Validation of a low-cost in-house HIV integrase inhibitor drug-resistance assay and characterization of the pol- integrase region from patients failing treatment at Kenyatta National Hospital Comprehensive Care Centre

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H56/11347/2018

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A thesis submitted in partial fulfilment of the requirements for the award of a Master of Science Degree in Medical Microbiology at the University of Nairobi

November 2022

ACKNOWLEDGEMENT

My utmost gratitude first to He who establishes the works of our hands and perfects all which concerns us; God. To Dr Frank Onyambu, for giving me the opportunity to work on this project. Thank you for your support and guidance right from the conceptualization and proposal development, laboratory and data analysis and through to the write up. To Dr Moses Masika for recommending me for this project, providing valuable feedback every step of the way and for keeping me accountable. To the late Dr Graeme Brendon Jacobs, rest his soul, for agreeing to be a collaborator on this work and providing excellent mentorship and prompt feedback. To Dr Marianne Mureithi for agreeing to step in as a supervisor, reviewing and helping improve my work. To the Molecular and Infectious Diseases Research (MIDR) Laboratory for allowing me to use their space and equipment to perform my laboratory analysis. Lastly, many thanks to all who took their time to read, critique and help shape and improve various aspects of this work; Rachael Gachogo, Dr Seth Inzaule, Dr Thibault Mesplède, Dr Susan Little and Dr Vainav Patel.

DECLARATION

Candidate Declaration

I hereby declare that this thesis and the entirety of the work contained herein is my original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted or presented this work in any institution for the award of any qualification

Vera Morangi Onwonga

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Signature:  Date: 28/11/2022

Supervisors Declaration

This thesis has been submitted with our approval as supervisors for examination according to the University of Nairobi regulations

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LIST OF ACRONYMS

ADR- Acquired Drug Resistance

AIDS- Acquired Immunodeficiency Disease

ART- Antiretroviral Therapy

ARV- Antiretroviral

BIC- Bictegravir

CAB- Cabotegravir

CCR5- C-C chemokine receptor type 5

CD4- Cluster of differentiation 4

cDNA- Complementary DNA

CNS- Central Nervous System

DRMs- Drug Resistance Mutations

DRTs- Drug Resistance Tests

DTG- Dolutegravir

EVG- Elvitegravir

GRTs- Genotypic Resistance Tests

HAART- Highly Active Antiretroviral Therapy

HIV- Human Immunodeficiency Virus

HIVDR- HIV Drug Resistance

HIVDRT- HIV Drug Resistance Testing

IAS- International AIDS Society

IN- Integrase

INSTI- Integrase Strand Transfer Inhibitor

KNH-CCC- Kenyatta National Hospital Comprehensive Care Center

LTRs- Long Terminal Repeats

MRVs- Minority Resistant Variants

MTCT- Mother-to-Child Transmission

NGS- Next Generation Sequencing

NRTI- Nucleoside/Nucleotide Reverse Transcriptase Inhibitor

NNRTI- Non- Nucleoside Reverse Transcriptase Inhibitor

PDR- Pre-treatment Drug Resistance

PI- Protease Inhibitor

PLHIV- People Living with HIV

PR- Protease

RAL- Raltegravir

RAMs- Resistance-associated mutations

RT- Reverse Transcriptase

RTI-Reverse Transcriptase Inhibitor

TDR- Transmitted Drug Resistance

WHO- World Health Organization

URTIs- Upper Respiratory Tract Infections

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Background: HIV drug resistance testing (DRT) plays an essential role in the surveillance of HIV drug resistance (HIVDR) and informs on the effectiveness of treatment regimens for people living with HIV (PLHIV). HIV DRT is costly and its access is limited especially in poor-resourced settings such as Kenya. With the recent inclusion of integrase strand transfer inhibitors (INSTIs) as part of the first-line regimen for all PLHIV, there arises a need for an affordable and effective INSTI drug resistance test. Further, there is a knowledge gap on the prevalence of INSTI resistance-associated mutations (RAMs) that may affect the utility of INSTIs. This study aims to validate an affordable in-house INSTI drug resistance test and characterize the pol-integrase region of INSTI-naïve treatment-experienced patients.

Broad objective: To validate an in-house INSTI HIV drug resistance test and characterize the HIV-1 pol-integrase region of INSTI-naïve patients experiencing first-line or second-line treatment failure at the Kenyatta National Hospital Comprehensive Care Centre (KNH CCC)

Study design: This was a laboratory-based cross-sectional study done to validate an in-house INSTI HIV drug resistance test and characterize the pol-integrase region of HIV-1 isolates from INSTI-naïve patients failing treatment

Methodology: The performance characteristics (accuracy, precision, reproducibility and amplification sensitivity) of an in-house HIV INSTI drug resistance assay were assessed using 36 plasma samples. Genetic analysis was performed on 87 plasma-derived samples from INSTI-naïve HIV-1 infected patients for characterization of the pol-integrase region. REGA HIV-1 subtyping tool was used for subtype analysis and Stanford HIV database for analysis of resistance-associated loci. The cost per test was estimated using an ingredient costing approach.

Results: The mean nucleotide and amino acid sequence identity between the in-house and reference assay was 99.5%, CI [99.21- 99.77] and 99.0%, CI [98.58, 99.42] respectively. The mean nucleotide sequence identity between replicates intra-assay (precision) and inter-assay (reproducibility) was 100% and 99.1% respectively. Amplification sensitivity for samples with VL> 1000 copies/mL was 100% and 50% for samples with VL <1000 copies/mL. Characterization of the pol-integrase region revealed genetic variability with 76% of the samples belonging to subtype A, 13% subtype D, 9% subtype C, 1% CRF10_CD and 1% subtype G. Drug resistance mutation analyses revealed the absence of major mutations and identified four accessory mutations (T97A, Q146QR, D232N, and T97TA) and two APOBEC mutations (E198K and R224Q). The estimated cost of providing the in-house INSTI-HIVDR test to a patient was $50.31 (reagents and consumables).

Conclusion: The in-house HIV-Integrase assay satisfied the validation criteria for an in-house genotyping assay and proved affordable, making it an attractive alternative to the costly commercially available INSTI drug resistance tests, especially for resource-poor settings. In INSTI-naïve patients, the occurrence of major mutations was uncommon and accessory mutations with no influence on drug susceptibility occurred more frequently indicating that INSTI treatment is likely to be effective and underscoring the need for continued genetic surveillance for HIV drug resistance.

CHAPTER 1: INTRODUCTION

1.1 Background

HIV remains a significant public health concern globally. On average, about 38.4 million people are living with HIV (PLHIV) worldwide and sub-Saharan Africa harbours two-thirds of this population (UNAIDS, 2022). Southern and East Africa are the most affected regions within sub-Saharan Africa and the number of PLHIV in Kenya was estimated at 1.4 million (Ministry of Health Kenya, 2021; UNAIDS, 2022). HIV lowers the heath-related quality of life for its victims and significantly retards the development of economies. The most affected countries are those in the low and middle-income (LMIC) category due to the strain placed on the health care systems and decreased productivity within the workforce (Dixon et al., 2002; Miners et al., 2014;Tawfik & Kinoti, 2006).

Over the past decade, the number of people accessing antiretroviral therapy (ART) has increased substantially, resulting in a 52% decrease in HIV-related deaths (UNAIDS, 2022). Despite the headway made thus far in combating the HIV epidemic, the emergence of HIV drug resistance (HIVDR) threatens this progress. The persistence of HIVDR will challenge the attainment of the 95-95-95 UNAIDS goal of having suppressed viral load in 95% of people on ART (UNAIDS, 2015). In a HIVDR prevalence survey carried out by the World Health Organization (WHO), there was an increase in the level of pre-treatment drug resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) exceeding the 10% recommended threshold and indicating the need to replace NNRTIs as a first-line in these countries (World Health Organization, 2019a). Integrase Strand Transfer Inhibitors (INSTIs) are the latest class of antiretroviral (ARV) drugs that bind the enzyme integrase (IN) and hinder its ability to incorporate the genome of the virus into the DNA of the infected host cell (Katz & Skalka, 1994; Vink & Plasterk, 1993). INSTIs have been demonstrated

to act against viruses exhibiting resistance to other drug classes (Schafer & Squires, 2010). Currently, there are five FDA-approved INSTIs; Raltegravir (RAL), Elvitegravir (EVG), Dolutegravir (DTG), Bictegravir (BIC) and Cabotegravir (CAB) (Mbhele et al., 2021). DTG is now the preferred first-line drug for HIV treatment in all populations, including women both pregnant and of childbearing potential (World Health Organization, 2019b). By early 2019, 75 LMICs had updated their national HIV treatment guidelines to include DTG in their HIV treatment package and with many more countries planning to follow suit, data on the existence or emergence of resistance to INSTIs is essential (World Health Organization, 2019b). Variability exists in the integrase gene both within and between subtypes and while some of these offer a fitness advantage for the virus, some are associated with the development of resistance (Geretti A. M. & Easterbrook, 2001; Herring et al., 2004). Resistance to INSTIs has been reported both in INSTI-naïve and experienced individuals (Brado et al., 2018a; Chang et al., 2016a; Doyle et al., 2015a; Mikasi et al., 2020). INSTI resistance is high in the first-generation INSTIs; RAL and EVG (Anstett et al., 2017; Blanco et al., 2011) but lower in second-generation INSTIs such as DTG which has a high genetic barrier and thus few reported cases of resistance (Llibre et al., 2015) Clinically relevant drug resistance mutations (DRMs) have been reported at positions 143, 148 and 155 of the integrase gene (Quashie et al., 2013). On the other hand, DRMs for DTG have not been well studied (Inzaule et al., 2019). Drug resistance testing for population-level monitoring and prevention of drug resistance should ideally be done prior to initiating ART, or following treatment failure, to help guide the best choice of ARV regimen, as is the case in resource-rich countries (Department of Health and Human Services, 2019; European AIDS Clinical Society, 2019). Such a scenario is, however, impractical in under-resourced countries for several reasons. Firstly, the costs associated with drug resistance tests (DRTs) are high and there is a shortage of human

resource capacity with the skill set to perform these tests. Secondly, there is a deficiency in molecular laboratory infrastructure coupled with the challenge posed by the complexity of cold chain sample and reagent management (Inzaule et al., 2016).

This study aims to validate a low-cost in-house developed INSTI drug resistance assay and to characterize the HIV pol-integrase region among INSTI-naïve patients experiencing first-line or second-line treatment failure in Kenya.

1.2 Rationale

The surge in HIVDR levels, poses a challenge in ending the HIV/AIDS epidemic. HIVDR results in poor treatment outcomes and increases the risk of HIV transmission and HIV-associated mortality. Access to individual-level HIVDR testing in resource-limited countries is hampered by the high cost of commercial genotyping assays, insufficient human resource capacity with the technical skill set required to perform these assays, and lack of training for clinicians to create demand of and utilize DRT tests. Validation of a low-cost in-house INSTI HIVDR assay will increase access to individual-level HIV drug resistance testing and improve treatment monitoring of patients on INSTI regimen in Kenya given that INSTIs are now part of the first-line ART. The in-house INSTI genotypic resistance test will aid in the identification of clinically relevant INSTI resistance mutations which informs regimen switches. The outcome will be improved care and treatment outcomes for HIV patients by guiding the best choice of treatment regimens and prevention of unwarranted switches to costly and less bearable alternative treatment regimens. Further, this study will contribute to the training of young Kenyan investigators on the performance of HIV drug resistance tests and the process of validating an in-house assay, all of which are skills that are needed in this region.

The genetic diversity of the pol-integrase region has not been well studied and there is a paucity of such data. Further, different subtypes have different susceptibilities to developing drug resistance. It is therefore important to perform studies that characterize and describe the genetic diversity of the pol-integrase region for HIV isolates from INSTI-naïve patients taking into consideration the different circulating subtypes. Such data will help highlight the occurrence of HIV drug resistance in Kenya if any. Furthermore, the majority of HIV research is performed on HIV subtype B which is majorly found in North America and Europe whereas the majority of the HIV epidemic is caused by non-B HIV subtypes. It is therefore paramount for additional research to be performed to describe the genetic diversity of non-B HIV isolates. Such data will also contribute significantly to the ongoing WHO surveillance on resistance to INSTIs in addition to driving conversation around the need for policy changes.

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1.3 Study Questions

1. Do the in-house assay's performance characteristics (accuracy, precision, reproducibility and amplification sensitivity) meet the acceptance criteria defined by the World Health Organization for a genotyping assay to be deemed valid?
2. What are the genetic characteristics of the HIV-1 pol-integrase region of treatment-experienced INSTI-naïve patients and does the region possess resistance-associated mutations that will affect response to an INSTI-based regimen?
3. How do the study-derived pol-integrase region sequences compare to those previously sequenced and deposited in the Los Alamos National Laboratory (LANL) database?
4. What is the cost of providing an HIV INSTI drug resistance test to a patient presenting with treatment failure to an INSTI-based regimen?

1.4 Study Objectives

1.4.1 Broad Objective

To validate an in-house INSTI HIVDR assay and to characterize the HIV-1 pol-integrase region from INSTI-naïve patients experiencing first-line or second-line treatment failure at Kenyatta National Hospital Comprehensive Care Centre (KNH CCC)

1.4.2 Specific Objectives

1. To assess the accuracy, precision, reproducibility and amplification sensitivity of an in-house HIV INSTI drug resistance assay using WHO guidelines for the validation of an in-house genotyping assay.

