guideline%2004092023%20signed%20WEB_1.pdf

32. Zhang Y, Guy R, Camara H, Applegate TL, Wiseman V, Treloar C, *et al*. Barriers and facilitators to HIV and syphilis rapid diagnostic testing in antenatal care settings in low-income and middle-income countries: A systematic review. BMJ Global Health. 2022;7(11):e009408.

33. Stafford IA, Berra A, Minard CG, Fontenot V, Kopkin RH, Rodrigue E, *et al*. Challenges in the contemporary management of syphilis among pregnant women in New Orleans, LA. Infectious Diseases in Obstetrics and Gynecology. 2019;220(1).

34. Dassah ET, Adu-Sarkodie Y, Mayaud P. Factors associated with failure to screen for syphilis during antenatal care in Ghana: A case control study. BMC Infectious Diseases. 2015;15:1-9.

35. Ndlovu N, Padarath A. District health barometer 2022/23. Durban, South Africa: Health Systems Trust; 2024 Feb [cited 2024 Jul 26]. Available from:

https://www.hst.org.za/publications/District%20Health%20Barometers/District%20Health%20Barometer_Complete%20Book_March.pdf.

36. Manabe YC, Namale G, Nalintya E, Sempa J, Ratanshi RP, Pakker N, *et al*. Integration of antenatal syphilis screening in an urban HIV clinic: A feasibility study. BMC Infectious Diseases. 2015;15:1-6.

37. de Voux A, Maruma W, Morifi M, Maduma M, Ebonwu J, Sheikh K, *et al*. Gaps in the prevention of mother-to-child transmission of syphilis: A review of reported cases, South Africa, January 2020–June 2022. Journal of Tropical Pediatrics. 2024;70(3).

38. Kufa T, Woldesenbet S, Cheyip M, Ayalew K, Kularatne R, Manda S, *et al*. Syphilis screening coverage and positivity by HIV treatment status among South African pregnant women enrolled in the 2019 Antenatal HIV Sentinel Survey. Scientific Reports. 2023;13(1):5322.

39. Qin J, Feng T, Yang T, Hong F, Lan L, Zhang C. Maternal and paternal factors associated with congenital syphilis in Shenzhen, China: A prospective cohort study. European Journal of Clinical Microbiology & Infectious Diseases. 2014;33:221-32.

40. Bocoum FY, Tarnagda G, Bationo F, Savadogo JR, Nacro S, Kouanda S, *et al*. Introducing onsite antenatal syphilis screening in Burkina Faso: Implementation and evaluation of a feasibility intervention tailored to a local context. BMC Health Services Research. 2017;17:1-10.

41. Mashamba-Thompson TP, Drain PK, Kuupiel D, Sartorius B. Impact of implementing antenatal syphilis point-of-care testing on maternal mortality in KwaZulu-Natal, South Africa: An interrupted time series analysis. Diagnostics. 2019;9(4):218.

42. Cohn J, Owiredu MN, Taylor MM, Easterbrook P, Lesi O, Francoise B, *et al*. Eliminating mother-to-child transmission of Human Immunodeficiency Virus, syphilis and hepatitis B in sub-Saharan Africa. Bulletin of the World Health Organization. 2021;99(4):287.

43. Dassah ET, Adu-Sarkodie Y, Mayaud P. Rollout of rapid point of care tests for antenatal syphilis screening in Ghana: Healthcare provider perspectives and experiences. BMC Health Services Research. 2018;18:1-12.

44. Nkamba D, Mwenechanya M, Kilonga AM, Cafferata ML, Berrueta AM, Mazzoni A, *et al*. Barriers and facilitators to the implementation of antenatal syphilis screening and treatment for the prevention of congenital syphilis in the Democratic Republic of Congo and Zambia: Results of qualitative formative research. BMC Health Services Research. 2017;17:1-11.

45. StatsSA. General household survey 2023. Pretoria, South Africa: Statistics South Africa (StatsSA); 2024 May [cited 2024 Jul 26]. Available from: https://www.statssa.gov.za/publications/P0318/P03182023.pdf.

46. Rocha AFB, Araújo MAL, Miranda AE, de Leon RGP, da Silva Junior GB, Vasconcelos LDPG. Management of sexual partners of pregnant women with syphilis in northeastern Brazil: A qualitative study. BMC Health Services Research. 2019;19:1-9.

47. Nakku-Joloba E, Kiguli J, Kayemba CN, Twimukye A, Mbazira JK, Parkes-Ratanshi R, *et al*. Perspectives on male partner notification and treatment for syphilis among antenatal women and their partners in Kampala and Wakiso districts, Uganda. BMC Infectious Diseases. 2019;19:1-13.

48. CDC. Sexually transmitted infections treatment guidelines 2021. Atlanta, USA: Centers for Disease Control and Prevention (CDC); 2024 Mar [cited 2024 Jul 26]. Available from: https://www.cdc.gov/std/treatment-guidelines/screening-recommendations.htm.

49. Manyahi J, Jullu BS, Abuya MI, Juma J, Ndayongeje J, Kilama B, *et al*. Prevalence of HIV and syphilis infections among pregnant women attending antenatal clinics in Tanzania, 2011. BMC Public Health. 2015;15:1-9.

50. Mutagoma M, Remera E, Sebuhoro D, Kanters S, Riedel DJ, Nsanzimana S. The prevalence of syphilis infection and its associated factors in the general population of Rwanda: A national household-based survey. Journal of Sexually Transmitted Diseases. 2016;2016(1).

51. Simiyu A, Atuheire CGK, Taremwa M, Ssali SN, Mwiine FN, Kankya C, *et al*. Sero-prevalence of syphilis and associated risk factors among pregnant women attending antenatal care at an urban-poor health centre in Kampala, Uganda: A cross-sectional study. The Pan African Medical Journal. 2024;47.

52. Benedetti KCSV, da Costa Ribeiro AD, de Sá Queiroz JHF, Melo ABD, Batista RB, Delgado FM, *et al*. High prevalence of syphilis and inadequate prenatal care in Brazilian pregnant women: A cross-sectional study. The American Journal of Tropical Medicine and Hygiene. 2019;101(4):761.

53. Genetu K, Abere K, Tachbele E. Magnitudes and correlates of Human Immunodeficiency Virus, hepatitis B virus, and syphilis among pregnant mothers attending antenatal care in Addis Ababa, Ethiopia. Infectious Diseases in Obstetrics and Gynecology. 2022(1):6156613.

54. StatsSA. Mid-year population estimates 2022. Pretoria, South Africa: Statistics South Africa (StatsSA); 2022 Jul [cited 2024 Jul 26]. Available from: https://www.statssa.gov.za/publications/P0302/P03022022.pdf.

55. UNAIDS. The path that ends AIDS: UNAIDS global AIDS update 2023. Geneva, Switzerland: Joint United Nations Programme on HIV/AIDS (UNAIDS); 2023 [cited 2024 Jul 26]. Available from: https://thepath.unaids.org/wp-content/themes/unaids2023/assets/files/2023_report.pdf.

56. Ren M, Dashwood T, Walmsley S. The intersection of HIV and syphilis: Update on the key considerations in testing and management. Topical Collection on Co-infections and Comorbidity. 2021;18:280-8.

57. Calegari LH, Friedrich L, Astolfi VR, Kerber JM, Andrades GS, Da Silva CH. The impact of maternal syphilis and associated factors on HIV vertical transmission. The Pediatric Infectious Disease Journal. 2022;41(7):563-5.

58. Getaneh Y, Khairunisa S, Husada D, Kuntaman K, Lusida MI. Burden of HIV, HBV and syphilis among children in urban Ethiopia: Community-based cross-sectional study. HIV Medicine. 2023;24(6):676-90.

59. Kinikar A, Gupte N, Bhat J, Bharadwaj R, Kulkarni V, Bhosale R, *et al*. Maternal syphilis: An independent risk factor for mother to infant Human Immunodeficiency Virus transmission. Sexually Transmitted Diseases. 2017;44(6):371-5.

60. de Macêdo VC, de Lira PIC, de Frias PG, Romaguera LMD, Caires SdFF, Ximenes RAdA. Risk factors for syphilis in women: Case-control study. Revista de Saude Publica. 2017;51:78.

61. Paixao ES, Ferreira AJ, Pescarini JM, Wong KL, Goes E, Fiaccone R, *et al*. Maternal and congenital syphilis attributable to ethnoracial inequalities: A national record-linkage longitudinal study of 15 million births in Brazil. The Lancet Global Health. 2023;11(11):e1734-e42.

62. Slogrove AL, de Beer ST, Kalk E, Boulle A, Cotton M, Cupido H, *et al*. Survival and health of children who are HIV-exposed uninfected: Study protocol for the CHERISH (Children HIV-Exposed Uninfected-Research to Inform Survival and Health) dynamic, prospective, maternal-child cohort study. BMJ Open. 2023;13(1):e070465.

63. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap): A metadata-driven methodology and workflow process for providing translational research informatics support. Journal of Biomedical Informatics. 2009;42(2):377-81.

64. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, *et al*. The REDCap consortium: Building an international community of software platform partners. Journal of Biomedical Informatics. 2019;95:103208.

65. Jafari Y, Peeling RW, Shivkumar S, Claessens C, Joseph L, Pai NP. Are Treponema pallidum specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? Evidence from a meta-analysis. PloS one. 2013;8(2):e54695.

