**Research Report**

# Factors associated with virologic failure among patients receiving antiretroviral therapy in Butha – Buthe district, Lesotho

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**Declaration**

I, Khotso Maile, Student Number: 850303, declare that this research report is my own work. I am submitting it in partial fulfilment of the requirements of the Master of Science in Epidemiology in the field of Epidemiology and Biostatistics at the University of the Witwatersrand, School of Public Health. This report had not been submitted before for any degree at any other university. Acknowledgements and required referencing conventions have been adhered to where thoughts and ideas of others have been used.

Date: 01 October 2019

Signature: KMaile

**Dedication**

I dedicate this work to my late mother Mapinki Maile, my sisters ‘Malesala and Teboho Maile who supported me throughout my studies.

**Abstract**

**Background**

A substantial number of patients in Lesotho fail to achieve viral load suppression. However, evidence on the predictors of viral load non – suppression is still scarce in the country. This study aims to determine predictors of virologic failure among patients receiving antiretroviral therapy in Butha – Buthe district 12 months after the introduction of routine viral load (VL) monitoring.

**Methods:**

The study was based on secondary data analysis of an on-going dynamic cohort of 4448 Butha – Buthe ART patients receiving VL monitoring during December 2015 to November 2016. Patients were selected for participation if they had been on ART for six or more months and had at least one viral load measurement. Medians (inter-quartile range (IQR)) and frequency tables were used to describe continuous and categorical variables respectively. Stepwise model building technique was used to develop the multivariable logistic regression model which was used for determining factors associated with virologic failure (≥1000copies/ml).

**Results:**

Of 4448 patients who participated in the study, the median age 43 years (IQR 35 – 53) and patients were predominantly females, 3073 (69.1%). The prevalence of virologic failure was 7.4%. In multivariable analysis, being on alternative 1$^{st}$ line ART regimen [(AZT + 3TC + EFV) or (ABC + 3TC + EFV)] [(Adjusted Odds Ratio (AOR) 3.67, 95% CI 2.16, 6.25; p<0.001)] increased the odds of virologic failure compared to being on the preferred 1$^{st}$ line (TDF + 3TC + EFV). Similarly, patients who were virologically monitored because they

were being targeted for immunological failure (AOR 8.25, 95% CI 1.08, 63.05; p=0.042) and for clinical failure (AOR 9.53, 95% CI 2.79, 32.57; p<0.001) had increased odds of virologic failure compared to routine viral load testers. Missing a single or more doses of ART in past 30 days (OR 2.91, 95% CI 1.45, 5.83; p=0.003) significantly increased the odds virologic failure compared to adhering well. But, being a breastfeeding mother (AOR 1.45, 95% CI 0.83, 2.53; p=0.187) was not associated with virologic failure.

**Conclusion:**

The study results identified some of the predictors of virologic failure in and groups at increased risk of virologic failure in Lesotho. Targeted interventions such as enhanced adherence counselling are recommended for these failure groups.

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**Nomenclature**

| | |
|---|---|
| 3TC | Lamivudine |
| ABC | Abacavir |
| AIDS | Acquired Immune Deficiency Syndrome |
| AOR | Adjusted odds ratios |
| ART | Antiretroviral therapy |
| ARV | Antiretroviral |
| ATV/r | Atazanavir/ritonavir |
| AZT | Azidothymidine |
| CAG | Community ART groups |
| CI | Confidence Interval |
| EAC | Enhanced adherence counselling |
| EFV | Efavirenz |
| HBV | Hepatitis B virus |
| HIV | Human Immunodeficiency Virus |
| HR | Hazard ratio |
| HIV RNA | Human Immunodeficiency Virus Ribonucleic Acid |
| IQR | Inter quartile range |
| LIS | Laboratory Information System |
| LPV/r | Lopinavir |
| MOH | Ministry of Health |
| NNRTIs | Non-nucleoside reverse transcriptase inhibitors |
| NRL | National Referral Laboratory |
| OR | Odds Ratio |

| | |
|---|---|
| Swiss TPH | Swiss Tropical and Public Health Institute |
| TB | Tuberculosis |
| TDF | Tenofovir |
| UNAIDS | United Nations Programme on HIV/AIDS |
| VIRAL LOAD | Viral load |
| WHO | World Health Organization |

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## Chapter 1: Introduction

This chapter begins with an overview of the burden of Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) globally, in East and Southern Africa as well as in Lesotho. It then provides a brief history of the provision of antiretroviral care in the country. This is then followed by a reviewed of published literature on factors associated with virologic outcomes. Finally, it describes the research question and objectives.

### 1.1 Background

In 2017, around 36.9 million people were HIV positive globally; 19.6 million of which were in Eastern and Southern Africa (1). Since the HIV and AIDS epidemic began, approximate 77.3 million individuals globally have been infected, resulting in about 35 million deaths (2). However, there had been a surge in antiretroviral treatment coverage in recent years which had resulted in decreases in AIDS related deaths (3). New HIV infections worldwide, declined by an estimated 400 000 from 2.2 million in 2010 to 1.8 million in 2017 (2). Even though new infections were declining globally, Eastern and Southern Africa had the highest number of new HIV infections (790, 000) in 2016 (3). About 5000 new infections occurs daily in the world, 64% them in Sub-Saharan Africa (3).

The prevalence of HIV in Lesotho is 25.6% among individuals aged 15 - 59 years, which is the second highest in the world (4). The prevalence is 30.4% among women and 20.8% among men (4). The study region Butha – Buthe district has the lowest HIV prevalence (17.8%) in the country (4). An estimated 306, 000 adults aged 15 – 59 years survives with HIV and AIDS in the Lesotho (4). Of those, approximately 41% were put on antiretroviral therapy by the end of 2014 (5). The annual HIV incidence among adult population (15 – 59 years) is approximately 1.47%. The incidence is highest among female (1.74%) than among male (1.22%) (4).

Health care services in the country are provided by a network of 372 health facilities at three levels, which are primary, secondary and tertiary levels. Butha – Buthe district have 16 health facilities comprising two hospitals, ten health centres, and four privately owned facilities. Since 2004, the Lesotho Ministry of Health (MOH) has provided care and treatment for HIV infected people through nurse based health care services (6). During this period, the criterion for initiating patients on ART was a CD4 cell count not exceeding 200 cells per $mm^3$. However, as of 2007 the was criteria was revised and increased to 350 cells per $mm^3$; and in 2012, a new strategy, option B+ was introduced to avert HIV transmission from mother to child (5).

As of 2014, the threshold for initiating adolescent and adult patients on ART in Lesotho was set as CD4 cell count not more than 500 cells per $mm^3$ (6). HIV infected pregnant and lactating women, HIV positive under five children, HIV positive partners in sero – discordant couples, HIV/TB and HBV/HIV co-infected patients are placed on lifelong ART treatment regardless of their CD4 count (6). Tenofovir (TDF) based regimen had been provided as an ideal first line treatment since 2010 in the country (5). This includes once daily single fixed dose combination of ARV tablets used for ART treatment in the country (6).

Based on the World Health Organization (WHO) recommendation, the MOH, in 2014, updated its guidelines to include the usage of routine viral load (VL) testing as the ideal technique for confirmation of ART treatment failure (6,7). In December 2015, routine viral load monitoring was initiated in Butha – Buthe district in a partnership between the MOH, Solidarmed and the Swiss Tropical and Public Health Institute (Swiss TPH). During the first six months of implementation, routine viral load testing was provided for Butha – Buthe hospital patients only. Patients from other facilities in the district were only considered for targeted viral load testing when they were suspected of treatment failure (6). However, beginning June 2016, routine viral load monitoring was rolled out to the other hospital

(Seboche) and the rest of the health centres in the district. Routine viral load testing services are not provided for private facilities. Prior to this viral load testing was conducted in the National Referral Laboratory (NRL) for patients with suspected treatment failure (targeted viral load monitoring) only. This was determined through clinical and immunological criteria.

### 1.2 Literature Review

Achieving the last 90 of the UNAIDS 90 90 90 targets, viral load suppression among ART patients, depends on a number of factors, of which receiving uninterrupted ART is critical (8–12). It also depend on the sustained use of viral load monitoring for confirmation of ART treatment success and for distinguishing between poor adherence and treatment failure (7,12). Thus minimizing unneeded switches to 2$^{nd}$ line antiretroviral regimens and reducing the hazards of drug resistance mutations (7).

Viral load suppression refers to viral load below a detection threshold using the viral assay, usually fewer than fifty copies per millilitre (7). Currently, the recommended WHO threshold for suppression is viral loads fewer than a thousand copies per millilitre (7). Recent studies had shown a strong association between good adherence and virologic suppression (13–16). Good adherence is defined as the accurate and timeous consumption of 95% to 105% of antiretroviral medication (5). A recent study conducted among 138 ART patients with unsuppressed viral load, indicated that enhance adherence counselling after failure led improve virologic outcomes (17).

Wide scale up of ART had been suggested to led to suppression, thus reducing the risk of HIV transmission at population level (18). Studies conducted in both low and middle income as well as high income settings had demonstrated that early ART initiation and well – timed

linkage to care were some of the key factors which led to shorter time to viral suppression (18). High frequency of health facilities visits by ART patients were also reported to be associated with quicker time to viral suppression (19).

Furthermore, clinical factors such as high patient weight (13), and longer duration on ART (13), as well as low viral load at ART commencement have been shown to predict viral load suppression (10). In addition, disclosures of one's own HIV status to their partners increase the odds of viral suppression compared to not disclosing (20).

A cohort of ART naïve minors aged above two years, had shown that initiating ART with lopinavir boosted with ritonavir increased likelihood of achieving viral load suppression than initiating ART with non-nucleoside reverse transcriptase inhibitors based regimens (NNRTIs) (21). Another study conducted among 137 adults patients with mean age 39, reported an association between first line regimen and viral suppression, but highlighted that poor virologic outcomes were experienced by 22% of individuals treated with (NNRTIs) (22).

