Factors affecting viral load suppression amongst pregnant women on second line ART in South Africa 2022.

Mr. Sbonelo Charles Chamane

Student no: 2726849

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of MSc Epidemiology (Epidemiology and Biostatistics)

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DECLARATION

I, Sbonelo Charles Chamane, declare that this research report is my own work. It is being submitted in partial fulfilment of the requirements for the degree Master of Epidemiology (Epidemiology and Biostatistics) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Sbonelo Charles Chamane

Student number: 2726849

Medical HREC CLEARANCE CERTIFICATE: NO. M240423

On the 17th day of June 2025

DEDICATION

This work is dedicated to my family, whose unwavering support, encouragement, and belief in me have been the foundation of my academic journey. To my mentors, I extend my deepest gratitude for their insightful guidance and inspiration.

Lastly, I dedicate this work to the communities affected by the issues explored in this report, with the hope that the findings will contribute to a deeper understanding and meaningful improvements in their lives.

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I would like to express my deepest gratitude to all those who have supported me throughout this research journey.

First and foremost, I would like to thank my family (Ntombikhona Chamane, Sindisiwe Chamane & Nkululeko Chamane) for their endless love, encouragement, and belief in me. Their support has been invaluable in helping me overcome challenges and stay focused on my goals.

I am deeply grateful to Dr. Tendesayi Kufa-Chakezha my supervisor, whose guidance and expertise have shaped the direction of this research. Dr Kufa-Chakezha's thoughtful feedback, constructive criticism, and encouragement have been pivotal to the success of this study.

I am deeply grateful to the Tiso Foundation for their generous funding of my master's studies. Their support has been crucial in enabling me to pursue this academic journey, and I truly appreciate their commitment to advancing education.

I would also like to sincerely acknowledge the National Institute for Communicable Diseases (NICD) for generously providing the secondary data used in this research. Their support has been instrumental in making this work possible.

ABSTRACT

Introduction: This study was a secondary analysis of data collected by The National Institute for Communicable Diseases (NICD) during the 2022 antenatal surveillance. It described the characteristics and distribution of pregnant women who were on second-line regimen, determine viral suppression levels among women on second-line antiretroviral therapy (ART), and factors associated with viral suppression in pregnant women.

Methods: The study included pregnant women aged 15-49 attending antenatal care who consented to the survey and had viral load and ART status data available. Analysis was done using STATA Standard Edition version 18.0. Descriptive statistics were employed to determine the distribution of sample characteristics using proportions and percentages. Included and excluded participants were compared and the Pearson's chi-square test was used to determine any statistical differences between the two groups. Univariable analysis was employed which was inclusive of all the variables and stratified by viral suppression, exploring two viral load thresholds (Suspected virologic failure (≥1000 copies/ml) and virally unsuppressed (≥50 copies/ml)). In the same univariable table, univariable logistic regression is presented with odds ratios (OR), 95% CI (Confidence Interval), and p-value (probability value). For the multivariable analysis ART regimen type was used as the primary exposure and viral suppression as the outcome variable. Six models were constructed. Model I and Model II were fitted using the DAGs minimal sufficient set of exposure variables and all variables considered as confounders were included in the models. Model I used VL ≥1000 copies/ml as the threshold for unsuppressed VL while Model II used VL ≥50 copies/ml as the threshold for unsuppressed VL. Model III and Model IV were multivariable logistic regression models fitted on factors that were significant at univariable logistic regression, with Model III using ≥1000 copies/ml and Model IV using ≥50 copies/ml thresholds. In both models III and IV, a category coded as missing for variables that had more than 5% of missing observations was included. Model V and Model VI were multivariable logistic regression models built on factors that were significant at univariable logistic regression, with Model V focussed on the a Suspected virologic failure (≥1000 copies/ml) and Model VI using the virally unsuppressed (≥50 copies/ml), and both these models use full case analysis of the logistic regression. Model VII and Model VIII were constructed as backwards stepwise logistic regression models stratified by ART regimen type (first-line and second-line, respectively), and focused on factors associated with suspected virologic failure (≥1000 copies/ml). All the analyses conducted in this study included survey weights and we used α =0.05.

Results: The study assessed 42 875 participants for eligibility for enrolment. Of those, 36 677 were eligible for enrolment with and final analysis included 8 606 participants aged 15-49 who had complete data on ART regimen and viral load. The majority of participants included in the secondary analysis were virally suppressed, on first-line ART, aged 25 to 34 years, of African race, attending follow-up visit. Moreover, most were in their second pregnancy, had one living baby, were in their third trimester not living together but in a relationship with the baby's father, partner was HIV positive, had one sexual partner, had negative syphilis test results, and started ART before pregnancy. The rate of virally unsuppressed pregnant women was 32.5% among those on first line and 45.0% among those on second-line regimen using the ≥50 copies/ml threshold. When comparing all the models fitted, the factors that were significant through all fitted models were regimen type, age groups, first Antenatal visit type, and HIV status of current sexual partner. However, some factors, such as gestational age group, were significant from Model I to Model IV, while the number of live births was significant in all these models except for Model III. Syphilis test results were only significantly associated with viral load status in Model IV.

Conclusion: The findings reveal that those on second-line ART, younger women, and those unaware of their partner's HIV status face higher odds of being virally unsuppressed. Additionally, women with multiple live births and those initiating ART during pregnancy also show increased odds of being virally suppressed. These results emphasise the need for a targeted intervention that strengthens adherence counselling and sexual reproductive health education for pregnant women on ART to improve viral suppression outcomes, particularly among youth aged 15 to 24 The study's contributions highlight the complex interplay of demographic, clinical, and social factors in achieving viral suppression, underscoring the importance of a multifaceted approach to HIV management in pregnant women.

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ABBREVIATIONS

ANC	Antenatal Clinic
ART	Antiretroviral therapy
DAG	Directed Acyclic Graphs
DTG	Dolutegravir
HIV	Human Immunodeficiency Virus.
InSTI	Integrase strand transfer inhibitors
NHC	National Health Council
NICD	Institute for Communicable Diseases
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NSP	National Strategic Plan
NVP	Nevirapine
OMR	Optical Mark Recognition
PLHIV	People living with HIV.
PrEP	Pre-exposure prophylaxis.
StatsSA	Statistics South Africa
TEE	Tenofovir disoproxil fumarate-Emtricitabine-Efavirenz
TLD	Tenofovir disoproxil fumarate-Lamivudine-Dolutegravir
UNAIDS	Joint United Nations Programme on HIV/Aid
VL	Viral Load
VLS	Viral load suppression

VT	Vertical Transmission
VTP	Vertical Transmission Programme
WHO	World Health Organization

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GLOSSARY

Antiretroviral Therapy (ART)
A combination of drugs used to treat HIV infection by stopping the virus from replicating in the body. ART helps reduce the viral load to undetectable levels and improves immune function.

Cross-sectional Study
An observational study that analyses data from a population at a specific point in time.

Dolutegravir (DTG)
A type of antiretroviral drug that belongs to the integrase strand transfer inhibitors (InSTIs) class, recommended as a preferred first-line treatment for HIV due to its high barrier to resistance.

HIV (Human Immunodeficiency Virus)
A virus that attacks the body's immune system and, if not treated, can lead to AIDS (Acquired Immunodeficiency Syndrome).

Integrase Strand Transfer Inhibitors (InSTIs)
A class of antiretroviral drugs that block the integrase enzyme HIV needs to insert its viral DNA into the host cell's DNA.

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
A class of antiretroviral drugs that inhibit reverse transcriptase, an enzyme crucial for HIV replication.

People Living with HIV (PLHIV)
Individuals who have been diagnosed with HIV, regardless of whether they are on treatment or not.

Pre-exposure Prophylaxis (PrEP)
A preventive treatment for HIV-negative individuals who are at high risk of acquiring HIV, involving the daily use of antiretroviral medications.

Tenofovir Disoproxil Fumarate-Emtricitabine-Efavirenz (TEE)
A fixed-dose combination of three antiretroviral drugs commonly used in first-line HIV treatment regimens.

Tenofovir-Lamivudine-Dolutegravir (TLD)
A fixed-dose combination antiretroviral regimen recommended as the preferred first-line treatment in many countries due to its efficacy, safety, and low resistance profile.

Viral Load (VL)
The amount of HIV RNA in a blood sample. It is used to monitor the effectiveness of ART. A lower viral load indicates better treatment outcomes.

Viral Load Suppression (VLS)
Achieving a viral load below the level of detection (usually less than 50 copies/ml), indicating effective ART and reduced risk of HIV transmission.

Vertical Transmission (VT)
The transmission of HIV from a mother to her child during pregnancy, childbirth, or breastfeeding.

Vertical Transmission Programme (VTP)
National or institutional strategies aimed at preventing mother-to-child transmission of HIV.

1 Chapter 1: INTRODUCTION

1.1 Background

According to Statistics South Africa (StatsSA), 932,138 births were registered with the Department of Home Affairs in 2023, of which 848,337 (91.0%) were for births that took place that year (1). According to the 2022 antenatal survey, 27.5% of pregnant women at the national level were living with HIV, reflecting a 2.5% decline since 2019 (2).

Pregnant women with HIV are at risk of transmitting HIV to their children, this is known as Vertical transmission (VT) (3). A systematic review focused on VT identified that in the absence of treatment, risk of transmission ranges between 15% to 30% during the gestational period or labour, while prolonged breastfeeding increased the transmission risk by 10% to 20%. In the absence of HIV treatment, transmission during pregnancy accounted for 60% of cases while 40% of the transmissions occurred during breastfeeding (4). The risk of transmission has been estimated to be 1% or less in women who are treated for HIV while those not treated for HIV range between 20 – 40% (3,5)

An antenatal care HIV prevalence survey is a crucial public health surveillance system aimed at assessing the prevalence of HIV and other health-related factors among pregnant women attending antenatal clinics in South Africa. During the data collection phase for the Antenatal Care HIV prevalence survey, from February to April of 2022, clinical practitioners used the 2019 ART Clinical Guidelines. Annexure 6.6 displays the timeline of ART clinical guidelines comparing 2019 to 2023. According to the 2019 Guidelines, a combination therapy of tenofovir disoproxil fumarate-emtricitabine-efavirenz (TEE) or tenofovir disoproxil fumarate-lamivudine-dolutegravir (TLD) was used as the preferred first-line regimen for pregnant and breastfeeding women. A resistance test for those failing on first line is required for individuals with HIV to be switched to second-line ART. Adults on a second-line regimen received a combination therapy of Zidovudine (AZT)/ Tenofovir (TDF) + Lamivudine (3TC)/ Emtricitabine (FTC) + Lopinavir/Ritonavir (LPV/r) or Atazanavir/ritonavir (ATV/r) or Dolutegravir (DTG) (6).

According to the 2023 ART Clinical Guidelines, the National Department of Health (NDoH) had included DTG-based regimens as first, second, and third-line regimens which were adopted from the World Health Organization (WHO) recommendations. New terminology for first line has been replaced by TLD 1, which is now given to patients on a DTG-containing regimen with no previous failure on any other regimen. TLD 2 is now the new terminology for second-line regimen and will refer to patients who have failed on an earlier regimen containing DTG. TLD remains the preferred first-line regimen, however, it will also be used as a second and third-line ART regimen in all adults including pregnant and breastfeeding women. Switching of patients to TLD is no longer viral load dependent. Investigations of neural tube defects caused by DTG have shown no increased risk associated with taking a DTG-containing regimen (6).

A patient is deemed as having virological failure if presenting two or more times at the clinical facility with a viral load of ≥ 1000 copies/ml after having taken a DTG/Protease inhibitor-containing regimen for two or more years with adherence greater than 80% (6). A resistance test for these patients is required for them to be switched to second-line ART (6,7).

When on ART, viral suppression is the main goal. An individual with a viral load of <50 copies/ml is considered virally suppressed (8); low-level viremia refers to those with a viral load between 50 to 999 copies/ml (10), whereas an individual with a viral load of ≥ 1000 copies/ml is unsuppressed (8). ART is not only essential for preventing VT and horizontal transmission to sexual partners, but also plays a critical role in protecting the health and well-being of pregnant women living with HIV (9).

Viral load (VL) test results act as a guide to clinical action and are a useful indicator of adherence to ART (10). Pregnant women who are newly initiated on ART and those reinitiated on a DTG-containing regimen and who have been pregnant for <28 weeks, have their viral load monitored 3 months after being initiated on ART. Second monitoring is done during delivery provided that the pregnant woman's first VL was <50 copies/ml. For those diagnosed with HIV before pregnancy, viral load monitoring is done on first antenatal clinic (ANC) visit, if the VL is <50 copies/ml monitoring will be done during delivery. Women who present themselves after 28 weeks or during delivery will get their first viral load test done during delivery and 10-12 weeks post-delivery. VL monitoring will further be done for all women on the 6th month postpartum and every 6 months thereafter for those who choose to breastfeed (11).