2. To identify subtypes and drug resistance-associated loci on the pol-integrase region for HIV-1 isolates from INSTI-naïve patients failing first-line or second-line treatment

3. To assess the phylogenetics of HIV-1 pol integrase gene sequences obtained in this study

4. To perform cost analysis for providing HIV INSTI DRT assay to patients failing an INSTI-based regimen.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to HIV

The Human Immunodeficiency Virus (HIV) is an RNA virus belonging to the family Retroviridae and the genus Lentivirus (Luciw, 1996). There are two types of genetically and antigenically distinct forms of HIV; HIV-1 and HIV-2 (Abbas et al., 2017). Both forms of HIV demonstrate homogeneity in their transmission mode, replication mechanism and clinical outcomes (Nyamweya et al., 2013). The distinction between the two lies in their transmissibility, geographical distribution and rate of progression to immunodeficiency (Nyamweya et al., 2013). HIV-1 is inherently more transmissible than HIV-2 and most HIV infections worldwide are linked to HIV-1 which is more geographically widespread than its counterpart HIV-2 that occurs in West Africa and countries with ties to West Africa such as Portugal (Campbell-Yesufu & Gandhi, 2011). Additionally, progression to Acquired Immunodeficiency Syndrome (AIDS) occurs faster in HIV-1 than in HIV-2 (Nyamweya et al., 2013). HIV-1 and HIV-2 are further classified into groups. HIV-1 has four groups designated M, N, O and P while HIV-2 has nine groups designated A-I (Myers et al., 1996; Plantier et al., 2009; Simon et al., 1998; Vallari et al., 2011; Visseaux et al., 2016). Group M of HIV-1 causes the bulk of the global HIV epidemic and exhibits a pronounced genetic diversity with 9 subtypes (A-D, F-H, J, and K) each exhibiting unique geographical distribution patterns and at least 98 circulating recombinant forms (Los Alamos National Library, 2018; Simon et al., 1998; Vallari et al., 2011). Subtype B prevails in the Americas and Europe while non-B subtypes mainly A, C and CRF02-AG, prevail on the African continent (Bbosa et al., 2019). Subtype A is the prevailing subtype in East Africa, including Kenya, although subtypes D and C have been found co-circulating in Kenya (Gounder et al., 2017). South Africa is predominated largely by subtype C while CRFs, specifically the CRF02-AG, occur chiefly in West

and West Central Africa. CRF01-AE predominates in Asia and in India subtypes A and C co-circulate (Bbosa et al., 2019)

2.2 HIV-1 genome

The hereditary material of HIV-1 is contained within two identical positive-sense single-stranded RNA molecules (Abbas et al., 2017). The information for the production of various viral components is contained in nine genes; *gag*, *pol* and *env* which code for viral structural proteins and enzymes: Tat, Nef, Rev, Vpu, Vpr, and Vif which code for regulatory proteins (Abbas et al., 2017). The Group-specific antigen (*gag*) gene codes for the structural proteins p17, p24 and p7 which assemble into the virus' outer core membrane, capsid protein and nucleocapsid, respectively (Seitz, 2016). Lying adjacent to the *gag* gene is the Polymerase (*pol*) gene from which the viral proteins Protease (PR), Reverse transcriptase (RT) and Integrase (IN) emanate (Seitz, 2016). PR cleaves Pr55Gag and Pr160GagPol, precursor proteins for the inner structural proteins and viral enzymes respectively (Seitz, 2016). RT generates complementary DNA (cDNA) molecules using the RNA genome as a template while IN prevents the DNA of the virus from integrating into that of the host cell (Seitz, 2016). The Envelope (*env*) gene follows the *pol* gene reading frame and codes for gp120 and gp41, the two glycoproteins that mediate viral attachment and membrane fusion, two events key in the establishment of infection in the target cell (Seitz, 2016).

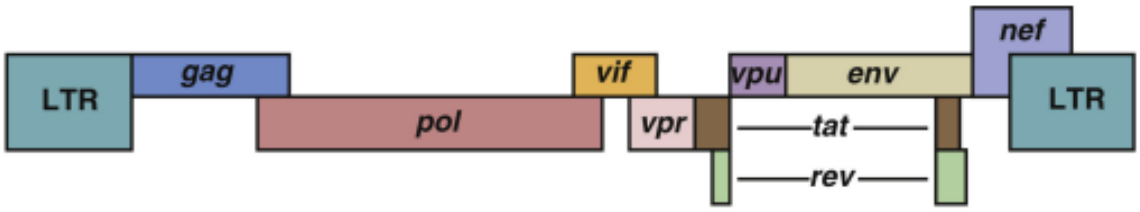

Figure 1: Illustration of the HIV-1 genome

HIV-1 genes are represented as blocks along the linear genome. Areas of overlap represent sequences shared between genes. The LTR on either side of the genome are sequences that bind transcription factors. LTR, long terminal repeat; gag, group-specific antigen; pol, polymerase; vif- viral infectivity factor; vpr, viral protein R; vpu, viral protein u; env, envelope; tat, transcriptional activator; rev, regulator of viral gene expression; nef, negative effector (Abbas et al., 2017).

2.3 The HIV life cycle

HIV first infects and replicates in a cell, typically a CD4+ T-lymphocyte or a macrophage (Abbas et al., 2017). This phenomenon is termed dual tropism and HIV is designated T-tropic or M-tropic depending on whether it infects a CD4+ T-lymphocyte or macrophage (Abbas et al., 2017). Early in the infection, HIV is M-tropic but a shift to T-tropic is seen as the infection progresses (Abbas et al., 2017). Viral attachment to the target cell is mediated by the HIV envelope glycoprotein gp120 which must bind the CD4 receptor and either of two chemokine co-receptors; CXCR4 or CCR5 for infection to be established (Abbas et al., 2017). The co-receptor to which HIV binds determines the HIV tropism; that is R5 tropic if it binds the CCR5, X4 tropic if it binds CXCR4 and R5X4 tropic if it binds both CXCR4 and CCR5 (Abbas et al., 2017). The binding of gp120 induces a molecular shape change in gp41 which results in the exposure of a fusion peptide that promotes coalescing of the viral membrane and the cell membrane of the afflicted cell

consequently resulting in entry of the HIV virion (Abbas et al., 2017). Viral entry causes activation of the viral enzymes, which initiate the viral reproduction cycle (Abbas et al., 2017). Once the nucleoprotein complex is disrupted, the RT enzyme begins to reverse transcribe the RNA genome to yield cDNA that is then translocated into the nucleus via a nuclear pore along with IN (Seitz, 2016). IN catalyzes a reaction that leads to cDNA insertion into the DNA of the infected cell to form a provirus that may remain latent in the infected cells for years but in most cases, the provirus uses the host cell machinery for transcription of its genome (Abbas et al., 2017). Transcription yields a single full-genome-length transcript, which undergoes alternative splicing to give the mRNAs that encode the various HIV proteins (Abbas et al., 2017). Translation of the mRNAs to the various viral proteins occurs in the cytoplasm in two stages; the early stage where regulatory proteins are formed and the late stage where structural proteins are formed (Abbas et al., 2017). Structural gene products occur as polyproteins, Pr160GagPol and Pr55Gag that must be proteolytically cleaved. Pr160GagPol, a *pol* gene product, is cleaved by PR to give the viral enzymes while Pr55Gag, a *gag* gene product, is cleaved to give the inner structural proteins required for viral assembly (Seitz, 2016). The viral genome is then packaged together with the viral enzymes and p7 protein forming a nucleoprotein complex that exits the cell by budding across the plasma membrane acquiring the envelope and host glycoproteins that ultimately form the envelope of the virus (Abbas et al., 2017).

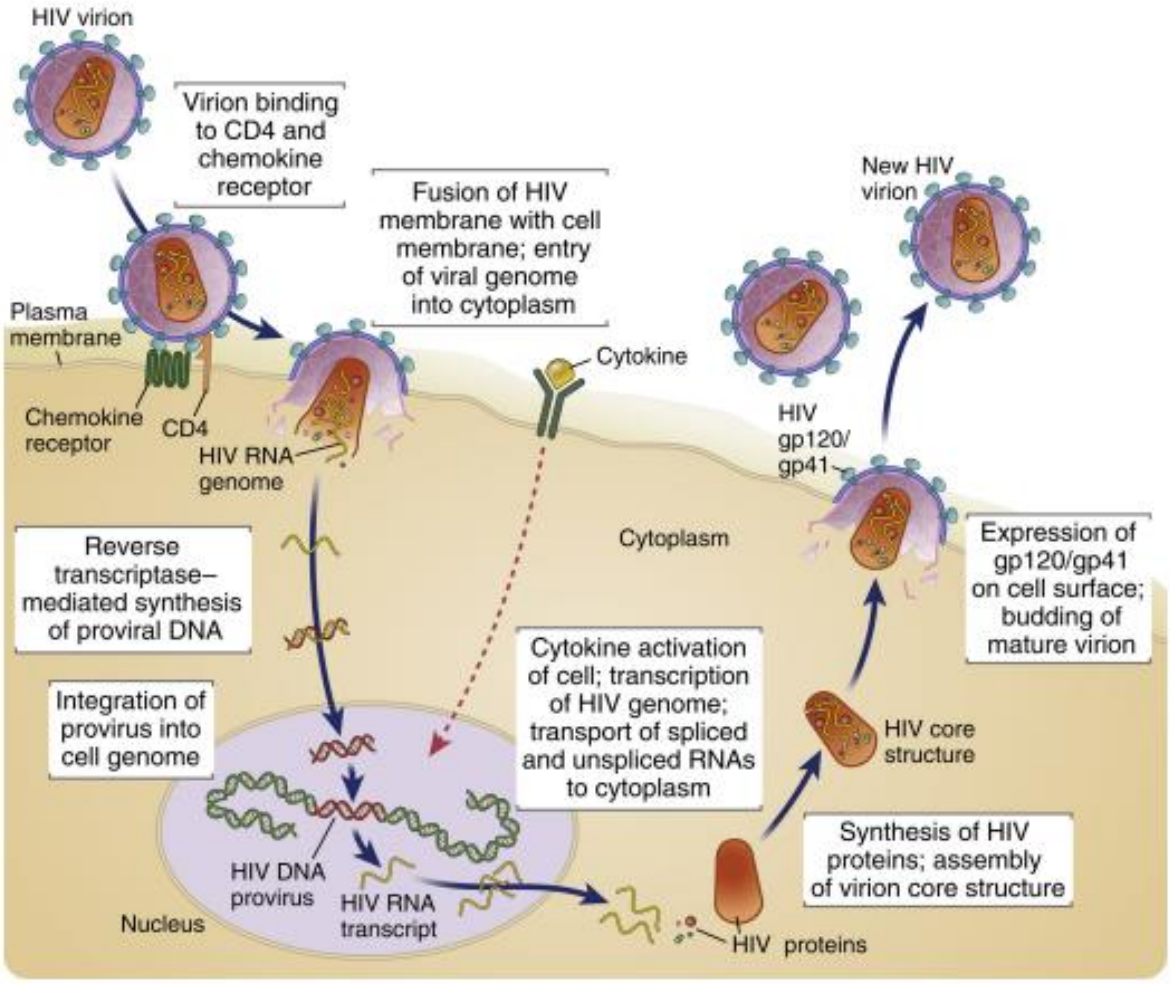

Figure 2: The life cycle of HIV

The lifecycle of HIV is shown sequentially from viral entry into the host cell to maturation and release of an infective virion. Antigens and cytokines, the activators of viral transcription, are not shown (Abbas et al., 2017).

2.4 Genetic variation

HIV-1 has a high replication and recombination level, which partly accounts for its observed extensive genetic variation (Santoro & Perno, 2013). Further, unlike other DNA polymerases, the HIV RT lacks an error correction mechanism, resulting in multiple wrongly incorporated

nucleotides during the chain extension process (Santoro & Perno, 2013). RT introduces nucleotide substitutions at a rate of approximately 10-4 per nucleotide per cycle of replication (Santoro & Perno, 2013). This, coupled with the high HIV-1 replication rate of approximately 10^9 viral particles per day, accounts for the sustained production of new HIV variants termed quasi-species (Santoro & Perno, 2013). Recombination events during the replication process may also contribute to the generation of either fitter forms or drug-resistant variants of HIV (Santoro & Perno, 2013).

2.5 Transmission and progression of HIV

HIV spreads mainly when infected seminal, vaginal fluids or rectal fluids come into contact with mucous membranes (Hladik & Mcelrath, 2008; Tebit et al., 2012). Transmission may also occur during blood transfusion and from mother to child (mother-to-child transmission (MTCT)) in-utero, at childbirth or while breastfeeding (Shaw & Hunter, 2012). Other transmission routes are needle-sharing in people who inject drugs (PWIDs) and percutaneous (Shaw & Hunter, 2012). Exposure to HIV does not necessarily translate to infection and the risk of acquiring HIV differs with the act of exposure. In a systematic review, the probability of acquiring HIV per act of exposure increased from oral intercourse to blood transfusion, with the lowest and highest risk respectively (Patel et al., 2014). Further, the receptive forms of intercourse bear a greater risk of HIV transmission than their insertive counterparts and receptive anal intercourse has the greatest risk (Patel et al., 2014). Routes such as needle-sharing in PWIDs and percutaneous ranked just lower than receptive anal intercourse while bites and spitting have negligible HIV acquisition risk (Patel et al., 2014). Most HIV infections occur at mucosal surfaces and result from unprotected sexual activity with an infected individual (Hladik & Mcelrath, 2008). The progression of HIV to AIDS can be viewed as occurring in three stages.

2.5.1 Stage I: Acute/Early infection

In this phase, the virus entering the body through a mucosal surface encounters dendritic cells, antigen-presenting cells (APCs), which capture, process and present HIV to $CD4^+$ T-lymphocytes at the lymph nodes (Abbas et al., 2017). The direct cell-to-cell contact established during antigen presentation may pass on HIV to the activated CD4+ T-cells (Abbas et al., 2017). HIV replicates within these $CD4^+$ T-cells causing their death and that of abortively infected bystander $CD4^+$ T-cells before moving into the bloodstream (Abbas et al., 2017). The occurrence of viremia coincides with the onset of acute HIV syndrome 2-4 weeks post-infection characterized by symptoms such as headache, fever, sore throat, generalized lymphadenopathy and body rashes (Miedzinski, 1992). Seeding of peripheral lymphoid tissue occurs as the virus disseminates and various cells present at peripheral lymphoid tissues, including helper T-cells and macrophages get infected (Abbas et al., 2017). The adaptive arm of the immune response then becomes activated and both anti-HIV antibodies (humoral arm) and cytotoxic T-cells (cell-mediated arm) mount an immune response to HIV and manage to partially control the infection which accounts for the considerable drop in viremia (Abbas et al., 2017).

2.5.2 Stage II: Chronic/ Clinical latency phase

The clinical latency phase is an asymptomatic phase marked by continuous low-level virus reproduction in the lymph nodes and spleen. The immune system weakens slowly but manages to fight off opportunistic microbes, thus, individuals in this phase are asymptomatic and may remain so for years (Abbas et al., 2017). Ultimately, as a result of sustained virus reproduction, infection of new cells and destruction of infected ones, there occurs a decline in CD4 count and when the count falls to lower than 200 cells/µl, a state of immunodeficiency, AIDS, arises (Abbas et al., 2017).

2.5.3 Stage III: AIDS

This is the final and often lethal phase of the HIV infection as CD4 counts are extremely low, rendering an individual severely immunosuppressed and susceptible to a broad array of opportunistic infections by microbes including *Toxoplasma gondii, Cryptosporidium parvum, Cryptococcus neoformans* and *Mycobacterium tuberculosis* as well as cancers such as Kaposi sarcoma among others (Abbas et al., 2017). Further, central nervous system (CNS) manifestations and wasting syndrome are also characteristic of this stage (Abbas et al., 2017).