In a recent cohort conducted among 836 pregnant women in Philadelphia, United States Of America, it was indicated that HIV diagnosis and ART initiation prior to pregnancy as well as adequate prenatal care increases the chances of achieving viral load suppression (23). Moreover, higher antenatal care attendance had been associated with the ability to suppress viral load (13). In another study conducted among 217 pregnant mothers on third trimester, it was shown that viral suppression was common among expectant mothers who initiated on ART at 14 weeks of pregnancy than among those who initiated ART at 28 weeks of pregnancy (13). Women initiated on lifelong ART during pregnancy (option B+) achieves higher rates of viral suppression, even several years after initiation (20).

Other studies had indicated that the benefits of early ART initiation, uninterrupted ART and timely linkage to care may be eroded by factors such as patients loss to follow up (24–26).

While others have shown that tuberculosis, WHO clinical stages three and four (27) and history of treatment interruptions (22) increases the risks of viral load failure among ART patients.

Viral load failure is the incapability of an ART patient to attain and sustain viral load below detection cut off point (28). The World Health Organisation defines it as two successive viral load above 1000 copies per millilitre taken within three months, with enhanced adherence counselling (7). However, the actual threshold for defining viral load failure has not yet been confirmed (7). As a result, researchers use different criterions for defining virologic failure. In this study, viral load failure is defined as a thousand or more plasma viral load copies per millilitre.

Studies had shown that the likelihood of having an unsuppressed viral load was high among underweight individuals (26). Similarly, having a low CD4 cell count (CD4 cell< 200 cell/$mm^3$) strongly predicts the inability to achieve viral suppression (29,30) and vice versa (31). In contrast, an association between elevated viral load and CD4 count as high as 500 cells/$mm^3$ had been shown in a cohort conducted among 1211 ART naïve patients (32).

Recent studies had suggested a strong association between failure to suppress and persistent low level viremia (viral load ranging from fifty to a thousand copies per millilitre) (27,33–35), especially among ART experienced individuals (35). Low level viremia does not only occur as results variations in assay as was previously suggested, but rather due to virus replication (27). However, no association was observed between very low level of viremia (viral load ranging from fifty to 199 copies per millilitre) and viral load failure (36).

In a cohort study conducted in Uganda, it was indicated that treatment with a combination: stavudine (d4T), lamivudine (3TC) and nevirapine (NVP) predicts viral load failure compared to treatment with a combination: zidovudine (ZDV), lamivudine (3TC) and

efavirenz (EFV) in adult patients (37). A systematic review assessing the harms and benefits of switching to tenofovir (TDF) based regimen among ART experienced patients, reported that using tenofovir (TDF) based regimen was not significantly associated with elevated viral load (38).

In addition, studies had demonstrated an association between high pill burden and poor adherence as well as virologic failure (20). Patients treated with didanosine – based regimens were highly likely to experience poor virologic outcomes compared to patients treated with lamivudine (3TC) – based regimens (39). Similarly, patients treated with boosted protease inhibitors based regimen were more likely to fail to suppress virally compared to those treated using NNRTIs (40).

Even though the longer duration on ART had been shown to be linearly related to viral suppression (31,41), a recent study reported an association between more time on ART and unsuppressed viral load (42). In contrast, another study conducted in rural Lesotho reported no association between time on ART and level of viral load (17).

A recent study had shown that patients residing in rural areas have poor adherence and poor virologic response than those residing in urban areas (43). Other studies had also reported higher odds of virologic failure among patients residing far away from the health facilities (44) and those receiving ART services at any other facility than major hospitals (39). Receiving ART services at public health facilities increases the odds of viral failure compared to receiving ART at private health facilities (26). It had also been demonstrated that missed clinic appointments among HIV patients were associated with failure to suppress (45).

Participation in sex trade had been identified as another risk factor of virologic failure (46). It had been demonstrated that financial insecurity among women and vehicle ownership

among males predicts failure (20). Moreover, psychological factors such as isolation from the society and stigmatization had also been suggested to be some of the predictors of virology failure (29). A recent study had revealed that restricted access to ART care because of incarceration was associated with suboptimal adherence to treatment which in turn led to poor virologic outcomes (47). Food insecurity and starvation predicts ART non-adherence and depression, stigma, substance abuse and HIV-related social stressors which results in elevated viral load (48). Persistent high viral load had also been shown to be predicted by illegal drugs use and heavy alcohol drinking (49) as well as tobacco use (20). Being unaware of one's own HIV status significantly increase the odds of viral load detectability than knowing one's own status (50).

A recent study conducted in England reported that lower socio – economic status and poor ART adherence were strongly associated and hence predicts elevated viral load (51). Being an unemployed migrant reduced the likelihood of initiating ART and thus results with elevated viral loads (40). Factors such as ethnicity and low educational attainment are other predictors of failure (17,46,47).

Studies had indicated that virologic failure was highly likely to occur among males than among females (30,32,41). However, another study reported no significant difference in virologic outcomes between females and males (29). Patients aged 40 years or younger were reported to be at a higher risk of experiencing viral load failure than were older individuals (30,45). A recent study conducted among children, highlighted that children were more likely to experienced virologic failure than adults and that among children male gender was an independent risk factor for elevated viral load (37). Another study conducted in Swaziland also indicated that poor virologic outcomes were most likely to occur among patients of younger age and those with advanced stage of HIV (19).

## 1.3 Problem Statement

Studies conducted in Lesotho prior to the implementation of routine viral load monitoring have reported viral load failure rates of 27.7% among children under the age of 16 years (11,52) and 7% among adults aged 16+ years (52,53). The recent LePhia survey reported the prevalence of viral load failure as 32.4% among adults aged 15 – 59 years in the country (4). Obviously, achieving 100% viral suppression among ART patients in Lesotho appears to be a challenge. Studies have also shown that individuals with detectable viral load were highly likely to develop resistance to treatment (54). This, in turn, threatens the long-term success of ART treatment.

Evidence on why some patients are not achieving viral load suppression in Lesotho is very scarce. This study therefore aims to identify factors associated with virologic failure among patients receiving ART, 12 months after the introduction of routine viral load testing in Lesotho (December 2015 to November 2016).

## 1.4 Justification

Given different ART options, free adherence counselling, improved CD4 count threshold for initiating patients on ART (6) and lately the introduction of test and treat (5); it is unclear why some patients are not able to achieve virologic suppression. Only a few studies on this topic have been conducted in Lesotho. As a result, evidence of the factors associated with virologic failure in this setting is scarce. Generating more evidence will provide more understanding of these factors and in turn inform policy on how to prevent virologic failure.

### 1.5.0 Question

What are the factors associated with virologic failure among patients receiving antiretroviral therapy 12 months after the introduction of routine viral load testing in Butha – Buthe district in Lesotho?

### 1.5.1 Aim

To determine factors associated with virologic failure among patients receiving antiretroviral therapy 12 months after the introduction of routine viral load testing in Butha – Buthe district in Lesotho.

### 1.5.2 Objectives

1. To describe socio-demographic and clinical characteristics among patients receiving antiretroviral therapy 12 months after the introduction of routine viral load testing in Butha – Buthe district.
2. To determine proportion of patients with virologic failure among patients receiving antiretroviral therapy 12 months after the introduction of routine viral load testing in Butha – Buthe district.
3. To identify factors associated with virologic failure among patients receiving antiretroviral therapy 12 months after the introduction of routine viral load testing in Butha – Buthe district.

## Chapter 2: Methodology

This chapter describes the methodology used in conducting the study. It begins with study design followed by a description of the study setting, population and sampling technique for selection of study participants. It then proceeds with the description of data collection methods and variables used in the multivariable analysis of factors associated with virologic failure. This is followed by a description of the data management and statistical analysis plan. It closes with a presentation of the ethical considerations.

### 2.1 Study design

The study was based on secondary data analysis of an on-going open cohort of Butha – Buthe ART patients receiving viral load monitoring. ART patients' viral load testing records in Butha – Buthe hospital over a 12-month period, beginning in December 2015 to November 2016 were extracted and analysed.

### 2.2 Study site

The study used routinely collected data from the viral load platform database in Butha – Buthe district hospital, located in the northern region of Lesotho. In 2014, the total number of individuals newly initiated on ART in the district was 1461, while the total number of those surviving with HIV and AIDS and were currently on ART was 5542 (55).

### 2.3 Study Population

The study population were ART patients in Butha - Buthe district. Inclusion criteria was that a patient should (i) have evidence of being on ART for six or more months (ii) have been tested for viral load at least once (iii) be registered on ART at Butha - Buthe district or have proof of being transferred to Butha - Buthe district from other districts health facilities and

obtained the district ART number to be able to receive viral load testing on condition that criteria (i) was met.

### 2.4 Sampling

No sampling technique was applied. All 4448 patients meeting the inclusion criteria were included.

### 2.5 Data collection method

The dataset used in this study was from the viral load platform database. All samples for viral load testing in the district are sent to Butha-Buthe hospital and their results entered into the viral load database.

Routine viral load testing is done at half a year after ART initiation, at one year and then every 12 months afterwards (6). After conducting viral load tests, results and patients' demographic and clinical information (which are found in viral load request form) are then entered into the MOH Laboratory Information System (LIS). Thereafter, data in the form of a .csv file (Excel 2010) is transferred to the viral load platform database, where it is crossed checked against the request forms and then stored. In case of missing or incomplete data, the health facility in question is contacted for verification.

During routine collection of viral load data, the viral load platform was visited on three occasions to conduct data verification before the data could be used for analysis. During each visit, the data was cross checked between the platform and (i) Butha-Buthe hospital laboratory information system (LIS) (ii) viral load testing registers and (iii) patients ART Cards. Patients ART numbers were used to link information from the three sources to viral load platform. Inconsistent and missing data were identified and traced back on viral load testing register and patients ART cards, if found, were corrected. In cases where the ART

card had similar inconsistent or incorrect information as the platform, the ART clinic staff was made aware so that they could correct such errors during the next time the patient visit the health facility.

