HSV-2 PREVALENCE AND HIV DIVERSITY AMONG CO-INFECTED FISHERMEN ALONG LAKE VICTORIA IN KISUMU COUNTY

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HSV-2 Prevalence and HIV Diversity among Co-Infected Fishermen along Lake Victoria in Kisumu County

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

Signature……Date…….

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This thesis has been submitted for examination with our approval as the university supervisors

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DEDICATION

I dedicate this thesis to my mother, Mrs. Sephirah Wanjiku Macharia who taught me to persevere and be prepared to face challenges with faith and humility.

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This thesis has been possible through God's favors. I would not have made it were it not for the tireless and sacrificial inputs of several individuals. I am particularly grateful to my supervisors Dr. Raphael Lihana and Dr. Caroline Ngugi for their continuous unreserved enlightening supervision and support.

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ABREVIATIONS AND ACRONYMS

AIDS Acquired Immunodeficiency syndrome

BLAST Basic Local Alignment Search Tool

CD4 Clusters of differentiation 4

CRFs Circulating Recombinant Forms

DNA Deoxyribonucleic Acid

DR Drug resistance

EDTA Ethylenediaminetetraacetic acid

ELISA Enzyme-linked Immunosorbent Assay

ERC Ethics and Review Committee

HIV Human immunodeficiency virus

HSV Herpes simplex virus

IgG Immunoglobin G

IQR Interquartile range

KAIS Kenya Aids Indicator Survey

KEMRI Kenya Medical Research Institute

Km Kilometers

LANL Los Alamos National Laboratory.

ml	Milliliters
mRNA	Messenger RNA
NF-B	Nuclear Factor beta
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
SD	Standard deviation
SIV	Simian Immunodeficiency virus
STIs	Sexually Transmitted Infections
TDR	Transmitted Drug Resistance
US$	United States dollar
UV	Ultra violet light
VL	Viral load

ABSTRACT

Herpes simplex virus type 2 (HSV-2) infections have been associated with a three- fold risk of Human Immunodeficiency Virus-1 (HIV-1) acquisition. The prevalence of HIV-1 and HSV-2 in the fishing communities along the shores of Lake Victoria in Kisumu has been reported to be high. This may contribute to the growing HIV epidemic in Kenya including the spread of transmitted drug resistance (TDR). This study aimed at finding the prevalence of HSV-2, HIV and HSV-2/HIV co-infection, circulating HIV subtypes and the factors associated with these infections. A cross sectional study was conducted where blood samples were obtained from 249 consenting fishermen from four beaches; Nyamware, Kichinjio, Dunga, and Kobudho of Lake Victoria. A questionnaire was administered to collect sociodemographic and related data. Blood samples were analyzed for HIV-1/HSV2 co-infection. The HSV-2 serology was performed using Kalon HSV type 2 enzyme- linked immunosorbent assay (ELISA). The HIV-1 serology was carried out according to local standards of practice in Kenya, using two parallel rapid assays (Alere *Determine*™ *HIV*-1/2 and Trinity Biotech Uni-Gold™), with a third ELISA - Vironostika HIV Uni-Form II Ag/Ab for resolving discrepancies. All HIV positive samples were tested for Drug resistance using an in-house HIV-1 *pol-RT* genotyping protocol. Of the 249 recruited fishermen (mean age 35.1 years), 104 (41.8%) were HIV/HSV-2 negative, 86 (34.5%) were HSV-2 mono-positive, 11 (4.4%) were HIV- 1 mono positive while 48 (19.3%) were HIV/HSV-2 co-infected. HIV-1 subtype A was most common 28/59 (47.5%). Subtypes D, C B, G accounted for 16/59 (27.1%), 3/59 (5.1%), 3/59 (5.1%) and 2/59 (3.4%), respectively. The CRF01_AE recombinant forms were also identified in 7/59 (11.9 %) participants. Low condom use, multiple partners, owning fishing net and being a fisherman was strongly associated with HIV, HSV-2 and HIV/HSV-2 co-infection. There is need for improved STI services and targeted behavioral interventions among this fishing community.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Human Immunodeficiency Virus type 1 (HIV-1) and HIV type 2 (HIV-2) are retrovirus causing human infection (King *et al.,* 2011). The HIV- 1 is genetically classified into four distinct groups namely: M, non - M, non - O (N), outlier (O) and P with group M responsible for the current global pandemic (Peeters *et al*., 2003; McCutchan, 2006).

Since recognition in 1981 of the acquired immune deficiency syndrome (AIDS), HIV has produced a worldwide epidemic. In many parts of Africa, HIV/AIDS is a major public health concern and cause of death. Africa is home to about 15.2% of the world's population yet sub-Saharan Africa alone, the hardest hit region, accounts for more than two-thirds of people living with HIV (UNAIDS, 2021). It is primarily spread through sexual exposure (WHO, 2017). Nonsexual HIV transmission can occur through transfusion with contaminated blood products, injection drug use, occupational exposure, or accidental needle sticks (Neupane & Mishra, 2014). HIV prevalence in Kenya also varied by region, with the highest prevalence in Siaya county at 26%, and lowest prevalence in the North Eastern region, Wajir County at 0.1%. Kenya has an estimated 44,800 new HIV infection among adults and about 8000 among children annually (NACC, 2018).

Due to the introduction of highly active antiretroviral therapy in the mid- 1990's HIV related morbidities have reduced and the lifespan of people living with HIV has also been prolonged (Wing, 2016). However, the broad use of multiple antiretroviral drugs has led to drug resistance which is a common cause of treatment failure and also to transmission of drug resistant viruses (Obiako, 2010).

Herpes simplex viruses type 2 (HSV-2 or human herpesvirus 2, HHV-2), are members of the *Herpesviridae* family and *Alphaherpesvirinae* subfamily (Davison, 2010). Herpes simplex type 2 virus (HSV-2) is one of the world's three most sexually

transmitted diseases and a leading cause of genital ulcers (Corey & Handsfield, 2000; Gupta *et al.*, 2007). It is a lifelong viral sexually transmitted infection (STIs) of global health importance. HSV has been considered the most common cause of sexually transmitted infections leading to ulcers (Hope-Rapp *et al*., 2010) although it leads to periodic recurrences of painful genital ulcers in symptomatic individuals; it is asymptomatic in about 80%–90% of individuals in whom viral reactivation and shedding occur (Mertz *et al*., 1992; Wald *et al*., 2000). Hence, HSV-2 transmissions can remain sustainable in the general public and the prevalence rates can reach very high levels if interventions to curb the infection are not in place.

In 2012, around 417 million (11.3%) people aged 15-49 years were infected with HSV-2 globally (Looker *et al.,* 2017). Hence, HSV-2 transmissions can remain sustainable in the general public and the prevalence rates can reach very high levels if interventions to curb the infection are not in place.

It has been reported that sexually transmitted diseases (STDs) facilitate the sexual spread of human immunodeficiency virus (HIV) infection (Wasserheit, 1992; Galvin *et al*., 2004; Valere *et al*., 2015; Beck *et al*., 2015). This is particularly strong for genital ulcer disease, with an increased 2 - 4-fold incidence of HIV in the presence of genital ulcers (Cameron *et al*., 1989; Dickerson *et al.,* 1996). Genital HSV-2 infection is considered as one of the major contributors to the sexual transmission and acquisition of HIV-1 (Wald and Link, 2002). Epidemiological evidence, biological Plausibility and interventional studies supporting the interaction between HIV and HSV-2 exist (Fleming and Wasserheit, 1999; Wald and Link, 2002; Celum, 2004). As a major co - factor of HIV-1 sexual transmission, the biological effect of HSV-2 includes: Herpetic ulcerations; viral shedding and in vitro synergestic interaction between HIV-1 and HSV-2 (Mbopi-Kéou *et al*., 2000; Baeten *et al*., 2004; LeGoff *et al*., 2007).

Sub-Saharan African countries in the past decade, has been marked with substantial scale up in the access to antiretroviral therapy (ART) (Jahn et al., 2008). Unfortunately, significant increase in patients failing ART and HIV resistance to some ART medications is being observed (Gupta *et al*., 2012). Consequently, cases of ART-naïve

individuals infected with TDR, associated with treatment failure is on the rise (Wittkop *et al.,* 2011). Studies across Kenya are beginning to show widespread resistance in ART-naïve persons (Chung *et al*., 2014; Budambula *et al.*, 2015). Data area however lacking among the fishermen in the western Kenya, a group at a significant risk of HIV infection. Against this backdrop, this study was to determine the prevalence of HIV, HSV-2, HIV-1/HSV-2 co-infection, HIV-1 subtypes, the drug resistance mutations and the factors influencing their transmission among fish workers along Nyamware, Kichinjio, Ndunga and Kobudho beaches in Kisumu County.