2.6 HIV clinical staging

WHO classifies HIV into four clinical stages and patients are placed into a stage if they exhibit at least one of the clinical conditions characteristic of that stage (Weinberg & Kovarik, 2010). WHO clinical stage one consists of asymptomatic individuals with generalized lymphadenopathy that persists for more than six months (World Health Organization, 2007). In the second clinical stage, individuals experience weight loss of a small degree, usually not exceeding 10% of the total body weight (World Health Organization, 2007). Upper respiratory tract (URT) infections such as sinusitis, tonsillitis and pharyngitis and minor mucocutaneous manifestations such as recurrent oral ulcerations and fungal nail emanate (World Health Organization, 2007). The disease progresses to the third clinical stage, and the clinical symptoms are more pronounced than in the previous stages. Individuals experience a drop in weight in excess of 10% of their total body weight in addition to neutropenia, chronic thrombocytopenia, unexplained anaemia, chronic diarrhoea of more than a month and oral manifestations such as oral candidiasis, oral leukoplakia, periodontitis and gingivitis (World Health Organization, 2007). Acute bacterial infections also tend to occur, ranging from pulmonary tuberculosis, pneumonia, and meningitis to bacteremia (World Health Organization, 2007). The fourth clinical stage is the last stage where AIDS occurs.

Individuals in this stage experience body wasting (HIV wasting syndrome) and opportunistic infections such as extrapulmonary tuberculosis, disseminated mycosis and non-tuberculous mycobacterial infection, HIV encephalopathy, cryptococcosis, cryptosporidiosis and malignancies such as Kaposi's sarcoma (World Health Organization, 2007).

2.7 The structure of integrase and the process of integration

The HIV enzyme IN is a product of the *pol* gene comprising 288 amino acids (Delelis et al., 2008). Structurally, it is composed of a core domain lying between an N-terminal domain and a C-terminal domain (Esposito & Craigie, 1999). The core domain holds the enzyme's active site in its DDE motif and spans amino acids 50 to 212 (Delelis et al., 2008). The C-terminal domain spans the amino-acids 213 to 288 and binds the viral DNA and host cell DNA to create a stable complex that facilitates the integration process (Delelis et al., 2008). The amino-acids 1 to 49 form the N-terminal domain which has zinc-binding capabilities through its HHCC motif (Delelis et al., 2008). The binding of zinc is important in assembling multimers, which are crucial for the integration process (Delelis et al., 2008). Integration is a two-step reaction. In the first reaction, an endo-nucleotide cleavage occurs at the 3' end LTRs of the viral DNA, an IN-catalyzed reaction that results in the loss of two nucleotides at the 3' end and an overhang at the 5'end (Delelis et al., 2008). Hydroxyl groups are also channelled in this step to aid in nucleophilic attack (Delelis et al., 2008). In the second step, the 5' overhang on the viral DNA is removed and 3' hydroxyl groups from the viral DNA cleave, in a staggered manner, the bonds between the sugar and phosphate molecules that form the backbone of the host cell DNA (Delelis et al., 2008). The 3' hydroxyl group of the viral DNA and the 5' phosphate end of the DNA of the infected cell then associate and a gap repair process mediated by the host cell machinery completes the integration cycle to give a provirus (Delelis et al., 2008).

2.8 HIV treatment and HIV drug classes

To date, HIV remains incurable. Management of HIV entails the administration of ART which represses the level of viral reproduction and reinstates the immune system to a functional state by improving CD4 counts (May et al., 2014). Consequently, HIV patients have an increased life expectancy and improved health-related quality of life (May et al., 2014). Access to ART has increased significantly over the past nine years. By the end of December 2021, 28.7 million people were accessing ART up from 7.8 million in 2010 (UNAIDS, 2021). The increase in ART access can be attributed to increased funding for HIV treatment and care programmes (Magomere et al., 2019). There are currently eight classes of FDA-approved HIV drugs (Kemnic & Gulick, 2022). Table 1 below lists the drug classes, their target and mechanism of action.

Table 1: HIV drug classes, drug target and mechanism of action

Drug Class	**Target**	**Mechanism of Action**
Fusion inhibitors	Envelope proteins	Disrupt fusion of viral and infected cell membranes
Co-receptor antagonists	CCR5 co-receptor	Competitively bind CCR5co-receptor
Nucleoside reverse transcriptase inhibitors	RT	Chain termination of growing DNA chain
Non-nucleoside reverse transcriptase inhibitors	RT	Bind active sites of RT
Protease inhibitors	Protease	Bind PR active sites
Integrase strand transfer inhibitors	Integrase	Hinder incorporation of viral DNA into DNA of an infected cell
Post-attachment inhibitors	CD4	Hinder viral access to co-receptors
Pharmacokinetic enhancers	Human CYP3A protein	Increase bioavailability of other HIV drugs

2.9 HIV treatment guidelines

HIV drugs are administered as a combination referred to as Highly Active Antiretroviral Therapy (HAART). Updated WHO guidelines recommend two NRTIs and an INSTI in the first-line treatment of adults and adolescents, including women both pregnant and of childbearing potential (World Health Organization, 2019b). The current first-line regimen for HIV treatment in adults and adolescents is TDF in combination with 3TC or FTC and DTG (World Health Organization, 2019b). Second-line ART regimen includes an optimized NRTI backbone and DTG for those failing a non-DTG-based regimen and boosted PIs and an optimized NRTI backbone for those failing a DTG-based regimen (World Health Organization, 2019b). In children, the preferred first-

line treatment is ABC in combination with 3TC and DTG while in infants AZT in combination with 3TC and RAL is used (World Health Organization, 2019b). Failure of the preferred first-line regimen in children would warrant a switch to AZT in combination with 3TC and a boosted PI either LPV/r or ATV/r while in infants a change to a regimen comprising AZT (or ABC) in combination with 3TC and DTG as a second-line regimen would be justified (World Health Organization, 2019b). Guidelines on initiation of therapy have been changing over the past decade. Previously, treatment initiation was based on the WHO clinical stage of HIV and immunological markers such as CD4 counts and total lymphocyte counts (World Health Organization, 2002, 2004, 2006, 2010, 2013). This was revised in the 2015 WHO guidelines that recommended initiation of treatment in all PLHIV without reference to their WHO clinical stage and CD4 count (World Health Organization, 2015).

2.10 HIV drug resistance

HIVDR is the ability of HIV to sustain the viral reproduction cycle even in the presence of ART due to changes in the genetic structure of the virus, usually due to mutations (World Health Organization, 2019a). Mutations can be primary or secondary (World Health Organization, 2001). Primary mutations arise first during therapy and result in a weakened drug-enzyme interaction; thus, higher amount of drug is required for enzyme inhibition to be achieved (World Health Organization, 2001). Secondary mutations on the other hand do not interfere with inhibitor binding but serve to improve the fitness of the virus that bears primary mutations and as such, they do not contribute significantly to the level of resistance (World Health Organization, 2001). HIVDR can be classified into acquired HIV drug resistance (ADR), transmitted drug resistance (TDR) or pre-treatment drug resistance (PDR) (World Health Organization, 2019a). ADR occurs in treatment-experienced individuals when viral replication is not completely suppressed and mutations that

confer drug resistance emerge (World Health Organization, 2019a). ADR may be an outcome of poor treatment adherence or structured treatment interruptions (Chimukangara et al., 2021; World Health Organization, 2019a). Further, individuals on a sub-standard treatment regimen may experience ADR (Chimukangara et al., 2021). On the other hand, TDR occurs in individuals who are treatment-naïve and typically results from infection with a virus bearing drug resistance mutations (World Health Organization, 2019a). PDR may occur in either treatment-naïve or experienced individuals who are initiating or re-initiating first-line treatment respectively and as such, PDR may be TDR or ADR or both (World Health Organization, 2019a)**.**

2.10.1 Mechanism of NRTI resistance

Two mechanisms can explain resistance to NRTIs. The first mechanism involves a mutation on the RT gene that permits the distinction of host cell deoxynucleoside triphosphates (dNTPs) from NRTIs resulting in the preferential incorporation of host cell dNTPs over the NRTIs (Asahchop et al., 2012). The mutations M184V, L74V, Q151M, and K65R are examples of such mutations (Asahchop et al., 2012). In the second mechanism, mutations termed primer unblocking mutations or thymidine analog mutations (TAMs) excise an NRTI tri-phosphate from the growing DNA chain by phosphorolysis and include D67N, K219Q/E, K70R M41L, L210W, and T215Y/F (Asahchop et al., 2012).

2.10.2 Mechanism of NNRTI resistance

NNRTIs exhibit their inhibitory action by binding the NNRTI-binding pocket situated within the p66 subunit of the enzyme RT (Asahchop et al., 2012). Resistance to NNRTIs results from mutations that impede the inhibitor-enzyme interaction (Asahchop et al., 2012). K103N is one such mutation that reduces susceptibility to NVP and EFV by preventing their entry into the binding pocket (Asahchop et al., 2012). Other mutations such as Y181C lead to drug resistance by

causing a reduced association between the NNRTI and the residues in the NNRTI-binding pocket (Asahchop et al., 2012). The size and conformation of the NNRTI-binding pocket may also be altered in some instances such as when a Y188L mutation occurs resulting in a structure that is unable to bind the NNRTI with high specificity (Asahchop et al., 2012). Other frequently occurring NNRTI mutations include L100I, K101E/P, V106A/M, G190A/S/E, and M230L all which cause resistance to NVP (Melikian et al., 2014). Cross-resistance is also a defining feature in this class of drugs (Asahchop et al., 2012).

2.10.3 Mechanism of PI Resistance

The mechanism of development of resistance to PIs is progressive and involves primary and secondary mutations that change the substrate-binding cleft of PR (Maarseveen & Boucher, 2006). The level of resistance to PIs increases with the co-occurrence of primary and secondary mutations and because the PIs share a great similarity in their structures, a virus with a RAM to one PI may be resistant to many other PIs (Maarseveen & Boucher, 2006). Further, the error-prone RT leads to the production of many mutations in the virus, some of which may confer resistance to PIs (Maarseveen & Boucher, 2006).

2.10.4 Mechanism of INSTI Resistance

One mechanism leading to the emergence of resistance is the absence of an error correction mechanism by RT and the subsequent emergence of viral variants which may carry mutations and prevail under conditions of incomplete viral suppression (Quashie et al., 2013). The drug-binding time and the concentration of bound drug at the active site influence the development of resistance (Hightower et al., 2011; Quashie et al., 2013). RAL and EVG have a higher propensity to develop resistance than their counterpart DTG which tends to have a longer binding time long after plasma clearance (Quashie et al., 2013). The possible effect of this observed phenomenon is the ability to

overcome resistance resulting from suboptimal treatment adherence (Quashie et al., 2013). Resistance may also develop due to differences between subtypes with different subtypes having different propensities to develop resistance (Santoro & Perno, 2013). INSTI resistance is high in the first-generation INSTIs; RAL and EVG (Quashie & Wainberg, 2012). Clinically relevant DRMs have been reported at positions 143, 148 and 155 of the integrase gene (Quashie et al., 2013). These primary mutations appear in drug-experienced individuals and occur independently (Quashie et al., 2013). Mutations conferring resistance to RAL include N155H, Q148H/R/K and Y143C/R (Quashie et al., 2013; Quashie & Wainberg, 2012). Additional mutations include E92Q and G140S/A which may occur with N155H and Q148H/R/K respectively (Quashie et al., 2013). N155H ± E92Q and Q148H/R/K ± G140SA confer resistance to EVG (Quashie et al., 2013). As can be seen, mutations for EVG and RAL cross-react and DRMs for DTG have not been well studied (Quashie et al., 2013). Nevertheless, an in vitro study pointed to T124A, R263K, S153Y, and L101Y as possible mutations for DTG (Quashie et al., 2013)

2.11 Drug resistance testing

Drug resistance testing may take on two approaches; phenotypic and genotypic. Phenotypic drug resistance testing measures the amount of ARV drug needed to repress viral replication in cell culture and reports it as half-maximal inhibitory concentration; (IC50) (Mayer et al., 2001). This is then compared to the IC50 of a reference strain susceptible to the test drug and expressed as a ratio termed 'fold change' (Mayer et al., 2001). Phenotypic DRTs are costly, have a high turnaround time, and are, therefore, not used routinely in clinical practice unless for complex clinical cases (Mayer et al., 2001). Genotypic drug resistance tests (GRTs) determine the nucleotide sequence of specific HIV genes that confer drug resistance, such as the *pol* gene, to identify DRMs of clinical importance (Mayer et al., 2001). DRMs are identified using specialized software tools that are also

capable of correctly detecting mixtures given the heterogenous nature of the virus population of an individual (Mayer et al., 2001). The Stanford HIV database is one such DRM interpretation tool that uses an in-built algorithm and the International AIDS Society (IAS) mutation list for DRM interpretations. The cost-effectiveness of GRTs is debatable and largely depends on the income group of the setting (Mayer et al., 2001). GRTs may also be done using next-generation sequencing (NGS). NGS overcomes the possibility of failing to detect minority resistant variants (MRVs) that occur below the 20% threshold (Parikh et al., 2017). Compared to Sanger population sequencing, NGS is both time and cost-efficient owing to the ability of pooling specimens for processing (Parikh et al., 2017). NGS also allows for scaling up (Parikh et al., 2017).

2.12 Assay validation

Routine DRT has not been realized in poor-resourced settings because of the high capital expenditure required to set up molecular laboratory infrastructure and the high patient test cost (Inzaule et al., 2016). In-house developed genotypic drug resistance tests reduce test costs by reduction of primers and amplification steps, use of more affordable open-source reagents, miniaturization of reagents, negotiating with suppliers for markdowns on reagents and consumables or working with not-for-profit manufacturers (Aitken et al., 2013; Inzaule et al., 2016; Magomere et al., 2019; Manasa et al., 2014; Zhou et al., 2011). Increased access to genotypic drug resistance tests will be valuable in providing public health surveillance data on HIVDR (World Health Organization, 2020). However, to ensure that data from various independent laboratories are of good quality and comparable, WHO set out guidelines for the validation of in-house genotypic tests (World Health Organization, 2020). Newly developed assays must undergo a full-scale validation where at least four parameters of accuracy, amplification sensitivity, precision and reproducibility are tested (World Health Organization, 2020).