Pregnant women who present to the ANC with an elevated viral load are subdivided into 2 groups. Those who have a VL 50 – 999 copies/ml have a VL test done every 8 – 10 weeks until it is < 50 copies/ml. For those who have a VL of ≥ 1000 copies/ml, their VL is repeated every

4 – 6 weeks. If the viral load drops by > 1 log (more than 90%) their viral load is repeated every 8 – 10 weeks. However, if their VL remains ≥ 1000 copies/ml after the 4 – 6 weeks period, considerations are made on switching the patient to second-line regimen (11). Delaying this clinical action places the patient at risk of opportunistic diseases and death (7,12).

This study aimed to describe the characteristics and the distribution of pregnant women who are on a second-line regimen and determine the factors associated with viral suppression in pregnant women on a second-line ART regimen compared to those on a first-line ART regimen.

1.2 Literature Review

With the ongoing means to eliminate HIV as a public health threat in South Africa by 2030 (13). The Vertical Transmission Programme (VTP) is a useful intervention that protects infants from acquiring HIV (14) and thus forestalls the spread of HIV (15). An unstable VTP introduces a potential for an increased transmission risk, this may include late diagnosis, delayed initiation to ART, and lack of effective prophylaxis for infants that are HIV exposed (16).

In 2018, a study focusing on sub-optimal adherence and elevated HIV load in pregnant women found that pregnant women had a low understanding of VTP but had a high level of understanding of the need for ART (17). Despite this 80% of women reported having missed at least one dose of ART on 30 days follow-up (17). Sub-optimal adherence, and unsuppressed viral load (≥1000 copies/mL) had been observed in pregnant women who had voiced out their concerns about taking ART during the perinatal period and unintended pregnancy (17).

A viral load monitoring analysis based on the 2019 South African National Antenatal Sentinel HIV Survey found that out of 37,116 pregnant women tested, 11,518 (31.03%) were HIV-positive based on laboratory testing, and among those who tested positive, 11,049 (95.9%) were already aware of their HIV status. The study estimated viral load monitoring to be 81.7% in pregnant women compared to previous studies which reported a 30% and 72% coverage of viral load monitoring. Pregnant women initiated on ART before pregnancy had higher viral suppression compared to those initiated on ART during pregnancy. Young women aged 15-24 years and primigravida were associated with not receiving viral load monitoring. The absence of training, insufficient information among health workers, and deviation from the guidelines were distinguished as the main reasons behind missed opportunities to perform a viral load test (18).

In sub-Saharan Africa, viral load suppression among pregnant women living with HIV remains suboptimal. Evidence indicates that only approximately 63.8% of pregnant and breastfeeding women achieve viral suppression, defined as having a viral load below 1,000 copies/mL (19). A systematic review titled "The viral load monitoring cascade in HIV treatment programmes in sub-Saharan Africa" synthesised findings from 35 studies across 14 African countries, revealing substantial variation in viral load monitoring coverage which ranged from 24.3% to 99.7%, with an average of 63.8% (20). This wide variability is largely attributed to systemic gaps in access to viral load testing, inconsistent implementation of treatment guidelines, and disparities in health infrastructure across and within countries (21,22).

Some of the factors associated with the high prevalence of unsuppressed viral load among pregnant women were linked to dependence for monetary gains from spouse and married or cohabitating (23). Lack of disclosure of HIV status may act as a potential barrier to attaining viral suppression and accounts for 11.7% in pregnant women, while 35% of the women do not know their partner's HIV status (23). These barriers open a window for possible reinfection and an unsuppressed viral load. High levels of viral load during ART initiation, poor adherence to treatment, and delayed initiation have also been identified as factors associated with an unsuppressed viral load (24).

Before a patient is switched to second-line regimen in South Africa, a thorough assessment for the cause of an unsuppressed viral load is carried out where a clinician checks for adherence problems, intercurrent infections, incorrect ART dosage, drug interactions, and drug resistance (6,7). However, these factors may be overlooked considering the lack of knowledge among healthcare workers (24,25) and deviation from clinical guidelines (24). A study conducted in South Africa exploring barriers to timely switching to second-line ART further identified resistance to switching to a new regimen and suboptimal facility management as barriers to providing essential care to individuals on ART (25).

Individuals on second-line ART have been linked to an elevated risk of being virally unsuppressed (26). However, a study focusing on second-line antiretroviral therapy and its long-term outcomes in South Africans observed an improvement in adherence levels after patients were switched to a second-line regimen (27). Women with WHO stage IV disease or a history of weight loss are at risk of failing second line treatment, even with a VL of <1000 copies/ml before the switch (28).

1.3 Research Question

Are pregnant women on second-line regimen achieving viral suppression and what are the factors that contribute to viral suppression for this group?

1.4 Problem Statement

VTP focuses on halting the spread of HIV by minimising the risk of child infection and lowering the incidence of HIV in infants, while also prioritising the health and well-being of pregnant women living with HIV by preventing disease progression, maintaining immune function, and supporting overall maternal health during pregnancy. Investigating factors associated with viral suppression is important in understanding the HIV epidemic and strengthening the interventions targeted at eliminating HIV in South Africa. Individuals on second line are those who have not been able to achieve viral load suppression whilst on first-line regimen, hence research focusing on viral suppression for second-line regimen patients is imperative for achieving UNAIDS targets.

1.5 Justification

South Africa is one of the countries severely affected by the HIV epidemic, given the high number of people living with HIV (PLHIV). A woman’s ability to achieve viral load suppression during pregnancy limits the risk of paediatric infection and thus contributes to the overall control and prevention of HIV. Understanding factors associated with viral load suppression in pregnant women will help strengthen and guide public health interventions toward the elimination of HIV as a public health threat in South Africa. Virological failure while on first-line ART necessitates the employment of a second-line regimen. Second-line

ART is intended for individuals who were unable to achieve viral suppression on first-line ART, second line may still face challenges like adherence impacting viral suppression. As the HIV epidemic matures in South Africa, there is an increasing number of individuals requiring second-line and third-line antiretroviral therapy. As of 2021, approximately 5.6 million people were receiving ART, with 3.4 million on first-line regimens, 145,000 on second-line therapy, and approximately 700 on third-line treatment (29,30). This study aimed to assess whether pregnant women on second-line ART attain viral suppression, considering various factors influencing an unsuppressed viral load. This research aligns with the VTP narrative, working towards reducing pediatric HIV incidence.

1.6 Aim

This study described at a national level the characteristics and the distribution of pregnant women living with HIV who were on second-line regimen and determined the factors associated with viral suppression in pregnant women on a second-line regimen compared to those on first-line regimen.

1.7 Objectives:

1. To describe the characteristics and the distribution of pregnant women living with HIV who were on first-line regimen and second-line regimen.
2. To compare viral load suppression levels among pregnant women living with HIV and taking second-line ART compared to those taking first-line ART.
3. To determine factors associated with viral suppression among pregnant women living with HIV and on second-line regimen compared to those on a first-line regimen.

2 Chapter 2: METHODS

Introduction

This chapter details the methodologies applied in this research. The chapter describes the cross-sectional design employed in the primary study, including the key variables used in the current study. It provides an overview of data management procedures and statistical analysis techniques used to investigate the relationship between variables. Furthermore, the chapter discusses the ethical approval process that ensured the study's adherence to research standards.

2.1 Study design:

This study was a secondary data analysis of cross-sectional survey data from the 2022 national antenatal care HIV prevalence survey. The cross-sectional design was appropriate because it provided a snapshot of viral load suppression and associated factors among pregnant women at a specific point in time, allowing for the estimation of prevalence and exploration of relationships between variables within the population.

2.2 Study Setting

This study was conducted in the South African public health facilities, where the majority of HIV-related and antenatal care is delivered. As of 2014, South Africa had approximately 3,841 public health care clinics, forming the backbone of HIV and maternal health service delivery (31). The study targeted women of reproductive age (15–49 years), who were the primary users of these services. Based on the 2022 Census, South Africa had an estimated population of 62.2 million, of which approximately 51.5% or 31.9 million were female. Females aged 15–49 years made up around 17.2 million women in South Africa, representing 27.8% of the total South African population or 54.0% of the total female population (32). This group forms the critical demographic for national HIV treatment, VTP, and viral load monitoring programmes implemented at PHC level.

2.3 Description of primary study

This secondary analysis used data from the 2022 national antenatal care HIV prevalence survey. Objectives of the primary study were the estimation of HIV incidence and prevalence among pregnant women, tracking the progress of South Africa towards the 95-95-95 established by the UNAIDS, determining the prevalence of unplanned pregnancy in women, and determining coverage of PrEP among HIV-negative pregnant women. Primary study inclusion criteria: Pregnant women aged 15-49 attending antenatal care for the first time or follow-up who consented to the survey. Participants were recruited regardless of their HIV status. Pregnant women who had visited the antenatal clinic more than once during the survey period were excluded to avoid sampling the same patient twice. A barcode sticker was used to avoid sampling the same patient twice. Study sites were 1595 public health clinics located in all 52 districts and nine provinces in the country. The data was collected by NICD between February to March of 2022. The planned sample size was 36015 pregnant women who would be sampled from the participating public health facilities. A nurse already employed at the sampled facility obtained informed consent, administered the survey, and had a brief interview with the participants, medical record review, and blood specimen collection. The data was collected as part of an antenatal sentinel surveillance which is carried out every 2 years. Data clerks at NICD used Optical Mark Recognition (OMR) to convert data collected on paper into an electronic database. Demographic data and laboratory - HIV test results- data were combined by the data managers using barcode stickers which acted as a patient's unique identifier. The data was then kept protected using OMR and TrakCare safety and security features which included access control and a firewall as a countermeasure against hackers and viruses. The data collection tool used in the primary study is available as Annexure 6.7

2.4 Description of the current study

2.4.1 Study population:

Pregnant women living with HIV enrolled in the 2022 ANC survey and were on ART.

Inclusion: Pregnant women aged 15-49 who were HIV positive and on ART

Exclusion: participants with incomplete data on HIV status and ART status.

2.4.2 Dependent variable

The main dependent variable or primary outcome was viral load status. This was collected from the measurement of viral load in the laboratory and was recorded as a continuous variable. The continuous variable was converted into two distinct dichotomous variables using different thresholds of viral suppression: Suspected virologic failure (≥1000 copies/ml) and virally unsuppressed (≥50 copies/ml). Virally unsuppressed was coded as 1 while virally suppressed was coded as 0 for both variables.

The figures below illustrate the transformation of the outcome variable into two distinct dichotomous variables.

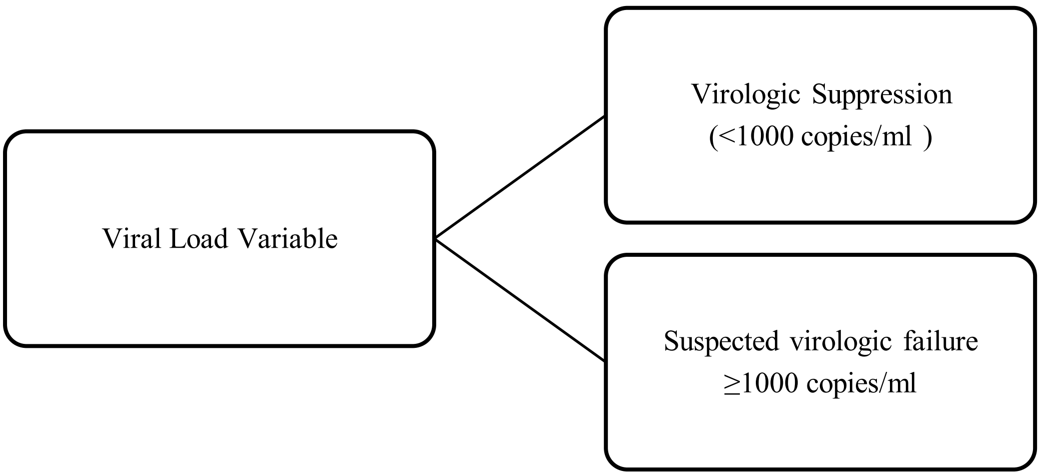


Figure 2.2 Schematic diagram depicting the transformation of the viral load variable exploring the ≥1000 copies/ml threshold.