1.2 Statement of the Problem

Herpes simplex virus 2 (HSV-2) and HIV represent a global health issue with estimated 512 million infected with HSV-2 while 36.7 million people were infected with HIV globally (Looker *et al*., 2017; "UNAIDS Data 2017", 2019). Approximately one million people have died of HIV-related causes worldwide (Looker *et al*., 2017; UNAIDS, 2019). An estimated 800 000 people in eastern and southern Africa acquired HIV in 2017, and an estimated 380 000 people died of AIDS-related illness. In Kenya, about 1.2 million adults and 104,000 children are infected with HIV (NASCOP, 2013). Currently, there is no cure for HIV infection (NIH: National Institute of Allergy and Infectious Diseases, 2019).

HSV-2 is a major coinfection among HIV infected people (Byrne, Gantt & Coombs, 2018). In 2012 there were an estimated 417 million people aged 15–49 years with prevalent HSV-2 infection globally (Looker *et al*., 2015). It is asymptomatic in about 80%–90% of individuals in whom viral reactivation and shedding occur undetected. Hence, HSV-2 transmissions can remain sustainable in the general public and the prevalence rates can reach very high levels if interventions to curb the infection are not in place (Anaedobe & Ajani, 2019).

The median economic cost per patient-year in Kenya was estimated at $248.91 (approximately $2011) for established adult ART patients ($120.72 with the cost of ARVs excluded) other expenses incurred includes infrastructure and training of staff and change of drug regimen to costlier ones (Larson *et al*., 2013; Centers for Diseases Control and Kenya Ministry of Health, 2013).

Research has shown that the spread of HIV is facilitated by sexually transmitted diseases (STDs) (Wasserheit, 1992; Galvin *et al*., 2004; Valere *et al*., 2015; Beck *et al*., 2015). The association of these STDs has also been documented (Cameron *et al*., 1989; Dickerson *et al.,* 1996). Herpes simplex virus type 2 (HSV-2) genital infection is considered to be a major co-factor that promotes both transmission and acquisition of HIV-1 (Wald and Link, 2002).

High incidence and prevalence of STI have been reported among the fishing community in Kisumu (Ng'ayo *et al*., 2008; Kwena *et al*., 2010). There is scarce data among this population on the prevalence of HIV, HSV-2, HIV-1/HSV-2 co-infection, HIV-1 subtypes, the drug resistance mutations and the factors influencing their transmission. Generating such data will significantly contribute to the understanding the course of the disease and its burden among the population, as well as inform alternative intervention strategies.

1.3 Justification of the study

The infection with HSV-2 infection crucially favors sexual transmission as well as acquisition of HIV-1 (Wald and Link, 2002). Transmission has been linked to specific HIV subtypes with some being more virulent than others. In Kenya, most HIV subtypes are A1, D, C, G, and their recombinants AD, AC, CRF02 AG, and CRF16 A2D (Lihana *et al*., 2006). In Sub Saharan Africa where the burden of HIV/AIDS is high, data are currently unavailable on the proportion of the HSV-2, HIV and their co-infection, the drug resistant pattern and the factors influencing their transmission. The need to continue evaluating interventions to manage and prevent HIV transmission is still urgent. These considerations demand for a suitable intervention strategy at community level (Schechter, 2002; Flemeing and Richardson, 2004). Fishermen in Kenya and elsewhere in the world constute a youthful group with high mobility (Allison and Seeley, 2004; Kissling *et al*., 2005). Culturally, fishermen along Lake Victoria are vulnerable to HIV and other STIs (Boerma *et al.,* 2003). Despite this, Kenya has had notable success in managing HIV and STIs (KAIS 2012; NASCOP, 2013) but there is scarcity of data on the disease burden including the circulating HIV subtypes and the HIV drug resistance mutations in this group.

1.4 Research Questions

1. What is the prevalence of HSV-2, HIV, and HIV/HSV-2 coinfection among fishermen in Kisumu, Kenya?
2. What are the circulating HIV-1 subtypes among fishermen in Kisumu, Kenya?
3. What are the HIV-1 drug resistant mutations among fishermen in Kisumu, Kenya?
4. What are the factors influencing the transmission HIV, HSV-2 and HSV-2/HIV co-infection among fishermen in Kisumu, Kenya?

1.5 General objective

To determine the HSV-2 prevalence and HIV diversity among HSV-2/HIV co-infected adult fishermen at Nyamware, Kichinjio, Dunga, and Kobudho beaches of Lake Victoria, Kenya.

1.5.1 Specific objectives

1. To determine the prevalence of HSV-2, HIV and HSV-2/HIV coinfection among fishermen at Nyamware, Kichinjio, Dunga, and Kobudho beaches of Lake Victoria, Kenya.
2. To establish the circulating HIV-1 subtypes among fishermen at Nyamware, Kichinjio, Dunga, and Kobudho beaches of Lake Victoria, Kenya.
3. To indentify the HIV-1 drug resistant mutation among fishermen at Nyamware, Kichinjio, Dunga, and Kobudho beaches of Lake Victoria, Kenya.
4. To assess the factors influencing transmission of HIV, HSV-2 and HSV-2/HIV co-infection among fishermen at Nyamware, Kichinjio, Dunga, and Kobudho beaches of Lake Victoria, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Origin of Human Immunodeficiency Virus

The HIV is a *lentivirus* and belongs to the family *Retroviridae* (Sonigo *et al*., 1985). Molecular clock and phylogenetic analyses estimate that HIV was introduced into the human population in the 1930s with a confidence gap of ± 20 years (Hahn *et al*., 2000; Korber *et al*., 2000). More recent reports demonstrate that wild chimpanzees can acquire AIDS such as SIV diseases (Sharp & Hahn, 2011). HIV-1 is thought to have been transmitted from common Chimpanzees, *Pan troglodytes troglodytes* (Gao *et al.,* 1999; Keele *et al*., 2006) while HIV-2 was most likely from sooty mangabey, *Cerocebus atys* (Hirsch *et al*., 1989; Gao *et al.,* 1992; Keele *et al*., 2006). Transmission in the human host of SIV strains is still common, and it remains unclear why HIV has become so predominant in its current form (Weiss and Wrangham, 1999; Kalish *et al*., 2005).

2.2 Classification of HIV

The HIV-1 and HIV-2 belong to the retrovirus family and the lentivirus subfamily (King *et al.,* 2011). The HIV- 1 is genetically classified into four groups as follows: M, non - M, non - O (N), outlier (O) and P (Peeters *et al*., 2003; McCutchan, 2006). The group M is known to account for the current global pandemic (Peeters *et al*., 2003; McCutchan, 2006). The HIV-2 strain is equally an important cause of disease in a number of regions in the world. It was predominant in West Africa but it has now spread to other parts of Africa, India, Europe, and the United States (Omobolaji *et al.,* 2011).

HIV-1 groups N, O and P are uncommon and by phylogenetic analysis the degree of their diversity is yet to be fully differentiated. However, group N seems to be phylogenetically equidistant from groups M and O (Spira *et al.,* 2003). Group M is currently divided into nine different subtypes (A-D, F-H, J and K) apart from 58 recombinant forms in circulation (http:/www.hiv.lanl.gov). Genetic variation within a

subtype is usually 8 to 17 percent, while variations between different subtypes are 17 to 35 percent (Korber *et al.,* 2001). The *env* gene shows the highest variation within the genome, while the *pol* gene, encoding for important viral enzymes, is the most conserved (Gaschen *et al*., 2002)

The HIV-1 Group M Subtype C causes the majority of all HIV-1 infections worldwide (Hemelaar *et al*. 2011). In another study, 85% of HIV-1 infections were reported as subtype B in Western and Central Europe while subtypes A, C and G, followed with 2% to 3% (Bannister *et al.,* 2006). Subtype B of HIV-1 occur frequently and especially in North America, Europe and Australasia (Hemelaar *et al*., 2011). Most HIV-1 subtypes are found in West and Central Africa, where HIV is thought to have originated through zoonosis (Marx, 2005).

Inter-subtype recombinants result from co-infection with two strains of HIV (Abecasis *et al,* 2013). Unique recombinant form (URFs) are inter-subtype recombinant genomes found only in one dually or multiply infected individual patient in which they arose. Circulating recombinant form (CRFs) are inter-subtype recombinant HIV-1 genomes transmitted to three or more people who are not epidemiologically related (Hemelaar *et al.,* 2011). There have been 79 CRFs and several URFs identified to date according to the Los Alamos HIV database. The wide range of recombinant types can influence the global HIV-1 pandemic (Lau & Wong, 2013).

2.3 The structure of HIV

The HIV virion has an icosahedral structure consisting of an outer membrane glycoprotein gp120, trans-membrane envelope components gp41, p18 inner membrane, p24 core protein, RNA, and enzyme reverse transcriptase, integrase, and protease (Briggs *et al*. 2003; Blumenthal, *et al.* 2012) (**Figure 2.1**). The RNA is single-stranded, positive sense and has a genome size of less than 10 kb (Fauci *et al*, 1998).