2.12.1 Accuracy

This is the degree of agreement between a measured value and a standard/ reference value (World Health Organization, 2020). For genotyping assays, nucleotide sequence similarity is the basis of comparison (World Health Organization, 2020). WHO recommends parallel sequencing of 20 plasma samples using the newly developed assay and a gold-standard method (World Health Organization, 2020). A comparison should then be made for the degree of similarity in the base calls and DRM calls from the two assays (World Health Organization, 2020). At least 90% of the base calls and DRM calls for each sample should not be less than 98% identical with non-matching mixtures counted as a difference or not less than 99% identical with non-matching mixtures not counted as a difference for the assay to be deemed accurate (World Health Organization, 2020).

2.12.2 Amplification Sensitivity

Amplification sensitivity of a genotyping assay is the percentage of both accurate and reproducible tests that an assay yields for a given range of viral loads (World Health Organization, 2020). Two approaches are defined, the choice of which depends on the availability of the specimens (World Health Organization, 2020). For samples with high viral load, testing of 10 specimens with 2 replicates each and for samples with low viral load, testing of 5 specimens with 4 replicates each is recommended (World Health Organization, 2020). In adjusting for the overlap between accuracy and sensitivity testing samples, duplicate testing of 10 specimens may be done provided they are representative of low and high viral loads such as a range of 1000-5000 copies/ml (World Health Organization, 2020). The defined acceptance criteria for specimens and replicates with a viral load between 1000 and 5000 copies/ml is a successful amplification of more than 90% of the samples and more than 95% for those with a viral load over 5000 copies/ml (World Health Organization, 2020).

2.12.3 Precision

This may be either intra or inter- assay precision. Intra-assay precision is the capability of a test to yield similar outcomes when the same sample is tested repeatedly in the same test run with variables such as the operator and critical reagent lots used being uniform (World Health Organization, 2020). Inter-assay precision is the capability of a test to yield similar outcomes when the same sample is tested repeatedly across multiple test runs and allowing for sources of variability such as the processing of the specimens and replicates in different batches, the use of different operators and different critical reagent lots across the runs and even spacing out the time between which the runs are executed (World Health Organization, 2020). For both tests, three different specimens and five replicates of each of these should be tested and pairwise comparisons of replicates from each specimen made for nucleotide sequence similarity (World Health Organization, 2020). The new assay is precise if 9 out of the possible 10 pairwise comparisons of replicates per sample are at least 98% identical (World Health Organization, 2020).

CHAPTER 3: METHODOLOGY

3.1 Study design

A laboratory-based cross-sectional study design was employed to characterize the HIV pol-integrase region of INSTI-naïve patients failing either first-line or second-line ART at Kenyatta National Hospital (KNH) Comprehensive Care Centre (CCC) between 1st January 2019 and 31st January 2020. Treatment failure was defined as having a plasma viremia of ≥ 1000 copies per ml after no less than 6 months on ART.

A validation study was undertaken to evaluate the performance characteristics of an in-house developed HIV INSTI DRT assay following pre-defined WHO guidelines for validating an in-house genotyping assay. Accuracy testing was done utilizing inter-laboratory assessment samples that served as our gold standard. The samples were from INSTI-experienced patients on a first-line regimen. Amplification sensitivity, precision and reproducibility assessments were performed using remnant plasma samples from routine viral load monitoring of INSTI-naïve patients attending KNH CCC.

3.2 Study site

The study was conducted at KNH CCC, an outpatient facility in Nairobi, Kenya, that serves residents of Nairobi and its outskirts and offers free extensive support to about 17,000 HIV seropositive individuals.

3.3 Sample size

All samples of patients presenting with either first-line or second-line treatment failure at KNH-CCC between 1st January 2019 and 31st January 2020 were genotyped for characterization of the pol-integrase region and determination of RAMs. The sample size for validating the in-house

genotyping assay was guided by WHO recommendations for the validation of an in-house genotyping assay as follows; 20 samples for accuracy, 10 for amplification sensitivity and 3 samples each for precision and reproducibility (World Health Organization, 2018).

3.4 Study population

The study population was patients failing either first-line or second-line treatment from the Centers of Excellence in HIV Medicine (CoEHM) program, a five-year (2010-2015) program funded by the USA President's Emergency Plan for AIDS and Relief (PEPFAR) and implemented at the KNH CCC to develop scope and building skills for practical and sustainable HIV prevention and treatment services. The main treatment regimen at this period included Tenofovir (TDF), Lamivudine (3TC) and Efavirenz (EFV); hence the study population were INSTI-naïve.

3.5 Sampling Technique

Remnant patient plasma samples were selected from the Molecular and Infectious Diseases Research (MIDR) laboratory archive using a consecutive sampling approach.

3.6 Inclusion criteria

1. Patients with viral load ≥1000 copies/ml.

2. Patients with medical records for the desired variables of age, sex, history of ART regimen and last VL

3.7 Exclusion criteria

1. Patients currently on or with prior exposure to an Integrase Strand Transfer Inhibitor.

3.8 Study Procedures

3.8.1 Patient and costing data abstraction

Patient information was abstracted from the Electronic Medical Records (EMR) system of KNH CCC by running queries and included the variables of age, date of birth, sex, last viral load, ART regimen history and last VL. Costing data was abstracted from laboratory records such as quotations for reagents and consumables, consignment notes and invoices

3.8.2 Viral RNA extraction

Viral RNA was extracted using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Briefly, 62.5 µL of Proteinase K, 500 µL Lysis Buffer and a 500 µL aliquot of the plasma sample were added into a 1.5ml tube and mixed by vortexing before incubating at 56°C for 15 minutes. To the lysate, 625 µL of absolute ethanol was added and the contents vortexed before a 5-minute incubation at room temperature. Purification of the RNA was done on a spin column and eluted into a final volume of 20µL.

3.8.3 cDNA synthesis and PCR amplification

A 5 µL aliquot of the RNA extract was denatured at 65°C for 10 minutes and used as a template for cDNA synthesis. cDNA synthesis and first-round amplification were performed using MyTaq^{TM} One-Step RT-PCR Kit (Bioline, USA) and two in-house developed primers in a 25µL reaction as outlined in Table 2 below:

Table 2: Preparation of MyTaqTM One-Step RT-PCR mix

Reagent	Volume (µL)
2x reaction mix	12.5 µL
Primer F (10µm)	1.0 µL
Primer R (10µm)	1.0 µL
Reverse Transcriptase	0.25 µL
RNAse Inhibitor	0.5 µL
DEPC-treated water	4.75 µL
RNA Template	5.0 µL
Final volume: 25µL	

The reaction proceeded through 45 minutes at 50°C for first-strand cDNA synthesis and 2 minutes at 94°C for enzyme inactivation and denaturation of the cDNA- RNA hybrid. Second strand synthesis and PCR amplification occurred across 40 cycles of denaturation at 94°C for 15 seconds, annealing at 50°C for 20 seconds and extension at 72°C for 2 minutes. The final extension was done at 72°C for 10 minutes. The reaction conditions are outlined in Table 3 below.

Table 3: RT-PCR Conditions

Temperature (ºC)	**Number of cycles**	**Time**
50	1	45minutes
94	1	2 minutes
94	40	15 seconds
50		20 seconds
72		2 minutes
72	1	10 minutes
4	1	30 minutes

Second-round amplification (nested PCR) for amplification of the pol-integrase region was performed using My Taq ™ Red Mix (Bioline, USA) and two in-house primers in a 25 µL reaction as outlined in Table 4 below:

Table 4: Preparation of My Taq ™ Red Mix

Reagent	**Volume (µL)**
My Taq Red Mix, 2x	12.5 µL
Primer F2 (10 µM)	1 µL
Primer R2 (10 µM)	1 µL
Water (ddH_2O)	8.5 µL
Template	2 µL
Final volume: 25 µL	

The reaction started with an initial denaturation at 94˚C for 4 minutes followed by 40 cycles of denaturation at 94˚C for 15 seconds, annealing at 50 ˚C for 20 seconds and extension at 72˚C for

2 minutes. The final extension was done at 72 ˚C for 10 minutes and cooled to 4 ˚C for downstream processing. The reaction conditions are outlined in Table 5 below.

Table 5: Nested PCR conditions

Temperature (ºC)	**Number of cycles**	**Time**
94	1	4 minutes
94	40	15 seconds
50		20 seconds
72		2 minutes
72	1	10 minutes
4	1	30 minutes

3.8.4 PCR product cleanup

For removal of residual primers and nucleotides, 5µL of PCR product was added to 2µL of ExoSAP-IT^{TM} reagent. The ExoSAP-IT^{TM} reagent comprises two enzymes; Exonuclease I which removes single-stranded oligonucleotides and Shrimp Alkaline Phosphatase which dephosphorylates nucleotides to hinder ligation to the newly synthesized DNA strand. The reaction mixture was incubated in a Veriti Thermocycler under the following conditions; 37ºc for 15 minutes for enzyme activity, 80º C for 15 minutes for inactivation of the enzymes and 4ºC for 5 minutes to cool the PCR products as outlined in Table 6 below.

Table 6: Enzymatic cleanup reaction conditions

Temperature (ºC)	**Number of cycles**	**Time in minutes**
37	1	15
80	1	15
4	1	5

3.8.5 Gel electrophoresis

Amplification of the pol-integrase region was confirmed on a 1% Agarose gel prepared by dissolving 1 gram of Agarose powder in 100ml of Tris-acetate EDTA (TAE) buffer and heating the mixture to aid in dissolution. The gel was stained by adding 4.5 µL of SYBR Safe and allowed to cool in an electrophoresis tank fitted with combs. TAE buffer was added to the tank and viral DNA (2µL) was loaded directly into the wells created by the combs before allowing for electrophoresis over 30 minutes. The gel was visualized under UV light from the UVITEC machine. Only samples giving 1.1kb bands as in the image below were subjected to cycle sequencing.

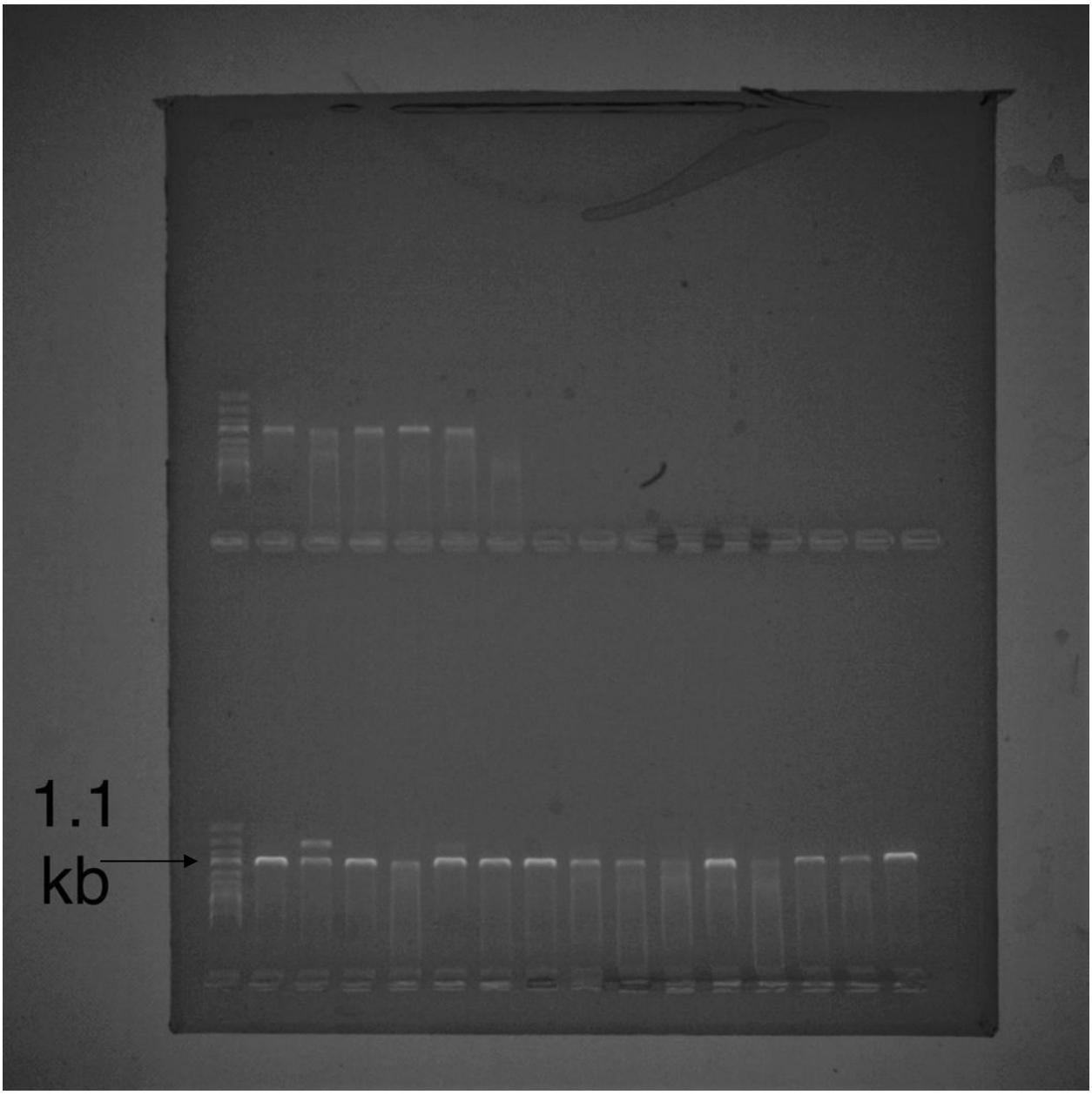

Figure 3: Agarose gel image showing 1.1kb HIV DNA bands

Primers specific for the pol-integrase region were used for the second round PCR. Electrophoresis on a 1% agarose gel was used to separate the PCR products. The DNA ladder was loaded onto the first wells on the left. Successful amplification was confirmed under UV light corresponding to the presence of a 1.1kb fragment.