### 2.5.1 Variables

All information was collected during patients visit for viral load testing and includes demographic information (sex, age and facility) and clinical data such as time on ART; adherence to ART treatment (missing single dose or more of ART during the past 30 days); regimen type (which is defined as a combination of antiretroviral drugs that are prescribed to ART patients); CD4 count; results; intermittent low level viremia; reason for viral load test; pregnancy and breastfeeding. The outcome variable was viral load failure which is defined as plasma viral loads above 1000 copies per millilitre. Variables included in analysis were presented in the following manner:

**Table 1: Variables from the viral load database**

| **Variable** | **Variable type** | **Variable information** |
|---|---|---|
| *Exposure Variable* | | |
| Patient identifier | Numeric | Patients unique identity |
| Sex | Binary | 1- Male and 2 - female |
| Facility | Categorical | 1-Butha - Buthe hospital, 2-Seboche hospital and 3-All health centres |
| Age (in years) | Continuous | Patients age in years |
| Time on current regimen (in years) | Continuous | (current date) – (date of current regimen initiation) |
| Adherence/Missed | Binary (No or Yes) | Whether patient miss a single or more |

| | | |
|---|---|---|
| dose(s) | | dose(s) of ART in the last 30 days<br><br>1-No and 2-Yes |
| Regimen type | Categorical | 1- Preferred 1st line regimen:<br><br>TDF + 3TC + EFV as fixed-dose combination<br><br>2 - Alternative 1st line regimens: (AZT + 3TC + EFV) or (ABC + 3TC + EVF) |
| CD4 lymphocyte cell / $mm^3$ | Continuous | |
| Viral load copies / ml | Continuous | |
| Intermittent low-level viremia | Continuous | Viral loads between 50 and 1000 copies per millilitre |
| Reason for viral load test | Categorical | 1-Routine, 2 - targeted clinical failure, 3 - targeted immunological failure, and 4 -repeat after EAC |
| Pregnant | Binary (No or Yes) | Whether a woman was pregnant or not<br><br>1 – No and 2 – Yes |
| Breastfeeding | Binary (No or Yes) | Whether a woman was breastfeeding or not<br><br>1 – No and 2 - Yes |
| *Outcome Variable* | | |
| Viral load failure | Binary (≥1000copies/ml or <1000copies/ml) | Whether viral load was 1000+ copies per millilitre<br><br>0 - <1000copies/ml and 1 - ≥1000copies/ml |

### 2.5.2 Data Management

Datasets in .csv format (excel 2010) were exported from the viral load platform and imported to STATA 13™ StataCorp LP (College Station, TX, USA) for the analysis. Data on the viral load platform was cross-checked against data on the LIS, ART registers and ART cards. Data that was not relevant to the study was excluded from the datasets. Logical checks were conducted to identify inconsistencies in the data. For example, data was checked and corrected where men were indicated as pregnant or breastfeeding. Range checks using histograms and box plots for continuous variables were conducted to check for outliers.

## 2.6 Statistical Analysis Plan

### 2.6.1 Descriptive statistics

Histograms were used for graphical depiction of continuous variables. Medians (with inter-quartile range [IQR]) were presented for continuous variables regardless of the distribution of the data because for normally distributed data, the mean is approximately equivalent to the median while for skewed data it is mandatory to report the median. Non-linearity test was also conducted for all continuous variables to confirm whether they were linearly related to viral load. Categorical data was described using frequency tables. The prevalence of viral load failure was calculated as the proportion of individuals with a viral load result of 1000 or more copies per millilitre during the one-year study period.

### 2.6.2 Inferential Statistics

In univariate analysis, simple logistic regression was used to determine the association between individual predictors and the outcome variable. Stepwise model building technique was used for developing the final logistic regression model. The Pearson chi-square goodness

of fit test was conducted to assess whether the model was a good fit. Multivariable logistic regression was then used to investigate factors associated with virologic failure.

### 2.7 Ethical Considerations

In this study, data from routine patients' medical records was used. As a result, patients were not required to consent. Data that were used were extracted from a password protected electronic viral load database in Butha - Buthe hospital. Permission was obtained from Solidarmed and Swiss Tropical and Public Health Institute for use of the data from the viral load database. Access to the database for use of data is restricted to certain members who have signed a confidentiality agreement. Unique numbers were used in place of patients' names and ART numbers to protect patients' identity. Only the study principal investigator and co-supervisors have access to the data. Ethical approval was obtained from Ethics committees of the Lesotho MOH and University of the Witwatersrand. The ethical certificate numbers were ID130-2017 and M170568 respectively.

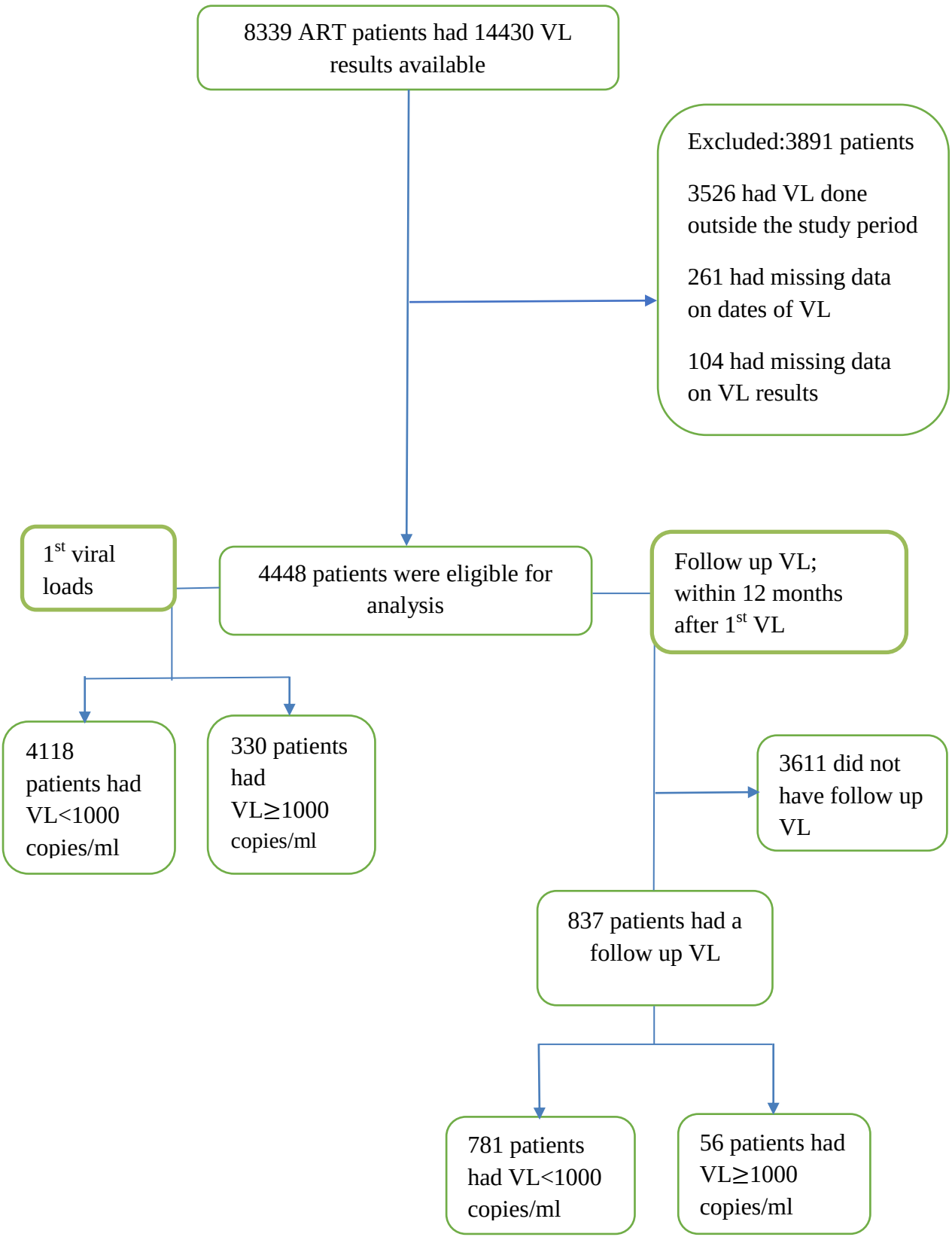
## Chapter 3: Results

The chapter begins with a flow chart of patients included and those excluded from the study, as well as the reasons of why they were included or excluded. This is then followed by a description of the baseline socio-demographic and clinical characteristics of the study participants. The proportions of patients with virologic failure are then presented. The final part of the chapter is a presentation of an association between virologic failure and socio-demographic and clinical characteristics.

### 3.1 Description of the patients' characteristics

Of 8339 ART patients with 14430 viral load results, 3891 (46.7%) patients did not meet the inclusion criteria: 3526 (42.3%) patients had viral loads done outside the study period (December 2015 to November 2016), while 261 (3.1%) patients viral loads which did not have dates of when they were conducted and data was missing on viral load results for 104 (1.2%) patients (Figure 1). A total of 4448 (53.3%) eligible patients were included in the study; of these 837 (10.0%) had follow up viral load measurements. Among the 4448 patients with first viral load results, 4118 (92.6%) had viral load <1000 copies per millilitre (suppressed) while 330 (7.4%) had viral load ≥1000 copies per millilitre (virologic failure). Of the 837 patients with follow up viral load, 781 (93.3) had viral load <1000 copies per millilitre while 56 (6.7%) had viral load ≥1000 copies per millilitre.

**Figure 1: Flow chart of patients on antiretroviral therapy in Butha - Buthe district included in the study**



### 3.2 Patients baseline socio–demographic and clinical characteristics

Patients' socio-demographic and clinical characteristics are presented in table 2 below. A total of 4448 patients received antiretroviral therapy in Butha – Buthe district between December 2015 and November 2016. Missing values were traced back to patients' files and where found were updated in the platform. A few missing data which could not be found in patients files were excluded from analysis. The median age of study participants was 43 (IQR 35 – 53) years. More females 3073 (69.1%) were in the study than males [1375(30.9%)]. Of the females, 67 (1.5%) were pregnant while 141 (3.2%) were breastfeeding. Majority of the patients 2606 (59.3%) were provided ART services at Butha – Buthe hospital.