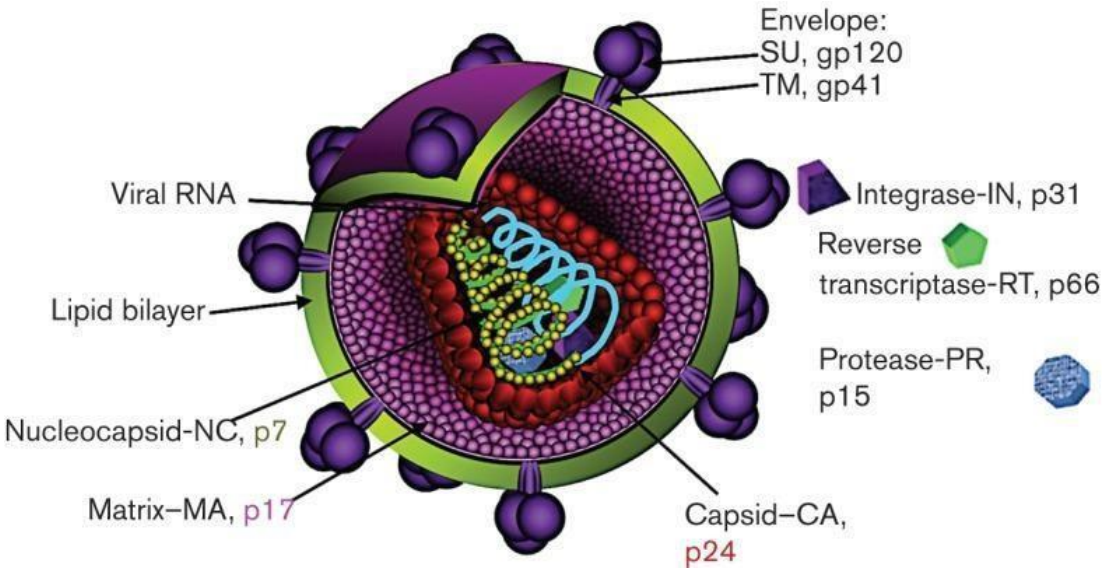


Figure 2.1: Diagram of mature HIV virion (Steckbeck *et al*., 2013)

2.3.1 The organization of HIV genome

The HIV genome consists of two identical single-stranded RNA molecules that are enclosed inside the core of the virus particle. The genome of the HIV provirus termed as proviral DNA is generated by way of the reverse transcription of the viral RNA genome into DNA, degradation of the RNA and integration of the double- stranded HIV DNA into the human genome (Craigie & Bushman, 2012). The HIV genome at each end has long terminal repeats (LTR) which are repetitive sequence of bases that serve some structural and regulatory purposes (Guangdi *et al*., 2015).

Three of the HIV genes, *gag, pol* and *env* are structural genes involved in development of structural proteins for new virus particles (Li & De Clercq, 2016). The *gag* gene is a group specific antigen gene that encodes capsid proteins P24 and nucleocapsid P7 and matrix protein P17 and other internal proteins. The *pol* gene is a polymerase gene encoding the viral enzymes protease, reverse transcriptase and integrase. Reverse transcriptase copies the virus RNA genome into DNA (Li & De Clercq, 2016). The protease processes proteins made from HIV genome and integrase gene integrates the DNA copy of HIV's genome into the host DNA. The *env* gene encodes *gp160* glycoprotein which is usually processed to give *gp120* an external glycoprotein and *gp41* a transmembrane glycoprotein (Rajarapu, 2014). The other six genes *tat, rev, nef, vif, vpr* and *vpu* are regulatory genes for proteins (Faust *et al.,* 2017). They are involved in control of the ability of HIV to infect cells and replication. The *tat* encodes transactivator protein and it positively stimulates transcription. The *rev* encodes a regulator of expression of viral protein and stimulates the production of HIV proteins but suppresses the expression of HIV's regulatory genes. The *nef* gene encodes the negative regulator protein which retards HIV replication. The *vif* is the virion infectivity factor and suppresses resistance to HIV infection by the host. The *vpr* encodes viral protein R and accelerates HIV protein production. The *vpu* encodes viral protein u and enhances the assembly of new virus particles and budding from the host cell. It's also involved in degradation of CD4 proteins (Fujita, 2013; Langer & Sauter, 2017).

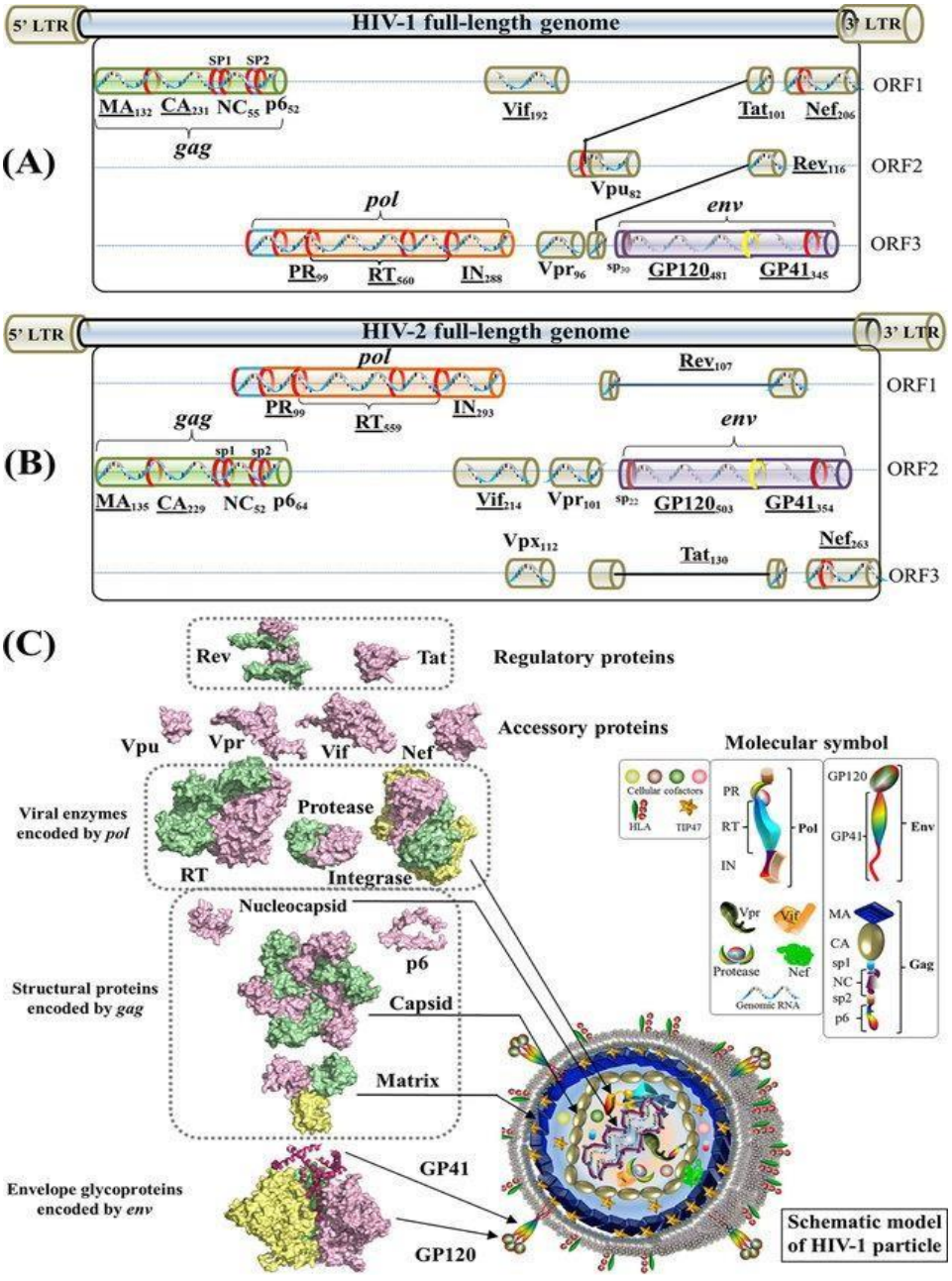


Figure 2.2: Gene maps and protein structures of HIV-1 and HIV-2. (Li et al., 2015)

2.4 The global epidemiology of HIV

2.4.1 The global prevalence of HIV

Since the beginning of the HIV pandemic, around 76.1 million people worldwide have been infected and about 39 million have died of AIDS related causes. Approximately 35 million people were living with HIV in 2016 globally (UNAIDS, 2017). In 2016, 1.8 million people became newly infected with HIV worldwide with 160,000 of this being children. In the same year 1 million people died in the world from AIDS related causes.