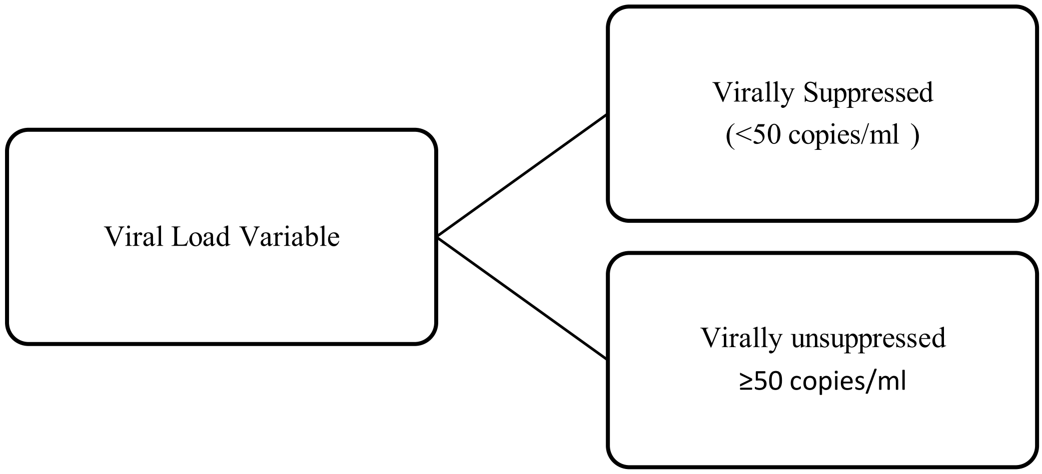


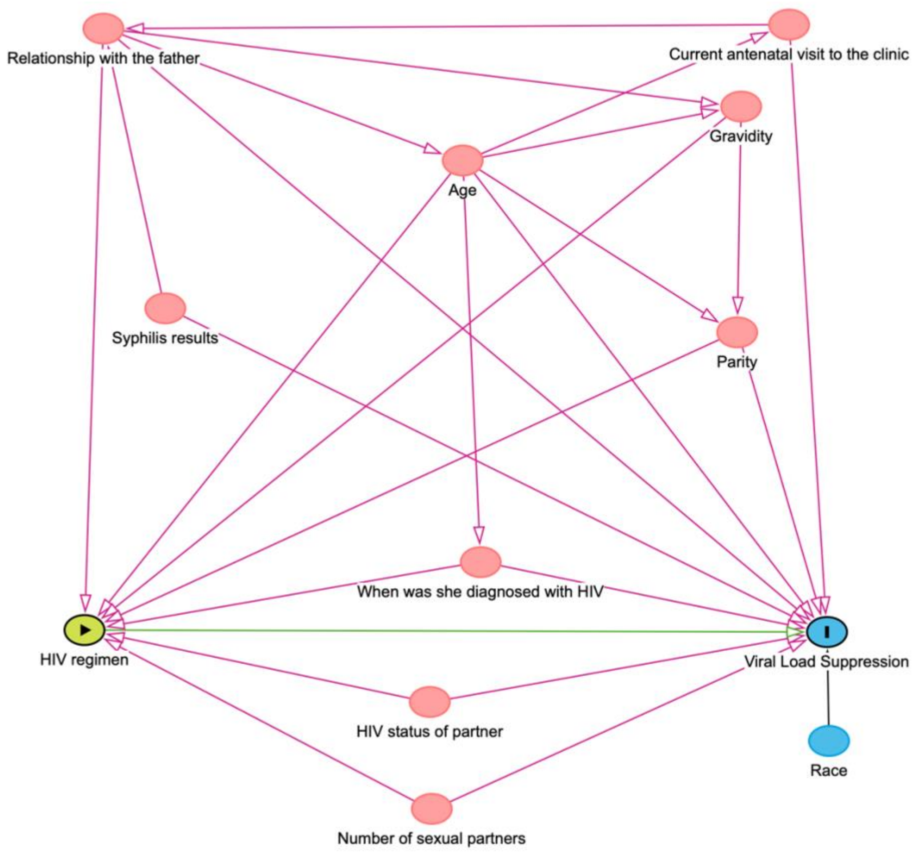
Figure 2.3 Schematic diagram depicting the transformation of the viral load variable exploring the ≥50 copies/ml threshold.

2.4.3 Independent variables

Being on second-line ART or not (ART regimen) was the primary exposure variable, and women who were on second line were given an exposed status while women who were on first-line ART were given an unexposed status and used as a comparison group.

Potential confounders: HIV regimen, age, race, current antenatal visit to the clinic, gravidity, parity, current gestational age in weeks, relationship with the father, HIV status of partner, number of sexual partners, syphilis results, HIV status from medical records, when was she diagnosed with HIV and when was the treatment started if HIV positive.

2.4.4 Directed acyclic graph.



All covariates included in the Directed Acyclic Graph were identified as distal factors that influenced viral load suppression indirectly, acting through adherence to ART.

Figure 2.4: A visual representation of distal causal relationships amongst demographic and clinical characteristics.

Figure 2.4 shows the Directed Acyclic Graph (DAG) which is a visual representation of distal causal relationships between demographic and clinical characteristics and viral suppression. These relationships have been identified in the literature (26,33,34). The outcome was established to be viral load suppression and the primary exposure to be regimen type. The proximal or immediate cause of unsuppressed viral load is no or low levels of ARV drugs and their metabolites in the blood.

The minimal sufficient set of confounding variables to adjust for included: age group, first antenatal visit type, number of pregnancies, number of live babies, gestational age group, relationship with father, HIV status of current sexual partner, number of sexual partners in the past 12 months, syphilis test result, timing of anti-retroviral therapy initiation.

2.4.5 Data management

Analysis was done using STATA Standard Edition version 18.0 (Stata Corporation, College Station, Texas, USA). The dataset was received as a CSV file. Before the data were analysed, the dataset was checked for missing data. Little's test was employed to assess if the data were missing at random. The Little's test included all variables listed as potential confounders under independent variables. Since the data were not missing at random, multiple imputation was not applicable (35). A missing category was added to all variables with more than 5% missing observations. Age and gestational age were categorised. Age was categorised into 15-24, 25-34 and 35-49, while gestational age was categorised into first trimester (week 0-13), second trimester (week 14-26) and third trimester (week 27-42).

2.5 Statistical analysis

2.5.1 Descriptive statistics

Descriptive statistics were employed to determine the distribution of sample characteristics using proportions and percentages. All variables included were categorical (binary, ordinal and nominal). The list of variables used included viral load suppression, regimen type, age group, first antenatal visit, race, number of pregnancies, number of live babies, gestational age group, relationship with father, HIV status of current sexual partner, number of sexual partners in the past 12 months, syphilis test result, and timing of anti-retroviral therapy initiation. A flow diagram (Figure 3.1) indicating inclusion and exclusion was created which also shows the distribution of viral suppression by ART regimen type. Included and excluded participants were compared and the Pearson's chi-square test was used to determine any statistical differences between the two groups.

2.5.2 Inferential statistics

Univariable analysis was employed, which was inclusive of all the variables and stratified by viral suppression, exploring two viral load thresholds (Suspected virologic failure (≥1000 copies/ml) and virally unsuppressed (≥50 copies/ml)), this was conducted to assess whether there were any differences between individuals who were excluded from the study and those who were included in the analysis. In the same univariable table, univariable logistic regression was presented with odds ratios (OR), 95% CI (Confidence Interval) and p-value (probability value).

Six multivariable models were constructed. Model I and Model II were fitted using the DAGs as the minimal sufficient set of exposure variables and all variables considered as confounders were included in the models. Model I used VL ≥1000 copies/ml as the threshold for unsuppressed VL while Model II used VL ≥50 copies/ml as the threshold for unsuppressed VL. Model III and Model IV were multivariable logistic regression models fitted on factors that were significant at univariable logistic regression, with Model III using ≥1000 copies/ml) and Model IV using ≥50 copies/ml) thresholds. In both models III and IV, a category coded as missing for variables that had more than 5% of missing observations was included. Model V and Model VI were multivariable logistic regression models built on factors that were significant at univariable logistic regression, with Model V focussed on the suspected virologic failure (≥1000 copies/ml) and Model VI using the virally unsuppressed (≥50 copies/ml), and both these models use full case analysis of the logistic regression. Model VII and Model VIII were constructed as backwards stepwise logistic regression models stratified by ART regimen type (first-line and second-line, respectively), and focused on factors associated with suspected virologic failure (≥1000 copies/ml). All the analyses conducted in this study considered survey weights and we used $\alpha = 0.05$.

2.6 Ethical approval

Ethical clearance was obtained from the University of Witwatersrand Human Research Ethics Committee in Johannesburg, South Africa (Medical HREC). Approval number: M240423 (Annexure 6.4). Approval for the use of the dataset was granted by The National Institute for Communicable Diseases (Annexure 6.5). The data was anonymised and password protected. It should be noted that no attempts were made to identify and/or contact any participants who were part of the survey, and the data was de-identified before it was given to the Primary Investigator. The anonymised dataset was securely stored on a password-protected institutional server and would be retained for a minimum of five years after completion of the current study, in accordance with data management and ethical guidelines.

3. Chapter 3: Results

This chapter describes the research findings, with an overview of the sociodemographic and clinical characteristics of the study participants. The chapter further examines the factors associated with viral load suppression by applying both univariable and multivariable logistic regression analyses.

3.1. Summary of inclusions and exclusions

The study assessed 42 875 pregnant women who were enrolled. Of those, 36 677 were considered eligible for inclusion in the study, while 6 198 were excluded due to either the absence of documented consent, pregnancy status or haemolysed blood samples. Among those eligible for inclusion in the study, 10 393 participants were considered eligible for secondary analysis of viral load suppression by ART regimen. The final secondary analysis comprised 8 606 participants, with 1 787 further excluded due to incomplete data on regimen type or viral load (Figure 3.1). The proportion of pregnant women who were suspected virologic failure (≥1000 copies/ml) on first-line ART was 10.9%, while it was 16.8% among those on second-line ART. A two-proportion power sample was calculated using STATA 18.0 with an alpha of 5% which yielded a power of 77.6% (Annexure 6.3). The viral load estimates presented in Figure 3.1 explore the ≥1000 copies/ml threshold VLS variable.

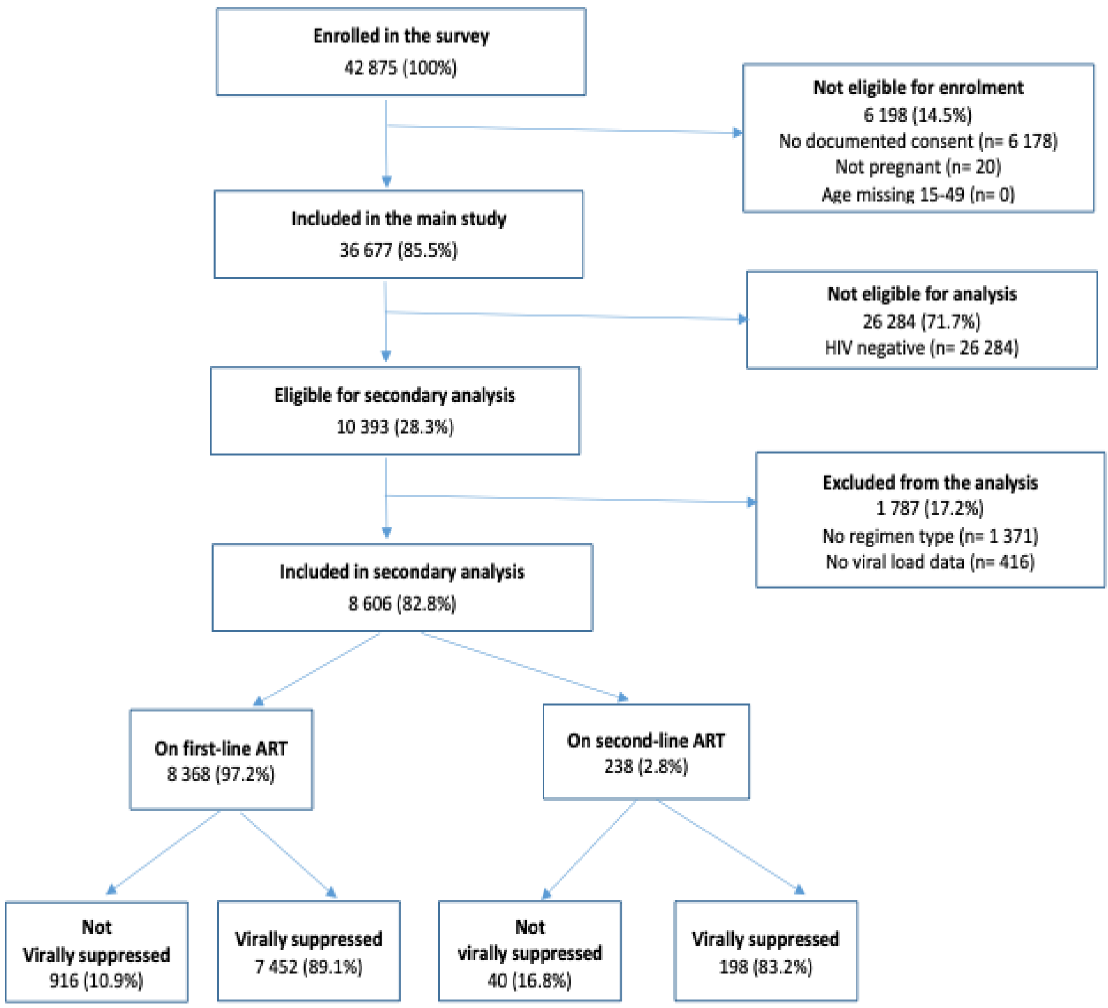


Figure 3.1: Study Flow

3.1 Socio-demographic and clinical characteristics of women included in the analysis

The majority of participants included in the secondary analysis were virally suppressed (88.5%), on first-line ART (97.3%), aged 25 to 34 years (57.0%), of African race (96.5%), attending follow-up visit (73.0%). Moreover, most were in their second pregnancy (29.5%), had one living baby (32.6%), were in their third trimester (45.9%) not living together but in a relationship with the baby's father (51.5%), partner was HIV positive (20.4%), had one sexual partner (49.9%), had negative syphilis test results (74.4%), and started ART before pregnancy (62.0%, Table 3.1).