Study patients had median CD4 lymphocyte of 222 (IQR 106 – 334) cells/$mm^3$. Patients were predominantly on the preferred 1$^{st}$ line (Tenofovir (TDF), Lamivudine (3TC) and Efavirenz (EFV)) [4239 (96.1%)] as fixed-dose combination. The median duration on antiretroviral therapy was 4.6 (IQR 2.6 – 7.0) years. While the median intermittent low - level viremia (among 488 patients with 50 - 999) was 132 (IQR 74.5 – 315.5) copies/ml. A majority of patients 4290 (97.9%) had not missed a single or more dose(s) of ART in the last 30 days. Almost all patients 4404 (99.0%) were provided testing for routine purpose.

**Table 2: Description of patients' socio–demographic and clinical characteristics (N= 4448)**

| **Characteristic** | **Number (n)** | **Percent (%)** |
|---|---|---|
| Age (in years), median (IQR) | 43 (35 - 53) | |
| Age groups | | |
| 18 - 19 | 46 | 1.0 |
| 20 - 24 | 155 | 3.5 |
| 25 - 29 | 304 | 6.8 |
| 30 - 34 | 598 | 13.4 |
| 35 - 39 | 688 | 15.5 |
| 40 - 49 | 1,198 | 26.9 |
| 50+ | 1,459 | 32.8 |

| | | |
|---|---|---|
| Sex | | |
| Female | 3073 | 69.1 |
| Male | 1375 | 30.9 |
| Facility | | |
| Butha – Buthe hospital | 2606 | 59.3 |
| Seboche hospital | 260 | 5.9 |
| All health centres | 1528 | 34.8 |
| CD4 cells / $mm^3$, median (IQR) | 222 (106 - 334) | |
| CD4 cells / $mm^3$ groups | | |
| <200 | 1901 | 45.5 |
| 200 - 349 | 1359 | 32.5 |
| 350 - 499 | 523 | 12.5 |
| 500+ | 397 | 9.5 |
| Regimen type | | |
| **Preferred 1st line:** TDF + 3TC + EFV | 4239 | 96.1 |
| **Alternative 1st line:** (AZT + 3TC + EFV) or (ABC + 3TC + EFV) | 173 | 3.9 |
| Time (in years) on ART, median (IQR) | 4.6 (2.6 – 7.0) | |
| Viral load results groups | | |
| <50 | 3670 | 82.5 |
| 50-999 | 488 | 10.1 |
| 1000+ | 330 | 7.4 |
| Intermittent low- level viremia, median (IQR) | 132 (74.5 – 315.5) | |
| Adherence (missed dose(s)) | | |
| No | 4290 | 97.9 |
| Yes | 92 | 2.1 |
| Reason for viral load test | | |
| Routine | 4404 | 99.0 |
| Targeted CF | 10 | 0.2 |
| Targeted IF | 29 | 0.7 |
| Repeat after EAC | 4 | 0.1 |
| Pregnant | | |
| No | 2991 | 67.5 |
| Yes | 67 | 1.5 |
| Male | 1375 | 31.0 |
| Breastfeeding | | |
| No | 2914 | 65.8 |
| Yes | 141 | 3.2 |
| Male | 1375 | 31.0 |

**Note**: 3TC: Lamivudine, ABC: Abacavir, ART: Antiretroviral therapy, ATV/r: Atazanavir/ritonavir, AZT: Azidothymidine, CF: Clinical Failure; EAC: Enhanced adherence counselling, EFV: Efavirenz, LPV/r: lopinavir, IQR: Inter quartile range, TDF: Tenofovir, VL: viral load

### 3.3 Proportion of ART patients with virologic failure

Overall, the proportion of patients with virologic failure was 7.4% with median age 37 (IQR 30 - 45) years which was lower than the overall median age. However, compared to patients with suppression, the median age was higher among those with suppression 43 (35 - 54) years than those with failure. The proportion of viral failure among females (7.8%); and was similar to the proportion of failure among males (7.3%). However, among patients on alternative 1$^{st}$ line regimen (AZT + TDF + EFV or ABC + 3TC + EFV) the proportion virologic failure was 19.1% while among patients on the preferred 1$^{st}$ line (TDF + 3TC + EFV) the proportion was failure was 6.8%. As expected, the median CD4 count [172 (65 – 303)] cells/mm$^3$ was lower among patient with virologic failure compared to those with suppression.

The proportion of failure among those who received ART services at Seboche hospital was 10.3% while amongst those who received ART services at Butha – Buthe hospital and at all health centres the proportions were 7.3% respectively. The proportion of virologic failure among patients who reported missing a single or more dose(s) of ART in the last 30 days (16.3%) was higher compared to the proportion of failure amid of those who reported good adherence (7.2%).

**Table 3: Characteristics of patients with viral load failure (N= 4448)**

| **Characteristic** | **Suppressed n (%)** | **Failure n (%)** |
|---|---|---|
| Overall | 4118 (92.6) | 330 (7.4) |
| Age (in years), median (IQR) | 43 (35 - 54) | 37 (30 - 45) |
| Age groups | | |
| 18 - 19 | 39 (84.8) | 7 (15.2) |
| 20 - 24 | 128 (82.6) | 27 (17.4) |
| 25 - 29 | 267 (87.8) | 37 (12.2) |
| 30 - 34 | 533 (89.1) | 65 (10.9) |
| 35 - 39 | 627 (91.1) | 61 (8.9) |
| 40 - 49 | 1125 (93.9) | 73 (6.1) |

| | | |
|---|---|---|
| 50+ | 1399 (95.9) | 60 (4.1) |
| Sex | | |
| Female | 1268 (92.2) | 107 (7.8) |
| Male | 2850 (92.7) | 223 (7.3) |
| Facility | | |
| Butha - Buthe hospital | 2417 (92.8) | 189 (7.3) |
| Seboche hospital | 232 (89.2) | 28 (10.8) |
| All health centres | 1416 (92.7) | 112 (7.3) |
| CD4 cells / $mm^3$, median (IQR) | 224 (110 – 337) | 172 (65 – 303) |
| CD4 cells / $mm^3$ groups | | |
| <200 | 1727 (90.9) | 174 (9.2) |
| 200 – 249 | 1275 (93.8) | 84 (6.2) |
| 350 – 499 | 499 (95.4) | 24 (4.6) |
| 500+ | 369 (92.9) | 28 (7.1) |
| Regimen type | | |
| **Preferred 1st line:** TDF + 3TC + EFV | 3951 (93.2) | 288 (6.8) |
| **Alternative 1st line:** (AZT+3TC+EFV) or (ABC+3TC+EVF) | 140 (80.9)) | 33 (19.1) |
| Time (in years) on ART, median (IQR) | 4.6 (2.6 – 7.0) | 4.8 (2.6 – 6.9) |
| Adherence (Missed dose(s)) | | |
| No | 3982 (92.8) | 308 (7.2) |
| Yes | 77 (83.7) | 15 (16.3) |
| Reason for viral load | | |
| Routine | 4092 (92.9) | 312 (7.1) |
| Targeted CF | 6 (60.0) | 4 (40.0) |
| Targeted IF | 16 (55.2) | 13 (44.8) |
| Repeat after EAC | 3 (75.0) | 1 (25.0) |
| Pregnant | | |
| No | 2780 (92.9) | 211 (7.1) |
| Yes | 57 (85.1) | 10 (14.9) |
| Male | 1268 (92.2) | 107 (7.8) |
| Breastfeeding | | |
| No | 2715 (93.2) | 199 (6.8) |
| Yes | 120 (85.1) | 21 (14.9) |
| Male | 1268 (92.2) | 107 (7.8) |

**Note**:3TC: Lamivudine, ABC: Abacavir, ART: Antiretroviral therapy, AZT: Azidothymidine, CF: Clinical failure, EAC: Enhanced adherence counselling, EFV: Efavirenz, IF: Immunological failure, TDF: Tenofovir, VL: Viral load

### 3.3.1 Subgroup analysis of patient with follow up viral loads

Of 837 patients with a follow up viral load measurement, 56 (6.7%) experienced virologic failure while 781 (93.3%) experienced virologic suppression. Of those with failure, 39

(69.6%) experienced virologic failure during their first viral load measurement, while 17(30.4%) experienced viral suppression during their first viral load measurement. Moreover, among those with failure, 92 (11.8%) experienced virologic failure during their first viral load measurement while 689 (88.2%) experienced viral suppression.

**Table 4: Subgroup analysis of patients with follow-up viral loads (N=837)**

| | **Follow up viral load n (%)** | |
|---|---|---|
| **First viral load** | **<1000 copies/ml** | **1000+ copies/ml** |
| **<1000 copies/ml** | 689 (88.2) | 17 (30.4) |
| **1000+ copies/ml** | 92 (11.8) | 39 (69.6) |
| **Total** | 781 (100.0) | 56 (100) |

### 3.4 Factors associated with virologic failure

The univariate and multivariable logistic regression models used to determine socio-demographic and clinical factors associated with virologic failure among study patients are depicted in Table 5 below. The study outcome was a dichotomous variable developed by categorizing the continuous HIV RNA variable in the following manner: All viral loads less than a thousand copies per millilitre were classified as virally suppressed while all viral loads 1000 or more copies per millilitre were classified as virologic failure. The odds ratios together with 95% confidence intervals and probability values (p-values) for each variable were also depicted.

Stepwise model building technique was used for selection of variables which were included in the final model. The level of significance used for selection of variables for the final multivariable model was p=0.20. The Pearson chi-square goodness of fit test was conducted to assess whether the final model was a good fit. The final multivariable logistic model had a Pearson chi-square goodness of fit of p=0.635 which indicated that the model had a good fit for the data used in this study. The following variables: pregnancy and time on ART were omitted in the final logistic model due to stepwise variable selection. While sex was omitted due to between terms collinearity. A Linear association between the HIV RNA and continuous variables was assessed. There was a statistically significant negative linear relationship between HIV RNA and both age and CD4 cell count. However, time on ART had a non-statistically significant negative linear relationship with the outcome.