In Africa, the eastern and southern countries remain most severely affected, with 19.4 million people living with HIV in 2016. Women account for 59% of this number and the incidence is especially high in adolescents and younger women (UNAIDS, 2017). Estimates of 790,000 people in Eastern and Southern Africa region were newly infected in 2016 accounting for almost 43% of the global total of new HIV infections. A total of 77,000 of these new HIV infections were children. People who died of AIDS related causes in the region were about 1 million in the same year (UNAIDS, 2017). Western and Central Africa were also heavily affected in 2016 with 6.1 million people living with HIV infection (UNAIDS, 2017).

Other areas significantly affected by HIV include Asia and the Pacific with 5.1 million people living with HIV and Latin America which had 1.8 million infected. Western and Central Europe and North America had 2.1 million people with HIV in 2016 while Eastern Europe and Central Asia had 1.6 million people infected. The Caribbean had 310,000 with the virus while Middle East and North Africa had 230,000 people with HIV (UNAIDS, 2017).

2.4.2 Prevalence of HIV in Africa

In many parts of Africa, HIV/AIDS is a major public health concern and cause of death. Africa is home to about 15.2% of the world's population yet sub-Saharan Africa alone, the hardest hit region, accounts for more than two-thirds of people living with HIV (UNAIDS, 2014). North Africa and countries in the Horn of Africa have lower

HIV prevalence rates (Ali-Akbar *et al.,* 2007; Bozicevic, 2013). Most children (94%) with HIV live in Sub-Saharan Africa and almost all of the region's nations have their national HIV prevalence greater than 1%. Countries in Southern Africa region are worst affected on the continent (Gouws *et al.*, 2008). South Africa has the highest number of people living with HIV in the world (5.6 million) while Swaziland has the highest prevalence in the world which is 26.0% (Abu-Raddad *et al*., 2010; Iliffe**,** 2006).

2.4.3 Prevalence of HIV in Kenya

Human immunodeficiency virus (HIV) remains a major health burden in Kenya despite numerous efforts to contain the scourge. HIV prevalence in 2015 was 5.9% in Kenya with 1,517,707 people living with the virus. HIV prevalence among females was higher in this year at 6.4% (775,939) compared to males at 5.5% (643,598). Children aged between 0 to 14 years had 105,213(6%) of them living with HIV in 2017 (NACC, 2018).

HIV prevalence in Kenya also varied by region, with the highest prevalence in Siaya county at 26%, and lowest prevalence in the North Eastern region, Wajir County at 0.1%. Kenya has an estimated 45,000 new HIV infection among adults and about 7978 among children annually (NACC, 2018).

2.5 Molecular epidemiology of Human Immunodeficiency Virus

2.5.1 The origin of HIV epidemic

The origin of HIV is thought to be from multiple zoonotic transmissions of the Simian Immunodeficiency Virus (SIV) from non-human primates to humans in West and Central Africa (Sharp & Hahn, 2011). There are more than forty different non- human primate species harboring SIV strains, each of which exhibits species- specificity (Aghokeng *et al*., 2010; Sharp & Hahn, 2011). HIV type 1 (HIV-1) groups M, N, O, and P; and HIV type 2 (HIV-2) groups A–H. While HIV-1 group M (Main) is responsible for the global HIV pandemic (https://www.hiv.lanl.gov/ components/sequence/ HIV/geo/geo.comp) (**figure 2.3**).

Not all HIV lineages have been identified and new cross-species transmissions may take place in the future. HIV-1 groups M and N originate directly, but independently, from SIV found in the chimpanzee *Pan troglodytes* (SIVcpz) in West–Central Africa (Gao *et al.,* 1999). Group N is confined to a small number of individuals in Cameroon, but group M spread to what is now Kinshasa in the Democratic Republic of the Congo, where the current worldwide HIV-1 epidemic is thought to have begun (Sharp & Hahn, 2011). Molecular clock computer programs used to estimate the time since the most recent common ancestors (tMRCAs) and evolutionary rates of various HIV lineages revealed that groups M and SIVcpz shared a common ancestor in 1853 (1799–1904), and cross-species transfer is therefore inferred to have taken place around this time (Wertheim & Worobey, 2009).

HIV infection is characterized by high levels of virus production and turnover (Vijayan, *et al*., 2017). The reverse transcription of viral RNA into DNA is highly prone to errors introducing on average one mutation for each viral genome transcribed (Coffin & Swanstrom, 2013). The high rate of HIV replication, combined with the high mutation rate that occur during the viral replication cycle ensures that patients harbour a complex and diverse mixture of viral genotypes (quasispecies), each differing by one or more mutations. Some of these mutations confer a selective advantage to the virus such as decreased susceptibility to antiretroviral drugs (Tang & Shafer, 2012; Pironti *et al*., 2017).

The two groups of HIV-2, A and B, are restricted in West Africa Visseaux *et al.,* 2016). HIV-1 group M viruses are more pathogenic and epidemiologically diverse and account for almost the entire global epidemic. HIV-1 group M is further divided into nine genetically distinct subtypes A, B, C, D, F, G, H, J, and K (Tongo, Dorfman & Martin, 2015). In addition, more than 91 circulating recombinant forms (CRFs) have been recognized so far (Abram *et al.,* 2010). The most important strains that cause the global epidemic are subtypes A, B, C, D, CRF01_AE and CRF02_AG (Lau & Wong, 2013; Patiño-Galindo & González-Candelas, 2017).

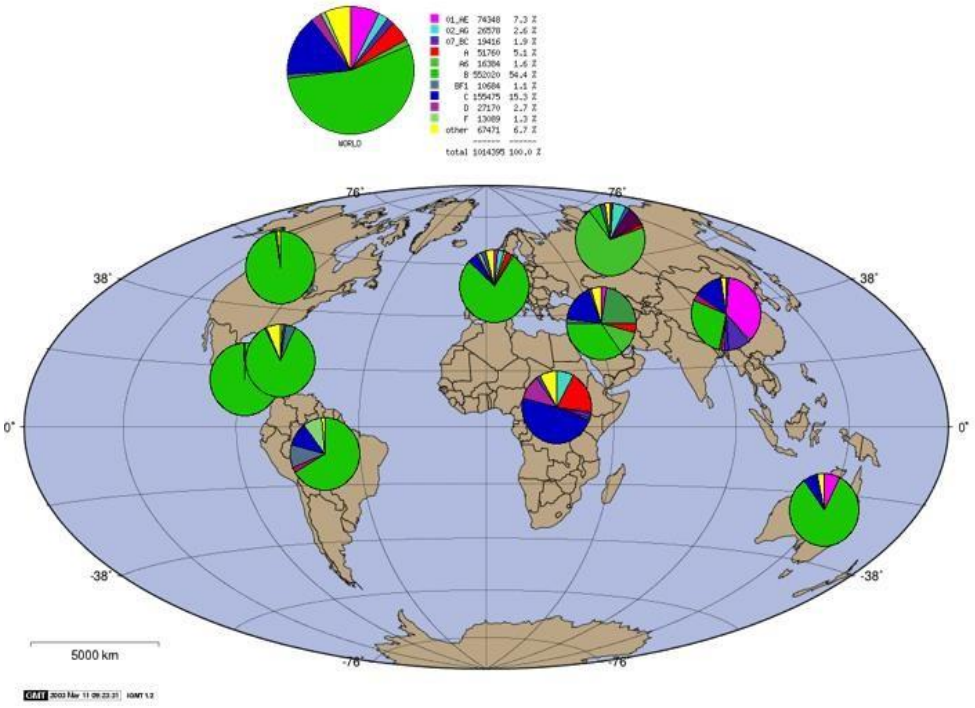


Figure 2.3: The current distribution of HIV-1 subtypes sequences according to distribution, population and spread across the continents. Adopted from the LANL HIV database

Source: (http://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp**)**

2.6 Transmission of HIV

The primary method of spread of HIV infection worldwide is through sexual exposure (WHO, 2017). HIV-1 is transmitted by sexual contact across mucosal surfaces, by maternal-infant exposure, and by percutaneous inoculation. It is speculated that CCR5-tropic viruses (R5 viruses) are in preference transmitted by all routes (Shaw & Hunter, 2012). In the areas of highest HIV prevalence globally, heterosexual intercourse is the primary mode of transmission, accounting for approximately 70% of the overall sexual transmission (Centers for Disease Control and Prevention, 2015). In rare cases nonsexual HIV transmission can occur through transfusion with contaminated blood products, injection drug use, occupational exposure, or accidental needle sticks (Neupane & Mishra, 2014). Transmission of HIV via blood or transplanted organs, including bone, is possible from about days 5- 6 after infection of the donor.

Mother-to-child transmission has been demonstrated from the 12th week of gestation, but transmission occurs predominantly (>90%) in the final trimester and particularly shortly before or during birth (Silasi *et al*., 2015). HIV can be transmitted via breast milk (Gray *et al*., 2011).