3.8.6 Cycle sequencing

Sanger sequencing was performed using the Big Dye™ Terminator v3.1 cycle sequencing kit and four in-house developed overlapping primers. The cycle sequencing mix was prepared for a 10 µL reaction as outlined in Table 7 below:

Table 7: Preparation of Big Dye™ Terminator cycle sequencing reaction mix

Component	**Quantity per reaction (µL)**
Big Dye™ Terminator 3.1 ready reaction mix	4
Primer (F1/F2/ F3/ F4) (10µM)	1
Water	4
Template	1
Final volume: 10 µL	

A two-stage cycle sequencing reaction was then performed in a Veriti Thermocycler beginning with 25 cycles of denaturation at 96ºC for 10 seconds, annealing at 50ºC for 5 seconds and an extension at 60ºC for 4 minutes. The second stage comprised a two-step one-cycle reaction; the first at 4ºC for 10 minutes and the second at 10ºC for 10 minutes. The reaction conditions are outlined in Table 8 below

Table 8: Cycle sequencing conditions

Temperature (ºC)	**Number of cycles**	**Time**
96	25	10 seconds
50		5 seconds
60		4 minutes
4	1	10 minutes
10	1	10 minutes

3.8.7 Purification of sequencing products

The sequencing reaction products were purified using the Big Dye XTerminator™ purification kit. A pre-mix solution of Big Dye X Terminator solution and SAM solution was prepared for a 10 µL as detailed in Table 9 below:

Table 9: Preparation of clean-up premix solution

Component	Volume per well
BigDye XTerminator Solution	10 µL
SAM Solution	45 µL
Total volume	55 µL

To each well containing the sequencing products, 55 µL of the premix solution was added, the plate rocked for 30 minutes and then centrifuged at 1000 x g for 2 minutes at room temperature before transferring 30µL of the purified sequenced products to a clean reaction plate and loading onto the ABI 3730 genetic analyzer (Applied Biosystem, CA, USA).

3.8.8 Cost analysis

Abstracted costing data was categorized into the laboratory processes for genotyping: extraction, PCR amplification, gel electrophoresis and sequencing. Microsoft Excel software version 16 (Microsoft, USA) was used for computing of the unit cost for the integrase assay. All costs were expressed in US dollars to facilitate comparison to commercially available integrase kits.

3.9 Data management

Abstracted patient data was stored in an electronic format on a database housed in a password-protected computer and backed up on a cloud-based server. All patient data were deidentified by

assigning unique study identification numbers in place of the CCC numbers routinely used for the identification of patients visiting the CCC clinic. A separate file containing patients' CCC numbers against their assigned unique identification numbers was kept in a password-protected computer file only accessible by the principal investigator and authorized individuals. Validation data (accuracy, precision and reproducibility) was analyzed on EMBOSS programs. Pairwise sequence alignment and subsequent determination of nucleotide and amino acid sequence similarity were performed on EMBOSS Needle. Descriptive statistics were used to summarize patient metadata and this was performed on the statistical software IBM SPSS Statistics Version 22. Data summaries were presented in form of tables and graphs. RECall software was used for the generation of consensus sequences from the raw AB1 files generated by the ABI 3730. RAMs were identified and interpreted by an in-built algorithm housed in the Stanford HIV database. Multiple sequence alignment for evolutionary analysis was performed on CLUSTAL X Version 2.1 and a neighbour-joining tree with bootstrapping for internal node support was constructed using Mega Version 11.

3.10 Quality assurance

Laboratory procedures were performed by a trained Medical Microbiologist with adherence to the developed and approved standard operating procedures and protocols. Maintenance of equipment was performed before every run and all equipment had undergone prior calibration. All nucleotide sequences were subjected to a quality check using an online HIV sequence quality control tool hosted on LANL (http://www.hiv.lanl.gov/content/sequence/QC//index.html).

3.11 Study results dissemination plan

The study results were presented to the Department of Medical Microbiology and Immunology, University of Nairobi and will be published in peer-reviewed journals.

3.12 Ethical considerations

Ethical approval for this study, including a waiver for informed consent, was obtained from the Ethical Review Committee of Kenyatta National Hospital-University of Nairobi and approved under reference number P263/05/2020. All laboratory procedures were conducted in conformance to the basic principles of good clinical and laboratory practice (GCLP).

CHAPTER 4: RESULTS

4.1 Validation of the in-house HIV INSTI drug resistance assay

Accuracy

The accuracy of the in-house assay was evaluated by paired comparison of nucleotide and amino acid sequences derived from genotyping of 20 plasma samples using the in-house assay and a reference assay. There was successful amplification of all 20 samples by both assays and the mean nucleotide sequence identity between the in-house and reference assay was 99.5%, CI [99.21-99.77], surpassing the predefined requirement for accuracy. The mean amino acid sequence identity between the two assays was 99%, CI [98.58, 99.42]. A side-by-side comparison of the 20 samples is outlined in Table 10 below and figure 4 below is a graphical depiction of the tabulated data.

Table 10: Paired comparison of nucleotide and amino acid sequences between the in-house and reference assay

Sequence ID	**Subtype**	**%Nucleotide Sequence Identity**	**%Amino acid Sequence Identity**
INT1	J	97.5	97.5
INT2	J	99.3	98
INT3	A	98.8	99.1
INT4	A	99.1	98.3
INT5	A	99.1	99.9
INT6	A	99.3	99.9
INT7	A	99.9	100
INT8	A	99.9	97.7
INT9	A	100	97.5
INT10	A	100	99.9
INT 11	A	99.5	100
INT 12	A	99.5	99.6
INT13	A	99.5	99.1
INT14	J	99.8	100
INT15	A	99.9	98.5
INT16	A	99.9	98.5
INT17	A	99.9	99.1
INT18	A	99.9	98.7
INT19	A	100	100
INT20	A	99	98.6
		$\overline{x}$: **99.5**	$\overline{x}$: **99**

x̄: Mean/ Average

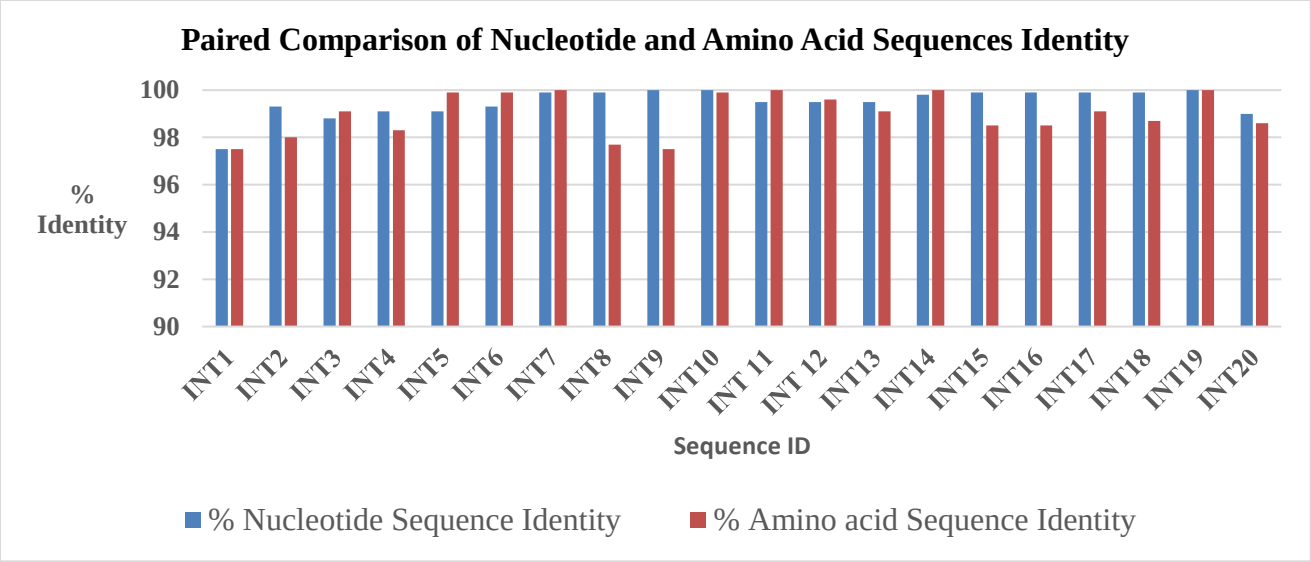

Figure 4: Paired Comparison of Nucleotide and Amino Acid Sequences Identity

x axis: % identity, y-axis: sequence ID, Blue bars represent % nucleotide sequence identity and red bars represent % amino acid sequence identity.

Discordance in base calling was observed in 7 samples at 11 positions, none of which were of clinical significance. Some of the discordances were attributed to differences in calling for mixed bases. The in-house assay picked up mixed bases in 15 samples while the reference assay detected mixed bases in 11 samples. Thus, positions with mixed bases were picked up with greater sensitivity by the in-house assay than by the reference assay as outlined in Table 11 below:

Table 11: Comparison of mixed bases detected by the reference and in-house assay

	No. of mixtures	
Sample ID	**Reference assay**	**In-house assay**
INT1	12	18
INT2	11	14
INT3	11	15
INT4	12	13
INT5	0	0
INT6	6	13
INT7	6	14
INT8	11	4
INT9	11	2
INT10	0	1
INT11	0	0
INT12	0	16
INT13	0	11
INT14	5	15
INT15	7	3
INT16	7	0
INT17	0	3
INT18	0	3
INT19	0	0
INT20	0	0

This difference was however not statistically significant as revealed by a Wilcoxon signed-rank test. Complete concordance in mutation calling was observed in the calling of the accessory DRM

T97A by both assays. Only two subtypes, A and J were identified from subtype analysis of the inter-laboratory assessment samples.

Precision (Intra-assay precision)

Precision assessment was done by genotyping 5 replicates of 3 samples in one test run. All replicates were successfully genotyped yielding 15 sequences. Comparison of pairs of replicates for each sample yielded 100% nucleotide sequence identity. Mutation analysis of the 15 sequences revealed the absence of INSTI RAMs.

Reproducibility (Inter-assay precision)

Reproducibility assessment was done by genotyping 5 replicates of 3 samples in separate test runs performed across different days. All replicates were successfully genotyped resulting in 15 sequences. The mean nucleotide sequence identity between pairs of replicates from the 3 samples ranged from 99.9%, CI [99.80, 99.92], 98.4%, CI [97.60, 99.22] to 99.2%, CI [98.79, 99.53]. INSTI RAMs were not detected in the replicates of the three samples upon mutation analysis.

Table 12 below gives a summary of the precision and reproducibility data for the in-house assay

Table 12: Reproducibility and precision data for the in-house assay

Sample ID	Subtype	Mean % nucleotide sequence identity	Replicate Tests				
			Number of Drug Resistance Mutations				
Reproducibility			A	B	C	D	E
RP1	A	99.9	0	0	0	0	0
RP2	A2	98.4	0	0	0	0	0
RP3	A	99.1	0	0	0	0	0
Precision							
PR1	CRF10_CD	100	0	0	0	0	0
PR2	A	100	0	0	0	0	0
PR3	A	100	0	0	0	0	0

Amplification sensitivity

Amplification sensitivity was assessed by duplicate testing of 10 plasma samples with a viral load range of 500 - 5000 copies/mL. There was successful amplification (100%) of all samples with VL >1000 copies/mL (n=8) and only one of the samples with VL <1000 copies/mL (n=2), was amplified. The collective amplification sensitivity of the assay was 90%. Table 13 below shows the amplification status of the10 plasma samples.

Table 13: Amplification sensitivity of the in-house assay

Sample ID	VL (copies/mL)	Amplification status
SN1	500	-
SN2	900	+
SN3	1030	+
SN4	1280	+
SN5	1395	+
SN6	1624	+
SN7	1678	+
SN8	1689	+
SN9	2577	+
SN10	4089	+

VL: Viral load

\+ : Passed Amplification

\- : Failed Amplification

4.2 Characterization of the pol-integrase region and analysis of RAMs

We successfully amplified and sequenced the IN gene of 87 plasma samples, 83 of which were complete for all the 288 amino acids of the IN gene. The remaining three samples had near full-length codon lengths; INTI031 (287 amino acids), INT032 (285 amino acids), INT034 (287 amino acids), and INT085 (285 amino acids). The majority of the plasma samples were collected in 2019 and of all analyzed sequences, 56% were from female subjects and 44% from male subjects. The median age and viral load at sampling were 21 years (IQR 15-43) and 31279 cp/mL (IQR 3389-59939) respectively. At the time of sampling, the majority of the patients were on a PI-based regimen and the median duration on ART was 59.6 ±35.4 months. The start regimen for most patients was an NNRTI-based regimen.

Subtype analysis

Subtype analysis identified 76% of the samples as subtype A, 9% as subtype C, 1% as CRF10_CD, 13% as subtype D and 1% as subtype G as depicted in figure 4 below.

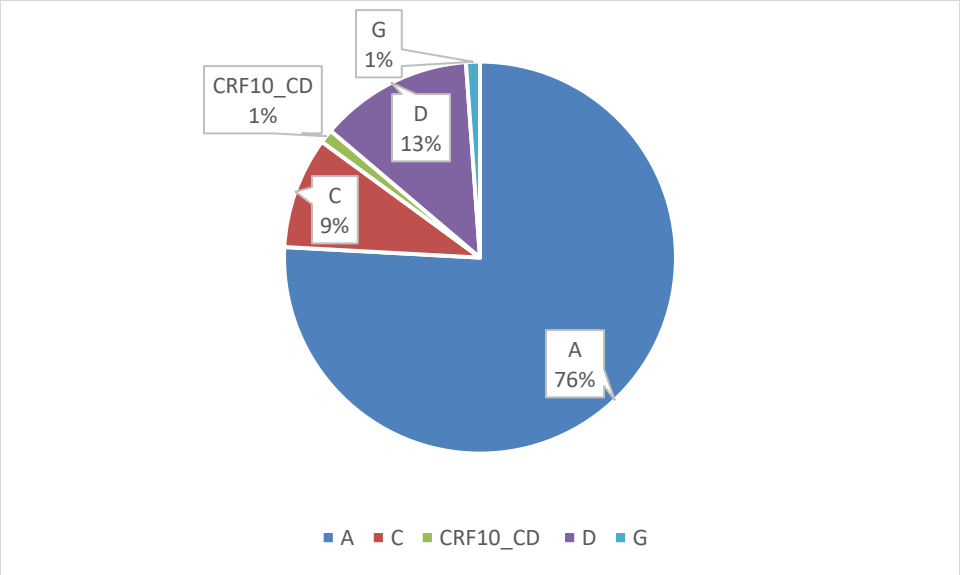

Figure 5: Percentage subtype distribution of test samples

The pre-dominant subtype is subtype A. Subtypes D and C occur at nearly equal frequencies. Subtypes G and the CRF_10_CD had the least occurrence frequency.

Mutation analysis

No major drug resistance mutations were detected. Four accessory mutations T97A, Q146QR, D232N, and T97TA were identified in four different samples all of which were subtype A. Two APOBEC mutations were present in 2 samples; INT017 (E198K) and INT038 (R224Q) of subtypes D and A respectively.

4.3 Phylogenetic analysis

The sequences clustered into four major clades indicating the diversity of the integrase gene. Most of the study sequences (%) were in the clade depicted in yellow. There was genetic relatedness between the study and database-derived sequences based on their location on the same node as depicted in Figure 6.