66. Mowbray Maternity Hospital Pharmacy. Syphilis POC test stockouts. Telephonic conversation: Kroon SM. 2025 Jan 24 (cited 2025 Jan 25).

67. Angel-Müller E, Grillo-Ardila CF, Amaya-Guio J, Torres-Montañez N. Diagnostic accuracy of rapid point-of-care tests for detecting active syphilis: A systematic review and meta-analysis. Sexually Transmitted Diseases. 2021;48(12):e202-e8.

68. Samuel O, Zewotir T, North D. Decomposing the urban–rural inequalities in the utilisation of maternal health care services: Evidence from 27 selected countries in sub-Saharan Africa. Reproductive Health. 2021;18:1-12.

69. Ebonwu J, Mumbauer A, Uys M, Wainberg ML, Medina-Marino A. Determinants of late antenatal care presentation in rural and peri-urban communities in South Africa: A cross-sectional study. PLoS One. 2018;13(3):e0191903.

70. StatsSA. Quarterly labour force survey: Quarter 2 2024. Pretoria, South Africa: Statistics South Africa (StatsSA); 2024 [cited 2024 Jul 26]. Available from: https://www.statssa.gov.za/publications/P0211/P02112ndQuarter2024.pdf.

71. Myer L, Phillips T, Manuelli V, McIntyre J, Bekker L-G, Abrams EJ. Implementation and operational research: Evolution of antiretroviral therapy services for HIV-infected pregnant women in Cape Town, South Africa. Journal of Acquired Immune Deficiency Syndromes. 2015;69(2):e57 – e65.
72. McDonald R, O'Callaghan K, Torrone E, Barbee L, Grey J, Jackson D, *et al*. Vital signs: Missed opportunities for preventing congenital syphilis—United States, 2022. Morbidity and Mortality Weekly Report. 2023;72.
73. Fekene DB, Bulto GA, Woldeyes BS, Dina GD, Negash KM. Determinants of adverse birth outcomes in the West Shewa Zone, Oromia, regional state, Ethiopia: Unmatched case-control study. Journal of Mother and Child. 2021;25(1):9-18.

APPENDICES

Appendix A – REDCap Data Collection Questionnaires

Maternal Enrolment

Record ID

_____

Data collector

_____

Visit code

- ◯ Antenatal
- ◯ Delivery
- ◯ 3 month
- ◯ 6 month
- ◯ 9 month
- ◯ 12 month
- ◯ 15 month
- ◯ 18 month
- ◯ 21 month
- ◯ 24 month
- ◯ 27 month
- ◯ 30 month
- ◯ 33 month
- ◯ 36 month

Are you enrolled in any other research study?

- ◯ Yes
- ◯ No
- ◯ Unknown

Describe the study

_____

Enrolment Information

Inskrywings inligting

Ulwazi lobhaliso

Cohort site

Kohort area

Indawo yeqela

- ◯ Breede Valley
- ◯ Gugulethu
- ◯ Khayelitsha

Maternal study id

Moederstudie id

Isazisi sokufunda komama

_____

Date of enrolment

Inskrywing datum

Usuku lobhaliso

_____

Enrolment site

Inskrywing area

Indawo yobhaliso

◯ Gugulethu CHC
◯ Khayelitsha Site B CDC
◯ Michael Mapongwana CDC
◯ Khayelitsha Community
◯ Other

If other, specify

indien ander, spesifiseer

Ukuba ngenye, cacisa

_____

Eligibility Criteria

Geskiktheids kriteria

Inqubo yokufaneleka

Gestational age at enrolment

Gestasie ouderdom by inskrywing

Ixesha lokukhulelwa kubhaliso

_____

Method used to calculate gestation age:

Metode wat gebruik was om swangerskap ouderdom te bereken:

◯ Ultrasound
◯ LNMP
◯ SFH
◯ Other

If other, specify

indien ander, spesifiseer

Ukuba ngenye, cacisa

_____

HIV status

MIV status

Isimo se HIV

◯ Positive
◯ Negative

Date of last HIV test

Datum van laaste MIV -toets

Umhla wovavanyo wokugqibela lwe HIV

_____

Date difference

_____
(From last HIV negative test till Today)

PLEASE ENSURE CONFIRMATION OF HIV NEGATIVE STATUS VERSEKER BEVESTIGING VAN 'N MIV-NEGATIEWE TOETS NCEDA UQINISEKISE NGOBUME BE HIV

Permanently resident within cohort site catchment area

Permanent woonagtig binne kohort terrein opvangsgebied

Ingaba ungumhlali ngokusisigxina kwindawo yeqela

◯ Yes
◯ No
◯ Uncertain

RECONSIDER ENROLMENT HEROORWEEG HIERDIE INSKRYWING CINGISISA UBHALISO

Has own cellphone for study contact

Het eie selfoon vir studie kontak

Uneselfowni yakhe yokuqhagamishelana neqela le study

◯ Yes
◯ No
◯ Uncertain

RECONSIDER ENROLMENT HEROORWEEG HIERDIE INSKRYWING CINGISISA UBHALISO

Informed consent provided

Ingeligte toestemming voorsien

Imvume enolwazi uyinikiwe

◯ Yes
◯ No

DO NOT PROCEED WITH QUESTIONING ON DATA COLLECTION
MOET NIE VOORTGAAN MET VRAE OOR DIE INSAMELING VAN DATA

MATERNAL ENROLMENT SOURCE

Upload Maternity Case Record source:

(Upload the entire booking page with SF plots)

NOTE*
Please obscure all personal identifying information.
Please indicate the PID on the uploaded file.

COMMENTS/NOTES

Additional comments

_____
("None" if no comments)

Maternity Case Record - Antenatal

Record ID

_____

Data abstraction date

_____

Data collector

_____

Visit code

- ◯ Antenatal
- ◯ Delivery
- ◯ 3 month
- ◯ 6 month
- ◯ 9 month
- ◯ 12 month
- ◯ 15 month
- ◯ 18 month
- ◯ 21 month
- ◯ 24 month
- ◯ 27 month
- ◯ 30 month
- ◯ 33 month
- ◯ 36 month

Booking Information

First antenatal visit date

_____

Gravidity

_____

Parity

_____

LNMP date

_____
(If unknown (09/09/1999))

Certain

- ◯ Yes
- ◯ No

(Are you certain about your LNMP date)

Any previous miscarriages?

- ◯ Yes
- ◯ No
- ◯ Unknown

Any previous TOP's

- ◯ Yes
- ◯ No
- ◯ Unknown

Any previous pre-term birth (< 37 weeks gestation)

- ◯ Yes
- ◯ No
- ◯ Unknown

Any previous intrauterine deaths/stillbirths	◯ Yes ◯ No ◯ Unknown
Any previous neonatal death (0-28 days old)	◯ Yes ◯ No ◯ Unknown
Any previous infant death (29 - 365 days old)	◯ Yes ◯ No ◯ Unknown
Any previous child death (1 - 5 years old)	◯ Yes ◯ No ◯ Unknown
Any previous multiple pregnancies?	◯ Yes ◯ No ◯ Unknown
Any previous pregnancy complications	◯ Yes ◯ No ◯ Unknown
PREGNANCY COMPLICATIONS:	
Hypertension	◯ Yes ◯ No ◯ Unknown
Diabetes	◯ Yes ◯ No ◯ Unknown
Cardiac dx	◯ Yes ◯ No ◯ Unknown
Tuberculosis	◯ Yes ◯ No ◯ Unknown
Other	◯ Yes ◯ No ◯ Unknown
Please describe	_____
Booking weight	_____ (in kg (with decimal) if unknown 99.9)
Booking height	_____ (in cm (with decimal) if unknown 99.9)

Booking muac

_____
(in cm (with decimal)if unknown 99.9)

Booking SFH

_____
(in cm (with decimal) If non-palpable 10.0)

Test result

If more than one test - choose the positive test result or if all positive choose the most recent test

RPR test date

_____
("09-09-1999' if unknown or not avaiable)

RPR test result

◯ Positive
◯ Negative
◯ Unknown

Blood group

◯ A
◯ B
◯ AB
◯ 0
◯ Unknown

Rhesus group

◯ Positive
◯ Negative
◯ Unknown

Ultrasound information

How many antenatal ultrasounds were done?

_____

Date of 1st ultrasound

_____

Gestation at 1st ultrasound (weeks, decimal for days)

_____

Expected day of delivery on 1st ultrasound

_____

Any abnormalities on ultrasound

◯ Yes
◯ No
◯ Unknown

Please describe

_____

Date of 2nd ultrasound

_____

Gestation at 2nd ultrasound in (weeks, decimal for days)

_____

Expected day of delivery on 2nd ultrasound

_____

Any abnormalities on 2nd ultrasound

◯ Yes
◯ No
◯ Unknown

Please describe

_____

Date of 3rd ultrasound

_____

Gestation at 3rd ultrasound (weeks, decimal for days)

_____

Expected day of delivery on 3rd ultrasound

_____

Any abnormalities on 3rd ultrasound

◯ Yes
◯ No
◯ Unknown

Please describe

_____

COMMENTS/NOTES

Additional comments

_____

Maternal HIV - Baseline

Record ID

_____

Data abstraction date

_____

Data collector

_____

Visit code

- ◯ Antenatal
- ◯ Delivery
- ◯ 3 month
- ◯ 6 month
- ◯ 9 month
- ◯ 12 month
- ◯ 15 month
- ◯ 18 month
- ◯ 21 month
- ◯ 24 month
- ◯ 27 month
- ◯ 30 month
- ◯ 33 month
- ◯ 36 month

Maternal HIV diagnosis

Do you have HIV?