2.7 HIV Life Cycle

The HIV life-cycle begins as it infects a new host cell (Shaw and Hunter, 2011) as shown in Figure 2.4. HIV infection starts by binding the CD4 receptor and either CCR5 or CXCR4 on the surface of CD4+ T-cells (Chen *et al*., 2012). HIV-1 may be classified as either CCR5 (R5), CXCR4 (X4) or dual tropic (R5X4) based on chemokine co-receptor use (Choe *et al*., 1996). HIV-1 surface protein gp120 binds either the β-chemokine receptor, CCR5 co-receptor (macrophage-tropic) or the α- chemokine receptor CXCR4 (T cell-tropic) on the CD4 receptor on helper T cells, macrophages and dendritic cells (Wu, 2011). This binding event leads to rearrangements of the GP120 Env sub-unit that ultimately lead to the repositioning of V3 loops and bridging sheet exposure essential for interactions with coreceptors (Wilen, 2012). Coreceptor - bound Env undergoes conformational modifications that expose the hydrophobic gp41 fusion peptide and then insert six helix bundles into the host cell membrane before folding. The latter is the driving force that brings closeness to the opposing membranes, creating a fusion pore (Melikyan, 2008).

The actual fusion occurs within minutes via pore formation in order to release the viral genome into the host cell cytoplasm. The viral RNA is reversed transcribed by viral enzyme reverse transcriptase into the complementary DNA (cDNA). Mutations can easily occur during this step (Hu *et al*, 2012). In conjunction with a DNA repair enzyme, a viral protein integrase inserts the viral double-stranded genome into gene rich, transcriptional DNA active domains that transforms the cell into a potentially viral producer at the middle of the infection. This is the latent phase of the HIV infection (Fanales-Belasio *et al*, 2013).

Different transcription factors are used to actively produce the virus. The main factor is the NF-B nuclear factor that is upregulated when T cells are activated (Pinzone *et al*, 2013). The viral particles that are produced leave the cell through the vesicles (Fauci *et al*, 1998; Simon *et al*, 2006). Cytoplasmic molecules of the producer cell and its surface lipid bilayer cell are integrated into the new viral particle; virons carry the mother cell's characteristics. Following the virus production, essential immune cells such as helper T cells (specifically CD4+ T cells), dendritic cells and macrophages become infected with HIV (Cunningham *et al*., 2010).

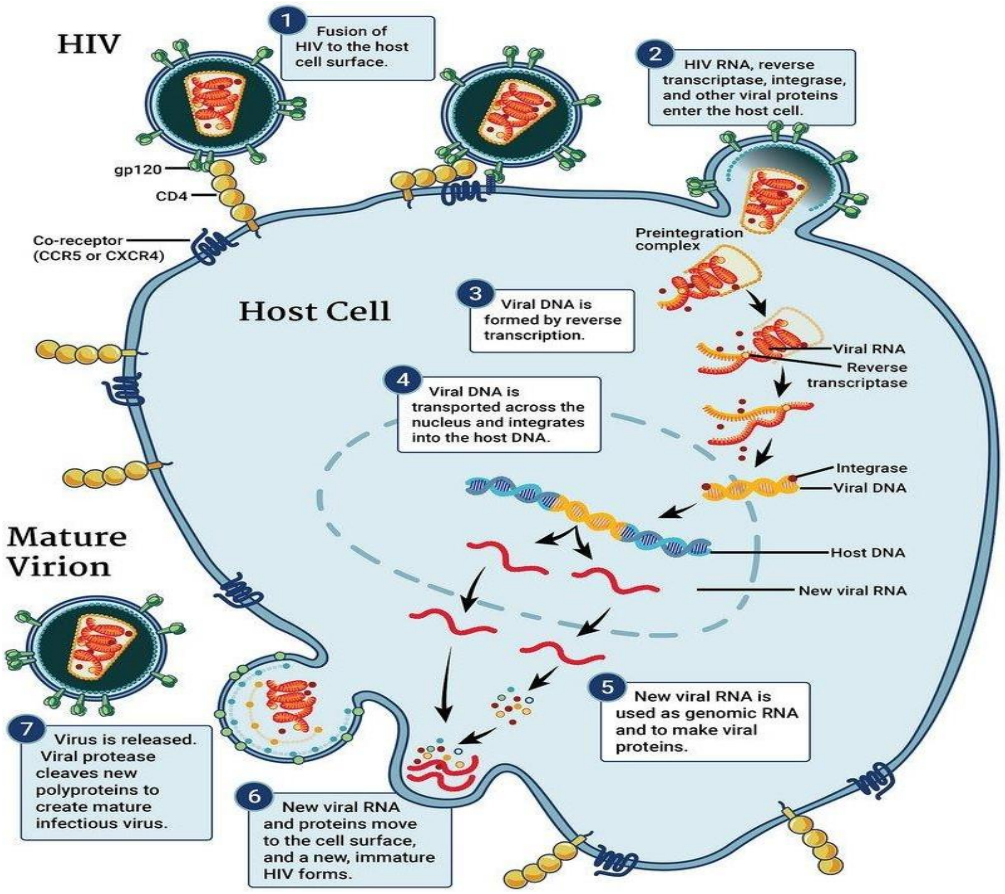


Figure 2.4: The HIV-1 replication cycle (NIAID, 2009)

2.8 Immunology and clinical manifestation of HIV

The innate immunity in HIV involves the Langerhans cells found in the foreskin and vaginal epithelia, T cells in rectal and vaginal epithelia; DCs, macrophages, and NK cells in subepithelial tissues. (Ackerman *et al*., 2012). The activation of macrophages leads to the production of CC chemokines RANTES, MIP-1α and MIP-1 β (Herbein & Varin, 2010). T cells give mucosal protection by producing Th1 AND th2 types of cytokines which lyse the cells infected by HIV (Saunders *et al*., 2011). In the oral mucosa, there are defensins which confers protection against HIV (Wilson *et al* 2013). APOBEC and APOBEC3G inhibit the replication of HIV (Koito and Ikeda, 2013).

After infection, p24 antigen appears in serum and disappears upon seroconversion (Cohen *et al*., 2010). Neutralising antibodies are produced which are directed towards the variable loop of GP120 (McMichael *et al*, 2010). The $CD8^+$ cells are activated when the viral antigen is presented by the MHC-1 molecules. They become CTLs and apoptosis kill the presenting cells. CD8 + belongs to and is the most effective cell - mediated immunity (Challa *et al.*, 2010).

Human Immunodeficiency Virus selectively infects and kills CD4 + T cells in vitro through a number of mechanisms, including non - infected cell apoptosis (Garg *et al*., 2012; Okoye and Picker, 2013), CD8 cytotoxic lymphocytes which identify infected CD4+T cells and kills them directly (Kumar, 2012). The number of CD4 + T cells continually decreases from 600 - 1200 to less than 500 cells/µl marking the onset of symptoms. Several conditions defining AIDS as a disease relate to CD4 + T cell levels. The first symptoms appear in the HIV-infected patient at approximately 500 cells / ml. The patient is susceptible to AIDS-defining opportunistic infections and neoplasms particularly Kaposi sarcoma and lymphomas when the number of CD4 + T - cells drops below 200 cells/µl (Tindall and Cooper, 1991; Fauci and Lane, 1997; Fauci *et al*, 1998; Simon *et al*, 2006).

The WHO system categorise adult patients into one of four hierarchical clinical stages ranging from stage 1 (asymptomatic) to stage 4 (AIDS). Patients are assigned to a particular stage when they demonstrate at least one clinical condition in that stage's

criteria. Patients remain at a higher stage after they recover from the clinical condition which placed them in that stage (Munthali *et al.,* 2014).

2.8.1 The HIV revised clinical staging and immunological classification of HIV-related disease in adults by WHO.

The WHO system groups adult patients into one of four hierarchical clinical stages ranging from stage 1 (asymptomatic) to stage 4 (AIDS). Patients are assigned to a particular stage when they demonstrate at least one clinical condition in that stage's criteria. Patients remain at a higher stage after they recover from the clinical condition which placed them in that stage (Malamba *et al*., 1999). These WHO categories apply to adults and adolescents 15 years-of-age and older.

The first stage includes patients who are asymptomatic or have persistent generalized lymphadenopathy (lymphadenopathy of at least two sites [not including inguinal] for longer than 6 months) are categorized as being in stage 1 (WHO, 2005).

Even in early HIV infection, patients may demonstrate several clinical manifestations. Clinical findings included in stage 2 (mildly symptomatic stage) are unexplained weight loss of less than 10 percent of total body weight and recurrent respiratory infections (such as sinusitis, bronchitis, otitis media, and pharyngitis), as well as a range of dermatological conditions including herpes zoster flares, angular cheilitis, recurrent oral ulcerations, papular pruritic eruptions, seborrhoeic dermatitis, and fungal nail infections (WHO, 2005).