In univariate analysis, age showed an association with virologic failure, but the odds of virologic failure were declining for yearly increase in age (OR 0.96, 95% CI 0.95, 0.97; p<0.001). No association was observed between time viral load failure and time on ART as yearly increase in time on ART did not show any effect in the odds of virologic failure (OR 1.00, 95% CI 0.96, 1.04; p=0.647). Similarly, being a male (OR 1.08, 95% CI 0.85, 1.37; p=0.537) did not have an effect on the odds of virologic failure compared to being a female.

The odds of virologic failure among patients receiving antiretroviral therapy at Seboche hospital (OR 1.54, 95% CI 1.04, 2.35; p=0.042) were 54% more than the odds of viral load failure among patients receiving antiretroviral therapy at Butha – Buthe hospital. However, for patients receiving ART therapy at all health centres (OR 1.01, 95% CI 0.79, 1.29; p=0.926) the odds of virologic failure were closer to the null and the confidence interval overlapped with the null. Thus, suggesting that receiving ART services at all health centres had no effect on the odds of virologic failure compared to receiving antiretroviral therapy at Butha – Buthe hospital.

CD4 cell count showed an association with virologic failure (OR 0.99, 95% CI 0.99, 1.00; p=0.001), but the odds of virologic failure were slightly decreasing with increasing CD4 cells/$mm^3$. Individuals on alternative $1^{st}$ line (AZT + TDF + EFV or ABC + 3TC + EVF) as fixed dose combinations (OR 3.23, 95% CI 2.17 – 4.81; p<0.001), were highly likely to have virologic failure compared to patients on the preferred $1^{st}$ line (TDF +3TC + EFV). Missing a single or more doses of ART in the 30 previous days (OR 2.52, 95% CI 1.43, 4.43; p<0.001) significantly increased the odds virologic failure compared to adhering well to antiretroviral therapy in the 30 past days.

The odds of virologic failure were greater among individuals whose reasons for viral load testing was targeted immunological failure (OR 10.66, CI 5.08, 22.35; p<0.001) and targeted clinical failure (OR 8.74, CI 2.45, 31.15; p=0.001) compared to routine testers. Though, being a repeat tester (OR 4.37, 95% CI 0.45, 42.15; p=0.202) increased the odds of viral load failure compared to routine testing, the relationship was not a statistically significant one. However, the confidence intervals were very wide indicating very lower precision of the odds ratios. Pregnant and or breastfeeding mothers were twice as likely to have virologic failure compared to non – pregnant mothers (OR 2.31, CI 1.16, 4.59; p=0.017) and non – breastfeeding mothers (OR 2.39, 95% CI 1.47, 3.88; p<0.001) respectively.

In multivariable logistic regression analysis, the odds of viral load failure remained unchanged for every yearly increase in age (AOR 0.95% CI 0.95, 0.96; p<0.001). But the odds were slightly reduced for any increase in CD4 cell/$mm^3$ (AOR 0.77, 95% CI 0.67, 0.92; p<0.001). Viral load failure among people on alternative $1^{st}$ line [(AZT + 3TC + EFV) or (ABC + 3TC + EFV) (AOR 3.67, 95% CI 2.16, 6.25; p<0.001)] was thrice as likely as viral load failure among those on the preferred $1^{st}$ line TDF+3TC+EFV. Similarly, viral load failure among patients who missed a single or more doses of ART in past 30 days (OR 2.91,

95% CI 1.45, 5.83; p=0.003) was twice as likely as the virologic failure among those who did not missed doses of ART.

Patients who were targeted for immunological failure (AOR 8.25, 95% CI 1.08, 63.05; p=0.042) and clinical failure (AOR 9.53, 95% CI 2.79, 32.57; p<0.001) were highly likely to have viral load failure compared to those who were monitored routinely. But, the confidence intervals were wide, suggesting a reduction in the precision of the odds ratios. The odds of viral load failure among breastfeeding mothers (AOR 1.45, 95% CI 0.83, 2.53; p=0.187) were 45% more than the odds of failure among non – breastfeeding mothers, even though the relationship was not statistically significant one.

**Table 5: Univariate and multivariable logistic regression analysis of factors associated with virologic failure (>1000 copies/ml)**

| **Characteristic** | **Univariate** | | **Multivariable** | |
|---|---|---|---|---|
| | **Unadjusted OR (95% CI)** | **P-value** | **Adjusted OR (95% CI)** | **P-value** |
| Age | 0.96 (0.95 – 0.97) | <0.001 | 0.95 (0.94 – 0.96) | <0.001 |
| Sex | | | | |
| Female | 1.00 | | | |
| Male | 1.08 (0.85 – 1.37) | 0.537 | | |
| Facility | | | | |
| Butha - Buthe hospital | 1.00 | | | |
| Seboche hospital | 1.54 (1.04 – 2.35) | 0.042 | | |
| All health centres | 1.01 (0.79 – 1.29) | 0.926 | | |
| CD4 cells / $mm^3$ | 0.99 (0.99 – 1.00) | 0.001 | 0.78 (0.67 – 0.92) | <0.001 |
| Regimen type | | | | |
| **Preferred 1st line:** TDF+3TC+EFV | 1.00 | | 1.00 | |
| **Alternative 1st line:** (AZT+3TC+EFV) or (ABC+3TC+EVF) | 3.23 (2.17 – 4.81) | <0.001 | 3.67 (2.16 – 6.25) | <0.001 |
| Time (in years) on ART | 1.00 (0.96 – 1.04) | 0.647 | | |
| Adherence (Missed dose(s)) | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 2.52 (1.43 – 4.43) | 0.001 | 2.91 (1.45 – 5.83) | 0.003 |
| Reason for viral load test | | | | |
| Routine | 1.00 | | 1.00 | |

| | | | | |
|---|---|---|---|---|
| Targeted CF | 8.74 (2.45 – 31.15) | 0.001 | 9.53 (2.79 – 32.57) | <0.001 |
| Targeted IF | 10.66 (5.08 – 22.35) | <0.001 | 8.25 (1.08 – 63.05) | 0.042 |
| Repeat after EAC | 4.37 (0.45 – 42.15) | 0.202 | 1.00 | |
| Pregnancy | | | | |
| No | 1.00 | | | |
| Yes | 2.31 (1.16 – 4.59) | 0.017 | | |
| Breastfeeding | | | | |
| No | 1.00 | | 1.00 | |
| Yes | 2.39 (1.47 – 3.88) | <0.001 | 1.45 (0.83 – 2.53) | 0.187 |

**Note:**3TC: Lamivudine, ABC: Abacavir, ART: Antiretroviral therapy, AZT: Azidothymidine, CF: Clinical failure, CI: Confidence interval, EAC: Enhanced adherence counselling, EFV: Efavirenz, IF: Immunological failure, IQR: Inter quartile range, OR: Odds ratio, TDF: Tenofovir, VL: Viral load

## Chapter 4: Discussion

The results of this study are discussed in this chapter. The aim of this study was to describe socio-demographic and clinical characteristics study participants, to determine the proportion of patients with virologic failure and to identify factors associated with virologic failure among patients receiving antiretroviral therapy 12 months after the introduction of routine viral load monitoring in Butha – Buthe district.

Overall, 7.4% of the study patients had viral loads failure. Similar results were reported by other studies conducted in the region (52,56). In contrast, the Lesotho population based HIV/AIDS impact assessment reported the virologic failure proportion of 31.5% in the study region (4). The low prevalence of unsuppressed viral load reported in the study may have been due to implementation of the "test and treat" strategy in 2016 in the country, which recommended ART initiation irrespective of CD4 count and WHO clinical staging (5), thus, leading to an increase in the number of stable individuals being initiated on ART. Or it may indicate that a substantial number of patients with virologic failure may have not been monitored at the time as routine viral load monitoring services were still very new in the country.

Contrary to other studies, this study reported that patients on alternative 1$^{st}$ line (AZT + 3TC + EFV or ABC + 3TC + EVF) had increased odds of virologic failure compared to those on the preferred first line (TDF + 3TC + EFV). This may have been due to poor adherence among those on alternative first line in the study region. In Lesotho, good adherence is defined as taking over 95% of medications accurately and timeously (5). This often led to a below detectable levels (< 40 copies per millilitre) decline in viral load in six months given over 95% of adherence to ART (5). A systematic review assessing the benefits and harms of switching to a TDF based regimen among ART patients reported no association between

taking a TDF based regimen and virologic failure (38). Another review comparing the harms and benefits of TDF based regimens to any other ART regimens, reported no difference between the groups on virologic failure (57). The ASSERT study comparing the safety of ABC and 3TC versus TDF and 3TC together administered with EFV, reported low virologic failure rates between both regimen types (58).

The findings of this study further revealed that missing a single or more doses of ART significantly increased the odds of virologic failure compared to no missed doses. Similarly, recent studies had demonstrated that missing doses of ART increased the risk of virologic failure (9,59–61), this eventually led to resistance to ART therapy (62). In Lesotho, poor adherence is not only caused by patients' forgetfulness of taking their medication, but by other factors such high patients burden on health care providers, rough terrain and long distances to health facilities coupled with unemployment no money for transportation which led to missed appointments, side effects caused by drugs and alcohol and substance abuse among others (5). Thus, indicating that the Ministry of Health may need to come up with differentiated models of care best suited to address patients' needs for ART refills, such as community ART dispensing and encouragement of patients to join community ART groups (CAGs). These models of care allows patients to take turns collecting medication at facilities, thus reducing high frequency and costs of visiting the facility per patient and improving treatment outcomes (63). The ministry may also need to prioritize resistance testing among patients with poor adherence.