Het jy MIV?

Ingaba unentsholongwane kagawulayo?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Yes, newly diagnosed

(This question will not be asked. Collect this data from the MCR.)

When were you diagnosed with HIV?

Wanneer was jy gediagnoseer met MIV?

Wafunyaniswa nini ukuba unentsholongwane kagawulayo?

_____

If only month and year known - put the date as 15; If only year known put day and month as 01(day) July(month)

Is this the exact date or estimated date?

Is dit die presiese datum of beraamde datum?

Ingaba lo ngowona mhla okanye umhla oqikelelweyo?

- ◯ Exact
- ◯ Estimated

Were you pregnant when you were first diagnosed with HIV?

Was jy swanger toe jy die eerste keer met MIV gediagnoseer was?

Ingaba ubukhulelwe ukuqala kokufunyaniswa ukuba unentsholongwane kagawulayo?

- ◯ Yes
- ◯ No
- ◯ Unknown

(either in this pregnancy or a previous pregnancy)

When was your last HIV test done?

Wanneer was jou laaste MIV toets gedoen?

Lwenziwe nini uvavanyo lwakho lokugqibela lwentsholongwane kagawulayo?

_____

If only month and year known - put the date as 15; If only year known put day and month as 01(day) July(month)

Is this the exact date or estimated date?

Is dit die presiese datum of beraamde datum?

Ingaba lo ngowona mhla okanye umhla oqikelelweyo?

◯ Exact
◯ Estimated

Participant's HIV status must be confirmed before proceeding with interview

Maternal ART history

When did you start antiretroviral therapy?

Wanneer het jy begin met antiretrovirale terapie?

Uqale nini ukuthatha ipilisi ART?

_____

If only month and year known - put the date as 15; If only year known put day and month as 01(day) July(month)

Is this the exact date or estimated date?

Is dit die presiese datum of beraamde datum?

Ingaba lo ngowona mhla okanye umhla oqikelelweyo?

◯ Exact
◯ Estimated

Were you pregnant when you first started antiretroviral therapy?

Was jy swanger toe jy die eerste keer antiretrivirale terapie begin het?

Ingaba ukuqala kwakho ipilisi ART ubukhulelwe?

◯ Yes
◯ No
◯ Unknown
(either in this pregnancy or previous pregnancy)

Are you still taking ART?

Neem jy steeds ART?

Ingaba usazithatha ipilisi ART?

◯ Yes
◯ No
◯ Unknown

Please follow SOP to reconnect mom to HIV care

When did you last pick-up your ART?

Wanneer het jy laas jou ART gaan afhaal?

Ugqibele nini ukuyothatha ipilisi zakho ART?

_____

If only month and year known - put the date as 15; If only year known put day and month as 01(day) July(month)

Is this an exact date or estimated date?

Is dit die presiese datum of beraamde datum?

Ingaba lo ngowona mhla okanye umhla oqikelelweyo?

- ◯ Exact
- ◯ Estimated

At which clinic do you follow-up for your HIV care?

By watter kliniek volg jy op vir jou MIV-sorg?

Ulandelela kweyiphi kliniki ukhathalelo lwakho lwentsholongwane kagawulayo?

- ◯ Worcester CDC
- ◯ De Doorns
- ◯ Empilisweni
- ◯ Orchard
- ◯ Sandhills
- ◯ Rawsonville
- ◯ Gugulethu CHC
- ◯ Nzame Zabantu
- ◯ Mzamomhle
- ◯ Hannan
- ◯ Nyanga
- ◯ Vuyani
- ◯ Masincedane
- ◯ Other
- ◯ None

Please follow SOP to reconnect mom to HIV care

If other, specify

Indien ander, spesifiseer

Ukuba enye, chaza

_____

Since you first started taking ART, have you ever stopped taking your ART for more than 7 days?

Vandat jy die eerste keer begin het met ART, het jy ooit opgehou om jou ART te neem vir meer as 7 dae?

Kuba sewuqalile ukuthatha ipilisi ART, ubuke wayeka ukuzithatha ipilisi zakho kangangentsuku ezisixhenxe?

- ◯ Yes
- ◯ No
- ◯ Unknown

How many times has it happened that you stopped taking your ART?

Hoeveel keer het dit gebeur dat jy opgehou het om jou ART te neem?

Kwenzeka kangaphi ukuba uyeke ukuthatha ipilisi ART zakho?

- ◯ once only
- ◯ more than once

What are the names of the ARV's you are currently taking?

Wat is die name van die ARV's wat jy tans neem?

Athini amagama weepilisi ARV's ozithathayo ngoku?

◯ TDF_3TC_DTG
◯ TDF_FTC_EFV
◯ TDF_3TC_EFV
◯ AZT_3TC_EFV
◯ TDF_FTC_NVP
◯ TDF_3TC_NVP
◯ AZT_3TC_NVP
◯ TDF_3TC_LPV/r
◯ AZT_3TC_LPV/r
◯ other
◯ TLD

(if newly initiated - What regimen did you receive? Please note* "Volutrip" and "Reydin are TLD. The option TLD have been removed, because the option "TDF_3TC_DTG" is TLD)

If other, specify

Indien ander, spesifiseer

Ukuba enye, chaza

_____

How many times a day do you take your ARV's?

Hoeveel keer ‘n dag neem jy jou ARV’s?

Uthatha kangaohi kwipilisi zakho ngosuku?

◯ once a day
◯ twice a day
◯ Unknown

How many pills do you take each time?

Hoeveel pille neem jy elke keer?

Uthatha ipilisi ezingaphi ngosuku?

◯ one pill
◯ 2 pills
◯ 3 pills
◯ more than 3 pills
◯ Unknown

Adherence

We are going to ask you some questions about your adherence to ARV treatment (ART).

Ons gaan jou ‘n paar vrae vra oor jou nakoming van ARV-behandeling (ART).

Sizakubuza imibuzo malunga nokubambelela kunyango lwe ARV.

In the last 30 days, on how many days did you miss at least one dose of any of your HIV medicines?

In die laaste 30 dae, op hoeveel dae het jy ten minste een dosis van enige van jou MIV-medisyne misgeloop?

Kwiintsuksu ezingamashumi amathathu ezigqithileyo, zingaphi iintsuku ophose umlinganiselo yenye yonyango lwakho lwentsholongwane kagawulayo?

_____

(Number of days (0-30))

In the last 30 days, how often did you take your HIV medicines in the way that you were supposed to?

In die laaste 30 dae, hoe gereeld het jy jou MIV-medisyne geneem op die manier waarop dit voorgeskryf word?

Kwintsuku ezimashumi amathathu ezigqithileyo, uwasele kangaphi amayeza akho wentsholongwane ka gawulayo ngendlela ebekufanele uwathatha ngawo?

- ◯ Never
- ◯ Rarely
- ◯ Sometimes
- ◯ Usually
- ◯ Almost always
- ◯ Always

In the last 30 days, how good a job did you do at taking your HIV medicines in the way that you were supposed to?

In die laaste 30 dae, hoe goed het jy jou MIV-medisyne geneem op die manier waarop dit voorgeskryf word?

Kwintsuku ezimashumi amathathu ezigqithileyo, wenze umsebenzi olunge kangakanani ngokuthatha amayeza akho wentsholongwane kagawulayo ngendlela ebekufanele ukuba uyenzile?

- ◯ Very poor
- ◯ Poor
- ◯ Fair
- ◯ Good
- ◯ Very good
- ◯ Excellent

How hard is it for you to take HIV medicines in a way you are supposed to?

Hoe moeilik is dit om jou MIV-medisyne te neem op die manier waarop dit voorgeskryf is?

Ingaba kunzima kangakanani kuwe ukuthatha amayeza akho ngendlela ebekufanele uwathatha ngawo?

- ◯ Extremely hard
- ◯ Very hard
- ◯ Somewhat hard
- ◯ Not very hard
- ◯ Not hard at all

Disclosure

We are going to ask you some questions about whom (if at all) you have disclosed to about your HIV status.

Ons gaan jou ‘n paar vrae vra oor om te bepaal met wie jy jou MIV-status bekend gemaak het.

Sizakubuza imibuzo malunga nomntu omchazeleyo ngobume bakho bentsholongwane ka gawulayo.

Have you told anyone about your HIV status, other than health professionals?

Behalwe gesondheidswerkers, het jy iemand van jou MIV-status vertel?

Sewuke waxelela umntu ngobume bakho bentsholongwane kagawulayo ngaphandle kooGqirha okanye ooMongikazi?

- ◯ Yes
- ◯ No

20. Which of these family members have you told about your HIV status? Please answer this question for each of the following: Example: Have you told your_____ that you are HIV-positive?

20. Watter van hierdie familielede het jy van jou MIV-status vertel? Beantwoord die vraag vir elk van die volgende, byvoorbeeld: Het jy al vir jou _____ gesê dat jy MIV-positief is?