In Stage three, disease progresses and additional clinical manifestations may appear. Those encompassed by the WHO clinical stage 3 (the moderately symptomatic stage) category are weight loss of greater than 10 percent of total body weight, prolonged (more than 1 month) unexplained diarrhea, pulmonary tuberculosis, and severe systemic bacterial infections including pneumonia, pyelonephritis, empyema, pyomyositis, meningitis, bone and joint infections, and bacteremia. Mucocutaneous conditions, including recurrent oral candidiasis, oral hairy leukoplakia, and acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis, may also occur at this stage (WHO, 2005).

The WHO clinical stage 4 (the severely symptomatic stage) designation includes all of the AIDS-defining illnesses. Clinical manifestations for stage 4 disease that allow presumptive diagnosis of AIDS to be made based on clinical findings alone are HIV wasting syndrome, *Pneumocystis pneumonia* (PCP), recurrent severe or radiological bacterial pneumonia, extrapulmonary tuberculosis, HIV encephalopathy, CNS toxoplasmosis, chronic (more than 1 month) or orolabial herpes simplex infection, esophageal candidiasis, and Kaposi's sarcoma (WHO, 2005).

Other conditions that should arouse suspicion that a patient is in clinical stage include cytomegaloviral (CMV) infections (CMV retinitis or infection of organs other than the liver, spleen or lymph nodes), extrapulmonary cryptococcosis, disseminated endemic mycoses (e.g., coccidiomycosis, penicilliosis, histoplasmosis), cryptosporidiosis, isosporiasis, disseminated non-tuberculous mycobacteria infection, tracheal, bronchial or pulmonary candida infection, visceral herpes simplex infection, acquired HIV-associated rectal fistula, cerebral or B cell non-Hodgkin lymphoma, progressive multifocal leukoencephalopathy (PML), and HIV-associated cardiomyopathy or nephropathy (WHO, 2005). Presence of these conditions unaccompanied by the AIDS-defining illnesses, however, should prompt confirmatory testing.

2.9 Diagnosis of HIV

There is a particular difference between two principles of identification: antibody and virus detection. A nucleic acid test (NAT) can be used to detect HIV RNA in the blood for approximately eleven days following infection.

2.9.1 Antibodies detection

This is the primary diagnostic test for HIV which is followed by a confirmation test in the case of a reactive test result. A commercially available enzyme-linked immunosorbent assay (ELISA or EIA) detects antibodies in the serum (Gan & Patel, 2013). Depending on the manufacturer, additional antigens derived from the reverse transcriptase and the p24 protein is included in the test systems. ELISA is not entirely

specific and a western blot (WB) method which identifies antibodies to specific components of HIV is employed. The WB can be positive, negative or indeterminate. Indeterminate results are neither positive nor negative, and usually reflect the beginning of seroconversion at the time of testing, or cross-reactivity. In these rare situations, retesting should be performed three weeks later. Only if the criteria for a positive Immunoblot/Western blot are fulfilled can the HIV infection be considered as confirmed (Hecht *et al*., 2011). But if an antibody test is reactive and a positive result for the specimen is obtained in the NAT with a sensitivity of less than 1,000 HIV genome copies/ml, the confirmatory immunoblot can be omitted and the HIV-infected individual be informed about the antibody and NAT results, advising the patient to seek specific medical attendance and treatment (Hecht *et al.*, 2011).

2.9.2 Virus Detection

P24 antigen detection is performed using a combination of polyclonal or monoclonal antibodies following the principle of the sandwich ELISA technique. These tests have the ability to detect HIV p24 with a sensitivity of 10 pg / ml. p24 antigen detection tests or combined antigen/antibody test systems (so-called 4th generation tests) are approved and commercially available (Mühlbacher *et al.*, 2012; Mitchell *et al*., 2013). A positive p24 test means that someone is HIV-positive. However, a negative p24 test can mean three things: the person is HIV-negative, the person is HIV-positive but that the test could not detect the p24 protein because the person was infected more than four to six weeks earlier or the levels of p24 antigen are too low to be detectable with current technologies (Tooley L., 2019)

2.9.3 Nucleic Acid Amplification Technology (NAT)

HIV infection can be diagnosed by determining the proviral DNA in the cells or the viral RNA genome in the plasma (Pasternak *et al*., 2013). For analysis of the viral load and the presence of HIV in blood donations, RNA is extracted from virus particles in plasma. Genome detection may be achieved either through direct amplification of the defined target sequences or through using probes with subsequent signal amplification. Depending on the source material, NAT enables the detection of 40-100 genome equivalents/ml of plasma in an individual donation (Roth, 2019). Nucleic acid testing

(NAT) systems have been developed and made commercially available that use primers binding preferentially and stringently to the genome of HIV-1 M: B; therefore, with a few exceptions, viruses of the type HIV-1 M: B are detected with the highest sensitivity. The more an HIV-1 nucleic acid sequence deviates from HIV-1 M: B, the lower the sensitivity. Depending on the test design and the target sequence, e.g. *gag* or LTR or *integrase* (IN), just HIV-1 of group M or also viruses from HIV-1 groups M, N, O and P can be detected (Masciotra *et al*., 2002; Mourez, Simon & Plantier, 2013).

2.10 HIV Treatment

2.10.1 Antiretroviral Therapy for HIV Infection

Antiretroviral therapy (ART) is the use of pharmacological agents (drugs) that have specific inhibitory effects on replication of HIV. Highly active antiretroviral therapy (HAART) comprises of multi anti-HIV drugs and is therefore able to act on different HIV targets (Arts & Hazuda, 2012). The current HAART regimen suppresses viral replication and maintains the immune system function. This has led to a decrease in AIDS opportunistic infections and deaths (Lu & Chen, 2010; Arts & Hazuda, 2012). There has been a lot of improvement in the ART over the year which has resulted to the current therapy being more effective, easier to take and with fewer side effects (Broder, 2010). Currently the Food and Drug Administration has approved 31 antiretroviral drugs (ARVs) to treat HIV infection ("Antiretroviral drugs used in the treatment of HIV infection", 2019).

Besides long-term side effects and suboptimal drug potency, the emergence of resistant virus and the necessity of perfect therapy adherence are major concerns for obtaining a sustained control of viral replication. HIV lacks proofreading enzymes to correct errors made when it converts its RNA into DNA via reverse transcription. Its short life-cycle and high error rate cause the virus to mutate rapidly, resulting in a high genetic variability of HIV which then leads to resistance to ARVs (Abram *et al*., 2019). To combat virus strains from becoming resistant to specific antiretroviral drugs, HIV therapy involves taking a combination of antiretroviral drugs known as highly active antiretroviral therapy (HAART) (Waters *et al.*, 2013). Combinations of antiretrovirals

create multiple obstacles to HIV replication to keep the number of offspring low and reduce the possibility of a superior mutation. If a mutation that conveys resistance to one of the drugs being taken arises, the other drugs continue to suppress reproduction of that mutation (Van, 2001). Examples of combined drugs include epzicom (ABC + 3TC), trizivir (ABC + AZT+3TC), combivir (AZT + 3TC) and truvada (TDF + FTC) ("Antiretroviral drugs used in the treatment of HIV infection", 2019).

2.10.2 Mechanisms of Action of HIV Antiretroviral Drugs

Antiretroviral drugs act by inhibiting viral enzymes that are critical to the HIV replication cycle while others act on the HIV host cell target (CCR5 or CCR4) which blocks the entry of the HIV virus into the cell. Currently, four classes of over 30 licensed antiretrovirals and combination regimens of these ARVs are in clinical use, including: reverse transcriptase inhibitors (RTIs) (e.g. nucleoside reverse transcriptase inhibitors, NRTIs; and non-nucleoside reverse transcriptase inhibitors, NNRTIs), protease inhibitors (PIs), integrase inhibitors and entry inhibitors such as fusion inhibitors and CCR5 antagonists (Lu & Chen, 2010).

2.10.3 Reverse Transcriptase (RT) Inhibitors

Nucleoside reverse transcriptase inhibitors (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI) are nucleoside and nucleotide analogues which inhibit reverse transcription (Maxwell *et al.,* 2012). NRTIs are chain terminators such that, once incorporated, they work by preventing the addition of other nucleosides because of the absence of a 3’ OH group and both act as competitive substrate inhibitors. Examples of NRTIs include zidovudine (AZT or ZDV) stavudine (d4T), didanosine (ddI), abacavir (ABC), lamivudine (3TC) emtricitabine (FTC) and tenofovir (Kalyan, 2013). Non-Nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase by binding to an allosteric site of the enzyme The NNRTIs interact with a specific 'pocket' site of HIV-1 RT that is closely associated with, but distinct from, the NRTI binding site. NNRTIs act as non-competitive inhibitors of reverse transcriptase (Balzarini, 2004; Jonckheere *et al*, 2000). NNRTIs examples include delavirdine (DLV), efavirenz (EFV) rilpivirine (RPV) Etravirine (ETR) and Nevirapine NVP (Kalyan, 2013; Mbuagbaw *et al.,* 2010). Mutations in the structure

of HIV-1 Reverse Transcriptase may lead to the development of drug-resistant strains of the virus. (Jonckheere *et al*., 2000; Castro *et al.*, 2006).