Even though this study reported overall low proportion of viral load failure, groups such as those with CD4 count <200 cells/mm$^3$, non-pregnant and non-breastfeeding mothers had significantly high proportions of viral load failure. This may have been due to poor adherence among these groups. Of great concern were the 10.1% with intermittent low levels of viremia (≥50 – 999 copies/ml), as studies had shown that patients with virologic blips were at an

higher risk of failing to suppress their viral loads and consequently developing antiretroviral drug resistance mutation (33,64). However, in a secondary analysis of the VACH Cohort, no association was observed between low levels of viremia and viral load failure, independent of the frequency of virologic blips (36). Nevertheless, in the VACH cohort the thresholds for low levels of viremia was set at (20 – 50 viral load copies per millilitre) and for viral load failure at viral loads >200 copies per millilitre (36). These were lower than the WHO cut off for viremia and failure (7). A cohort assessing the association of different levels of low level viremia (50 – 499 viral load copies per millilitre) with viral load failure, the occurrence of AIDS, and mortality among HIV infected individuals, reported that low levels of viremia predicted viral load failure but did not predict the occurrence of AIDS event or death (34). These suggests that there may be a need to revise the current WHO threshold of viremia (HIV RNA in the range 50 to 1000 copies per millilitre) and virologic failure (1000 or more copies of viral load per millilitre) to much lower thresholds as in the VACH study to minimize the risk of AIDS and mortality.

The study results further demonstrated that the odds of unsuppressed viral load were significantly reduced amongst individuals with CD4 cells count exceeding 200 cells/$mm^3$ compared to those with low CD4 count. This results were consistent with those reported by other studies (29,31,32). Other studies have also shown that CD4 count less than 200 cells/$mm^3$ increased the risk of viral load failure (29) and death (31,65). However, in a recent study conducted among 1211 ART naïve individuals, virologic failure was associated with CD4 count in the range (351 – 500 cells/$mm^3$) (32). This might have been caused by delayed ART initiation as patients were, at that period, initiated on ART at CD4 count threshold of 350 cells/$mm^3$. Thus, placing them at risk of having elevated viral load even though their CD4 were above 350 cells/$mm^3$.

Even though majority of patients in this study received viral load for routine purposes, being targeted for immunological failure increased the odds of virologic failure compared to routine testing. This emphasizes the importance of using routine viral load monitoring because immunological failure is not as good in determining treatment failure (66). A similar study conducted Uganda reported that patients re-tested due to being suspected treatment failures recorded highest rates of non-suppression (67). The Lesotho guidelines for HIV & AIDS care and treatment indicates that for any viral load measure beyond a thousand copies per millilitre, improved adherence counselling sessions aimed at assessing any issues hindering adherence must be prioritized; Any untreated opportunistic infections or co-infections must be identified and the use of any traditional medicine which may interact with ART must be confirmed (5).

Although sex did not predict virologic failure in this study, we found that males had slightly increased odds of virologic failure compared to female. This was consistent with some studies (68,69) though others found it differently (references??). In Lesotho, more males than females are heavy alcohol drinkers who adheres poorly to their treatment (70). Heavy alcohol drinking (49) as well as tobacco use (20) predicts viral load failure. In a study assessing whether sex was associated with virologic failure among 2303 Burkinabe adult patients, it was reported that being a male was strongly associated with elevated viral load (30,68).

This study indicated that older age significantly decreased the odds of virologic failure. Recent studies had shown that younger individuals fail to suppress because they delay to start ART treatment (71), had lower adherence (72) and fears stigma attached to the treatment compared to older patients (73). Thus, indicating there may be a need for the ministry of health to come up with differentiated ART care models which may be best suited for improving the health outcomes of younger patients. Studies had also reported that the odds of virologic failure were higher among patients aged 40 years and below (30,40) than among

older individuals. Others have demonstrated that the odds of unsuppressed viral load declines as patients ages (67). In contrast, a prospective cohort of 1563 ART patients in Lesotho found no association was between age and virologic outcomes (17).

Although the effect of duration on ART on virologic outcomes had been investigated, conflicting results had been reported. Similar to a recent study conducted in Lesotho (17), no was association was observed between time on ART and virologic failure in this study. However, longer duration on ART had been shown to reduce the risk of virologic failure (31,74) while shorter duration on ART is associated with elevated viral load (41).

In this study, no significant association was observed between breastfeeding (at multivariable analysis level) and virologic failure. However, pregnancy strongly increased the odds of virologic failure (at univariate analysis level) compared to non-pregnancy. This may be due to good adherence during breastfeeding and knowledge as well as understanding of prevention of infection to infants by mothers. A recent cross sectional study in Uganda reported that pregnancy and breastfeeding were protective against virologic failure (67). The Lesotho guidelines for treatment and care of patients on antiretroviral therapy recommends that every HIV infected individual be put on ART irrespective of their CD4 count and WHO clinical staging (5). Nevertheless, factors such as multi-parity, initiating ART at third trimester and treatment interruptions increases the chances of virologic failure among pregnant mothers (15).

This study had several limitations. It may be subject to selection bias as it included majority of ART patients from Butha-Buthe hospital and may have left out some potential study participants from other facilities who may have not received their viral loads test measurements during the study period. The database did not have information on other important predictors of viral load failure such educational attainment, employment, marital

status, etc; as a result, their effect on the outcome may have been missed. A one year follow up time was short, and most individuals did not have a second viral load test result at the time of analysis. As a result, it was impossible to conduct survival analysis on time to virologic failure.

The study suggested an overall virologic failure prevalence of 7.4%, indicating an achievement of the last 90 in the UNAIDS 90 90 90 cascade at least among study patients retained in care. However, as majority of patients in the study were from Butha – Buthe hospital, therefore, the result of the study may not be generalisable to entire population surviving with HIV and AIDS in the country. A recent nationwide survey conducted in Lesotho in 2017 reported much higher viral load failure prevalence of 31.5% in Butha – Buthe district (4). This is threefold higher than the proportion reported in this study. The differences in proportions of virologic failure may have been due to differences in study designs employed by the two studies. The former was a cohort relying on patients to visit the health facilities and could have been affected by poor health seeking behaviour. While the latter was cross sectional in design and had an added advantage of reaching more HIV positive individuals as it was conducted at community level.

The results of this study may be of assistance to the Ministry of Health in identifying groups who are at great risk of virologic failure among patients on receiving antiretroviral therapy. This will provide the Ministry opportunities to prioritize and strengthen adherence measures among the various population groups.

### 4.1 Conclusion and recommendations

In conclusion, the results demonstrated that the prevalence of virologic failure was 7.4% among study patients, suggesting a success towards achieving the last 90 of the UNAIDS 90 90 90 targets. However, about 10% of patients among those with viral loads less than a

thousand copies per millilitre had virologic blips. The results further demonstrated that patients on alternative 1st line (AZT+3TC+EFV or ABC+3TC+EFV), those who reported missing doses of ART in past 30 days, those being targeted for immunological and clinical failure were at increased risk of virologic failure. Targeted interventions such as enhanced adherence counselling are recommended for these failure groups. Further research may be necessary to explore and determine causes of virologic failure among these population groups to provide more definite conclusions.

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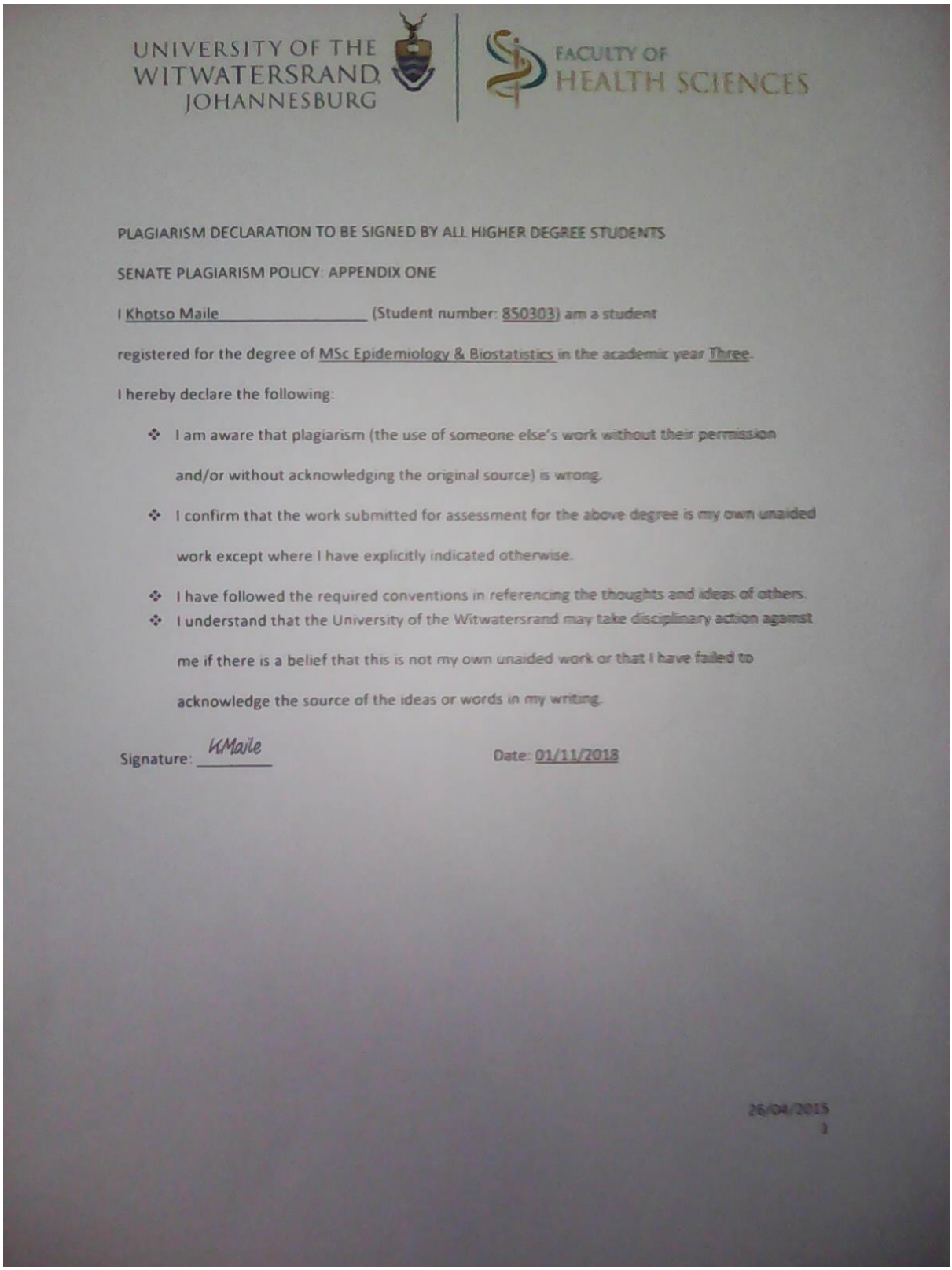
## Appendix 1: Plagiarism declaration report



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 Khotso Maile (Student number: 850303) am a student registered for the degree of MSc Epidemiology & Biostatistics in the academic year Three.