20. Ngabaphi kwaba balandelayo kusapho lwakho oke wabaxelela ngobume bakho bentsholongwane kagwulayo? Nceda uphendule lombuzo ngokulandelayo: Umzekelo: Uxelele_____ukuba unentsholongwane kagawulayo?

a. Husband

a. Eggenoot

a. Umyeni

◯ Yes
◯ No
◯ N/A: No Husband

---

b. Boyfriend or partner

b. Kêrel of lewensmaat

b. Isoka, iqabane

◯ Yes
◯ No
◯ N/A: No Boyfriend/partner

---

c. Mother

c. Ma

c. UMama

◯ Yes
◯ No
◯ N/A: No Mother

---

d. Father

d. Pa

d. UTata

◯ Yes
◯ No
◯ N/A: No Father

---

e. Sister

e. Suster

e. USisi

◯ Yes
◯ No
◯ N/A: No Sister

---

f. Brother

f. Broer

f. uBhuti

◯ Yes
◯ No
◯ N/A: No Brother

---

g. Daughter

g. Dogter

g. Intombi yakho

◯ Yes
◯ No
◯ N/A: No Daughter

---

h. Son

h. Seun

h. Unyana

◯ Yes
◯ No
◯ N/A: No Son

---

i. Uncle

i. Oom

i. Malume

◯ Yes
◯ No
◯ N/A: No Uncle

---

j. Aunt

j. Tante

j. Makazi

◯ Yes
◯ No
◯ N/A: No Aunt

Maternal Health - Baseline

Record ID

_____

Data abstraction date

_____

Data Collector

_____

Visit code

- ◯ Antenatal
- ◯ Delivery
- ◯ 3 month
- ◯ 6 month
- ◯ 9 month
- ◯ 12 month
- ◯ 15 month
- ◯ 18 month
- ◯ 21 month
- ◯ 24 month
- ◯ 27 month
- ◯ 30 month
- ◯ 33 month
- ◯ 36 month

We are going to ask you a few questions about your health.

Ons gaan jou 'n paar vrae oor jou gesondheid vra.

Sisakubuza imibuzo embalwa malunga nempilo yakho.

TB History

During your current pregnancy, has a doctor or nurse told you that you have TB?

Tydens jou huidige swangerskap, het 'n dokter of verpleegster jou gesê dat jy TB het?

Ngelixesha lokukhulelwa kwakho, ingaba uGqirha okanye uMongikazi ebeke wakuxelela ukuba unesifo sephepha?

- ◯ Yes
- ◯ No

When did you receive this TB diagnosis?

Wanneer het jy hierdie TB-diagnose ontvang?

Ingaba uzixelelwe nini iziphumo zakho zesifo lephepha?

_____
(DD-MM-YYYY)

Where in your body was the TB (e.g. lungs or other location)?

Waar in jou liggaam was die TB (bv longe of ander plek)?

Sasiphi esisifo sephephe emzimbheni wakho?

- ◯ Lungs
- ◯ Brain
- ◯ Unknown
- ◯ Other
(Place in body)

Did you receive treatment for TB?

Het jy TB behandeling ontvang?

Walufumana unyango sesifo sephepha?

◯ Yes
◯ No

Other than during this pregnancy has a doctor or nurse ever told you that you have TB?

Anders as hierdie swangerskap het 'n dokter of verpleegster ooit vir jou gesê dat jy TB het?

Ngaphambi kokuba ukhulelwe ubuke waxelelwa nguGqirha okanye uMongikazi ukuba unesifo esphepha?

◯ Yes
◯ No

When did you receive this TB diagnosis (not during this pregnancy)?

Wanneer het jy hierdie TB-diagnose ontvang (nie tydens hierdie swangerskap nie)?

Ingaba uxelelwe nini ukuba unesifo sephepha(ngaphambi ukhulelwe)?

_____
(DD-MM-YYYY)

Did you receive treatment for TB when last diagnosed?

Het jy behandeling vir TB ontvang toe jy laas gediagnoseer was?

Ingaba walufumana unyango lwesifo sephepha xa wagqibela ukufunyaniswa?

◯ Yes
◯ No

How many times in total have you been treated for TB?

Hoeveel keer in totaal is jy vir TB behandel?

Kukangaphi unyangwa isifo sephepha?

_____
(Number of times)

Where did you receive your TB treatment?

Waar het jy jou TB-behandeling ontvang?

Walufumana phi unyango lwesifo sephepha?

_____
(Name of clinic)

How long was your TB treatment the last time you were diagnosed?

Hoe lank was jou TB-behandeling die laaste keer toe jy gediagnoseer was?

Ibilixesha elingakanani unyango lwakho kwisifo sephepha emveni bekexelele unaso?

◯ 6 months
◯ 8 months
◯ 9 months
◯ Ongoing
◯ Other
◯ Don't know

Other, specify

Ander, spesifiseer

Ezinye, cacisa

_____

Hospitalization History

Have you been hospitalized during this pregnancy?

Was jy tydens hierdie swangerskap in die hospitaal opgeneem?

Ukhe walaliswa esibhedlele ngelixesha ukhulelweyo?

- ◯ Yes
- ◯ No
- ◯ Unknown

If only month and year known - put the date as 15; If only year known put day and month as 01(day) July(month)

When were you hospitalized?

Wanneer was jy gehospitaliseer?

Ubulaliswe nini esibhedlele?

_____

Is this the exact date or estimated date?

Is dit die presiese of beraamde datum?

Ingaba lomhla nguwo ngqo okanye ngowuqikelelayo?

- ◯ Exact
- ◯ Estimated

To which hospital were you admitted?

By watter hospitaal is jy opgeneem?

Ubulaliswe kwesiphi isibhedlele?

- ◯ Worcester Provincial Hospital
- ◯ Brewelskloof Hospital
- ◯ Tygerberg Hospital
- ◯ Mowbray Maternity Hospital
- ◯ Groote Schuur Hospital
- ◯ Other

If other, specify

Indien ander, spesifiseer

Ukuba esinye, cacisa

_____

What was the reason for your hospitalization?

Wat was die rede vir jou hospitalisasie?

Ibiyintoni isizathu sokulaliswa kwakho esibhedlele?

_____

Were you hospitalized for a complication due to your pregnancy or something not related to your pregnancy?

Was jy gehospitaliseer vir 'n komplikasie as gevolg van jou swangerskap of iets wat nie verband hou met jou swangerskap?

Ubulaliswe esibhedlele ngenxa yengxaki edibenisene nokukhulelwa okanye into engahambiselani nokukhulelwa?

- ◯ pregnancy-related
- ◯ not pregnancy-related
- ◯ Unknown

Pre- exposure Prophylaxis

Have you ever taken PrEP?
- ◯ Yes
- ◯ No
- ◯ Unknown

Have you taken PrEP during this pregnancy?
- ◯ Yes
- ◯ No
- ◯ Unkown

Are you currently taking PrEP?
- ◯ Yes
- ◯ No
- ◯ Unknown

COVID

Have you had a positive COVID test?

Het jy 'n positiewe COVID-toets gehad?

Uke wavavanywa for Covid wafumaniseka unayo?
- ◯ Yes
- ◯ No
- ◯ Don't know

Date of positive COVID test?

Datum van positiewe COVID-toets?

Umhla owafumaniseka une Covid?

_____

Is this the exact or estimated date?

Is dit die presiese of geskatte datum?

Ingaba lona ngowonamhla okanye ngumhla oqikelelweyo?
- ◯ Exact
- ◯ Estimated

Have you received the COVID vaccine?

Het jy die COVID-entstof ontvang?

Ulufumene ugonyo lwe Covid?
- ◯ Yes
- ◯ No
- ◯ Don't know

Did you receive the vaccine as part of a research study trial or through the standard national program?

Het jy die entstof ontvang as deel van 'n navorsing studie of deur die standaard nasionale program?

Ulifumene ugonyo lokukhusela njengenxalenye yesifundo sophando okanye ngenqubo yesizwe emiselweyo?
- ◯ COVID study trial
- ◯ Standard national program

Vaccine type?

Tipe entstof?

Uhlobo lokugonya?
- ◯ J&J
- ◯ Pfizer
- ◯ Other

Specify

Spesifiseer

Cacisa

_____

How many doses did you receive?

_____

Date COVID vaccine received?

Datum COVID entstof ontvang?

Umhla wokugonywa?

_____ (IF UNKNOWN 09-09-1999)

Date 2nd dose received?

Datum 2de dosis ontvang?

Umhla wedosi yesibini efunyenwe?

_____ (IF UNKNOWN 09-09-1999)

Date 3rd dose received?

Datum 3rde dosis ontvang?

_____ (IF UNKNOWN 09-09-1999)

Date 4th dose received?

Datum 4de dosis ontvang?

_____ (IF UNKNOWN 09-09-1999)

Date 5th dose received?

Datum 5de dosis ontvang?

_____ (IF UNKNOWN 09-09-1999)

Food Insecurity

In the past week, were there days that you did not have a meal?

In die afgelope week, was daar dae wat jy nie 'n maaltyd gehad het nie?

Kwiveki edlulileyo, bezikhona imini ubuke awabina kutya?

◯ Yes
◯ No

How many times in the past week have you gone without a meal?

In die afgelope week, hoeveel keer het jy sonder 'n maaltyd gegaan?

Zingaphi intsuku kwiveki edlulileyo uye awafumana kutya?