Table 3.1: Demographic and clinical characteristics of pregnant women included and excluded in the secondary analysis (N=10 393)

Variables	Included (N=8606)			Excluded (N=1787)			p-value
	n	%	95% CI	n	%	95% CI	
Viral load suppression							
Virally unsuppressed	956	11.5	10.9-12.0	332	27.6	25.4-30.0	<0.001
Virally suppressed	7650	88.5	88.0-89.1	925	72.4	70.0-74.6	
Regimen type							
First-line ART	8368	97.3	97.0-97.5	410	98.4	97.1-99.2	0.074
Second-line ART	238	2.7	2.5-3.0	6	1.6	0.8-2.9	
Age group							
15-24	1573	17.9	17.2-18.6	462	25.0	23.4-26.7	<0.001
25-34	4888	57.0	56.1-57.9	929	52.4	50.5-54.3	
35-49	2145	25.1	24.3-25.9	396	22.6	20.9-24.3	
First Antenatal visit							
First visit	2227	27.0	26.0-28.1	663	40.2	37.9-42.4	<0.001
Follow-up visit	6200	73.0	71.9-74.0	1077	59.8	57.6-62.1	
Race							
African	8283	96.5	96.1-96.8	1685	94.6	93.8-95.4	<0.001
Other	307	3.4	3.1-3.7	97	5.1	4.4-5.9	
Missing	16	0.2	0.1-0.3	5	0.3	0.1-0.6	
Number of pregnancies							
Once	1164	13.2	12.6-13.8	343	18.4	17.0-19.9	<0.001
Twice	2564	29.5	28.7-30.3	530	29.0	27.4-30.8	
Three times	2497	29.4	28.6-30.2	417	23.9	22.4-25.6	
Four or more times	2170	25.6	24.8-26.4	381	22.3	20.8-24.0	
Missing	211	2.3	2.1-2.6	116	6.3	4.9-8.0	
Number of live babies							
None	1548	17.6	16.9-18.3	404	21.6	20.2-23.2	<0.001
One	2816	32.6	31.8-33.4	562	31.7	29.9-33.4	
Two	2450	28.8	28.0-29.6	411	23.3	21.7-24.9	
Three	1159	13.7	13.1-14.3	215	12.2	11.0-13.5	
Four or more	552	6.4	6.0-6.8	110	6.6	5.6-7.7	
Missing	81	0.9	0.7-1.1	85	4.6	3.5-6.1	
Gestational age group							
First trimester	867	9.9	9.3-10.4	208	11.6	10.4-12.9	<0.001
Second trimester	3185	36.6	35.7-37.4	633	36.0	34.0-38.2	
Third trimester	3905	45.9	44.9-47.0	666	37.0	34.8-39.2	
Missing	649	7.6	7.1-8.2	280	15.4	13.2-17.8	
Relationship with father							
Married	1125	13.4	12.8-14.1	196	11.7	10.4-13.1	0.020
Living together/co-habiting	2442	30.5	29.5-31.4	489	29.1	27.2-31.0	
Not living together but in a relationship	4663	51.5	50.5-52.6	1010	53.7	51.7-55.7	
No relationship	306	3.8	3.5-4.2	74	4.5	3.7-5.5	

Missing	70	0.8	0.7-1.0	18	1.0	0.6-1.5	
HIV status of current sexual partner							
HIV positive	1713	20.4	19.3-21.6	234	13.9	12.4-15.5	<0.001
HIV negative	993	12.2	11.5-13.0	301	17.9	16.3-19.6	
Don't know his/their status	1242	14.8	13.9-15.7	326	18.2	16.6-20.0	
Don't have a sexual partner	52	0.6	0.5-0.8	15	0.8	0.5-1.2	
Missing	4606	52.0	49.9-54.1	911	49.3	46.7-51.9	
Number of sexual partners in the past 12 months							
More than one sexual partner	233	2.7	2.3-3.0	60	3.2	2.6-4.0	<0.001
One sexual partner	4142	49.9	47.9-52.0	983	56.8	54.1-59.4	
Missing	4231	47.4	45.3-49.5	744	40.0	37.4-42.6	
Syphilis test result							
Positive	320	3.4	3.1-3.8	51	2.7	2.1-3.4	<0.001
Negative	6528	74.7	73.5-75.8	1103	59.5	57.0-62.0	
Missing	1758	21.9	20.7-23.1	633	37.8	35.3-40.3	
Timing of anti-retroviral therapy initiation							
Before pregnancy	5399	62.0	60.8-63.2	614	33.0	31.0-35.1	<0.001
During pregnancy	1890	22.3	21.4-23.3	212	12.0	10.6-13.5	
Missing	1317	15.6	14.8-16.5	961	55.0	52.7-57.2	

Table 3.1 further shows that statistically significant differences were observed between the included and excluded groups. Excluded pregnant women were more likely to be virally suppressed (72.4%), be aged 25-34 years (52.4%), of African race (94.6%), attending follow-up visits (59.8%), in their third pregnancy (23.9%), have two living babies (23.3%), in their third trimester (37.0%), living together but not married (29.1%). Additionally, they were more likely, to have an HIV positive sexual partner (13.9%), negative syphilis test results (59.5%), and started ART before pregnancy (33.0%) and had one sexual partner (56.8%).

3.2. Determinants of viral suppression

In univariable analyses, the odds of being a suspected virologic failure (VL ≥1000 copies/ml) were significantly higher among second-line ART users compared to those on first-line ART [OR=1.56 (95% CI: 1.18–2.06), p=0.002]; participants aged 15 to 24 years compared to those aged 35 to 49 years [OR=2.15 (95% CI: 1.84–2.52), p<0.001]; those attending their first antenatal visit compared to follow-up visits [OR=3.51 (95% CI: 3.12–3.96), $p<0.001$]; those who were classified as other racial group compared to Black African groups [OR=1.41 (95% CI: 1.10–1.81), $p=0.006$]; those who did not know the HIV status of their current sexual partner compared to those whose partner was HIV positive [OR=3.13 (95% CI: 2.63–3.73), $p<0.001$]; and those who started ART during pregnancy compared to those who started before pregnancy [OR=1.75 (95% CI: 1.53–2.01), $p<0.001$]. However, the odds of being suspected virologic failure were significantly lower among those who had been pregnant twice [OR=0.75 (95% CI: 0.64–0.89), $p=0.001$], three times [OR=0.71 (95% CI: 0.60–0.84), $p<0.001$], and four or more times [OR=0.60 (95% CI: 0.50–0.71), $p<0.001$] compared to those who had only been pregnant once; those with one [OR=0.75 (95% CI: 0.65–0.87), $p<0.001$], two [OR=0.66 (95% CI: 0.56–0.77), $p<0.001$], and three [OR=0.61 (95% CI: 0.50–0.74), $p<0.001$] living babies compared to those with none; and those in their third trimester [OR=0.60 (95% CI: 0.50–0.73), $p<0.001$] compared to the first trimester (Table 3.2).

Table 3.2: A univariable logistic regression of factors associated with viral suppression among included HIV-positive participants aged 15 years and older, N= 8 606.

	Suspected virologic failure (≥1000 copies/ml)							Virally unsuppressed ≥50 copies/ml)					
Variables	**N**	**%**	**95% CI**	**Odds Ratio**	**95% CI**		**p-value**	**%**	**95% CI**	**Odds Ratio**	**95% CI**		**p-value**
Regimen type													
First-line ART	8368	11.3	10.8-11.9	Ref				32.5	31.7-33.4	Ref			
Second-line ART	238	16.6	13.2-20.8	1.56	1.18	2.06	0.002	45.0	40.0-50.0	1.69	1.38	2.08	<0.001
Age group													
15-24	1573	18.3	16.7-19.9	2.15	1.84	2.52	<0.001	39.5	37.5-41.6	1.33	1.19	1.49	<0.001
25-34	4888	10.3	9.6-11.0	1.10	0.95	1.27	0.190	30.8	29.7-31.9	0.91	0.83	0.99	0.034
35-49	2145	9.4	8.4-10.5	Ref				32.9	31.3-34.6				
First Antenatal visit													
First visit	2227	22.3	20.8-23.8	3.51	3.12	3.96	<0.001	42.9	41.1-44.7	1.82	1.67	1.99	<0.001
Follow-up visit	6200	7.5	7.0-8.1	Ref				29.2	28.3-30.1	Ref			
Race													
African	8283	11.3	10.8-11.9	Ref				32.7	31.8-33.5	Ref			
Other	307	15.3	12.4-18.7	1.41	1.10	1.81	0.006	38.7	34.5-43.0	1.30	1.08	1.56	0.006
Number of pregnancies													
Once	1164	15.0	13.4-16.8	Ref				38.4	36.1-40.7	Ref			
Twice	2564	11.8	10.8-12.8	0.75	0.64	0.89	0.001	33.0	31.5-34.5	0.79	0.70	0.88	<0.001
Three times	2497	11.1	10.1-12.2	0.71	0.60	0.84	<0.001	32.2	30.7-33.7	0.76	0.68	0.86	<0.001
Four or more times	2170	9.6	8.6-10.6	0.60	0.50	0.71	<0.001	30.7	29.1-32.3	0.71	0.63	0.80	<0.001
Number of live babies													
None	1548	14.8	13.4-16.3	Ref				37.2	35.2-39.3	Ref			
One	2816	11.5	10.6-12.6	0.75	0.65	0.87	<0.001	33.1	31.7-34.5	0.83	0.75	0.93	0.001
Two	2450	10.2	9.3-11.3	0.66	0.56	0.77	<0.001	30.5	29.1-31.9	0.74	0.66	0.83	<0.001
Three	1159	9.6	8.3-11.1	0.61	0.50	0.74	<0.001	30.3	28.2-32.4	0.73	0.64	0.83	<0.001
Four or more	552	12.5	10.3-15.0	0.82	0.65	1.05	0.120	36.2	32.8-39.7	0.96	0.81	1.13	0.607
Gestational age group													

First trimester	867	13.6	11.8-15.7	Ref				36.1	33.6-38.8	Ref			
Second trimester	3185	13.4	12.4-14.4	0.98	0.82	1.17	0.810	35.9	34.5-37.3	0.99	0.87	1.13	0.873
Third trimester	3905	8.7	8.0-9.5	0.60	0.50	0.73	<0.001	29.4	28.3-30.5	0.74	0.65	0.83	<0.001
Missing	649	16.3	14.1-18.8	1.23	0.97	1.56	0.082	35.3	32.4-38.3	0.96	0.82	1.14	0.671
Relationship with father													
Married	1125	10.3	8.9-11.9	Ref				31.8	29.8-34.0	Ref			
Living together/co-habiting	2442	11.0	10.1-12.0	1.07	0.89	1.29	0.473	31.0	29.6-32.5	0.96	0.86	1.08	0.523
Not living together but in a relationship	4663	11.9	11.1-12.8	1.18	0.98	1.42	0.083	34.1	32.9-35.3	1.11	0.99	1.24	0.072
No relationship	306	13.3	10.6-16.7	1.34	0.98	1.82	0.066	33.2	29.4-37.3	1.06	0.87	1.30	0.556
HIV status of current sexual partner													
HIV positive	1713	7.7	6.7-8.8	Ref				28.9	27.0-30.8	Ref			
HIV negative	993	8.7	7.4-10.3	1.15	0.91	1.44	0.235	29.9	27.7-32.3	1.05	0.91	1.21	0.488
Don't know his/their status	1242	20.7	18.9-22.6	3.13	2.63	3.73	<0.001	43.1	40.8-45.4	1.86	1.65	2.11	<0.001
Don't have a sexual partner	52	9.9	5.2-17.8	1.31	0.65	2.64	0.442	28.7	20.7-38.2	0.99	0.64	1.54	0.963
Missing	4606	11.0	10.3-11.8	1.49	1.26	1.76	<0.001	32.3	31.1-33.5	1.18	1.05	1.31	0.004
Number of sexual partners in the past 12 months													
More than one sexual partner	233	13.5	10.3-17.6	Ref				34.3	29.5-39.4	Ref			
One sexual partner	4142	11.8	11.0-12.7	0.86	0.62	1.19	0.364	33.4	32.1-34.6	0.96	0.76	1.21	0.731
Missing	4231	11.0	10.2-11.8	0.79	0.57	1.09	0.153	32.3	31.1-33.5	0.91	0.73	1.15	0.439
Syphilis test result													
Positive	320	10.1	7.8-13.0	Ref				34.5	30.7-38.6	Ref			
Negative	6528	9.1	8.5-9.7	0.89	0.66	1.19	0.422	30.5	29.5-31.4	0.83	0.69	1.00	0.050
Missing	1758	19.8	18.3-21.5	2.19	1.62	2.97	<0.001	40.8	38.9-42.8	1.31	1.08	1.59	0.007
Timing of anti-retroviral therapy initiation													
Before pregnancy	5399	7.2	6.7-7.8	Ref				28.0	27.1-29.0	Ref			
During pregnancy	1890	12.0	10.8-13.2	1.75	1.53	2.01	<0.001	34.3	32.6-36.2	1.34	1.23	1.48	<0.001
Missing	1317	27.8	25.7-29.9	4.95	4.37	5.62	<0.001	50.2	47.7-52.6	2.59	2.33	2.88	<0.001