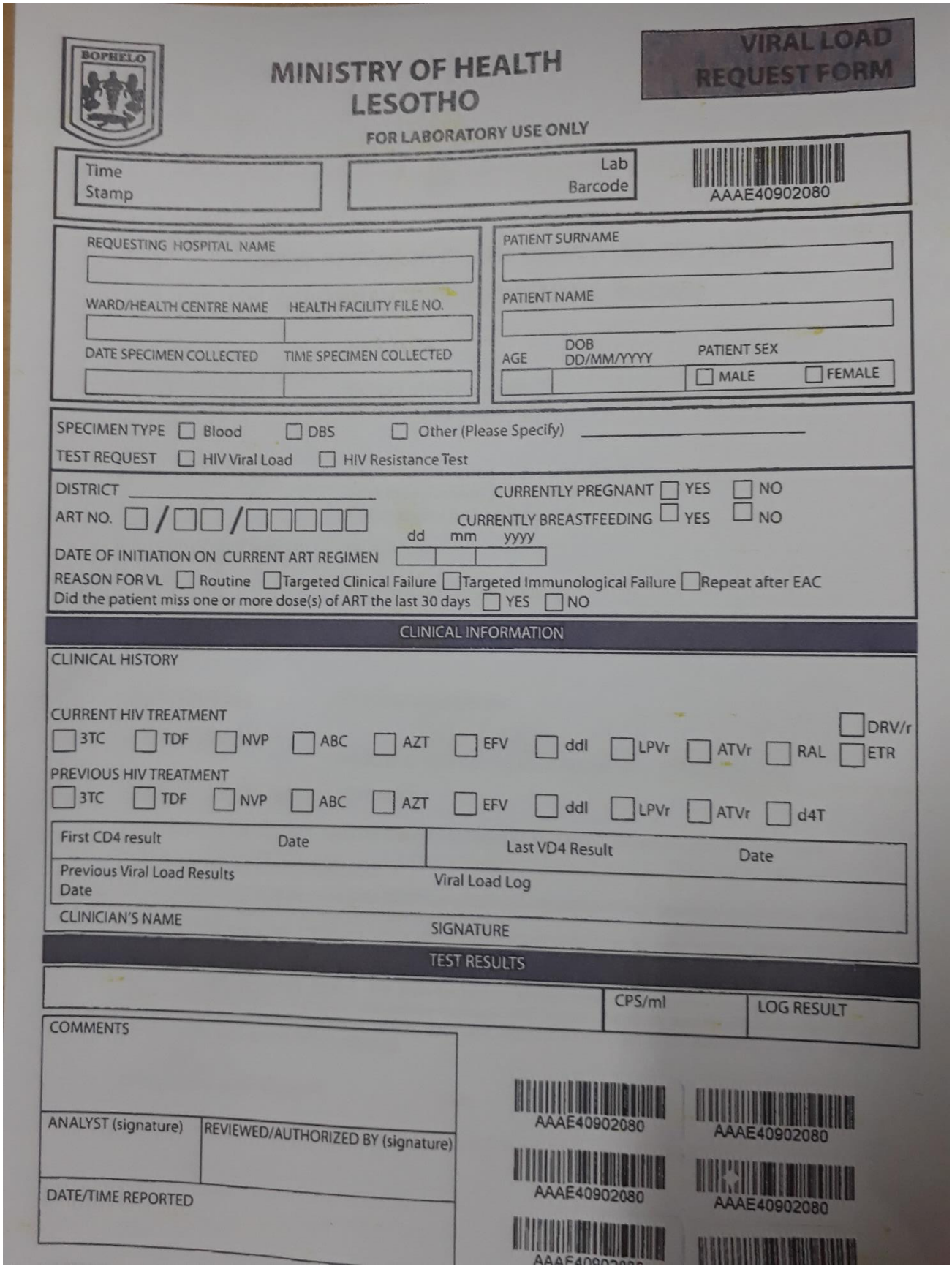
I hereby declare the following:

- 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.

Signature: KMaile Date: 01/11/2018

26/04/2015
1

**Appendix 2: Viral load request form (Data collection form)**



BOPHELO

MINISTRY OF HEALTH
LESOTHO

VIRAL LOAD REQUEST FORM

FOR LABORATORY USE ONLY

Time Stamp

Lab Barcode

AAAE40902080

REQUESTING HOSPITAL NAME

WARD/HEALTH CENTRE NAME | HEALTH FACILITY FILE NO.

DATE SPECIMEN COLLECTED | TIME SPECIMEN COLLECTED

PATIENT SURNAME

PATIENT NAME

AGE | DOB DD/MM/YYYY | PATIENT SEX ☐ MALE ☐ FEMALE

SPECIMEN TYPE ☐ Blood ☐ DBS ☐ Other (Please Specify) _____

TEST REQUEST ☐ HIV Viral Load ☐ HIV Resistance Test

DISTRICT _____

ART NO. ☐/☐☐/☐☐☐☐☐

CURRENTLY PREGNANT ☐ YES ☐ NO

CURRENTLY BREASTFEEDING ☐ YES ☐ NO

DATE OF INITIATION ON CURRENT ART REGIMEN dd mm yyyy

REASON FOR VL ☐ Routine ☐ Targeted Clinical Failure ☐ Targeted Immunological Failure ☐ Repeat after EAC

Did the patient miss one or more dose(s) of ART the last 30 days ☐ YES ☐ NO

CLINICAL INFORMATION

CLINICAL HISTORY

CURRENT HIV TREATMENT

☐ 3TC ☐ TDF ☐ NVP ☐ ABC ☐ AZT ☐ EFV ☐ ddI ☐ LPVr ☐ ATVr ☐ RAL ☐ DRV/r ☐ ETR

PREVIOUS HIV TREATMENT

☐ 3TC ☐ TDF ☐ NVP ☐ ABC ☐ AZT ☐ EFV ☐ ddI ☐ LPVr ☐ ATVr ☐ d4T

First CD4 result | Date | Last VD4 Result | Date

Previous Viral Load Results Date | Viral Load Log

CLINICIAN'S NAME | SIGNATURE

TEST RESULTS

CPS/ml | LOG RESULT

COMMENTS

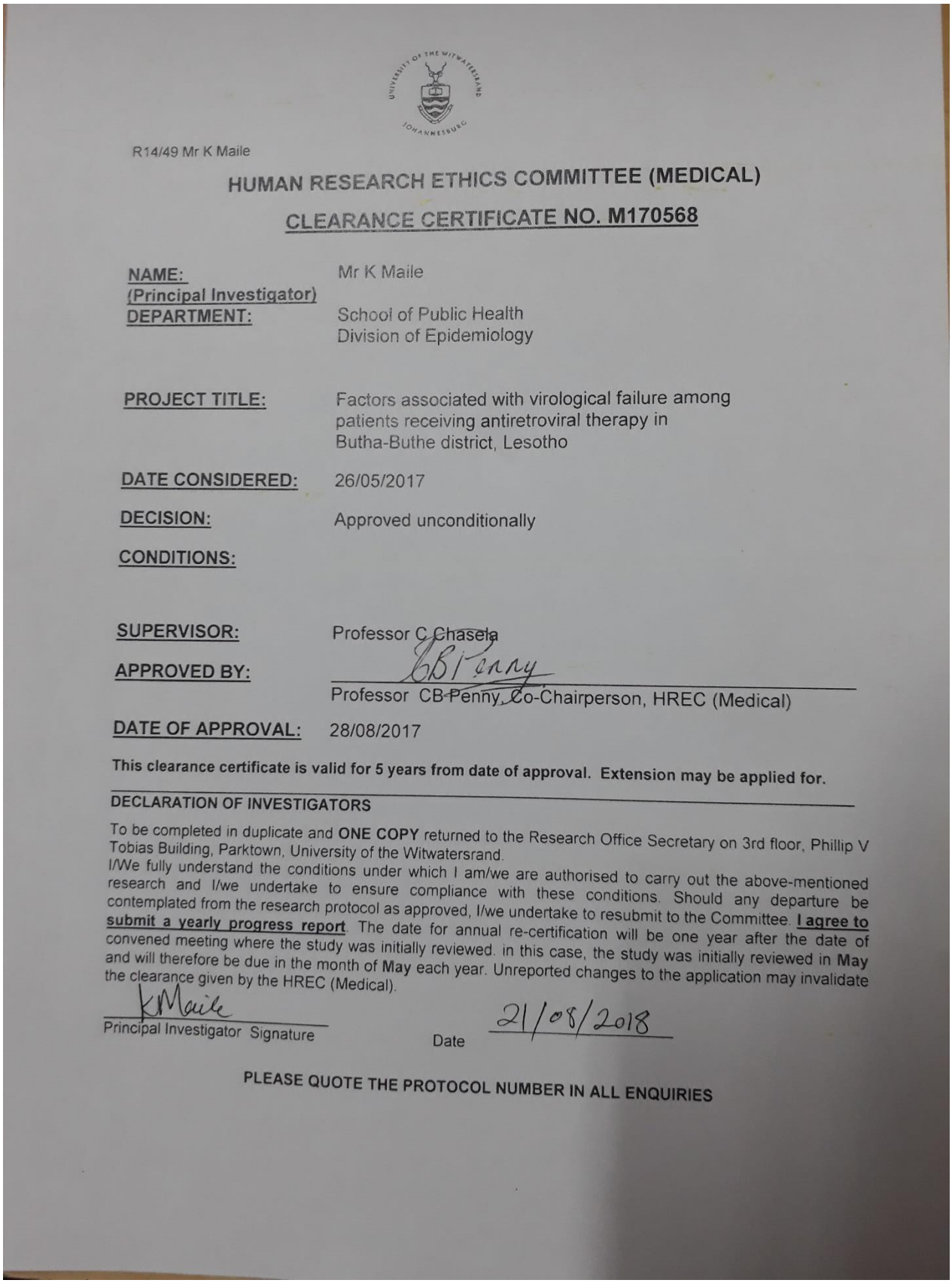
ANALYST (signature) | REVIEWED/AUTHORIZED BY (signature)