_____

Social And Household Information

Record ID

_____

Date of data abtraction

_____

Data Collector

_____

Visit code

- ◯ Antenatal
- ◯ Delivery
- ◯ 3 month
- ◯ 6 month
- ◯ 9 month
- ◯ 12 month
- ◯ 15 month
- ◯ 18 month
- ◯ 21 month
- ◯ 24 month
- ◯ 27 month
- ◯ 30 month
- ◯ 33 month
- ◯ 36 month

MATERNAL INFORMATION

1. What is your date of birth?

1. Wat is jou geboortedatum?

1. Uthini umhla wakho wokuzalwa?

_____
(DD-MM-YYYY)

2. What language do you speak at home?

2. Watter taal praat jy tuis?

2. Uthetha oluphi ulwimi ekhaya?

- ◯ isiXhosa
- ◯ isiZulu
- ◯ Afrikaans
- ◯ English
- ◯ Other

Specify other

Spesifiseer ander

Chaza olunye

_____

3. What was the highest grade you completed at school?

3. Wat was die hoogste graad wat jy op skool voltooi het?

3. Leliphi elona banga eliphezulu owaligqibayo eskolweni?

_____

Did you study anything further after matric?

Het jy ná matriek verder gestudeer?

Ikhona enye into oyifundileyo emveni kwematriki?

◯ Yes
◯ No
◯ Don't know

4. Are you currently working and/or studying?

4. Werk jy tans en/of studeer jy?

4. Ingaba uyaphangela okanye uyafunda?

◯ Yes
◯ No

5. If yes, which one of the following best describes what you do?

5. Indien ja, watter een van die volgende beskryf die beste wat jy doen?

5. Ukuba ngu ewe, ingaba ngeyiphi enye kwezi zilandelayo echaza oyenzayo?

◯ Employed full-time
◯ Employed part-time
◯ Informal job/hawker
◯ Attending school/learner
◯ Attending tertiary education (University/College)

(Choose only one)

6. Do you earn an income?

6. Verdien jy 'n inkomste?

6. Ingaba urhola umvuzo?

◯ Yes
◯ No

7. How much income do you earn per month?

7. Hoeveel verdien jy per maand?

7. Ingaba urhola kangakanani ngenyanga?

◯ Less than R1000 per month
◯ R1001 to R5000 per month
◯ R5001 to R10000 per month
◯ R10001 to R15000 per month
◯ More than R15000 per month

8. Do you currently receive any social assistance in the form of grants?

8. Ontvang jy tans enige maatskaplike hulp in die vorm van toelaes?

8. Ingaba okwangoku ufumana naluphi na uncedolwentlalo ngohlobo lweesibonelelo?

◯ Yes
◯ No

9. What type of government grant do you receive?

9. Watter tipe regeringstoelaag ontvang jy?

9. Luhlobo olunjani lwesibonelelo ka rhulumente olufumanayo?

☐ Children's grant
☐ Disability grant
☐ Care Dependency grant
☐ Other

Are you receiveing a children's grant for this child in this study?

Ontvang jy 'n kindertoelaag vir die kind in hierdie studie?

◯ Yes
◯ No
◯ Unknown

10. Number of Children's grant

10. Aantal kindertoelae

10. Inani lesibonelelo sabantwana

_____

10. Number of Disability grants

10. Aantal Gestremdheidstoelaes

10. Inani lesibonelelo saba khubazekileyo

_____

10. Number of Care Dependency grants

10. Aantal Sorg Afhanklikheid toelaes

10. Inani lesibonelelo saba xhomekekileyo

_____

10. Specify other grants

10. Spesifiseer ander toelaes

10. Cacisa ezinye izibonelelo

_____

10.Number of [shi_spec_other] grants

_____

HOUSEHOLD INFORMATION

11. What kind of home do you live in?

11. In watter soort huis woon jy?

11. Uhlala kwikhaya elinjani?

- ◯ Shack informal dwelling
- ◯ Wendy in yard
- ◯ Brick house
- ◯ Brick Flat in yard
- ◯ Flat/apartment in block
- ◯ Other

11. Other, specify

11. Ander, spesifiseer

11. Elinye, cacisa

_____

12. Does your house have the following:

12. Het jou huis die volgende:

12. Ingaba indlu yakho inazo ezizinto zilandelayo:

12a. A toilet inside

12a. 'n Toilet binne

12a. Indlu yangasese ngaphakathi

- ◯ Yes
- ◯ No
- ◯ Don't know

12b. A toilet outside - not shared with other households

12b. 'n Toilet buite - nie gedeel met ander huishoudings nie

12b. Indlu yangasese ngaphandle, engasetyenziswa lelinye ikhaya

- ◯ Yes
- ◯ No
- ◯ Don't know

12c. A toilet outside - shared with other households

12c. 'n Toilet buite - gedeel met ander huishoudings

12c. Indlu yangasese, esetyenziswa lelinye ikhaya

◯ Yes
◯ No
◯ Don't know

12d. Running water inside

12d. Lopende water binne

12d. Amanzi phakathi endlini

◯ Yes
◯ No
◯ Don't know

12e. Running water outside - not shared with other households

12e. Lopende water buite - nie met ander huishoudings gedeel nie

12e. Amanzi ngaphandle angasetyenziswa ngelinye ikhaya

◯ Yes
◯ No
◯ Don't know

12f. Running water outside - shared with other households

12f. Lopende water buite - gedeel met ander huishoudings

12f. Amanzi ngaphandle asetyenziswa lelinye ikhaya

◯ Yes
◯ No
◯ Don't know

12g. Electricity inside

12g. Elektrisiteit binne

12g. Umbane phakathi endlini

◯ Yes
◯ No
◯ Don't know

12h. A refrigerator

12h. 'n Yskas

12h. Ifriji

◯ Yes
◯ No
◯ Don't know

12i. A television

12i. 'n Televisie

12i. Umabonakude

◯ Yes
◯ No
◯ Don't know

12g. A vehicle

12g. 'n Voertuig

12g. Imoto

◯ Yes
◯ No
◯ Don't know

---

13. How many adults (>18 years) live at your house?

13. Hoeveel volwassenes (>18 jaar) woon by jou huis?

13. Bangaphi abantu abadala abahlala endlini yakho?

_____
(Including yourself if you are 18 and older)

14. How many children (< 18 years) live at your house?

14. Hoeveel kinders (< 18 jaar) woon by jou huis?

14. Bangaphi abantwana abahlala endlini yakho?

_____
(Including yourself if you are younger than 18)

Fathers Information

15. Does the father of this child with which you are pregnant live with you?

15. Leef die pa van hierdie kind waarmee jy swanger is, saam met julle?

15. Ingaba utata walomntwana umkhulelweyo uhlala naye?

◯ Yes
◯ No
◯ Don't know

16. Are you in a relationship with the father of the child?

16. Is jy in 'n verhouding met die pa van die kind?

16. Usenobudlelwane notata womntwana?

◯ Yes
◯ No
◯ Don't know

17. Does the father of this child support you financially?

17. Ondersteun die pa van hierdie kind jou finansieel?

17. Ingaba utata womntwana uyakuxhasa ngezemali?

◯ Yes
◯ No
◯ Don't know

18. Does the father of this child support you emotionally?

18. Ondersteun die pa van hierdie kind jou emosioneel?

18. Ingaba utata womntwana uyakuxhasa ngokwasemphefumlweni?

◯ Yes
◯ No
◯ Don't know

COMMENTS/NOTES

Please write notes and or any other comments here

_____

Appendix B – UCT HREC Approval 251/2024

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

E-52 – Room46, E-Floor, Old Main Building
Groote Schuur Hospital
Observatory 7925
Email: hrec-submissions@uct.ac.za
Website: https://health.uct.ac.za/home/human-research-ethics

18 April 2024

HREC REF: 251/2024

Dr E Kalk
Centre for Infectious Disease Epidemiology & Research
Public Health & Family Medicine-FHS
Email: emma.kalk@uct.ac.za
Student: DRJMAX001@myuct.ac.za

Dear Dr Kalk

PROJECT TITLE: MATERNAL SYPHILIS IN A RURAL AND URBAN COMMUNITY IN WESTERN CAPE, SOUTH AFRICA: A CROSS-SECTIONAL ANALYSIS-SUB-STUDY LINKED TO 723/2021-(MPHIL MATERNAL AND CHILD HEALTH-MAXINE JAYD DE ARAUJO)

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

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

Approval is only granted for one year until the 30 April 2025.

Please submit a progress report, using the standardised Annual Progress Report Forms (FHS016) **or** (FHS 017) if the study continues beyond the approval period. Please submit a Standard Closure form (FHS 010) when the study has been completed, this includes after publication or thesis submission and final completion.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Maxine Jayd de Araujo will also be involved in this study.

Please quote the HREC REF 251/2024 in all your correspondence.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

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

Yours sincerely

Signed by candidate

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix C – SU CHERISH HREC Approval N20/08/084

Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT

forward together
sonke siya phambili
saam vorentoe

Approval Letter
Progress Report

18/09/2023

Project ID: 17206

Ethics Reference No: N20/08/084

Project Title: CHERISH-1

Dear Mrs E Schoeman

We refer to your request for an extension/annual renewal of ethics approval dated 22/08/2023 11:46.

The Health Research Ethics Committee reviewed and approved the annual progress report through an expedited review process.

The approval of this project is extended for a further year.

Approval date: 29 September 2023

Expiry date: 28 September 2024

Kindly be reminded to submit progress reports two (2) months before expiry date.

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your Project Id 17206 and ethics reference number N20/08/084 on any documents or correspondence with the HREC concerning your research protocol.

Please note that for studies involving the use of questionnaires, the final copy should be uploaded on Infonetica.