When comparing the univariable analyses at the two VLS thresholds, several differences were observed. At the ≥50 copies/ml threshold, being pregnant twice or more and having one to three living babies was associated with significantly lower odds of being virally unsuppressed, having a negative syphilis test result and being aged 25 to 34 years compared to 35 to 49 years. These factors were not significant at the ≥1000 copies/ml threshold (Table 3.2).

Table 3.3: Factors independently associated with viral suppression among HIV-positive participants aged 15 years and older (constructed using a DAG*)

	Model I (VL ≥1000 copies/ml) (N=8351)				**Model II (VL ≥50 copies/ml) (N=8351)**			
Variables	**aOR**	**95% CI**		**p-value**	**aOR**	**95% CI**		**p-value**
Regimen type								
First-line ART	Ref							
Second-line ART	2.32	1.68	3.21	<0.001	2.01	1.61	2.51	<0.001
Age group								
15-24	2.15	1.77	2.60	<0.001	1.21	1.06	1.39	0.004
25-34	1.13	0.97	1.33	0.116	0.92	0.83	1.01	0.083
35-49	Ref							
First Antenatal visit type								
First Visit	3.21	2.74	3.77	<0.001	1.66	1.48	1.86	<0.001
Follow-up visit	Ref							
Number of live babies								
None	Ref							
One	1.06	0.89	1.26	0.527	0.97	0.86	1.10	0.648
Two	1.04	0.85	1.27	0.690	0.90	0.79	1.02	0.102
Three	1.01	0.79	1.30	0.917	0.90	0.78	1.05	0.197
Four or more	1.49	1.11	1.98	0.007	1.17	0.97	1.41	0.107
Gestational age group								
First trimester	Ref							
Second trimester	1.46	1.20	1.77	<0.001	1.20	1.04	1.37	0.010
Third trimester	1.39	1.10	1.75	0.005	1.08	0.93	1.25	0.322
Missing	1.30	1.01	1.67	0.043	0.99	0.83	1.18	0.907
HIV status of current sexual partner								
HIV positive	Ref							

HIV negative	1.04	0.82	1.33	0.728	1.06	0.91	1.24	0.417
Don't know his/their status	2.18	1.80	2.65	<0.001	1.60	1.41	1.82	<0.001
Don't have a sexual partner	1.29	0.61	2.71	0.502	1.04	0.67	1.63	0.859
Missing	1.06	0.82	1.38	0.654	1.02	0.86	1.21	0.791
Timing of anti-retroviral therapy initiation								
Before pregnancy	Ref							
During pregnancy	2.08	1.79	2.42	<0.001	1.45	1.31	1.60	<0.001
Missing	4.00	3.51	4.57	<0.001	2.33	2.10	2.60	<0.001
Syphilis test result								
Positive	Ref							
Negative	1.03	0.74	1.43	0.858	0.89	0.73	1.08	0.225
Missing	1.30	0.93	1.83	0.125	1.01	0.82	1.24	0.956
Relationship with the father								
Married	Ref							
Living together/co-habiting	1.12	0.92	1.37	0.244	0.99	0.88	1.12	0.880
together but in a relationship	1.07	0.88	1.30	0.484	1.07	0.95	1.20	0.276
No relationship	1.01	0.73	1.41	0.941	0.96	0.78	1.19	0.714
Missing	1.34	0.66	2.72	0.416	1.22	0.74	2.02	0.435
Number of sexual partners in the past 12 months								
More than one sexual partner	Ref							
One sexual partner	1.06	0.76	1.47	0.733	1.09	0.86	1.38	0.457
Missing	1.37	0.94	2.00	0.101	1.23	0.95	1.60	0.112

*DAG = A directed acyclic graph (DAG) is a conceptual tool used to represent causal relationships in a visual graph format.

Table 3.3 shows that In Model I (VL ≥1000 copies/ml), the odds of being a suspected virologic failure were significantly higher among second-line ART users compared to those using first-line ART [aOR=2.32 (95% CI: 1.68–3.21), p=<0.001]; those aged 15 to 24 years compared to 35 to 49 years [aOR=2.15 (95% CI: 1.77–3.77), p=<0.001]; among those attending their first antenatal visit compared to follow-up visits [aOR=3.21 (95% CI: 2.74–3.77), p=<0.001]; those with four or more living babies compared to those who had none [aOR=1.49 (95% CI: 1.11–1.98), p=0.007]; in their second trimester [aOR=1.46 (95% CI: 1.20–1.77), p=<0.001] and third trimester [aOR=1.39 (95% CI: 1.10–1.67), p=0.005] compared to the first trimester; those who did not know the HIV status of their current sexual partner compared to those whose sexual partner was HIV positive [aOR=2.18 (95% CI: 1.80–2.65), p=<0.001]; and among those who started ART during pregnancy compared to those who started before pregnancy [aOR=2.08 (95% CI: 1.79–2.42), p=<0.001].

In Model II (VL ≥50 copies/ml), the odds of being virally unsuppressed were significantly higher among second-line ART users compared to those using first-line ART [aOR=2.01 (95% CI: 1.61–2.51), p=<0.001]; those aged 15 to 24 years compared to 35 to 49 years [aOR=1.21 (95% CI: 1.06–1.39), p=0.004]; among those attending their first antenatal visit compared to follow-up visits [aOR=1.66 (95% CI: 1.48–1.86), p=<0.001]; in their second trimester [aOR=1.20 (95% CI: 1.04–1.37), p=0.010] compared to the first trimester; those who did not know the HIV status of their current sexual partner compared to those whose sexual partner was HIV positive [aOR=1.60 (95% CI: 1.41–1.82), p=<0.001]; and among those who started ART during pregnancy compared to those who started before pregnancy [aOR=1.45 (95% CI: 1.31–1.60), p=<0.001] (Table 3.3).

When comparing Model I and Model II, some variables were significant in one model but not in the other. In Model I, having four or more living babies compared to none was significantly associated with higher odds of being virally unsuppressed [aOR=1.49 (95% CI: 1.11–1.98), p=0.007], but this association was not significant in Model II [aOR=1.17 (95% CI: 0.97–1.41), p=0.107]. Similarly, being in the third trimester compared to the first trimester was significant in Model I [aOR=1.39 (95% CI: 1.10–1.67), p=0.005], but this association did not remain significant in Model II [aOR=1.08 (95% CI: 0.93–1.25), p=0.322] (Table 3.3).

Table 3.4: Factors associated with viral suppression among HIV-positive participants aged 15 years and older (constructed using variables that were significant in the univariable analyses)

	Model III (VL ≥1000) copies/ml) (N=8339)				**Model IV (VL ≥50 copies/ml) (N=8339)**			
Variables	**aOR**	**95% CI**		**p-value**	**aOR**	**95% CI**		**p-value**
Regimen type								
First-line ART	Ref							
Second-line ART	2.30	1.66	3.18	<0.001	2.31	1.67	3.20	<0.001
Age group								
15-24	2.15	1.78	2.61	<0.001	2.15	1.77	2.61	<0.001
25-34	1.15	0.98	1.34	0.088	1.15	0.98	1.35	0.084
35-49	Ref							
First Antenatal visit type								
First Visit	3.57	3.08	4.15	<0.001	3.18	2.72	3.73	<0.001
Follow-up visit	Ref							
Race								
African	Ref							
Other	1.38	1.04	1.84	0.028	1.41	1.05	1.88	0.021
Number of live babies								
None	Ref							
One	1.08	0.90	1.29	0.398	1.07	0.89	1.27	0.474
Two	1.07	0.88	1.31	0.490	1.05	0.86	1.28	0.618
Three	1.05	0.82	1.34	0.702	1.02	0.80	1.31	0.874
Four or more	1.52	1.14	2.02	0.005	1.47	1.10	1.96	0.009
Gestational age group								
First trimester	Ref							
Second trimester	1.46	1.20	1.77	<0.001	1.46	1.20	1.77	<0.001
Third trimester	1.37	1.09	1.73	0.006	1.38	1.10	1.74	0.005
Missing	1.29	1.01	1.66	0.043	1.29	1.01	1.66	0.045
HIV status of current sexual partner								
HIV positive	Ref							
HIV negative	1.05	0.82	1.34	0.685	1.05	0.82	1.34	0.699
Don't know his/their status	2.17	1.79	2.63	<0.001	2.17	1.79	2.63	<0.001
Don't have a sexual partner	1.22	0.57	2.58	0.606	1.25	0.59	2.63	0.558
Missing	1.32	1.11	1.58	0.002	1.32	1.11	1.57	0.002
Timing of anti-retroviral therapy initiation								
Before pregnancy	Ref							

During pregnancy	2.08	1.79	2.42	<0.001	1.04	0.75	1.45	0.797
Missing	3.96	3.47	4.52	<0.001	1.32	0.94	1.86	0.106
Syphilis test result								
Positive	Ref							
Negative					2.09	1.79	2.42	<0.001
Missing					3.93	3.44	4.48	<0.001

Models are constructed using variables that were significant in the univariable analyses and had missing as a category on variables that had more than 5% of missing observations

In Model III (VL ≥1000 copies/ml), the odds of being a suspected virologic failure were significantly higher among second-line ART users compared to those using first-line ART [aOR=2.30 (95% CI: 1.66–3.18), p=<0.001]; those aged 15 to 24 years compared to 35 to 49 years [aOR=2.15 (95% CI: 1.78–2.61), p=<0.001]; among those attending their first antenatal visit compared to follow-up visits [aOR=3.57 (95% CI: 3.08–4.15), p=<0.001]; those who were classified as other racial group compared to Black African groups [aOR=1.38 (95% CI: 1.04–1.84), p=0.028]; those with four or more living babies compared to those who had none [aOR=1.52 (95% CI: 1.14–2.02), p=0.005]; in their second trimester [aOR=1.46 (95% CI: 1.20–1.77), p=<0.001] and third trimester [aOR=1.37 (95% CI: 1.09–1.73), p=0.006] compared to the first trimester; those who did not know the HIV status of their current sexual partner compared to those whose sexual partner was HIV positive [aOR=2.17 (95% CI: 1.79–2.63), p=<0.001]; and among those who started ART during pregnancy compared to those who started before pregnancy [aOR=2.08 (95% CI: 1.79–2.42), p=<0.001].

In Model IV (VL ≥50 copies/ml), the odds of being virally unsuppressed were significantly higher among second-line ART users compared to those using first-line ART [aOR=2.31 (95% CI: 1.67–3.20), p=<0.001]; those aged 15 to 24 years compared to 35 to 49 years [aOR=2.15 (95% CI: 1.77–2.61), p=<0.001]; among those attending their first antenatal visit compared to follow-up visits [aOR=3.18 (95% CI: 2.72–3.73), p=<0.001]; those who were classified as other racial group compared to Black African groups [aOR=1.41 (95% CI: 1.05–1.88), p=0.021]; those with four or more living babies compared to those who had none [aOR=1.47 (95% CI: 1.10–1.96), p=0.009]; in their second trimester [aOR=1.46 (95% CI: 1.20–1.77), p=<0.001] and third trimester [aOR=1.38 (95% CI: 1.10–1.74), p=0.005] compared to the first trimester; those who did not know the HIV status of their current sexual partner compared to those whose sexual partner was HIV positive [aOR=2.17 (95% CI: 1.79–2.63), p=<0.001]; and those with a negative syphilis test result compared to those with a positive one [OR=2.09 (95% CI: 1.79–1.42), p=<0.001]

In comparing Model III and Model IV, some variables were significant in one model. In Model IV, a negative syphilis test result compared to a positive one was significantly associated with higher odds of being virally unsuppressed [aOR=2.09 (95% CI: 1.79–2.42), p<0.001], but this association was not significant in at univariable level used to construct Model III, hence the variable was not included in the full multivariable Model III. Additionally, starting ART during pregnancy compared to before pregnancy was significant in Model III [aOR=2.08 (95% CI: 1.79–2.42), p<0.001], but this association was not significant in Model IV [aOR=1.04 (95% CI: 0.75–1.45), p=0.797].