2.10.4 Protease Inhibitors

Protease inhibitors interfere with the protease enzyme by blocking it yet it is important in production of mature virions upon budding from the host membrane. Mostly these drugs prevent the cleavage of *gag* and *pol* precursor proteins and thus virus particles produced in their presence are defective and mostly non-infectious (Wensing, 2010). Examples of HIV protease inhibitors are lopinavir (LPV), indinavir (IDV), nelfinavir (NFV), amprenavir (APV) fosamprenavir (FOS-APV), atazanavir (ATV), darunavir (DRV), ritonavir (RTV), saquinavir (SQV), tipranavir (TPV) and ritonavir. Maturation inhibitors have a similar effect by binding to *gag*. Resistance to some protease inhibitors is high. Second generation drugs have been developed that are effective against otherwise resistant HIV variants (Gulick, 2003).

2.10.5 Fusion/Entry Inhibitors

This interferes with the binding, merging and entry of the virus into the host cell membrane by blocking one of several targets. HIV requires binding to both the CD4 molecule and a co receptor to enable entry into the cell. The chemokine receptors CXCR4 and CCR5 are used as the main co-receptors (Schols, 2004). An example of this drug is maraviroc which works by targeting CCR5. This co-receptor is located on human helper T-cells though there can be a shift in tropism which allows HIV to target an alternative co-receptor such as CXCR4. Some individuals may have mutations in the CCR5 delta gene resulting in a non-functional CCR5 co-receptor and resulting in resistance or slow progression of the disease (Sharon & Lieberman- Blum, 2008). Another example of this drug is Fuzeon (T20) which is a peptide drug and works by preventing fusion of the virus with the host membrane (Bai, 2013).

2.10.6 Integrase Inhibitors

Integrase enzyme is inhibited by integrase inhibitors. Examples of these drugs are raltegravir (RAL), dolutegravir (DTG) and elvitegravir (EVG) (Quashie, 2013). These three integrase inhibitors bind to the catalytic core (amino acids 50-212) of the integrase enzyme and target the integration process at the strand transfer step (Pinar &Anderson, 2014).

2.11 Development of Drug Resistance

Due to the introduction of highly active antiretroviral therapy in the mid- 1990's HIV related morbidities have reduced and the lifespan of people living with HIV has also been prolonged (Wing, 2016). However, the broad use of multiple antiretroviral drugs has led to drug resistance which is a common cause of treatment failure and also to transmission of drug resistant viruses (Obiako, 2010). HIV-1 is known to possess a high mutation rate and recombination of high frequency and the outcome of this could be rapid emergence of drug-resistant variants due to the viral replication not being inhibited sufficiently (Pinar and Anderson, 2014). There are also other factors that have been associated with emergence of drug-resistant HIV viruses including patient adherence to drugs, drug pharmacological factors and host immune response pressure (Obiako, 2010; Weber *et al.,* 2011).

To monitor disease progression in HIV infected individuals and measure ARV treatment success, viral replication in blood (plasma viral load) is measured and also the levels of $CD4^+$ T cells. A rise in viral load above 200 copies/ml might be a sign of emergence of drug resistance mutations (Paredes & Clotet, 2010). Prior to designing new antiretroviral regimens after treatment failure, detection and quantification of drug resistance have to be done (Weber *et al.,* 2011). Drug resistance testing which involves genotyping and phenotyping techniques are crucial in the management of antiretroviral therapy since they guide the clinician in finding better drug choices and combinations with minimum risk of drug resistance (Obiako, 2010; Paul & Jorden, 2003).

2.12 Global Prevalence of HIV Drug resistance

In 2018, 23.3 million people living with HIV were accessing antiretroviral therapy globally (UNAIDS, 2019). The numbers of those initiating treatment and those maintained on treatment are expected to increase in order to achieve global targets of epidemic control. However, the increase in numbers of people on treatment comes with the challenge of drug resistance (Zhou *et al*., 2016).

Pretreatment drug resistance to NNRTI was predicted to have reached different levels in 2016. In East Africa it had reached 15.5%, Latin America had 15%,11% in Southern Africa and 7.2% in West and Central Africa (WHO, 2017). Prevalence of NNRTI resistance exceeding 10% in a country requires a public health response according to WHO (WHO, 2017). Surveys were conducted on pretreatment drug resistance by WHO between 2014 and 2016. This indicated that all low- and middle- income countries had HIV drug resistance prevalence approaching or above the 10% mark. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) resistance ranged from 8.1% in Cameroon to 15.4% in Uganda. HIV drug resistance prevalence of any drug class was 9.8% in Brazil and 23.4% in Nicaragua (WHO, 2017).

2.12.1 Types of HIV drug resistance

Human Immunodeficiency Virus (HIV) drug resistance occurs when there is a change (mutation) in the genetic structure of HIV (Abram *et al.*, 2014). This interferes with the blocking of replication of the virus by antiretroviral drugs. The emergence of drug resistant virus poses a risk of ARV drugs becoming partially or fully inactive (Tang & Shafer, 2012). HIV drug resistance is divided into three main categories (WHO, 2017). First is the acquired HIV drug resistance (ADR) which emerges due to viral replication in patients receiving ARV drugs. This occurs mainly because of medication nonadherence and suboptimal treatment (De Luca & Zazzi, 2015). Secondly there is transmitted HIV drug resistance (TDR) which happens when uninfected ARV naive individuals are infected with HIV that already has drug resistance mutations (De Luca & Zazzi,2015). Thirdly is the pretreatment HIV drug resistance (PDR) which is detected at initiation of first line ART or at re initiation of first line ART. Pretreatment drug resistance can either be transmitted or acquired drug resistance and it can also be

both (Kityo C., 2017).

2.12.3 Genotypic and Phenotypic Measurement of Drug Resistance

Genotyping tests mainly comprise polymerase chain reaction (PCR) amplification and nucleotide sequencing of protease, reverse transcriptase and integrase genes allowing the detection of resistance associated mutations in the genome (Pinar & Anderson, 2014). Phenotyping is a more direct method that involves testing the ability of a patient derived virus to replicate in the presence of antiretroviral drugs in a cell-based assay (Weber *et al.,* 2011). HIV is grown in peripheral blood mononuclear cells maintained in RPMI 1640 medium in the presence of different concentrations of antiretroviral drugs and thereafter viral growth is observed and 50% tissue culture infective dose ($TCID_{50}$) is determined (Weber *et al.,* 2011).

2.13 Herpes simplex virus type 2 (HSV-2)

2.13.1 Classification of HSV- 2

Herpes simplex viruses type 2 (HSV-2 or human herpesvirus 2, HHV-2), are members of the *Herpesviridae* family and *alphaherpesvirinae* subfamily (Davison, 2010; Sharma *et al*., 2016).

2.13.2 The genome of Herpes Simplex Virus 2

Herpes Simplex Virus 2 is a 152,261-bp linear double-stranded GCrich (G+C, 68%) DNA sequence (Kieff *et al*, 1971). The DNA is packaged into nucleocapsid proteins that form an icosahedral structure in the center of the virion (Dai and Zouh 2018; Yuang *et al.,* 2018). The core nucleocapsid is separated from the outer membrane layer by a proteineous tegument, which comprise several viral and host proteins, but also viral mRNAs (Roizman and Pellet, 2001). Several trans membrane (glyco) proteins span the outer membrane (Chowdhury *et al*., 2013).

2.13.3 Occurrence and epidemiology of HSV-2

Herpes simplex type 2 virus (HSV-2) is one of the world's three most sexually transmitted diseases and a leading cause of genital ulcers (Corey & Handsfield, 2000; Gupta *et al.*, 2007). In 2012, around 417 million (11.3%) people aged 15-49 years were infected with HSV-2 globally (Looker *et al.*, 2017). In 2016, it was estimated that HSV-2 in the United States the serological prevalence was 11.9 percent in persons aged 14-49 years (McQuillan *et al*., 2018) while in Europe; it was approximately 4-24 percent, South-East Asia (8%) and Western Pacific (8%) (Looker *et al*., 2017).

However, the prevalence of HSV-2 varies between different selected populations (Looker *et al*., 2008). For instance, STD clinic attendees in Peru, had HSV-2 prevalence of 83% while blood donors in London UK had showed a sero-prevalence of 8% (Corey *et al.*, 2004). In pregnant women in Australia, it was 12.5 % (Mendel *et al.* 2000).