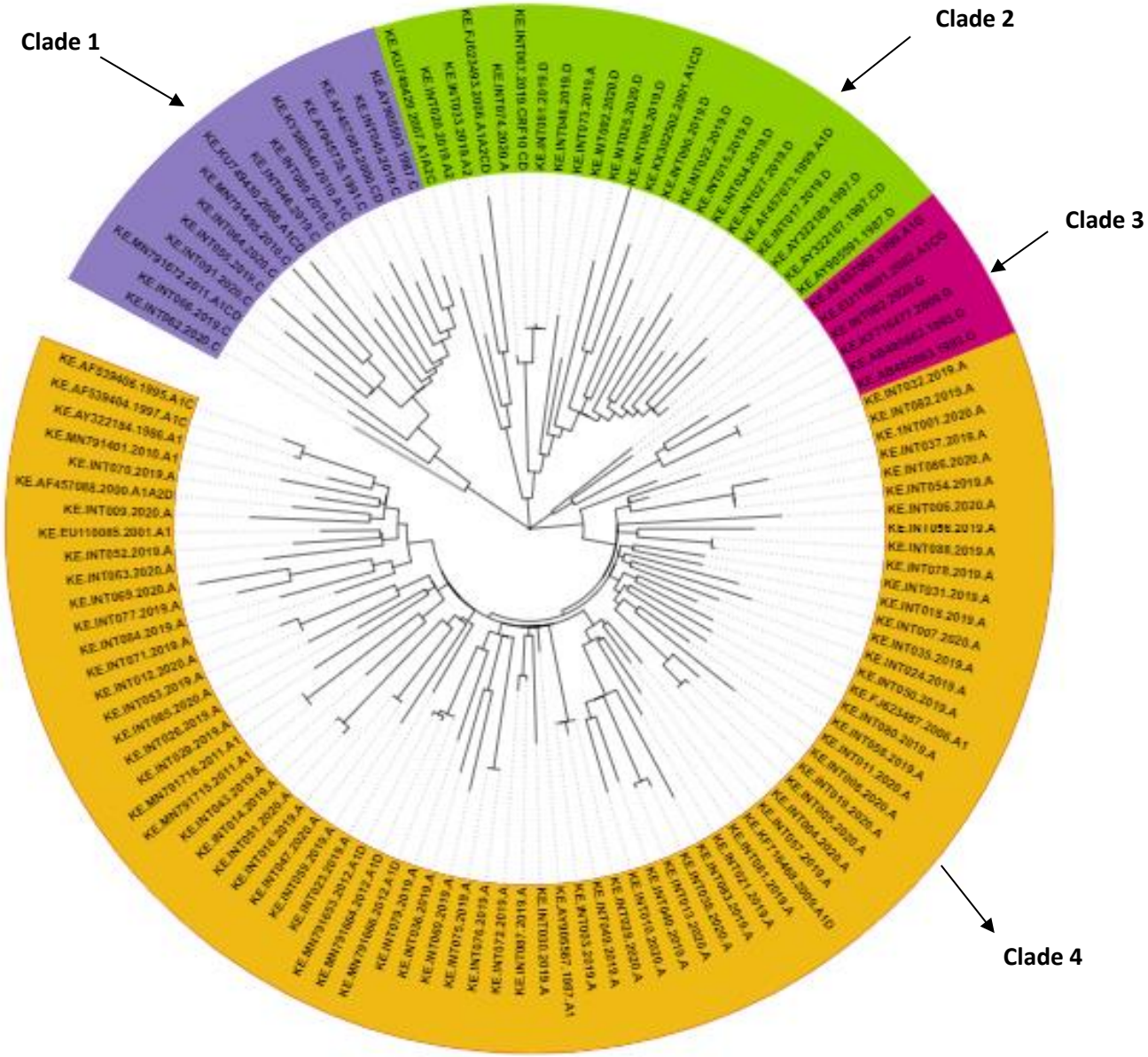

Figure 6: Phylogenetic analysis of the study and database-derived HIV-1 integrase sequences

Analysis of 120 pol-integrase nucleotide sequences;87 study-derived and 33 database-derived. The maximum likelihood phylogenetic tree was constructed using the MEGA 11 software package using a General Time Reversible model. The tree clustered into four clades represented above as different colored ranges.

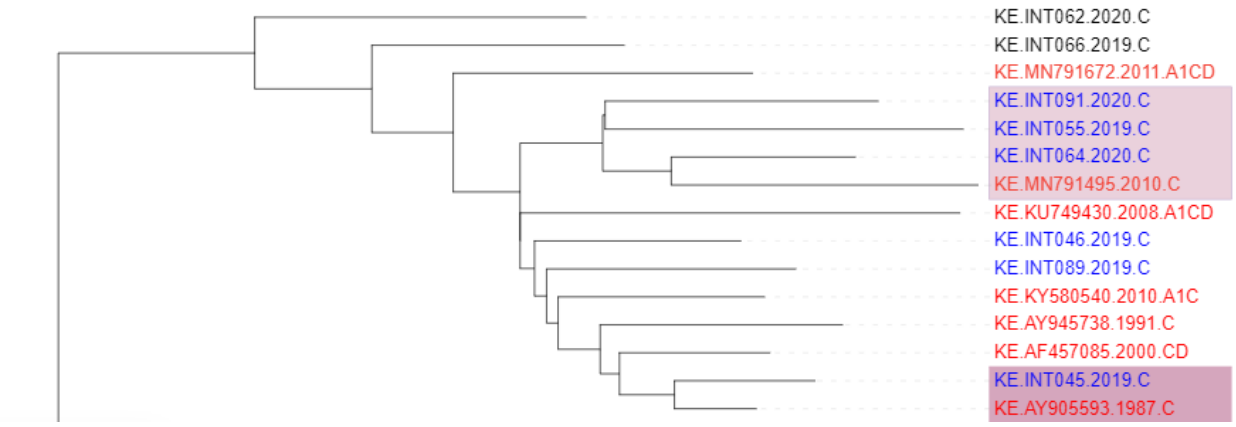

Figure 7: Phylogenetic tree section comparing study and database-derived sequences

Sequences in red are database-derived and blue, study-derived. Highlighted sections show clustering of the two sets of sequences on the same node indicating genetic relatedness.

4.4 Cost analysis

The cost per test for providing an INSTI HIVDR test was estimated to be $50.31 with PCR amplification and sequencing accounting for the bulk of the cost at $26.31 (52.30%) and $18.52, (36.81%) respectively. The costs of extraction and gel electrophoresis were $4.10 (8.15%) and $1.37 (2.7%). Table 14 below outlines the cost analysis for the in-house assay depicting the laboratory processes and the accompanying cost of reagents and consumables.

Table 14: Cost analysis of HIV-Integrase assay for each laboratory process (US$)

Laboratory processes	**$ Cost of reagents and consumables**	**% cost**
RNA Extraction	4.10	8.15
PCR Amplification	26.31	52.30
Gel Electrophoresis	1.36	2.72
Sequencing	18.52	36.81
Total	50.31	100

CHAPTER 5: DISCUSSION

Following the recent inclusion of INSTIs as part of the first-line regimen in all PLHIV and their now widespread use, there arises a need for an affordable and effective INSTI drug resistance test for surveillance of HIVDR to INSTIs and evaluation of the effectiveness of this treatment regimen in PLHIV (Gachogo et al., 2020; World Health Organization, 2017). HIV DRT is costly and its access is limited especially in under-resourced settings and cannot be used for all patients failing first-line treatment (Chaturbhuj et al., 2014; Manasa et al., 2014; Zhou et al., 2011). For this reason, population-based surveys are recommended as a HIVDR monitoring tool (World Health Organization, 2017). Various strategies have been employed in an attempt to make DRT more accessible and affordable, including but not limited to modification of commercial DRT assays (Magomere et al., 2019), reducing the number of amplification steps or primers required (Manasa et al., 2014) and development of in-house DRT assays (Chaturbhuj et al., 2014; Hearps et al., 2009; Inzaule et al., 2013; Seatla et al., 2019; To et al., 2013; Van Laethem et al., 2008; Zhou et al., 2011). However, most of these in-house assays have been developed for the RT and PR region. In this study, we demonstrate the performance characteristics of a low-cost in-house HIV DRT assay targeting the pol-integrase region using WHO guidelines to validate a genotyping test (World Health Organization, 2020). Further, we characterize the pol-integrase region of INSTI-naïve treatment-experienced patients and perform a cost analysis of providing DRT to patients failing an INSTI-based regimen. In summary, our in-house HIV-integrase assay satisfied the validation criteria for an in-house genotyping assay and proved affordable making it an attractive alternative to the costly commercially available INSTI drug resistance tests, especially for poor-resourced settings. Genetic diversity in the pol-integrase region was observed and the study sequences clustered with the database-derived sequences showing a degree of genetic relatedness. No major

RAMs were found, but accessory mutations with no influence on drug susceptibility and APOBEC mutations were observed.

Validation of a low-cost in-house HIV INSTI drug resistance assay

Our in-house assay was comparable to the reference assay as there was a high degree of similarity in the nucleotide and amino acid sequences generated by both assays for the same samples. Further, there was concordance in the detection of the T97TA mutation. Studies comparing the performance of in-house assays to commercial integrase genotyping assays, Viroseq™ Integra48 kit and Celera RUO, have reported similar comparability findings (Seatla et al., 2019; To et al., 2013). T97TA is a polymorphic accessory mutation that occurs infrequently in treatment-naïve patients but is also selected in treatment-experienced patients as in our assessment samples (Abram et al., 2017). The assessment samples lacked sufficient representation of Group-M subtypes as only subtypes A and J were identified during subtype analysis. Virological Quality Assurance (VQA) panels with comprehensive subtype coverage would serve as a better source of assessment samples and show our assay's versatility. The slight differences in base calling at positions with mixed bases by the two assays could be attributed to erroneous nucleotide incorporation by the enzyme *Taq* polymerase, binding of primers in a discriminatory manner, presence of numerous viral variants, poor sequence quality and variations in the criteria for calling of mixed bases (Chaturbhuj et al., 2014). The high average nucleotide sequence similarity of the replicates assessed intra and inter-assay (precision and reproducibility respectively) demonstrate the reliability of our in-house assay. Similar high reproducibility rates for an in-house assay have been obtained in previous studies (Hearps et al., 2009). In drug resistance testing for patients with treatment failure, it is important to be able to successfully amplify samples with VL>1000 copies/mL. Treatment failure is defined as having persistent VL>1000 copies/mL after

determining and addressing the cause of high VL. Our assay successfully amplified all samples with VL>1000 copies/mL (n=8, 100%) and can therefore potentially be useful in RLS to detect INSTI HIVDR. Similar findings of amplification sensitivity have been reported for an in-house assay (Seatla et al., 2019). Failure of our in-house assay to amplify a sample with VL of 500 copies/mL illustrates the inability of our assay to detect INSTI resistance in patients with low-level viremia effectively. Amplification failure can result from low quality of RNA, low VL and nucleotide changes at primer binding sites (Korn et al., 2009; Van Laethem et al., 2008).

Characterization of the pol-integrase region and analysis of drug resistance-associated loci

We successfully amplified and sequenced the IN gene of 87 plasma samples, 83 of which were complete for all the 288 amino acids of the IN gene. The remaining four samples had near full-length codon lengths; INTI031 (287 amino acids), INT032 (285 amino acids), INT034 (287 amino acids) and INT085 (285 amino acids). The majority of the plasma samples were collected in 2019 and of all analyzed sequences, 56% were from female subjects and 44% from male subjects. The median age and viral load at sampling were 21 years (IQR 15-43) and 31279 cp/mL (IQR 3389-59939) respectively. At the time of sampling, the majority of the patients were on a PI-based regimen and the mean duration on ART was 59.6 ±35.4 months. The start regimen for most patients was an NNRTI-based regimen. Subtypes A and D were the pre-dominating subtypes among the study sequences occurring at a frequency of 76% and 13% respectively. Comparable findings were reported for a similar Kenyan study for the same population with subtypes A and D prevailing at frequencies of 78.8% and 15.2% respectively (Mabeya et al., 2020). Non-B subtypes are the dominant subtypes within the African region with subtype A being prevalent in East Africa and subtypes A and D co-circulating within Kenya (Bbosa et al., 2019; Gounder et al., 2017). No major INSTI RAMs were identified in the study sequences, suggesting the role of selective drug

pressure on their emergence and the improbability of transmission of drug resistance in populations naïve to INSTIs. While other studies reported similar findings for INSTI-naïve patients (Acharya et al., 2020; Arruda et al., 2010; Brado et al., 2018b; Doyle et al., 2015b; Mabeya et al., 2020; Nyamache et al., 2012; Parczewski et al., 2012) some studies have reported the presence of major INSTI RAMs for the same population (Chang et al., 2016b; Mikasi et al., 2020). The polymorphic (T97A/T97TA) and non-polymorphic (D232N and Q146QR) accessory mutations present in 5% of the sequences have little influence on INSTI susceptibility. T97A is the most commonly occurring accessory mutation and has been observed in several studies involving INSTI-naïve subjects (Acharya et al., 2020; Mabeya et al., 2020; Nyamache et al., 2012; Parczewski et al., 2012). In the presence of a major mutation, accessory mutations may result in drug resistance, thus there is a need for genetic surveillance even in light of the increasing use of INSTI-based therapy (World Health Organization, 2001)

Phylogenetic analysis of study-derived and database-derived sequences

The topology of the phylogenetic tree constructed from the study-derived and database-derived sequences revealed genetic relatedness between these two sets of sequences and reflected the existence of genetic variability in the pol-integrase region. Genetic variability may lead to the preferential development of viral variants with an increased propensity to drug resistance subsequently affecting the clinical use of INSTIs (Rhee et al., 2016; Santoro & Perno, 2013).

Cost analysis

Our in-house assay proved an affordable alternative to commercial integrase genotyping assays for monitoring HIVDR to INSTIs. The estimated cost of providing the in-house INSTI-HIVDR test to a patient was $50.31 (reagents and consumables). This cost is considerably lower in

comparison to a commercial HIVDR assay, Viroseq™ HIV-1 Integrase RUO Genotyping Kit (Celera Corporation, USA), which costs about $147 (reagents only) (Seatla et al., 2019). Similar trends have been observed in cost analysis of other in-house integrase genotyping assays (Seatla et al., 2019; To et al., 2013).