Table 3.5: Factors associated with viral suppression among HIV-positive participants aged 15 years and older (constructed using a complete case analysis)

	Model V (VL≥1000 copies/ml) (N=3041)				**Model VI (VL ≥50 copies/ml) (N=2481)**			
Variables	**aOR**	**95% CI**		**p-value**	**aOR**	**95% CI**		**p-value**
Regimen type								
First-line ART	Ref				Ref			
Second-line ART	2.60	1.57	4.32	<0.001	2.23	1.20	4.17	0.012
Age group								
15-24	1.57	1.10	2.23	0.012	1.63	1.06	2.49	0.025
25-34	1.00	0.75	1.32	0.982	1.01	0.72	1.42	0.966
35-49	Ref				Ref			
First Antenatal visit type								
First Visit	2.21	1.68	2.92	<0.001	1.81	1.25	2.62	0.002
Follow-up visit	Ref				Ref			
Race								
African	Ref				Ref			
Other	2.39	1.60	3.57	<0.001	2.94	1.92	4.51	<0.001
Number of live babies								
None	Ref				Ref			
One	0.94	0.67	1.33	0.733	0.90	0.60	1.35	0.610

Two	0.94	0.66	1.34	0.748	0.88	0.58	1.34	0.557
Three	0.82	0.53	1.25	0.353	0.97	0.57	1.64	0.911
Four or more	1.36	0.81	2.31	0.247	1.59	0.83	3.06	0.162
Gestational age group								
First trimester	Ref				Ref			
Second trimester	1.20	0.85	1.69	0.308	1.08	0.66	1.76	0.770
Third trimester	1.18	0.80	1.75	0.407	1.07	0.63	1.80	0.809
HIV status of current sexual partner								
HIV positive	Ref				Ref			
HIV negative	0.98	0.74	1.30	0.891	0.95	0.68	1.32	0.762
Don't know his/their status	1.74	1.36	2.23	<0.001	1.68	1.26	2.24	<0.001
Don't have a sexual partner	0.91	0.31	2.65	0.865	1.08	0.36	3.24	0.890
Timing of anti-retroviral therapy initiation								
Before pregnancy	Ref				Ref			
During pregnancy	2.03	1.65	2.50	<0.001	1.87	1.46	2.40	<0.001
Syphilis test result								
Positive	Ref				Ref			
Negative					1.15	0.65	2.04	0.632

Models were constructed using a complete case analysis

In Model V (VL ≥1000 copies/ml), the odds of being a suspected virologic failure were significantly higher among second-line ART users compared to those using first-line ART [aOR=2.60 (95% CI: 1.57–4.32), p=<0.001]; those aged 15 to 24 years compared to 35 to 49 years [aOR=1.57 (95% CI: 1.10–2.23), p=<0.012]; among those attending their first antenatal visit compared to follow-up visits [aOR=2.21 (95% CI: 1.68–2.92), p=<0.001]; those who were classified as other racial group compared to Black African groups [aOR=2.39 (95% CI: 1.60–3.57), p=<0.001]; those who did not know the HIV status of their current sexual partner compared to those whose sexual partner was HIV positive [aOR=1.74 (95% CI: 1.36–2.23), p=<0.001]; and among those who started ART during pregnancy compared to those who started before pregnancy [aOR=2.03 (95% CI: 1.65–2.50), p=<0.001].

In Model VI (VL ≥50 copies/ml), the odds of being virally unsuppressed were significantly higher among second-line ART users compared to those using first-line ART [aOR=2.23 (95% CI: 1.20–4.17), p=0.012]; those aged 15 to 24 years compared to 35 to 49 years [aOR=1.63 (95% CI: 1.06–2.49), p=0.025]; among those attending their first antenatal visit compared to follow-up visits [aOR=1.81 (95% CI: 1.25–2.62), p=0.002]; those who were classified as other racial group compared to Black African groups [aOR=2.94 (95% CI: 1.92–4.51), p=<0.001]; those who did not know the HIV status of their current sexual partner compared to those whose sexual partner was HIV positive [aOR=1.68 (95% CI: 1.26–2.24), p=<0.001]; and among those who started ART during pregnancy compared to those who started before pregnancy [aOR=1.87 (95% CI: 1.46–2.40), p=<0.001].

In comparing Model V (VL ≥1000 copies/ml) and Model VI (VL ≥50 copies/ml), no factors were significantly associated with unsuppressed VL in only one model. All the significant associations observed in Model V were also present in Model VI.

When comparing all the models fitted with regimen type as an independent variable, the factors that were significant through all fitted models were the regimen type, age groups, first Antenatal visit type and HIV status of the current sexual partner. However, some factors, such as gestational age group, were significant from Model I to Model IV, while the number of live births was significant in all these models except for Model III. Syphilis test results were only significantly associated with viral load status in Model IV.

Table 3.6. presents a multivariate backwards stepwise logistic regression of factors associated with suspected virologic failure (VL ≥1000 copies/ml) among pregnant women on first-line and second-line art regimens. In both Model VII (first-line regimen) and Model VIII (second-line regimen), an increase maternal age was significantly associated with lower odds of failure in both models. Compared to women aged 15–24 years, those aged 25–34 years had significantly lower odds [Model VII: aOR=0.52; 95% CI: 0.43–0.63; p<0.001]. Additionally, the odds of being suspected as virologic failure were lower for women aged 35–49 years in both models [Model VII: aOR=0.47; 95% CI: 0.37–0.60; p<0.001; Model VIII: aOR=0.23; 95% CI: 0.10–0.53; p=0.001]. Women presenting for follow-up antenatal visits were less likely

to be classified as suspected virologic failures compared those attending their first visit [Model VII: aOR=0.30; 95% CI: 0.24–0.37; p<0.001]. Those initiated on ART during pregnancy had higher odds of suspected failure compared to initiating treatment before pregnancy [Model VII: aOR=2.28; 95% CI: 1.85–2.81; p<0.001].

Table 3.6. Factors Associated with suspected virologic failure (VL ≥1000 copies/ml) among pregnant women on first-line and second-line art regimens (constructed using a backwards stepwise logistic regression)

	Model VII (First regimen) (N=8190)				**Model VIII (Second regimen) (N=237)**			
Variables	**aOR**	**95% CI**		**p-value**	**aOR**	**95% CI**		**p-value**
Age group								
15-24	Ref				Ref			
25-34	0.52	0.43	0.63	<0.001	–	–	–	–
35-49	0.47	0.37	0.60	<0.001	0.23	0.10	0.53	0.001
First Antenatal visit type								
First Visit	Ref							
Follow-up visit	0.30	0.24	0.37	<0.001	–	–	–	–
Race								
African	Ref							
Other	1.43	0.94	2.17	0.094	–	–	–	–
Gestational age group								
First trimester	Ref				Ref			
Second trimester	1.46	1.12	1.90	0.005	1.86	0.82	4.20	0.135
Third trimester	1.40	1.04	1.88	0.028	–	–	–	–
Missing	1.29	0.92	1.80	0.143	–	–	–	–
Syphilis test result								
Positive	Ref							
Negative	–	–	–	–	–	–	–	–
Missing	1.27	1.04	1.54	0.016	–	–	–	–
Timing of anti-retroviral therapy initiation								
Before pregnancy	Ref							
During pregnancy	2.28	1.85	2.81	<0.001	–	–	–	–
Missing	4.48	3.74	5.37	<0.001	–	–	–	–
Number of live babies								
None	Ref				Ref			
One	–	–	–	–	–	–	–	–
Two	–	–	–	–	2.35	0.78	7.05	0.128
Three	–	–	–	–	6.53	1.83	23.27	0.004

Four or more	1.37	0.99	1.91	0.060	8.39	1.92	36.62	0.005
Missing	0.21	0.06	0.67	0.009	4.62	0.52	40.74	0.168
Relationship with father								
Married					Ref			
Living together/co-habiting	–	–	–	–	–	–	–	–
Not living together but in a relationship	–	–	–	–	3.23	1.33	7.84	0.010
No relationship	–	–	–	–	8.06	0.34	189.27	0.195

Models are constructed using a backwards stepwise logistic regression with the probability of removal of 20%

Women with three and four or more live children had higher odds of suspected failure compared to those with one child [Model VII: aOR=6.53; 95% CI: 1.83–23.27; p=0.004; Model VIII: aOR=8.39; 95% CI: 1.92–36.62; p=0.005, respectively]. Moreover, women in their second [Model VII: aOR=1.46; 95% CI: 1.12–1.90; p=0.005] and third trimesters [Model VII: aOR=1.40; 95% CI: 1.04–1.88; p=0.028] had significantly higher odds of suspected virologic failure than those in their first trimester. Lastly, in Model VIII, women who were not living with their partner but still in a relationship had increased odds of failure compared to married women [aOR=3.23; 95% CI: 1.33–7.84; p=0.010]. Model VII and VIII which present virologic failure stratified by regimen type present consistent findings with the previous models (Model I to Model VI) which adjusted for regimen type as an independent variable in the models.

Among all the significant variables identified, the direction and level of significance were consistent. Age was the only variable that remained significant across both models. In Model VII, which focused on individuals on a first-line regimen, significant predictors included the type of first antenatal visit, gestational age group, and timing of antiretroviral therapy (ART) initiation. In contrast, Model VIII, which examined those on a second-line regimen, found significance in the number of live babies and the relationship with the father. Notably, Model VIII had the smallest sample size (N = 237) due to the limited number of individuals on a second-line regimen. This smaller sample likely contributed to the wider confidence intervals observed in that model

4. Chapter 4: Discussion

This chapter discusses the findings of this study and further outlines the limitations, recommendations, opportunities for future research and conclusion.

4.1. Summary of findings

In this secondary analysis which aimed to describe the characteristics and the distribution of pregnant women who were on second-line regimen and determine the factors associated with viral suppression among those on second-line regimen compared to those on first-line regimen only 2.3% were on second-line regimen. Across viral load thresholds of ≥50 copies/ml and ≥1000 copies/ml, a significant proportion of pregnant women on second-line antiretroviral therapy were virally unsuppressed: 45.0% and 16.6% respectively. All fitted models showed that second-line ART users consistently showed a higher likelihood of being virally unsuppressed. Other factors associated with viral suppression in this population were the age of participants, first antenatal visit type, race group, gestational age, HIV status of current sexual partner, the number of live babies, the syphilis test result and timing of ART initiation.

The effect of regimen type on viral suppression

Studies focusing on viral suppression among Pregnant women living with HIV have found lower rates of virologic suppression among those on a second-line regimen compared to those on a first-line regimen (23,36) and amongst the broader population (26,33,34). A study investigating protease inhibitor-based antiretroviral therapy in treatment-naive HIV-1-infected patients identified a lower suppression rate amongst participants on protease inhibitor-based regimen, commonly used in second-line treatment compared to nucleoside reverse transcriptase inhibitors (commonly used in first-line regimen) (37). The Guideline for Vertical Transmission Prevention of Communicable Infections published by the South African NDoH specifies that all pregnant women and those currently breastfeeding, who are HIV positive need to be switched to a DTG-containing regimen (38). These findings stress the importance of optimizing regimen choices to enhance viral suppression and reduce vertical transmission risks.

The effect of age group on viral suppression

In this study, age was significantly associated with viral suppression. Youth aged 15 to 24 years consistently showed a higher likelihood of being virally unsuppressed across all models compared to those aged 35-49. Similar studies that focussed on viral suppression among HIV-positive individuals have observed lower rates of virologic suppression amongst the youth (39–41). These results are further supported by the 2022 antenatal survey of South Africa which found that pregnant women aged 15 to 19 years followed by those aged 20 to 24 had a higher likelihood of being virally unsuppressed compared to those aged 35–49 years (42). The South African National HIV Prevalence, Incidence, Behaviour and Communication Survey (2017) found that young women aged 15 to 24 years had the lowest rates of viral suppression at 47.0% compared to all other age groups (39). The findings suggest that age is a significant determinant of viral suppression, with younger pregnant women exhibiting a higher risk of being virally unsuppressed compared to their older counterparts.