Yours sincerely,

Ms Brightness Nxumalo
Coordinator: Health Research Ethics Committee 2 (HREC 2)

National Health Research Ethics Council (NHREC) Registration Number:
REC-130408-012 (HREC1)•REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)•IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by the World Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects; the South African Department of Health (2006).Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition); as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes and Structures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

Appendix D – UCT CHERISH HREC Approval 723/2021

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	28.02.2025
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee	Signed by candidate	Date Signed	10/2/2024

Note: Please email this form and supporting documents (if applicable) in a combined pdf file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

RESEARCH ETHICS COMMITTEE
- 8 FEB 2024
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	08 February 2024		
HREC REF Number	723/2021	Current Ethics Approval was granted until	28 February 2024
Protocol title	**Children HIV Exposed Uninfected - Research to Inform Survival and Health (CHERISH)- Dynamic Cohort**		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	X No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Mary-Ann Davies		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Department / Office Internal Mail Address	Centre for Infectious Disease Epidemiology & Research SPH Room 5.42 Falmouth Building UCT Faculty of Health Sciences, Anzio Road Observatory, 7925

1.1 Does this protocol receive US Federal funding?	☐ X Yes	☐ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ X No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	***Description***	***New fee (Vat Incl.)***	***tick ✓***
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	✓
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Contact person	
Telephone number	
Email Address	
2. **Internal Journal Billing:**	
Fund Number:	471794
Cost Centre Number:	PPH1324
Account Holder Name:	Mary-Ann Davies
Division of Account Holder:	CIDER

2. List of documentation for approval

Fhs016

3. Protocol status (tick ✓)

☐X	Open Enrolment
☐	Closed to enrolment (tick ✓)
☐X	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	556
Number of participants enrolled, since last HREC Progress report (continuing review)	310
Additional number of participants still required	194

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	**32**

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

6. Cumulative summary of participants

Total number of participants who provided consent	**556**
Number of participants determined to be ineligible (i.e. after screening)	29
Number of participants currently active on the study	529
Number of participants completed study (without events leading to withdrawal)	0
Number of participants withdrawn at participants' request (i.e. changed their mind)	9
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	14
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	6

1. One participant moved ou of the country and is no longer using the numbers she gave us. The next of kin confirmed that she went back to her country for good.
2. The other 5 participants gave wrong addresses, are no longer using their numbers and their next of kin's were not available for a period of a year after completing baseline visit.

Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	5

All 5 infants were infected with HIV by their moms and were excluded.

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
The research project involves enrolling two groups: pregnant women living with HIV and pregnant women without HIV. We are nearing completion of recruitment for the HIV-positive group, requiring only 4 more participants to reach our target of 250. Concurrently, recruitment for the HIV-negative group will continue until we reach 500 participants. No new issues have arisen since our previous report.

8. Protocol violations and exceptions (tick ✓ all that apply)

☐✓	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

9. Amendments (tick ✓ all that apply)

☐ ✓	No Prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
N/A

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ X Yes	☐ No	☐ Not applicable
If yes, please describe:		
All participants are screened for AUDIT, EPDS, Fagerstrom test and Self report questionnaire and whenever there is a need to refer a referral letter is completed. The participant always gets a referral letter on the same day of the visit. If they are suicidal the team ensures that they are seen on the same day but if the participant is not high risk and prefers to be seen on a different day arrangements are made between the counsellor and the participant. Infant with HIV infection referred for treatment and moms who default on taking their treatment are referred to the clinic.		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	☐ ✓ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☐ ✓ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ ✓ Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

	DSMB report attached	☐ Yes	☐ No	☐ ✓ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☐ ✓ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
☐✓	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ ✓ Yes	☐ No		☐ Not Applicable – N/A
If yes, please complete the following:			
Insurer's name:	UCT Sponsorship and Insurance for Clinical Research		
Policy no.		*Coverage Period:	**2023/07/01 – 2025/06/31**
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☐ ✓ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI	Signed by candidate	Date	08/02/2024

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix E – WC Health Research Committee Approval WC_2021_09_007

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 0866: fax: +27 21 483 6058
5th Floor, Norton Rose House,, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_202109_007
ENQUIRIES: Dr Sabela Petros

Francie van Zijl Drive
Tygerberg
7505
Cape Town
South Africa

For attention: Prof Amy Slogrove, Prof Mary-Ann Davies, Prof Andrew Boulle, Prof Mark Tomlinson, Prof Mark Cotton, Dr Moleen Dzikiti, Dr Emma Kalk, Dr Ushma Mehta

Re: CHERISH (Children Highly Exposed - Research to Inform Survival and Health)

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact **Dr Danie Theron (023 348 1304)** to assist you with any further enquiries in accessing the following sites:

DE DOORNS CLINIC
EMPILISWENI (WORCESTER) CLINIC
WORCESTER CDC

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted and the constraints caused by the Covid-19 epidemic above are respected and adhered to.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final feedback (**Annexure 9**) within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. In the event where the research project goes beyond the *estimated completion* date which was submitted, researchers are expected to complete and submit a progress report (**Annexure 8**) to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
4. The reference number above should be quoted in all future correspondence.

Yours sincerely

Signed by candidate

DR M MOODLEY
DIRECTOR: HEALTH INTELLIGENCE
DATE: 30/11/2021
CC

Appendix F – Informed Consent Form Gugulethu Site

TITLE OF THE RESEARCH PROJECT:	**Children HIV Exposed Uninfected - Research to Inform Survival and Health (CHERISH)- Dynamic Cohort**
REFERENCE NUMBER:	**723/2021**
ADDRESS:	**Professor Mary-Ann Davies** **Centre for Infectious Disease Epidemiology & Research; SPHFM Room 5.42 Falmouth Building** **UCT Faculty of Health Sciences, Anzio Road Observatory, 7925**
CONTACT NUMBER:	**021 406 6051**

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary,** and you are free to decline to participate. If you say no, this will not affect your health care. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at the University of Cape Town** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council Ethical Guidelines for Research.

What is this research study all about?

We want to find out why some babies might be more likely to grow and develop poorly or get sick and maybe die in the first 3 years of life.

- This study is being done at the Gugulethu Midwife Obstetric Unit (MOU). The same study is also being done at partaking health care facilities in the Breede Valley. We will be asking about 1800 women and their babies to participate, 900 in Gugulethu and 900 in Breede Valley.
- By participating, you will help us to learn why some women's babies are more likely to need to go into the hospital, more likely to grow poorly, more likely to not reach their developmental milestones or more likely to die in the first 3 years of life than other women's babies. You will help us also to learn if women who are ill have babies who are more likely to experience these adverse outcomes – needing to go into hospital, grow more slowly, not reach their developmental milestones or more likely to die.

Funding for the study:

- The study is funded externally by the United States NIH (National Institutes of Health) and long term follow up visits for the study are reliant on continuous funding.

Why have you been invited to participate?

- If you do not have HIV, you and your baby are being asked to participate in this study because we would like to learn how often and why babies of women without HIV experience these adverse outcomes in the first 3 years of life.
- If you do have HIV, you and your baby are being asked to participate in this study because your baby, even though he/she is HIV negative may have seen some of the HIV in your blood while you were pregnant, and we would like to learn how often and why babies who see HIV virus like this experience these adverse outcomes of needing to be hospitalized, growing slowly, developing slowly or dying in the first 3 years of life. You and your baby are also being asked to participate in this study because we would like to learn more about the positive and negative effects on your baby of the antiretroviral drugs you take to keep yourself healthy.

What will your responsibilities be?

- If you agree to participate in this study, we will do an interview with you while you are still pregnant to ask you questions about your pregnancy, your physical and mental health, your home circumstances and your habits such as smoking, alcohol and drug use.
- We will collect information about your health and your pregnancy from your maternity case record (pregnancy book) and from your hospital folder if you are admitted during your pregnancy or at any stage before your baby reaches 3 years of age.
- We will collect information about your baby from their hospital folder at the time of birth and if they are admitted to hospital at any stage before they reach 3 years of age.
- We will use electronic healthcare data about you and your baby that is routinely collected by the Western Cape Government Department of Health to see if you or your baby have been admitted to hospital, received any treatment or had any laboratory investigations done during your pregnancy and up until your baby reaches 3 years of age.
- We will look in the National Health Laboratory Database at the results of any tests you have had done during pregnancy or that you or your child have done if you or your baby are hospitalized. This will include any HIV tests, CD4 tests or HIV viral load tests on either you or your baby.
- We will keep in contact with you by telephone every 3 months until your baby turns 3 years old; that is when your baby is close to 3, 6, 9, 15, 18, 21, 27, 30, 33 months. During these telephone calls we will ask you about your mental and physical health and medications, about the baby's health and any medications received, about whether you are still breastfeeding and what foods the baby is eating, whether the baby has received immunizations recently, or whether the baby has been in the hospital recently. We will send a small amount of airtime to the cell phone number we have for you right before we plan to make the call, to ensure that you are able to receive the telephone call from the CHERISH team.
- To help us keep in contact with you we will need your address and a telephone number to reach you. It would also be helpful if you could provide us with the address and telephone number of somebody else close to you that we can contact if we are unable to reach you, for example if your telephone number has changed or if you have moved.
- You and your baby will need to make a visit to the Gugulethu MOU when your baby reaches 12 months (1 year), 24 months (2 years0 and 3 years of age. We will help you with the transport for this visit and will contact you beforehand to agree on the date and time of the visit and the transport arrangements to attend the visit.
- At this visit to the Gugulethu MOU in Cape Town when your baby is 12, 24 months and 3 years of age, we will examine your baby and measure his/her growth. We will check in detail your baby's developmental milestones during these visits. We will perform the Development Screening

Questionnaire (DSQ) at the 12 – and 24 Month visit. At the 3 years visit we will do a detailed neurodevelopmental assessment. We will ask you questions about your and your baby's health, what you have been feeding your baby and your home circumstances. We will record some information from the Road to Health Book.