Conclusion

The in-house HIV-Integrase assay met the validation acceptance criteria and proved affordable, highlighting its potential use as an alternative to commercial INSTI tests in resource-limited settings. In INSTI-naïve patients, the occurrence of accessory mutations with no influence on drug susceptibility was common with no major resistance mutations suggesting the absence of pre-treatment INSTI drug resistance and consequently the effectiveness of INSTI treatment in Kenya.

Study limitations

The study samples were drawn from a single Comprehensive Care Centre thus, the findings of the above study cannot be extrapolated to the larger PLHIV-1 population. This being a retrospective study, missing data on certain variables of interest for some patients was a drawback. Thus, the interpretation of drug resistance needs to be taken with caution.

Recommendations

- Conduct a wider study with sampling at different clinics serving PLHIV across geographical locations in Kenya
- Perform a prospective study to address the drawback of missing data variables and ensure real-time capture of variables of interest.
- Conduct continuous genetic surveillance to provide useful HIVDR data for policy makers and implementers

REFERENCES

Abbas, A., Lichtman, A., & Pillai, S. (2017). Cellular and Molecular Immunology. In *Journal of Chemical Information and Modeling* (9th ed., Vol. 8, Issue 9). Elsiever. https://doi.org/10.1017/CBO9781107415324.004

Abram, M. E., Ram, R. R., Margot, N. A., Barnes, T. L., White, K. L., Callebaut, C., & Miller, M. D. (2017). Lack of impact of pre-existing T97A HIV-1 integrase mutation on integrase strand transfer inhibitor resistance and treatment outcome. *PLOS ONE*, 1–25. https://doi.org/10.1371/journal.pone.0172206

Acharya, A., Tagny, C. T., Mbanya, D., Fonsah, J. Y., Nchindap, E., Kenmogne, L., Jihyun, M., Njamnshi, A. K., & Kanmogne, G. D. (2020). Variability in HIV-1 integrase gene and 3'-polypurine tract sequences in Cameroon clinical isolates, and implications for integrase inhibitors efficacy. *International Journal of Molecular Sciences*, *21*(5). https://doi.org/10.3390/ijms21051553

Aitken, S. C., Bronze, M., Wallis, C. L., Stuyver, L., Steegen, K., Balinda, S., Kityo, C., Stevens, W., & Wit, T. F. R. de. (2013). A Pragmatic Approach to HIV-1 Drug Resistance Determination in Resource-Limited Settings by Use of a Novel Genotyping Assay Targeting the Reverse Transcriptase-Encoding Region Only. *Journal of Clinical Microbiology*, *51*(6), 1757–1761. https://doi.org/10.1128/JCM.00118-13

Anstett, K., Brenner, B., Mesplede, T., & Wainberg, M. A. (2017). HIV drug resistance against strand transfer integrase inhibitors. In *Retrovirology* (Vol. 14, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12977-017-0360-7

Arruda, L.̃Bárbara, Fonsesca, L. A. M., Duarte, A. J. S., & Casseb, J. (2010). Genetic Diversity on the Integrase Region of the pol Gene among HIV Type 1-Infected Patients Naive for Integrase Inhibitors in São Paulo City, Brazil. *AIDS Research and Human Retroviruses*, *26*(1). https://doi.org/10.1089/aid.2009.0179

Asahchop, E., Wainberg, M., Sloan, R., & Tremblay, C. (2012). Antiviral Drug Resistance and the Need for Development of New. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5000–5008. https://doi.org/10.1128/AAC.00591-12

Bbosa, N., Kaleebu, P., & Ssemwanga, D. (2019). HIV subtype diversity worldwide. *Current Opinion in HIV and AIDS*, *14*(3), 153–160. https://doi.org/10.1097/COH.0000000000000534

Blanco, J. L., Varghese, V., Rhee, S. Y., Gatell, J. M., & Shafer, R. W. (2011). HIV-1 integrase inhibitor resistance and its clinical implications. In *Journal of Infectious Diseases* (Vol. 203, Issue 9, pp. 1204–1214). https://doi.org/10.1093/infdis/jir025

Brado, D., Obasa, A. E., Ikomey, G. M., Cloete, R., Singh, K., Engelbrecht, S., Neogi, U., & Jacobs, G. B. (2018a). Analyses of HIV-1 integrase sequences prior to South African national HIV-Treatment program and available of integrase inhibitors in Cape Town, South Africa. *Scientific Reports*, *8*(1), 1–9. https://doi.org/10.1038/s41598-018-22914-5

Brado, D., Obasa, A. E., Ikomey, G. M., Cloete, R., Singh, K., Engelbrecht, S., Neogi, U., & Jacobs, G. B. (2018b). Analyses of HIV-1 integrase sequences prior to South African national HIV-Treatment program and available of integrase inhibitors in Cape Town, South Africa. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-22914-5

Campbell-Yesufu, O. T., & Gandhi, R. T. (2011). Update on human immunodeficiency virus (HIV)-2 infection. *Clinical Infectious Diseases*, *52*(6), 780–787. https://doi.org/10.1093/cid/ciq248

Chang, S. Y., Lin, P. H., Cheng, C. L., Chen, M. Y., Sun, H. Y., Hsieh, S. M., Sheng, W. H., Su, Y. C., Su, L. H., Chang, S. F., Liu, W. C., Hung, C. C., & Chang, S. C. (2016a). Prevalence of Integrase Strand Transfer Inhibitors (INSTI) Resistance Mutations in Taiwan. *Scientific Reports*, *6*, 1–9. https://doi.org/10.1038/srep35779

Chang, S. Y., Lin, P. H., Cheng, C. L., Chen, M. Y., Sun, H. Y., Hsieh, S. M., Sheng, W. H., Su, Y. C., Su, L. H., Chang, S. F., Liu, W. C., Hung, C. C., & Chang, S. C. (2016b). Prevalence of Integrase Strand Transfer Inhibitors (INSTI) Resistance Mutations in Taiwan. *Scientific Reports*, *6*. https://doi.org/10.1038/srep35779

Chaturbhuj, D. N., Nirmalkar, A. P., Paranjape, R. S., & Tripathy, S. P. (2014). Evaluation of a Cost Effective In-House Method for HIV-1 Drug Resistance Genotyping Using Plasma Samples. *PLOS ONE*, *9*(2), 1–8. https://doi.org/10.1371/journal.pone.0087441

Chimukangara, B., Lessells, R. J., Singh, L., Grigalionyte, I., Yende-Zuma, N., Adams, R., Dawood, H., Dlamini, L., Buthelezi, S., Chetty, S., Diallo, K., Duffus, W. A., Mogashoa, M., Hagen, M. B., Giandhari, J., de Oliveira, T., Moodley, P., Padayatchi, N., & Naidoo, K. (2021). Acquired HIV drug resistance and virologic monitoring in a HIV hyper-endemic setting in KwaZulu-Natal Province, South Africa. *AIDS Research and Therapy*, *18*(1). https://doi.org/10.1186/s12981-021-00393-5

Delelis, O., Carayon, K., Saïb, A., Deprez, E., & Mouscadet, J. F. (2008). Integrase and integration: Biochemical activities of HIV-1 integrase. *Retrovirology*, *5*, 1–13. https://doi.org/10.1186/1742-4690-5-114

Department of Health and Human Services. (2019). *Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV*. https://aidsinfo.nih.gov/guidelines

Dixon, S., Mcdonald, S., & Roberts, J. (2002). The impact of HIV and AIDS on Africa ' s economic development. *British Medical Journal* , 232–234. https://doi.org/10.1136/bmj.324.7331.232

Doyle, T., Dunn, D. T., Ceccherini-Silberstein, F., de Mendoza, C., Garcia, F., Smit, E., Fearnhill, E., Marcelin, A. G., Martinez-Picado, J., Kaiser, R., Geretti, A. M., Calvez, V., Malet, I., Pino, M., Santos, J. R., & Clotet, B. (2015a). Integrase inhibitor (INI) genotypic resistance in treatment-naive and raltegravir-experienced patients infected with diverse HIV-1 clades. *Journal of Antimicrobial Chemotherapy*, *70*(11), 3080–3086. https://doi.org/10.1093/jac/dkv243

Doyle, T., Dunn, D. T., Ceccherini-Silberstein, F., de Mendoza, C., Garcia, F., Smit, E., Fearnhill, E., Marcelin, A. G., Martinez-Picado, J., Kaiser, R., Geretti, A. M., Calvez, V., Malet, I., Pino, M., Santos, J. R., & Clotet, B. (2015b). Integrase inhibitor (INI) genotypic resistance in treatment-naive and raltegravir-experienced patients infected with diverse HIV-1 clades. *Journal of Antimicrobial Chemotherapy*, *70*(11), 3080–3086. https://doi.org/10.1093/jac/dkv243

Esposito, D., & Craigie, R. (1999). HIV integrase structure and function. *Advances in Virus Research*, *52*, 319–333. https://doi.org/10.1016/S0065-3527(08)60304-8

European AIDS Clinical Society. (2019). *European AIDS Clinical Society Guidelines*. https://www.eacsociety.org/files/guidelines-10.0_final_2_2.pdf

Gachogo, R. W., Mwai, D. N., & Onyambu, F. G. (2020). Cost analysis of implementing HIV drug resistance testing in Kenya : a case study of a service delivery site at a tertiary level hospital in Kenya. *F1000Research*, 1–16. https://doi.org/https://doi.org/10.12688/f1000research.23379.1

Geretti, A. M., & Easterbrook, P. (2001). Antiretroviral resistance in clinical practice. *International Journal of STD& AIDS* , *12*(3), 145–153. https://doi.org/10.1258/0956462011916938

Gounder, K., Oyaro, M., Padayachi, N., Zulu, T. M., de Oliveira, T., Wylie, J., & Ndung'u, T. (2017). Complex subtype diversity of HIV-1 among drug users in Major Kenyan cities. *AIDS Research and Human Retroviruses*, *33*(5), 500–510. https://doi.org/10.1089/aid.2016.0321

Hearps, A. C., Greengrass, V., Hoy, J., & Crowe, S. M. (2009). An HIV-1 integrase genotype assay for the detection of drug resistance mutations. *Sexual Health*, *6*(4), 305–309. https://doi.org/10.1071/SH09041

Herring, B. L., Cunningham, A. L., & Dwyer, D. E. (2004). Potential Drug Resistance Polymorphisms in the Integrase Gene of HIV Type 1 Subtype A. *AIDS Research and Human Retroviruses* , *20*(9), 1010–1014. https://doi.org/10.1089/aid.2004.20.1010

Hightower, K. E., Wang, R., Deanda, F., Johns, B. A., Weaver, K., Shen, Y., Tomberlin, G. H., Iii, H. L. C., Broderick, T., Sigethy, S., Seki, T., Kobayashi, M., & Underwood, M. R. (2011). Dolutegravir (S / GSK1349572) Exhibits Significantly Slower Dissociation than Raltegravir and Elvitegravir from Wild-Type and Integrase Inhibitor-Resistant. *Antimicrobial Agents and Chemotherapy* , *55*(10), 4552–4559. https://doi.org/10.1128/AAC.00157-11

Hladik, F., & Mcelrath, M. J. (2008). Setting the stage : host invasion by HIV. *Nature Reviews Immunology* , *8*, 447–457. https://doi.org/10.1038/nri2302

Inzaule, S., Hamers, R. L., Doherty, M., Shafer, R. W., Bertagnolio, S., & Wit, T. F. R. De. (2019). Personal View Curbing the rise of HIV drug resistance in low-income and middle-income countries : the role of dolutegravir-containing regimens. *The Lancet Infectious Diseases*, *3099*(18), 1–7. https://doi.org/10.1016/S1473-3099(18)30710-2

Inzaule, S., Ondoa, P., Peter, T., Mugyenyi, P. N., Stevens, W. S., Wit, T. F. R. de, & Hamers, R. L. (2016). Affordable HIV drug-resistance testing for monitoring of antiretroviral therapy in sub-Saharan Africa. *The Lancet Infectious Diseases*, *3099*(16). https://doi.org/10.1016/S1473-3099(16)30118-9

Inzaule, S., Yang, C., Kasembeli, A., Nafisa, L., Okonji, J., Oyaro, B., Lando, R., Mills, L. A., Laserson, K., Thomas, T., Nkengasong, J., & Zeh, C. (2013). Field evaluation of a broadly sensitive HIV-1 In-house genotyping assay for use with both plasma and dried blood spot specimens in a resource-limited country. *Journal of Clinical Microbiology*, *51*(2), 529–539. https://doi.org/10.1128/JCM.02347-12

Katz, R. A., & Skalka, A. M. (1994). The Retroviral Enzymes. *Annual Review of Biochemistry* , *63*, 133–173. https://doi.org/10.1146/annurev.bi.63.070194.001025

Kemnic, T., & Gulick, P. (2022). *HIV Antiretroviral Therapy* . StatPearls Publishing, Treasure Island (FL).

Korn, K., Weissbrich, B., Henke-Gendo, C., Heim, A., Jauer, C. M., Taylor, N., & Eberle, J. (2009). Single-Point Mutations causing more than 100-Fold Underestimation of Human Immunodeficiency Virus Type 1 (HIV-1) Load with the Cobas TaqMan HIV-1 Real-Time PCR Assay. *Journal of Clinical Microbiology*, *47*(4), 1238–1240. https://doi.org/10.1128/JCM.02204-08

Llibre, J. M., Pulido, F., García, F., Deltoro, M. G., Blanco, J. L., & Delgado, R. (2015). Genetic Barrier to Resistance for Dolutegravir. *AIDS Reviews*, *17*, 56. https://pubmed.ncbi.nlm.nih.gov/25472016/

Los Alamos National Library. (2018). *HIV Circulating Recombinant Forms* .

Luciw, P. A. (1996). *Human immunodeficiency viruses and their replication* (3rd ed., Vol. 2). Lippincott-Raven.