The effect of the first visit type on viral suppression

This study found that women who presented themselves for the first antenatal visit had a higher likelihood of being virally unsuppressed compared to those who came for a follow-up visit. This relationship was consistent throughout all fitted models. The findings suggest that these pregnant women may have started ART but disengaged from care or were recently diagnosed HIV positive. Starting the ANC clinic early could be prompted by the perceived risk of VT among those who had disengaged from ART. This perception aligns with findings suggesting that women prioritize VT prevention once pregnant (43), but gaps in ART retention and adherence programs that fail to fully engage and support women before pregnancy persist (43,44). Advancing ART retention programs towards these non-pregnant women is a need to prevent a lack of adherence and promote viral suppression before pregnancy.

The effect of race group on viral suppression

The findings revealed that those classified as Other racial groups (Whites, Indian/Asian and Coloured) had a higher likelihood of being virally unsuppressed. There is limited information on viral suppression by different racial groups among pregnant women. South African population-based survey study that investigated the disparity in virological suppression amongst individuals of different races, refutes these findings and suggests that black individuals have a higher likelihood of being virally unsuppressed compared to other races (45). The rate of suppression in the 2017 SABSSM survey among South African individuals by race was estimated to be 87.3% among Black Africans and 92.7% among those classified as Other population group (45). Although the two studies are invaluable in assessing viral load suppression in South Africans it should be noted that the difference in population and the methodological techniques hinders fair comparability between the two studies, also there were relatively few women enrolled from other races. The SABSSM is a population-based survey that is comprised of a nationally representative sample and data is collected at both the individual level and household level, while the ANC survey focuses on pregnant women as the study population and data is collected only at an individual level. Additionally, SABSSM used small layer areas as a sampling unit compared to the ANC survey that used public facilities as a sampling unit. However, both studies use laboratory facilities to test for HIV and viral load levels in their study participants.

The effect of gestational age at enrolment on viral suppression

This study revealed that the relationship between viral load suppression and gestational age varied based on how the model was fitted and the viral load threshold used in the model. The multivariable model built using a DAG showed that those in their second and third trimesters had a higher likelihood of being virally unsuppressed which is consistent with previous findings (46), except for Model II when it comes to third trimester which was not statistically significant. However, no significance was observed in the full case analysis multivariable models. One study reported that viral suppression did not vary by gestational age (24). However, other studies have observed such variation, suggesting that viral suppression rates can fluctuate across trimesters (46,47). These findings underscore the complexity of achieving viral suppression during pregnancy and suggest that model specifications and data handling can influence observed relationships. These findings further highlight the need to increase monitoring of viral load suppression in later stages of pregnancy. This entails enhancing adherence counselling and more frequent viral load testing.

The effect of the HIV status of a current sexual partner on viral suppression

The UNAIDS emphasises the use of barrier methods and other preventative techniques for individuals who are unaware of their sexual partner's HIV status (48). Pregnant women who did not know their sexual partner's status had a higher likelihood of being virally unsuppressed across all fitted models. The study investigating HIV viral load non-suppression and associated factors among pregnant and postpartum women in rural north-eastern South Africa found that women who were unaware of their partner's HIV status had increased odds of viral non-suppression (23), thus supporting the findings of this study. The lack of awareness among pregnant women regarding their partner's HIV status suggests poor communication and a lack of adequate support from their partners.

The effect of the number of live babies on viral suppression

A Pearson chi-square test was used to test for collinearity (49) between gravidity and parity (43). An association between gravidity and parity was observed $p<0.001$. One of the recommended techniques to handle collinearity is to remove one of the variables and retain the one which provides the most information (49,50). In this study, parity was retained as it provided more information compared to gravidity (Table 1). Those with four or more live babies had consistently higher odds of being virally unsuppressed on Model I, II and III compared to those who had no live babies. Limited research looking at this association is available. One possible explanation for the observed trend is that high-parity women may experience unique barriers to maintaining viral suppression. Although they may have been on

ART longer, other factors such as caregiving demands, limited resources, and potential lapses in consistent healthcare engagement could interfere with optimal adherence.

The effect of the syphilis test result on viral suppression

This study showed that syphilis status was associated with viral load status of the pregnant women although this relationship was only significant in model IV with a VL ≥50 copies/ml threshold. Those who tested negative for syphilis had a higher likelihood of being virally unsuppressed compared to those who tested positive. These results are counterintuitive and may be related to more intensive monitoring and treatment for women diagnosed with syphilis during pregnancy. Research conducted in KwaZulu-Natal, South Africa, found that pregnant women who have ever tested positive for an STI were more likely to achieve viral suppression compared to those who have not (51). While other research did not observe any statistical difference in VL by syphilis status (46). Research often focuses more broadly on the impact of other sexually transmitted infections (STIs) on viral suppression, highlighting that co-infections can complicate HIV management (52,53).

The effect of the timing of ART initiation on viral suppression

The two most critical factors in predicting perinatal VT are the maternal HIV viral load and the timing of ART initiation (54). This research further revealed that pregnant women who initiated ART during pregnancy had a higher likelihood of being virally unsuppressed compared to those who initiated ART before pregnancy. These results were consistent through all the models fitted, except for Model IV. A previous study focussing on the progress towards the UNAIDS 95-95-95 targets among pregnant women in South Africa, that utilised both the 2017 and 2019 antenatal HIV sentinel survey found that pregnant women who were initiated ART during pregnancy had a lower odd of being virally suppressed, thus supports the findings of this study (55). Early initiation allows for better management of viral loads(56,57). Women who begin ART during pregnancy may have a shorter duration to achieve viral suppression compared to those who start earlier. A need to increase awareness and access to preconception care for women of reproductive age, particularly those at higher risk for HIV. This includes counselling on reproductive health and the importance of ART. Unintended pregnancies play a pivotal role in this issue. A Systematic Review and Meta-analysis found that unintended pregnancy was significantly higher in HIV-positive women than for HIV-negative women (58). Unintended pregnancies are often associated with late booking for antenatal care, which delays ART initiation among women not previously on ART or those who have disengaged from care. A need for better access to contraception for HIV-positive women to mediate the risk of unplanned pregnancy.

4.2. Interventions to improve viral suppression during pregnancy

Researchers have recommended encouraging early antenatal care bookings and early initiation of antiretroviral therapy within the general population could enhance viral suppression rates among pregnant women (24). Studies have assessed the combined interventions focused on enhancing participants' knowledge of prescribed ART through individualized counselling or group sessions, educating women about the HIV risk of VT and the importance of ART adherence (59,60). A study assessing the challenges in VTP and antiretroviral adherence in northern KwaZulu-Natal, South Africa found that sub-optimal adherence to antiretroviral therapy was attributed to misconceptions, domestic violence, poverty, and issues related to disclosure and stigma (59). Two studies examined the impact of male partner involvement on women's adherence to VTP services, including initiation, adherence, and continuation of ART. Interventions involved male partners accompanying pregnant female partners to antenatal clinics for HIV testing and counselling. The male partner provided support to his female partner to initiate, adhere to, and continue taking ART during pregnancy (61,62). While increasing male attendance at antenatal clinics is necessary, it is not enough to boost VTP uptake in South Africa. Increasing HIV knowledge and encouraging active male participation through psychoeducational interventions could be a more effective strategy(60). A randomised control trial exploring the interventions aimed at improving VT prevention by integrating routine antenatal care and ART services in one clinic. The randomised control trial found that the service integration improved maternal HIV care enrolment and HAART uptake, but VTP utilization outcomes remained poor and postnatal service use remained poor across both arms (63).

4.3. Limitations and Strengths

The secondary analysis included only pregnant women who attended antenatal care in public institutions and thus the results obtained from the analysis may not be generalizable to the entire population of pregnant women in South Africa especially those who attended in private institutions for antenatal checks. The researcher acknowledges that not all variables associated with the viral load suppression were included in the data collection and thus will not form part of the analysis, such as the use of mental health status, psychosocial support, substance abuse, adherence, etc. The cross-sectional nature of the study design limits the ability to establish causal relationships as the outcome and exposure are measured concurrently (64).

Statistical differences were observed between the included and excluded groups regarding several key variables. Included participants had a higher proportion of virally suppressed individuals compared to excluded participants, potentially overestimating the prevalence of viral suppression in the overall population. Such disparities introduce selection bias, potentially affecting the generalisability of the findings. This study used some self-reported explanatory variables such as the HIV status of the current sexual partner. The use of self-reported variables introduces the possibility of response bias as a form of social-desirability bias, where participants want to look good to the investigator (65). This can be mediated by introducing a way to measure these variables such as testing for the sexual partner.

Missing data presented an additional limitation. Various models were applied to assess the impact of missing data on the estimates. Models III and IV included missing categories for variables with over 5% missing data, with a sample size of approximately 8339, while Models V and VI were limited to complete cases, reducing the sample size (Model V with 3041 and Model VI with 2481 participants). The presence of missing data introduced the potential for residual bias, even after model adjustments. If regimen type was inconsistently represented across models due to missing data, this may affect the accuracy and reliability of the estimates.

One other notable limitation of this analysis is the presence of wider confidence intervals, particularly in Model VIII. This model had a smaller sample size (N = 237), due to the relatively limited number of individuals receiving second-line antiretroviral therapy. The reduced statistical power in this subgroup may have led to less precise estimates, as reflected in the wider confidence intervals, and may have limited the detection of potentially meaningful associations. Additionally, both Model VII and Model VIII were developed using stepwise regression procedures. While stepwise methods can be useful for variable selection, they also carry inherent limitations, including the risk of overfitting, biased coefficient estimates, and the potential exclusion of variables that are theoretically important but did not meet strict statistical entry criteria. However, it was selected due to the small number of sample size for Model VIII which prevented the model from converging.

This study draws on a large, nationally representative dataset, enabling the findings to be generalised to the wider population of pregnant women attending public antenatal care in South Africa. The use of survey weights throughout the analysis further enhances the representativeness of the results. Additionally, this study employed rigorous statistical methods, including the use of multiple multivariable models to account for missing data and assess the robustness of the findings. Finally, the research addressed an important public health priority by focusing on maternal viral suppression in the context VTP, contributing valuable insights toward achieving the UNAIDS 95-95-95 targets.

4.4. Conclusion

In conclusion, this secondary analysis provided valuable insights into the factors influencing viral load suppression among pregnant women on ART in South Africa. This study showed that pregnant women on second-line ART are achieving viral suppression, though at lower rates compared to those on first-line ART. The findings reveal that those on second-line ART, younger women, and those unaware of their partner's HIV status face higher odds of being virally unsuppressed. Additionally, women with multiple live births and those initiating ART during pregnancy also show an increased risk of being virally unsuppressed. These results emphasise the need for a targeted intervention to improve viral suppression outcomes among pregnant women, particularly among high-risk groups identified in this research. Addressing these disparities is crucial for achieving the UNAIDS 95-95 targets and improving maternal and child health outcomes. The study's contributions highlight the complex interplay of demographic, clinical, and social factors in achieving viral suppression, underscoring the importance of a multifaceted approach to HIV management in pregnant women.

4.5. Recommendations

- Strengthen adherence counselling and sexual reproductive health education for pregnant women on ART, especially those on second-line regimen. This could help in managing and mitigating the risks such as VT and inter-partner transmission of HIV.
- Advance youth-targeted interventions which target education, mental health support, and adherence counselling could improve viral suppression rates and aid in lowering teenage pregnancy.
- Strengthen ART programs for non-pregnant women by providing tailored education and counselling during family planning.
- Increase HIV screening for women in their reproductive age who present in health institutions for health-related checkups.
- Implement targeted research and intervention strategies that seek to identify the underlying factors contributing to viral load disparities among different racial groups. Key areas of investigation should include access to healthcare, socioeconomic status, cultural attitudes towards HIV, and the availability of supportive services.
- Educational programmes already offered to pregnant women should emphasise the importance of knowing their partner's HIV status. Additionally, the education programmes should integrate the health and wellness conversations particularly targeting U=U as a key topic. Integrating partner testing and counselling into routine antenatal care could be beneficial.
- The unexpected finding regarding syphilis status and VL underscores the need for further investigation into the relationship between STI status and HIV management. Ensuring robust screening and treatment for syphilis and other STIs remains critical for pregnant women.

- Overall, improving ART adherence is crucial for achieving and maintaining viral suppression. Implementing comprehensive adherence support programmes, including counselling, education, and access to resources, will be key to improving ART outcomes for pregnant women and the overall success of the VTP.