- At the 12-, 24- and 36-month visits we will do a HIV rapid test on your baby. This will be done by obtaining 2-3 drops of blood via a heel prick. If the HIV rapid test result is uncertain (does not give a positive or negative result) or gives a positive result, we will follow up with an HIV PCR test at 12 and 24 months or a second HIV rapid test at 36 months. For the HIV PCR test we will obtain a small amount of blood (less than a teaspoon) from your child via venepuncture (drawing blood) and send it to the Laboratory (NHLS) to confirm the result of the HIV rapid test. We will provide initial post-test counselling and support to you and also refer your child to the health care facility you usually attend, for further management.
- We will measure your length and mid upper arm circumference as well as weigh you at the month 12-, 24- and 3-year visits.
- *Mothers living without HIV:*

We will do a HIV rapid test (via a finger prick) at the 12-, 24- and 36-month visits on you. If the HIV rapid test result is uncertain (does not give a positive or negative result) or gives a positive result, we will follow up with a second HIV rapid test to confirm the result. If the second HIV rapid test is also positive, we will provide initial post-test counselling and support to you and also refer you to your health care facility for further management.

Will you benefit from taking part in this research?

- As part of this study, you will have contact with the CHERISH study team every 3 months who will be available to answer your questions, provide guidance on any concerns you have about you and your child and assist you in seeking healthcare for you or your child if it is necessary.
- At 12, 24 months and 3 years of age your child will receive a full examination including of his/her growth and development. Should anything worrying be picked-up during these examinations the CHERISH study team will arrange for the necessary evaluations or referrals for more investigations or treatment if needed.
- We hope that the results of this study will help to improve the quality of the health care that is given to other babies like yours in the future.

Are there any risks involved in your taking part in this research?

- It is very unlikely that you will be harmed by participating in this study.
- It is possible that other participants in this study or other members of your community may find out that you and your baby are participating in this study. We will try very hard to keep your privacy and make sure that this does not happen. We will not share your information with anyone outside of the CHERISH team.

If you do not agree to take part, what alternatives do you have?

- If you choose not to participate in this study you and your baby will not be treated any differently by the staff of the hospital or clinic where you are delivering your baby, or by the staff of the clinic where you take your baby for her/his childhood health check-ups. In other words, you and your baby will not be harmed if you choose not to participate in this study.
- If you choose to join this study but then decide, for any reason, that you do not want to participate in the study anymore, you may stop participating (withdraw) at any time. You can stop participating by telling the study counsellor or nurse that you would like to withdraw. Withdrawing from the study will

not change the treatment that you and your baby receive from staff at the clinics or hospitals where you go for health care.

Who will be able to see your medical records?

- The information that we collect about you and your baby will be securely stored at Gugulethu MOU at the University of Cape Town. To keep your privacy, you and your baby will be given a study number so that none of the information that we collect about you, except for your contact information and this consent form, will have your names on it.
- Only the staff and researchers working on this study will be able to see the information about you and your baby. If results from this study are shown to other researchers or written about in scientific magazines your names will not be used.

What will happen in the unlikely event of some form of injury occurring as a direct result of your taking part in this research study?

Only a very small amount of blood will be obtained either via heel prick (child), finger prick (adult) or by venepuncture. Your child or you might experience a little bit of pain and discomfort when these procedures are performed. The collection of blood are low risk procedures. No other investigations are being performed as part of this study, and you will not be receiving any treatment as part of this study.

Will you be paid to take part in this study and are there any costs involved?

- You will not be paid to take part in this study.
- We will pay for the cost of your transport to and from the Gugulethu MOU for you and your baby's study visit and we will reimburse you for your time to come to the visit (about R250).
- You will also be offered a small amount of cell phone airtime (about R30) at the time of the telephone contact every month so that the study team is able to contact you.

Is there anything else that you should know or do?

- Once your baby is born, we will go through this consent process again and ask you to provide consent for continuation of yourself and your baby in the CHERISH study.
- This study is being conducted by Stellenbosch University together with the University of Cape Town and with support from Harvard University (in the United States of America). The information that is collected from you and your baby might be sent to the researchers at the University of Cape Town and Harvard University as well as Stellenbosch University for analysis.
- To better understand these child outcomes (hospitalization, growth, reaching their milestones and survival) in children born to women with and without HIV it could be beneficial if the information you have shared with us about you and your child could also be shared with other researchers across the world. If you agree for your and your child's information that we have collected during the CHERISH study to be shared with other researchers, this would be done only after all of your and your child's identifying information has been removed and only through secure public data repositories approved by the University of Cape Town Health Research Ethics Committee. Use of this data for any analyses would need to first be approved by a Health Research Ethics Committee, like the Committee at Stellenbosch University that has approved this CHERISH study. You can still participate fully in the CHERISH student even if you do not wish to share your and your child's data in a secure public data repository.

- At the visit when your baby reaches 3 years of age, we might ask you whether you are willing to continue on the CHERISH study for longer. The information about any continuation of CHERISH and the reasons for this will be discussed with you and you will have the opportunity to **voluntarily** consent to the participation of you and your child in longer follow-up. You will also be quite free to choose not to continue to participate in longer follow-up and you and your child will not be treated any differently should you choose not to continue.
- You can contact Prof Mary-Ann Davies at 021 406 6051 if you have any further queries or encounter any problems.
- You can contact the Chair of UCT Health Research Ethics Committee, Prof Marc Blockman at 021 406 6338 if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.
- You will be given a copy of this information and consent form to keep for yourself.

Declaration by participant

By signing below, I ……. agree to take part in the research study entitled *"CHERISH-1 Dynamic Cohort"*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions, and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

☐ I **DO NOT** want my or my child's data to be shared in a secure public data repository.

☐ I **DO NOT** want to be contacted to participate in future research related to the CHERISH study after my child turns 3 years of age

Signed at (*place*) .....…..…… on (*date and time*) ……...……...

....
Signature of participant **Signature of witness** (if needed)

Declaration by investigator/Recruiter

I *(name)* ……...…… declare that:

- I explained the information in this document to …….
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..... on (*date and time*) ……...……

Signature of investigator/Recruiter.....

Declaration by interpreter

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) ……...……... using the language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) .....….. on (*date and time*) ……...……

.....
Signature of interpreter

Reconsent by participant at time of birth of baby for ongoing participation of mother and baby

Declaration by participant

By signing below, I ……..……. agree to take part in the research study entitled *"CHERISH-1 Dynamic Cohort" and provide consent for my baby, ……..[name] to be included in this study.*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions, and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

☐ I **DO NOT** want my or my child's data to be shared in a secure public data repository.

☐ I **DO NOT** want to be contacted to participate in future research related to the CHERISH study after my child turns 3 years of age

Signed at (*place*) .....…..…… on (*date and time*) ……...…….

....
Signature of participant **Signature of witness** (if needed)

Declaration by investigator/Recruiter

I *(name)* ……...…… declare that:

- I explained the information in this document to ……...
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..... on (*date and time*) ……...…….

Signature of investigator/Recruiter.....

Declaration by interpreter

I *(name)* ……...…… declare that:

- I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) ……...……. using the language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....….. on (*date and time*) ……...……

.....
Signature of interpreter

Appendix G – Informed Consent Form Breede Valley Site

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

CHERISH Dynamic Cohort

REFERENCE NUMBER: N20/08/084

PRINCIPAL INVESTIGATOR: Dr. Amy Slogrove

ADDRESS: Worcester Campus of Stellenbosch University, 1 Durban St, Worcester, 6850

CONTACT NUMBER: 023 346 7817 / 079 8933269

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect your health care. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council Ethical Guidelines for Research.

What is this research study all about?

We want to find out why some babies might be more likely to grow and develop poorly or get sick and maybe die in the first 3 years of life.

- This study is being done at partaking health care facilities in the Breede Valley. The same study is also being done in Cape Town at Gugulethu Community Health Centre. We will be asking about 1800 women and their babies to participate, 900 in the Breede Valley and 900 in Gugulethu.
- By participating, you will help us to learn why some women's babies are more likely to need to go into the hospital, more likely to grow poorly, more likely to not reach their developmental milestones or more likely to die in the first 3 years of life than other women's babies. You will help us also to learn if women who are ill have babies who are more likely to experience these adverse outcomes – needing to go into hospital, grow more slowly, not reach their developmental milestones or more likely to die.

Funding for the study:

- The study is funded externally by the United States NIH (National Institutes of Health) and long term follow up visits for the study are reliant on continuous funding.

Why have you been invited to participate?

- If you do not have HIV, you and your baby are being asked to participate in this study because we would like to learn how often and why babies of women without HIV experience these adverse outcomes in the first 3 years of life.
- If you do have HIV, you and your baby are being asked to participate in this study because your baby, even though he/she is HIV negative may have seen some of the HIV in your blood while you were pregnant, and we would like to learn how often and why babies who see HIV virus like this experience these adverse outcomes of needing to be hospitalized, growing slowly, developing slowly or dying in the first 3 years of life. You and your baby are also being asked to participate in this study because we would like to learn more about the positive and negative effects on your baby of the antiretroviral drugs you take to keep yourself healthy.