DATE/TIME REPORTED

AAAE40902080 AAAE40902080

AAAE40902080 AAAE40902080

## Appendix 3: Ethical clearance - Wits University



R14/49 Mr K Maile

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**

**CLEARANCE CERTIFICATE NO. M170568**

| | |
|---|---|
| **NAME:** (Principal Investigator) | Mr K Maile |
| **DEPARTMENT:** | School of Public Health<br>Division of Epidemiology |
| **PROJECT TITLE:** | Factors associated with virological failure among patients receiving antiretroviral therapy in Butha-Buthe district, Lesotho |
| **DATE CONSIDERED:** | 26/05/2017 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | Professor C Chasela |
| **APPROVED BY:** | CB Penny<br>Professor CB Penny, Co-Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 28/08/2017 |

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

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand.

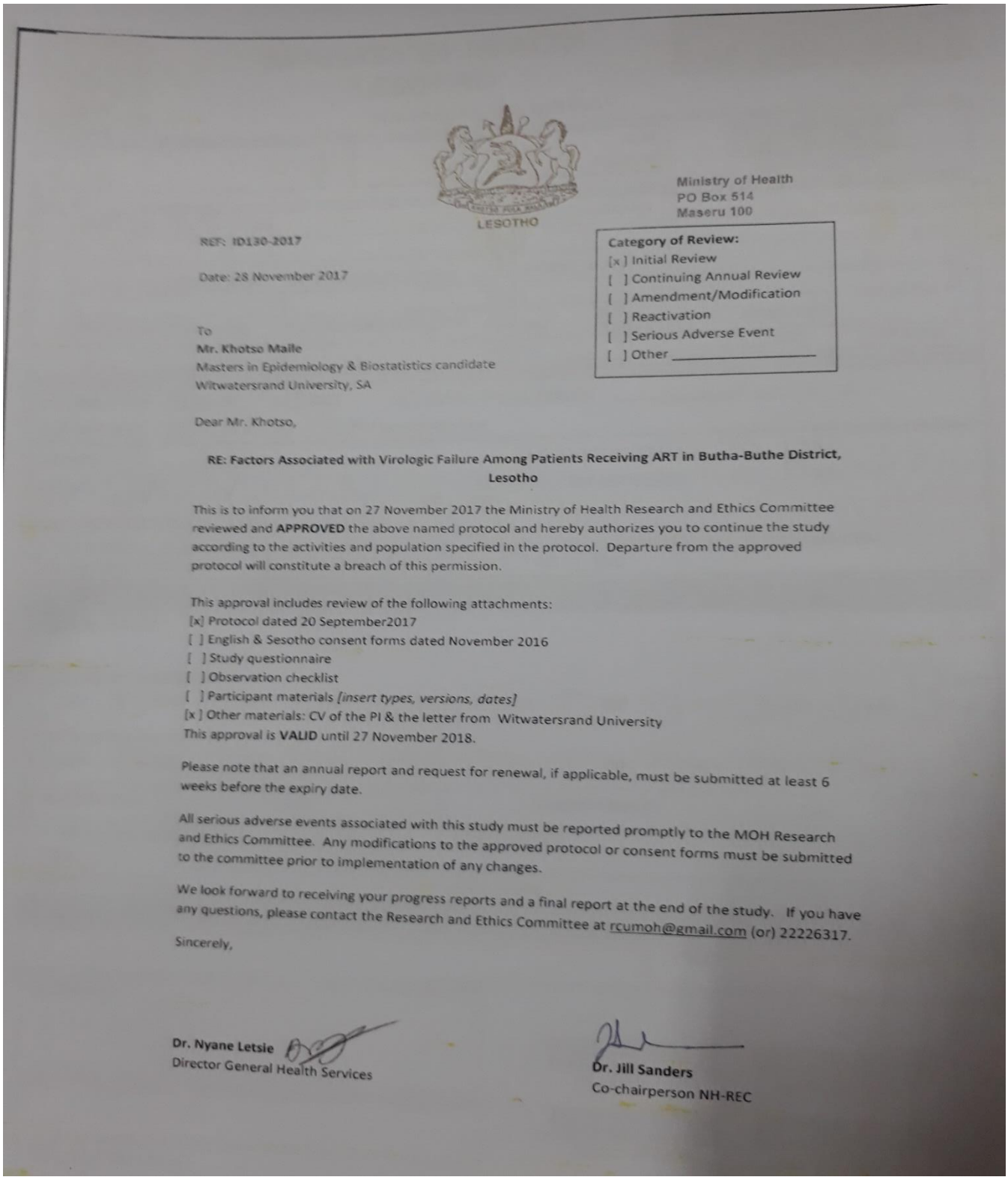
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

K Maile
Principal Investigator Signature

Date 21/08/2018

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

## Appendix 4: Ethical clearance – Ministry of Health Ethics Committee



LESOTHO

Ministry of Health
PO Box 514
Maseru 100

REF: ID130-2017

Date: 28 November 2017

**Category of Review:**
[x ] Initial Review
[ ] Continuing Annual Review
[ ] Amendment/Modification
[ ] Reactivation
[ ] Serious Adverse Event
[ ] Other _____

To
**Mr. Khotso Maile**
Masters in Epidemiology & Biostatistics candidate
Witwatersrand University, SA

Dear Mr. Khotso,

**RE: Factors Associated with Virologic Failure Among Patients Receiving ART in Butha-Buthe District, Lesotho**

This is to inform you that on 27 November 2017 the Ministry of Health Research and Ethics Committee reviewed and **APPROVED** the above named protocol and hereby authorizes you to continue the study according to the activities and population specified in the protocol. Departure from the approved protocol will constitute a breach of this permission.

This approval includes review of the following attachments:
[x] Protocol dated 20 September2017
[ ] English & Sesotho consent forms dated November 2016
[ ] Study questionnaire
[ ] Observation checklist
[ ] Participant materials *[insert types, versions, dates]*
[x ] Other materials: CV of the PI & the letter from Witwatersrand University
This approval is **VALID** until 27 November 2018.

Please note that an annual report and request for renewal, if applicable, must be submitted at least 6 weeks before the expiry date.

All serious adverse events associated with this study must be reported promptly to the MOH Research and Ethics Committee. Any modifications to the approved protocol or consent forms must be submitted to the committee prior to implementation of any changes.

We look forward to receiving your progress reports and a final report at the end of the study. If you have any questions, please contact the Research and Ethics Committee at rcumoh@gmail.com (or) 22226317.

Sincerely,

**Dr. Nyane Letsie**
Director General Health Services

**Dr. Jill Sanders**
Co-chairperson NH-REC

## Appendix 5: Data use agreement

Swiss TPH
Swiss Tropical and Public Health Institute
Schweizerisches Tropen- und Public Health-Institut
Institut Tropical et de Santé Publique Suisse

Assoziiertes Institut der Universität Basel

**Medical Services and Diagnostics**
Clinical Research Unit

**Niklaus Labhardt, MD, MIH, DTM&H**
Principal Investigator of "towards 90-90-90" project

n.labhardt@unibas.ch
T +41 61 284 8255/4 direkt
M +41 79 870 1859

**Agreement between**

Swiss Tropical and Public Health Institute (Tracy Glass and Niklaus Labhardt)

**and**

Khotso Maile, Ministry of Health of Lesotho

Butha Buthe, 07 March 2016

**Introduction**

Khotso Maile subscribed for a MSc in Epidemiology at the University of Witwatersrand, Johannesburg, South Africa in 2015. The research consortium of the proposal "Improving the HIV care cascade in Lesotho: Towards 90-90-90 - A research collaboration with the Ministry of Health" (IZ07Z0_160876/1 R4D Open Call Grant) has agreed to support K. Maile in completing his MSc studies. The "Towards 90-90-90" proposal entails several trials and studies: K. Maile will conduct his master thesis on the introduction of routine viral load monitoring in Butha-Buthe, using data from the project viral load database (https:lstowards909090.org).

**Deliverables Swiss TPH (Tracy Glass and Niklaus Labhardt):**

- Fees of subscription for the whole year of 2016 and 2017 at University of Witwatersrand and the cost of a maximum of six courses taking place in 2016 or 2017 to complete the MSc will be covered by the R4D grant.
- To support cost for travel, accommodation, and course-material K. Maile receives a lump sum of ZAR 6'500.- per course-module attended at Witwatersrand University.
- No other cost related to courses will be covered.
- This lump sum will be transferred to his account prior to each course.
- For his thesis K. Maile will use the data set from the lstowards909090 database on viral load monitoring
- Tracy Glass, principal investigator of the viral load database, will take the lead in writing a protocol for submission to the Ethics Committee in Lesotho
- Tracy Glass will supervise K. Maile for conduction and publication of his thesis.

**Appendix 6: Turnitin report**