Maarseveen, N., & Boucher, C. (2006). Resistance to protease inhibitors. In A. Geretti (Ed.), *Antiretroviral Resistance in Clinical Practice*. Mediscript. https://www.ncbi.nlm.nih.gov/books/NBK2250/

Mabeya, S., Nyamache, A., Ngugi, C., Nyerere, A., & Lihana, R. (2020). Characterization of HIV-1 Integrase Gene and Resistance Associated Mutations Prior to Roll out of Integrase Inhibitors by Kenyan National HIV-Treatment Program in Kenya. *Ethiopian Journal of Health Sciences*, *30*(1), 37–44. https://doi.org/10.4314/ejhs.v30i1.6

Magomere, E. O., Nyangahu, D. D., Kimoloi, S., Webala, B. A., Ondigo, B. N., & Onyambu, F. (2019). Performance characteristics of a modified HIV-1 drug resistance genotyping method for use in resource-limited settings [version 1 ; peer review : 2 approved]. *F1000Research*, 1–13. https://doi.org/https://doi.org/10.12688/f1000research.20083.1

Manasa, J., Danaviah, S., Pillay, S., Padayachee, P., Mthiyane, H., Mkhize, C., Lessells, R. J., Seebregts, C., Wit, T. F. R. de, Viljoen, J., Katzenstein, D., & Oliveira, T. de. (2014). An Affordable HIV-1 Drug Resistance Monitoring Method for Resource Limited Settings. *Journal of Visualized Experiments*, *March*, 1–13. https://doi.org/10.3791/51242

May, M. T., Gompels, M., Delpech, V., Porter, K., Orkin, C., Kegg, S., Hay, P., Johnson, M., Palfreeman, A., Gilson, R., Chadwick, D., Martin, F., Hill, T., Walsh, J., Post, F., Fisher, M., Ainsworth, J., Jose, S., Leen, C., … Glabay, A. (2014). Impact on life expectancy of HIV-1 positive individuals of CD4R cell count and viral load response to antiretroviral therapy. *AIDS*, *28*(8), 1193–1202. https://doi.org/10.1097/QAD.0000000000000243

Mayer, K. H., Hanna, G. J., & D'Aquila, R. T. (2001). Clinical Use of Genotypic and Phenotypic Drug Resistance Testing to Monitor Antiretroviral Chemotherapy. *Clinical Infectious Diseases*, *32*(5), 774–782. https://doi.org/10.1086/319231

Mbhele, N., Chimukangara, B., & Gordon, M. (2021). HIV-1 integrase strand transfer inhibitors: a review of current drugs, recent advances and drug resistance. In *International Journal of Antimicrobial Agents* (Vol. 57, Issue 5). Elsevier B.V. https://doi.org/10.1016/j.ijantimicag.2021.106343

Melikian, G. L., Rhee, S. Y., Varghese, V., Porter, D., White, K., Taylor, J., Towner, W., Troia, P., Burack, J., DeJesus, E., Robbins, G. K., Razzeca, K., Kagan, R., Liu, T. F., Jeffrey Fessel, W., Israelski, D., & Shafer, R. W. (2014). Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: Implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. *Journal of Antimicrobial Chemotherapy*, *69*(1), 12–20. https://doi.org/10.1093/jac/dkt316

Miedzinski, J. (1992). Early clinical signs and symptoms of HIV Infection. *Canadian Family Physician*, *38*, 1401–1410. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2146153/

Mikasi, S. G., Gichana, J. O., van der Walt, C., Brado, D., Obasa, A. E., Njenda, D., Messembe, M., Lyonga, E., Assoumou, O., Cloete, R., Ikomey, G. M., & Jacobs, G. B. (2020). HIV-1 Integrase Diversity and Resistance-Associated Mutations and Polymorphisms Among Integrase Strand Transfer Inhibitor-Naive HIV-1 Patients from Cameroon. *AIDS Research and Human Retroviruses*, 1–14. https://doi.org/10.1089/aid.2019.0264

Miners, A., Phillips, A., Kreif, N., Rodger, A., Speakman, A., Fisher, M., Anderson, J., Collins, S., & Hart, G. (2014). Health-related quality-of-life of people with HIV in the era of combination antiretroviral treatment : a cross-sectional comparison with the general population. *The Lancet HIV*, *1*(1), e32–e40. https://doi.org/10.1016/S2352-3018(14)70018-9

Ministry of Health Kenya. (2021). *Kenya World AIDS Day Progress Report 2013-2021*. https://nacc.or.ke/information-on-hiv/

Myers, G., Korber, B., Mellors, J. W., Jeang, K.-T., & Wain-Hobson, S. (1996). *Human Retroviruses and AIDS; A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences*. https://doi.org/10.2172/463607

Nyamache, A. K., Muigai, A. S., Ng'ang'a, Z., & Khamadi, A. S. (2012). HIV-1 genetic diversity and naturally occurring polymorphisms in HIV-1 Kenyan isolates Integrase gene: Implications for integrase inhibitors. *AIDS Research and Human Retroviruses*, *28*(8). https://doi.org/10.1089/aid.2011.0122

Nyamweya, S., Hegedus, A., Jaye, A., Rowland-Jones, S., Flanagan, K., & Macallan, D. (2013). Comparing HIV-1 and HIV-2 infection: Lessons for viral immunopathogenesis. *Reviews in Medical Virology*. https://doi.org/10.1002/rmv

Parczewski, M., Bander, D., Urbańska, A., & Boroń-Kaczmarska, A. (2012). HIV-1 integrase resistance among antiretroviral treatment naive and experienced patients from Northwestern Poland. *BMC Infectious Diseases*, *12*. https://doi.org/10.1186/1471-2334-12-368

Parikh, U. M., McCormick, K., van Zyl, G., & Mellors, J. W. (2017). Future technologies for monitoring HIV drug resistance and cure. *Current Opinion in HIV and AIDS*, *12*(2), 182–189. https://doi.org/10.1097/COH.0000000000000344

Patel, P., Borkowf, C. B., Brooks, J. T., Lasry, A., Lansky, A., & Mermin, J. (2014). Estimating per-act HIV transmission risk : a systematic review. *AIDS*, *28*(10), 1509–1519. https://doi.org/10.1097/QAD.0000000000000298

Plantier, J.-C., Leoz, M., Dickerson, J. E., de Oliveira, F., Cordonnier, F., Lemée, V., Damond, F., Robertson David L., & Simon, F. (2009). A new human immunodeficiency virus derived from gorillas. *Nature Medicine*, *15*(8), 871–872. https://doi.org/10.1038/nm.2016

Quashie, P. K., Mesplède, T., & Wainberg, M. A. (2013). Evolution of HIV integrase resistance mutations. *Current Opinion in Infectious Diseases*, *26*(1), 43–49. https://doi.org/10.1097/QCO.0b013e32835ba81c

Quashie, P. K., & Wainberg, M. A. (2012). Resistance to HIV integrase inhibitors. *Current Opinion in HIV and AIDS*, *7*(5), 401–408. https://doi.org/10.1097/COH.0b013e328356db89

Rhee, S.-Y., Sankaran, K., Varghese, V., Winters, M. A., Hurt, C. B., Eron, J. J., Parkin, N., Holmes, S. P., Holodniy, M., & Shafer, R. W. (2016). HIV-1 Protease, Reverse Transcriptase, and Integrase Variation. *Journal of Virology*, *90*(13), 6058–6070. https://doi.org/10.1128/jvi.00495-16

Santoro, M. M., & Perno, C. F. (2013). HIV-1 Genetic Variability and Clinical Implications. *ISRN Microbiology*, *2013*, 1–20. https://doi.org/10.1155/2013/481314

Schafer, J. J., & Squires, K. E. (2010). Integrase inhibitors: A novel class of antiretroviral agents. In *Annals of Pharmacotherapy* (Vol. 44, Issue 1, pp. 145–156). https://doi.org/10.1345/aph.1M309

Seatla, K. K., Choga, W. T., Mogwele, M., Diphoko, T., Maruapula, D., Mupfumi, L., Musonda, R. M., Rowley, C. F., Avalos, A., Kasvosve, I., Moyo, S., & Gaseitsiwe, S. (2019). Comparison of an in-house 'home-brew' and commercial ViroSeq integrase genotyping assays on HIV-1 subtype C samples. *PLoS ONE*, *14*(11), 1–14. https://doi.org/10.1371/journal.pone.0224292

Seitz, R. (2016). Human Immunodeficiency Virus (HIV). *Transfusion Medicine and Hemotherapy*, *43*(3), 203–222. https://doi.org/10.1159/000445852

Shaw, G. M., & Hunter, E. (2012). HIV transmission. *Cold Spring Harbor Perspectives in Medicine*, *2*(11). https://doi.org/10.1101/cshperspect.a006965

Simon, F., Mauclére, P., Roques, P., Loussert-Ajaka, I., Müller-Trutwin, M. C., Saragosti, S., Georges-Courbot, M. C., Barré-Sinoussi, F., & Brun-Vézinet, F. (1998). Identification of a

new human immunodeficiency virus type 1 distinct from group M and group O. *Nature Medicine , 4*.

Tawfik, L., & Kinoti, S. N. (2006). *The impact of HIV / AIDS on the health workforce in developing countries*. http://www.who.int/hrh/documents/Impact_of_HIV.pdf

Tebit, D. M., Ndembi, N., Weinberg, A., & Quiñones-Mateu, M. E. (2012). Mucosal Transmission of Human Immunodeficiency Virus. *Current HIV Research , 10*(1), 3–8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3744389/

To, S. W., Chen, J. H., Wong, K., Chan, K. C., Ng, H., Wu, H., Tszhin, J., & Yam, W. (2013). Performance comparison of an in-house integrase genotyping assay versus the ViroSeq TM Integra48 , and study of HIV-1 integrase polymorphisms in Hong Kong. *Journal of Clinical Virology*, *58*(1), 299–302. https://doi.org/10.1016/j.jcv.2013.06.040

UNAIDS. (2015). *FastTrack-ending the AIDS epidemic by 2030*. https://www.unaids.org/sites/default/files/media_asset/JC2686_WAD2014report_en.pdf

UNAIDS. (2021). Global HIV & AIDS statistics — Fact sheet | UNAIDS. *Unaids*, 2020–2022. https://www.unaids.org/en/resources/fact-sheet

UNAIDS. (2022). *Global HIV & AIDS statistics- Fact sheet*. https://www.unaids.org/en/resources/fact-sheet

Vallari, A., Holzmayer, V., Harris, B., Yamaguchi, J., Ngansop, C., Makamche, F., Mbanya, D., Kaptué, L., Ndembi, N., Gürtler, L., Devare, S., & Brennan, C. A. (2011). Confirmation of Putative HIV-1 Group P in Cameroon. *Journal of Virology*, *85*(3), 1403–1407. https://doi.org/10.1128/jvi.02005-10

Van Laethem, K., Schrooten, Y., Covens, K., Dekeersmaeker, N., De Munter, P., Van Wijngaerden, E., Van Ranst, M., & Vandamme, A. M. (2008). A genotypic assay for the amplification and sequencing of integrase from diverse HIV-1 group M subtypes. *Journal of Virological Methods*, *153*(2), 176–181. https://doi.org/10.1016/j.jviromet.2008.07.008

Vink, C., & Plasterk, R. H. A. (1993). The human immunodeficiency virus integrase protein. *Trends in Genetics , 5*(2), 2591–2596. https://doi.org/10.1016/0168-9525(93)90107-S

Visseaux, B., Damond, F., Matheron, S., Descamps, D., & Charpentier, C. (2016). HIV-2 Molecular Epidemiology. *Infection, Genetics and Evolution*, *46*, 233–240. https://doi.org/10.1016/j.meegid.2016.08.010

Weinberg, J. L., & Kovarik, C. L. (2010). The WHO Clinical Staging System for HIV/AIDS. *American Medical Association Journal of Ethics*, *12*, 202–206. https://doi.org/10.1001/virtualmentor.2010.12.3.cprl1-1003

World Health Organization. (2007). WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children. In *World Health Organization*. https://apps.who.int/iris/handle/10665/43699

World Health Organization. (2001). HIV/AIDS Antiretroviral Newsletter. In *World Health Organization Regional Office for the Western Pacific* (Issue 5). https://apps.who.int/iris/handle/10665/272896

World Health Organization. (2002). Scaling up Antiretroviral Therapy in Resource-Limited Settings: Guidelines for a Public Health Approach. In *IAPAC monthly* (Vol. 8, Issue 6). https://doi.org/10.1097/00019048-200203000-00012

World Health Organization. (2004). Scaling Up Antiretroviral Therapy in Resource-Limited Settings: Treatment Guidelines for a Public Health Approach. In *World Health Organization, Geneva*. https://apps.who.int/iris/handle/10665/42932

World Health Organization. (2006). *Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach*. https://doi.org/10.1093/ofid/ofx156

World Health Organization. (2010). Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach. In *Health (San Francisco)*. https://apps.who.int/iris/handle/10665/44379

World Health Organization. (2013). *Consolidated Guidelines on the use of Antiretroviral Drugs for Treating and Preventing HIV Infection*. https://www.who.int/publications/i/item/9789241505727

World Health Organization. (2015). *Guideline on When to Start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for HIV* (Issue September). https://apps.who.int/iris/handle/10665/186275

World Health Organization. (2017). *Global Action Plan on HIV Drug Resistance 2017-2021* (Issue July 2017). https://www.who.int/hiv/pub/drugresistance/hivdr-action-plan-2017-2021/en/

World Health Organization. (2018). *Recommended Methods for Validation of an In-House Genotyping Assay for Surveillance of HIV Drug Resistance*. https://www.jstor.org/stable/pdf/resrep27859.13.pdf

World Health Organization. (2019a). *HIV Drug Resistance Report*. https://www.who.int/publications/i/item/WHO-CDS-HIV-19.21

World Health Organization. (2019b). *Update of Recommendations on First- and Second-line Antiretroviral Regimens* (Issue July). https://www.who.int/hiv/pub/arv/arv-update-2019-policy/en/

World Health Organization. (2020). *WHO HIVResNet HIV DRUG RESISTANCE LABORATORY OPERATIONAL FRAMEWORK*. file:///C:/Users/user/Downloads/9789240009875-eng%20(4).pdf

Zhou, Z., Wagar, N., Devos, J. R., Rottinghaus, E., Diallo, K., Nguyen, D. B., Ugbena, R., Wadonda-kabondo, N., Mcconnell, M. S., Zulu, I., Nkengasong, J., & Yang, C. (2011). Optimization of a Low Cost and Broadly Sensitive Genotyping Assay for HIV-1 Drug Resistance Surveillance and Monitoring in Resource-Limited Settings. *PLOS ONE*, *6*(11), 1–10. https://doi.org/10.1371/journal.pone.0028184

APPENDICES

Ethical Clearance

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14th September 2020

Vera Morangi Onwonga
Reg. No.H56/ 11347/2018
Dept.of Medical Microbiology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Vera

RESEARCH PROPOSAL – VALIDATION OF A LOW-COST INTEGRASE INHIBITOR DRUG RESISTANCE ASSAY AND CHARACTERIZATION OF THE HIV INTEGRASE GENE FROM PATIENTS AT KENYATTA NATIONAL HOSPITAL COMPREHENSIVE CARE CENTRE (P263/05/2020)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 14th September 2020 – 13th September 2021.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*)
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website**http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M' L. CHINDIA
SECRETARY, KNH-UoN ERC

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The Chairperson, KNH- UoN ERC
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