What will your responsibilities be?

- If you agree to participate in this study, we will do an interview with you while you are still pregnant to ask you questions about your pregnancy, your physical and mental health, your home circumstances and your habits such as smoking, alcohol and drug use.
- We will collect information about your health and your pregnancy from your maternity case record (pregnancy book) and from your hospital folder if you are admitted during your pregnancy or at any stage before your baby reaches 3 years of age.
- We will collect information about your baby from their hospital folder at the time of birth and if they are admitted to hospital at any stage before they reach 3 years of age.
- We will use electronic healthcare data about you and your baby that is routinely collected by the Western Cape Government Department of Health to see if you or your baby have been admitted to hospital, received any treatment or had any laboratory investigations done during your pregnancy and up until your baby reaches 3 years of age.
- We will look in the National Health Laboratory Database at the results of any tests you have had done during pregnancy or that you or your child have done if you or your baby are hospitalized. This will include any HIV tests, CD4 tests or HIV viral load tests on either you or your baby.
- We will keep in contact with you by telephone every 3 months until your baby turns 3 years old; that is when your baby is close to 3-,6,-,9-,15-,18-,21-,27-,30-, 33 months. During these telephone calls we will ask you about your mental and physical health and medications, about the babies health, medications received by your baby and your experience of giving these medications to your baby, about whether you are still breastfeeding and what foods the baby is eating,

whether the baby has received immunizations recently, or whether the baby has been in the hospital recently. We will send a small amount of airtime to the cell phone number we have for you right before we plan to make the call, to ensure that you are able to receive the telephone call from the CHERISH team.

- To help us keep in contact with you we will need your address and a telephone number to reach you. It would also be helpful if you can provide us with the address and telephone number of somebody else close to you that we can contact if we are unable to reach you, for example if your telephone number has changed or if you have moved.
- You and your baby will need to make a visit to the Research Site at Worcester Campus of Stellenbosch University, or at the Gugulethu site in Cape Town, when your baby reaches 12 months (1 year), 24 months (2 years) and 3 years of age. We will help you with the transport for this visit and will contact you beforehand to agree on the date and time of the visit and the transport arrangements to attend the visit. The in-person visits to the study site will take approximately 3 hours.
- At these visits to the Research Site at Worcester Campus of Stellenbosch University, when your baby is 12 –, 24 months and 3 years of age, we will examine your baby and measure his/her growth. We will also check in detail your baby's developmental milestones during these visits. We will perform the Development Screening Questionnaire (DSQ) at the 12 – and 24 Month visit. At the 3 years visit we will do a detailed neurodevelopmental assessment. We will also ask you questions about your and your baby's health, what you have been feeding your baby and your home circumstances. At the 12-month visit we will also ask you about support received from family and the community in taking care of the baby, your experience with caregiving and whether it poses any burden on you and about your experience with the health care services received for your child. We will record some information from the Road to Health Book.
- At the 12-, 24- and 36-month visits we will do a HIV rapid test on your baby. This will be done by obtaining 2-3 drops of blood via a heel prick. If the HIV rapid test result is uncertain (does not give a positive or negative result) or gives a positive result, we will follow up with an HIV PCR test at 12 and 24 months or a second HIV rapid test at 36 months. For the HIV PCR test we will obtain a small amount of blood (less than a teaspoon) from your child via venepuncture (drawing blood) and send it to the Laboratory (NHLS) to confirm the result of the HIV rapid test. We will provide initial post-test counselling and support to you and also refer your child to the health care facility you usually attend, for further management.
- We will measure your length and mid upper arm circumference as well as weigh you at the month 12-, 24- and 3-year visits.
- *Mothers living without HIV:*

We will do a HIV rapid test (via a finger prick) at the 12-, 24- and 36-month visits on you. If the HIV rapid test result is uncertain (does not give a positive or negative result) or gives a positive result, we will follow up with a second HIV rapid test to confirm the result. If the second HIV rapid test is also positive, we will provide initial post-test counselling and support to you and also refer you to your health care facility for further management.

Will you benefit from taking part in this research?

- As part of this study, you will have contact with the CHERISH study team every 3 months who will be available to answer your questions, provide guidance on any concerns you have about you and your child and assist you in seeking healthcare for you or your child if it is necessary.
- At 12-, 24 months and 3 years of age your child will receive a full examination including of his/her growth and development. Should anything worrying be picked-up during these examinations the CHERISH study team will arrange for the necessary evaluations or referrals for more investigations or treatment if needed.
- We hope that the results of this study will help to improve the quality of the health care that is given to other babies like yours in the future.

Are there any risks involved in your taking part in this research?

- It is very unlikely that you will be harmed by participating in this study.
- It is possible that other participants in this study or other members of your community may find out that you and your baby are participating in this study. We will try very hard to keep your privacy and make sure that this does not happen. We will not share your information with anyone else.

If you do not agree to take part, what alternatives do you have?

- If you choose not to participate in this study you and your baby will not be treated any differently by the staff of the hospital or clinic where you are delivering your baby, or by the staff of the clinic where you take your baby for her/his childhood health check-ups. In other words, you and your baby will not be harmed if you choose not to participate in this study.
- If you choose to join this study but then decide, for any reason, that you do not want to participate in the study anymore, you may stop participating (withdraw) at any time. You can stop participating by telling the study counsellor or nurse that you would like to withdraw. Withdrawing from the study will not change the treatment that you and your baby receive from staff at the clinics or hospitals where you go for health care.

Who will be able to see your medical records?

- The information that we collect about you and your baby will be securely stored at the Worcester Campus of Stellenbosch University. To keep your privacy, you and your baby will be given a study number so that none of the information that we collect about you, except for your contact information and this consent form, will have your names on it.
- Only the staff and researchers working on this study will be able to see the information about you and your baby. If results from this study are shown to other researchers or written about in scientific magazines your names will not be used.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

- Only a very small amount of blood will be obtained either via heel prick (child), finger prick (adult) or by venepuncture. Your child or you might experience a little

bit of pain and discomfort when these procedures are performed. The collection of blood are low risk procedures. No other investigations are being performed as part of this study and you will not be receiving any treatment as part of this study.

Will you be paid to take part in this study and are there any costs involved?

- You will not be paid to take part in this study.
- We will pay for the cost of your transport to and from the Research Site at the Worcester Campus of Stellenbosch University, for you and your baby's study visits and we will reimburse you for your time to come to the visits. (about R300).
- You will also be offered a small amount of cell phone airtime (about R30.00) at the time of the telephone contact every 3 months so that the study team is able to contact you.

Is there anything else that you should know or do?

- This study is being conducted by Stellenbosch University together with the University of Cape Town and with support from Harvard University (in the United States of America). The information that is collected from you and your baby might be sent to the researchers at the University of Cape Town and Harvard University as well as Stellenbosch University for analysis.

- To better understand these child outcomes (hospitalization, growth, reaching their milestones and survival) in children born to women with and without HIV it could be beneficial if the information you have shared with us about you and your child could also be shared with other researchers across the world. If you agree for your and your child's information that we have collected during the CHERISH study to be shared with other researchers, this would be done only after all of your and your child's identifying information has been removed and only through secure public data repositories such as the DASH repository of the United States National Institute of Child Health and Human Development. Use of this data for any analyses would need to first be approved by a Health Research Ethics Committed, like the Committee at Stellenbosch University that has approved this CHERISH study. You can still participate fully in the CHERISH student even if you do not wish to share your and your child's data in a secure public data repository.
- At the visit when your baby reaches 3 years of age we might ask you whether you are willing to continue on the CHERISH study for longer. The information about any continuation of CHERISH and the reasons for this will be discussed with you and you will have the opportunity to **voluntarily** consent to the participation of you and your child in longer follow-up. You will also be quite free choose not to continue to participate in longer follow-up and you and your child will not be treated any differently should you choose not to continue.
- You can contact Dr. Amy Slogrove at 023 346 7817 if you have any further queries or encounter any problems.
- You can contact the Health Research Ethics Committee at 021 938 9207 if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.

- You will be given a copy of this information and consent form to keep for yourself.
- Once your baby is born we will go through this consent process again and ask you to provide consent for continuation of yourself and your baby in the CHERISH study.

Declaration by participant

By signing below, I …………………………………..………… agree to take part in the research study entitled *“CHERISH”*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

☐ I **DO NOT** want my or my child’s data to be shared in a secure public data repository.

☐ I **DO NOT** want to be contacted to participate in future research related to the CHERISH study after my child turns 3 years of age

Signed at (*place*) ....….......……… on (*date and time*) ………...……..

....

Signature of participant **Signature of witness** (if needed)

Declaration by investigator/Recruiter

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..... on (*date and time*) ……...……..

Signature of investigator/Recruiter.....

Reconsent by participant at time of birth of baby for ongoing participation of mother and baby

Declaration by participant

By signing below, I ……..……. agree to take part in the research study entitled *"CHERISH" and provide consent for my baby, ……...[name] to be included in this study.*

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

☐ I **DO NOT** want my or my child's data to be shared in a secure public data repository.

☐ I **DO NOT** want to be contacted to participate in future research related to the CHERISH study after my child turns 3 years of age

Signed at (*place*) .....…..……. on (*date and time*) ……...……..

.....	.....
Signature of participant	**Signature of witness** (if needed)

Declaration by investigator/Recruiter

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) .....…..... on (*date and time*) ……...……..

Signature of investigator/Recruiter.....