4.6. Suggestions for future research

Future research should include longitudinal studies comparing the long-term efficacy of first-line versus second-line ART regimens in pregnant women. Such studies would provide crucial insights into how these regimens perform over the duration of pregnancy and the postpartum period, helping to inform and refine treatment guidelines. Additionally, there is a pressing need to develop and evaluate targeted interventions specifically for younger pregnant women aged 15-24 years. This age group demonstrates a higher likelihood of being virally unsuppressed, and tailored interventions could address their unique challenges and improve their ART adherence and outcomes.

Addressing racial disparities in ART outcomes is another critical area for future research. Investigating the underlying causes of these disparities and developing targeted interventions can help ensure equitable access to ART and support services, ultimately improving health outcomes across different racial groups. Another important focus should be on examining how different trimesters impact viral load suppression, taking into account various ART regimens and levels of adherence. Understanding this relationship will enable the optimization of ART management throughout pregnancy.

Finally, exploring the impact of partner HIV status awareness on ART adherence and viral suppression is essential. Research in this area could highlight how improving knowledge and communication about partner HIV status influences ART outcomes, particularly for pregnant women who are unaware of their partner’s HIV status. Addressing these key areas will significantly contribute to enhancing ART effectiveness and overall health outcomes for pregnant women living with HIV.

Declaration

We acknowledge the use of Grammarly Inc (Version 1.96.0.0) as a language editing tool to refine grammar, punctuation, and overall writing style in this report. The software is accessible at https://www.grammarly.com

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6. Annexure

6.1. Plagiarism declaration report

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I __**Sbonelo Charles Chamane**__ (Student number: __**2726849**__) am a student registered for the degree of __**MSc Epidemiology and Biostatistics**__ in the academic year __**2024**__.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: __[signature]__ Date: __**02 February 2025**__

6.2. Turnitin report signed by supervisor

MSc Report final version for Turnitin.docx

Supervisors Statement:

I have reviewed the student's work and can confirm that there is no plagiarism. The Turnitin report highlights technical terms and phrases that cannot be altered without altering accuracy-

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6.3. Two-proportion power calculation

```
. power twoproportions 0.109 0.168, n1(8368) n2(238) alpha(0.05)

Estimated power for a two-sample proportions test
Pearson's chi-squared test
H0: p2 = p1  versus  Ha: p2 != p1

Study parameters:

        alpha =    0.0500
            N =     8,606
           N1 =     8,368
           N2 =       238
        N2/N1 =    0.0284
        delta =    0.0590  (difference)
           p1 =    0.1090
           p2 =    0.1680

Estimated power:

        power =    0.7762
```

6.4. Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Mr Sbonelo Charles Chamane M240423 MED24-01-403

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M240423 MED24-01-403

NAME: (Principal Investigator)	Mr Sbonelo Charles Chamane
STUDENT/STAFF NO:	2726849
DEGREE:	MSc Epidemiology and Biostatistics
DEPARTMENT:	South Africa
PROJECT TITLE:	Factors affecting viral load suppression amongst pregnant women on second line Antiretroviral therapy in South Africa 2022
DATE CONSIDERED:	01/03/2024
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Mr Tendesayi Kufa-Chakezha
APPROVED BY:	Paul Ruff Prof P Ruff, Chair, HREC (Medical)
DATE OF APPROVAL:	22/04/2024 **EXPIRY DATE:** 22/04/2029

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

DECLARATION OF INVESTIGATORS

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to Committee. **I agree to submit a yearly progress report** in July each year until study is closed. Failure to submit annual report will result in ethics clearance certificate suspension. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application invalidate the clearance given by the HREC (Medical). Email signed copy of this ethics clearance certificate prior to commencing with the study hrec-medical.researchoffice@wits.ac.za

Chamane 23/04/2024

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

6.5. Approval to use the dataset

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

Centre for HIV and Sexually Transmitted Infections
1 Modderfontein Road, Sandringham, 2031
Tel: 011 386 6400 Fax:
Reference:

23rd January 2024

Mr. Sbonelo Chamane
1 Biccard Street
Braamfontein
2017

Dear Mr. Chamane,

Re: Permission to use the 2022 ANC survey data for research project

I confirm the receipt of your request to use data from the 2022 ANC survey for your research project titled "Factors affecting viral load suppression amongst pregnant women on second-line ART in South Africa, 2022." I am pleased to grant you access to the data you require for this analysis. I appreciate your commitment to handling the data with the utmost confidentiality and ensuring its use solely for the specified research purpose.

Please contact Dr Tendesayi Kufa-Chakezha, our Senior Epidemiologist to arrange the transfer of this data. Please note that this access is granted on condition that you provide ethical clearance from the Wits Human Research Ethics Committee. It is crucial to adhere to all ethical standards and guidelines in the pursuit of your research.

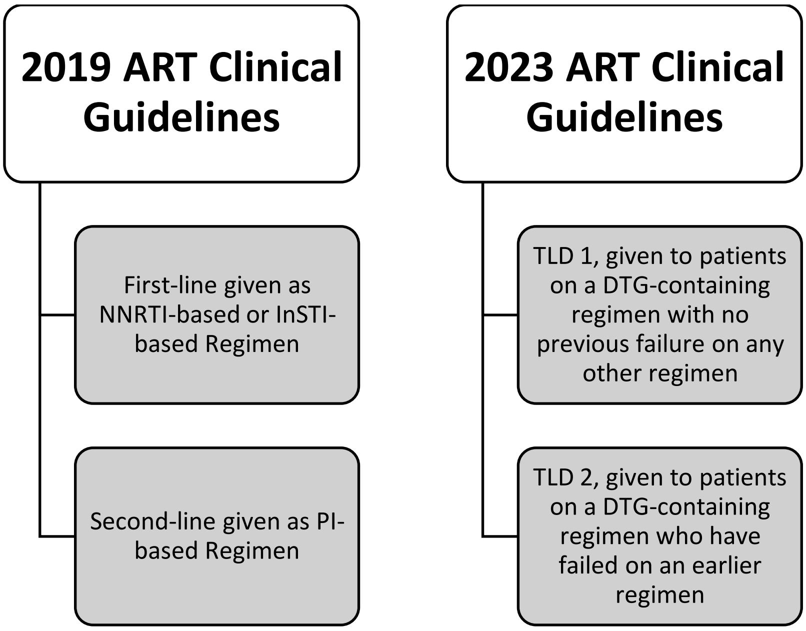
I wish you success in your analysis, and I look forward to the valuable insights your study may contribute to the field.

If you have any further questions or require additional support, feel free to reach out.

Best regards,

Prof Adrian Puren
Head of Centre for HIV and STIs

6.6. Timeline of ART clinical guidelines



6.7. Data collection form used for primary study

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

EPISODE NO. BARCODE FOR LAB USE

NATIONAL 2019 ANTENATAL SENTINEL HIV SURVEY, SOUTH AFRICA

GAUTENG

GEOGRAPHIC

A. GEOGRAPHIC INFORMATION

A1. Province: Eastern Cape ☐ Free State ☐ Gauteng ☑ KwaZulu-Natal ☐ Limpopo ☐ Mpumalanga ☐ Northern Cape ☐ North West ☐ Western Cape ☐

A2. District: Ekurhuleni ☐ Johannesburg ☐ Sedibeng ☐ Tshwane ☐ West Rand ☐

A3. Name of Sentinel Clinic ______

A4. Facility Code (see back of the cover page for code) ______ A5. Client Folder / HPRN Number ______

SPECIMEN

B. SPECIMEN INFORMATION

B1. Collection Date D D M M 2019 B2. Test Requested ☑ HIV Elisa ANS

C. Survey enrolment questions ***(Note: the following section should be completed for all women including those who refuse to participate in the survey)***

C1. Are you pregnant? Yes ☐ No ☐

C2. How old are you (in years)? ☐☐

C3. What is your race? African ☐ Asian ☐ Coloured ☐ White ☐ Other ☐

C4. What is your relationship with the father of the child?

Married ☐

Living together/co-habiting ☐

Not living together but in a relationship ☐

No relationship ☐

C5. Is this her first antenatal clinic visit? (review medical record):

Yes ☐ No 2nd visit ☐ No 3rd visit ☐ No ≥ 4th visit ☐

D. Consent for participating in current survey

It has been explained to me and I understand what the antenatal survey is about, and I agree to participate in the survey

Yes ☐ No ☐

Participant signature Date Nurse's signature Date

E. Consent for storage and future use of your information and blood sample

I agree to the use of blood specimen for future studies Yes ☐ No ☐

Participant signature Date Nurse's signature Date

Nurses name (please print as it appears on ID): ______

F. Demographic and clinical information

F1. How old is the father of this baby? ☐☐ Don't know ☐

F2. Is the father of this baby older than you by 5 years or more? Yes ☐ No ☐ Don't know ☐

F3. What is your highest completed level of education? None ☐ Primary ☐ Secondary ☐ Tertiary ☐

F4. How many times have you been pregnant including this current pregnancy? 1 (one) ☐ 2 (two) ☐ 3 (three) ☐ 4 (four) and above

F5. How many live babies have you delivered? 0 (zero) ☐ 1 (one) ☐ 2 (two) ☐ 3 (three) ☐ 4 (four) and above

F6. Gestational age at today's visit (in weeks) (review medical record) ☐☐

F7. Gestational age at first antenatal care booking (in weeks) (review medical record) ☐☐

F8. Just before I became pregnant:

- I intended to get pregnant ☐
- My intention kept changing ☐
- I did not intend to get pregnant ☐

F9. In the month that I became pregnant:

- I/we were not using contraception ☐
- I/we were using contraception but not on every occassion ☐
- I/we were always using contraception but knew the method had failed (i.e. broke, moved, cameoff, came out, not worked etc) at least once ☐
- I/we were always using contraception ☐

G. HIV status

G1. Have you been tested for HIV before this pregnancy, if yes when?

- Never tested before this pregnancy ☐
- Tested <= 1 year before this pregnancy ☐
- Tested 1-2years before this pregnancy ☐
- Tested >2 years before this pregnancy ☐

G2. What is the participant's latest HIV status (from her medical records)?

- No HIV status recorded ☐
- Participant refused testing ☐
- Test not offered ☐
- Negative from test done in previous ANC visit ☐
- Negative from test done today ☐
- HIV positive ☐
- Discrepant result ☐

G3. If HIV positive, when was she first diagnosed HIV positive?

Positive before this pregnancy ☐ Tested positive during previous antenatal visit ☐ Tested positive today ☐

G4. If reported HIV positive, is she on ARVs? If yes when did she start (review medical record):

- Yes, before pregnancy (i.e. already on ART at time of first antenatal visit) ☐
- Yes, initiated today ☐
- Yes, initiated in prior antenatal visit at 1st trimester ☐
- Yes, initiated in prior antenatal visit at 2nd trimester ☐
- Yes, initiated in prior antenatal visit at 3rd trimester ☐
- No ☐

G5. If taking ARVs, which ARV regimen is she taking (review medical record):

Dolutegravir (DTG)-based regimen (TLD) ☐ Efavirenz (EFV)-based regimen ☐ Second line regimen ☐

Other (specify)________________

H. Viral load testing (if reported HIV positive complete this section by reviewing the participant's medical record)

H1. Is there a record showing viral load (VL) test was done for the participant during pregnancy?

Yes ☐ No ☐ Not due for viral load ☐

H2. If VL was done, has the VL test result been documented? Yes ☐ No ☐

H3. If VL is documented, what is the most recent VL result from test done during this pregnancy?

<=49 copies/ml ☐ 50-999 copies/ml ☐ >=1000 copies/ml ☐

H4. Date of most recent VL test DD/MM/YYYY

I. If the participant is HIV negative ask the following question:
This section is about pre-exposure prophylaxis (PrEP). PrEP is a pill taken by HIV negative people to reduce the risk of getting HIV

I1. In the past 12 months, I had:

More than 1 sexual partners ☐ Had only 1 partner ☐

I2. My current sexual partner(s) is/are:

HIV positive ☐ HIV negative ☐ Don't know his/their status ☐ Don't have sexual partner ☐

I3. Do you consider yourself to be at risk of getting HIV: Yes ☐ No ☐

I4. Have you ever heard of PrEP before today? Yes ☐ No ☐

I5. Have you been taking PrEP before this pregnancy? Yes ☐ No ☐

I6. Are you currently taking PrEP? Yes ☐ No ☐

J. SYPHILIS TESTING

J1. Is there a record showing syphilis test was done for the participant during this pregnancy? Yes ☐ No ☐

J2. If syphilis test was done, which type of test was done RPR ☐ point of care rapid test ☐

J3. If syphilis test was done, what is the result? Positive ☐ Negative ☐ Pending (in lab) ☐ Not in file ☐

J4. If the result was positive, did the patient receive treatment for syphilis? Yes ☐ No ☐

J5. If she did receive treatment, was this Benzathine Penicilin (Bicillin)? Yes ☐ No ☐

This questionnaire will be re-designed to fit in one page (back to back print) and to make it readable on OMR scanner