The prevalence of HSV-2 in the various populations of African women, ranges from 57% among migrants in Tanzania, 26.6% for adolescent and adult cohort in Western, Kenya, 15.2% for rural Malawian female adolescents (Sudfeld *et al*., 2013; Norris *et al*., 2017; Akinyi *et al*., 2017). In a survey of Kenyans aged 15–64 who completed interviews and tested for HIV and herpes, the national prevalence of HSV-2 among adults (15–64 years) were estimated to be 5.6 percent (KAIS, 2012), with a broad gender and geographical variation.

2.13.4 Transmission of HSV-2

Herpes Simplex Virus-2 is transmitted through contact with genital surfaces of an infected partner. In women, the risk of acquiring HSV2 appears to be greater than in men (Mertz *et al.*, 1992; Mertz, 1993; Koelle *et al*., 2000). This may be associated with higher recurrences of HSV2 in infected men (approximately 20% more than in females) (Benedetti *et al.*, 1994), biological factors, for example women's greater and vulnerable surfaces of the mucosa (Duriux-Smith *et al.*, 1992; Nicolosi *et al*., 1994; Carpenter *et al*., 1999;), Or perhaps differences in symptom awareness and their reporting in men and women. Most transmission of HSV is caused by asymptomatic

shedding (Mertz *et al*., 1992; Wald *et al*., 2000).

2.13.5 The lifecycle of Herpes Simplex Virus 2

Entry of HSV-2 is initiated by binding of glycoprotein B (gB) and gC to the extracellular matrix and binding of the gD to a second receptor followed by fusion of the membrane. This releases the nucleocapsid and the tegument proteins and RNA to the cytosol (Buch *et al.,* 2017).

In the herpesvirus lifecycle, three subsequent types of genes can be distinguished temporally during the lytic phase; immediate early (IE) genes comprise the tegument proteins and directly transcribed genes, early (L) genes follow the IE genes and late (L) genes are expressed thereafter. All three sets of genes have a specific function in the herpesvirus life cycle. The IE proteins are primary focused on the quick onset of viral genome transcription and early immune evasion, whereas the late genes are more concerned with the generation and trafficking of new virus particles (McGeoch *et al*., 2006; Harness *et al*., 2014).

The origin-binding protein pUL9 initiates replication by loading 68 the viral replisome complex onto origins of replication in the viral genome (Chouljenko, 2014). The replisome consists of a virally encoded Deoxyribonucleic acid (DNA) polymerase composed of pUL30 and pUL42, a helicaseprimase composed of pUL5, pUL8 and pUL52, and the single-stranded DNA-binding protein pUL29 (ICP8) (Muylaert *et al*., 2011).

Viral DNA replication proceeds using a rolling circle mechanism, and newly synthesized viral DNA is used as the template for transcription of late (gamma) genes primarily encoding structural proteins that are utilized for the formation of progeny virus. The host RNA polymerase II transcribes the viral genes while the synthesis of the viral protein takes place in the cytoplasm (Muylaert *et al*., 2011).

The assembly of HSV-2 capsids takes place in the nuclei of infected cells in large nuclear inclusion bodies that form approximately four hours after initial infection and persist for the duration of infection (Brown *et al.,* 2011).

2.13.6 Pathogenesis of HSV-2

When HSV-2 infects the body, cytolytic replication is initiated at the entry site through the skin or mucosal surfaces in the epithelial cells (Schiffer and Corey, 2013). The histological view of epithelial cells reveals virions as inclusion bodies. HSV-2 leads to the fusion and formation of multi - nucleated giant cells (Gupta *et al.*, 2007). Cell damage causes epithelial cells in the skin to detach and form blisters filled with fluid containing cellular debris, inflammatory cells and progeny virion (Gupta, Warren and Wald, 2007; Wang *et al.,* 2018). The HSV-2 penetrates the dermis and enters peripheral sensory nerve ends which innervate the infected cells therefore inducing an inflammatory response. The nucleocapsid, which contains the double-stranded DNA genome, is taken up by the sensory nerve axons and transported in the sensory nerve root ganglia in a retrograde manner to the neural soma (Antinone & Smith 2010; Koyuncu *et al*. 2013). It is believed that neuronal HSV-2 infection does not cause cell death (Yu and He, 2016).

In the sensory ganglion, the latent phase of HSV-2 infection occurs where it can persist for the infected person's life (Nicoll *et al*., 2012). The genome of HSV-2 is retained in an unintegrated latent state, with the expression of few proteins but without active or cytotoxic replication (Grinde, 2013). It is not yet fully understood the mechanism for establishing and maintaining latency (Thellman and Triezenberg, 2017). The ability of the virus to avoid recognition by the host's immune system facilitates latency in immune - privileged sites and actively suppressing the immune response of the host (Jean *et al.*, 2001; Gupta *et al.*, 2007).

All HSV-2 infected people apparently have latent virus in the nervous ganglia, whether they are symptomatic or not (Grinde, 2012). Recurring episodes occur when latent HSV-2 reactivates in the infected neurons and is transported back to the mucosal or skin surface of the peripheral nerves where it exits at the nerve end of any axon branch and causes lesions (Smith, 2012). The reactivation and replication of latent HSV-2 is triggered by specific conditions, including local trauma (e.g. surgery or UV light), and systemic stimuli (e.g. immunosuppression or fever). However, in most cases it is not possible to identify precipitating factors and the triggers are poorly understood (van

Lint *et al*., 2004; Gupta *et al*., 2007).

Herpes Simplex Virus 2 reactivation may result in clinically evident mucosal disruption (i.e.viral shedding recurrence) or viral shedding may occur without clinically recognized symptoms (Tronstein *et al.*, 2011). People who are HSV-2 positive shed HSV-2 intermittently, and HSV-2 transmission, both sexually and vertically, usually occurs during subclinical shedding (Mertz *et al.*, 1992; Gupta *et al*., 2004). Depending on the host factors, the clinical and subclinical reactivation rate varies greatly from one person to another. The HSV-1 infection may provide a small protection margin against HSV-2 infection; in contrast, HSV-2 infection appears to protect against acquisition of HSV-1 (Gupta *et al*., 2007).

2.13.7 Immunology and clinical manifestations of HSV-2 infection

The immune response against HSV involves both innate and adaptive immune mechanisms (Truong *et al*., 2019) There is production of type I interferon (IFN), comprising largely of IFNα and IFNβ (Ali *et al.*, 2019). In addition, multiple cell types have been shown to contribute to the innate immune responses to HSV *in vivo*. Among the most important of these cell types are natural killer (NK) cells, whose role in anti-HSV immunity involves cytokine production, recognition, and killing of virally infected cells, and plasmacytoid dendritic cells (pDCs), whose primary role involves type I IFN production *in vivo* (Schusteret *et al*., 2011; Tognarelli *et al*., 2019)

In addition, the adaptive immune response has been shown to play important roles in disease progression, latency and control of virus spread. Neutralizing antibody levels have been inversely correlated with the gravity of the disease (Khodai *et a*l., 2011). The cellular response is highly involved in antiviral defense, with CD8+ T cells playing an important role in this process, largely through the production of IFN γ (Chew, Taylor & Mossman, 2009). A minor role for CD4+ T cells has been described, with these cells providing some degree of protection in the absence of other immune effectors (Chew, Taylor & Mossman, 2009).

In general, HSV has been considered the most common cause of sexually transmitted infections leading to ulcers (Hope-Rapp *et al*., 2010). Genital disease caused by HSV-2 is clinically indistinguishable from that caused by HSV-1 (Dungan, 2010). Infections of the central nervous system (CNS) and the sense organs may manifest as encephalitis, meningitis, myelitis, or retinitis (HSV-1, HSV-2), and in immunocompromised patients, disseminated infections with visceral manifestations of the lungs, liver, or esophagus (HSV-1, HSV-2) may occur. Additionally, neonatal or congenital (very rarely) herpes (HSV-2, HSV-1) results from perinatal or prenatal (intrauterine) infection among pregnant women or neonates (Arvin, 2007; Straface *et al*., 2012)

2.13.8 Diagnosis of HSV-2 Infection

a) Serotyping

Western blotting (WB) assays have been described for serologic detection and differentiation of HSV-1 and -2. Whole-antigen preparations from HSV-1 or -2-infected cell lines are separated by electrophoresis, adsorbed to a nitrocellulose gel, and exposed to patient serum. HSV status is determined by banding patterns specific to HSV-1 or -2 (Wald *et al*., 2003).

Early enzyme-linked immunosorbent assays (ELISAs) utilize a whole-antigen preparation from HSV-1- or HSV-2-infected cell lines. These assays are sensitive (92 to 100%) and are less expensive, are easier to perform, and provide significantly faster turnaround than Western Blot. Newer serologic methods incorporate type- specific assays based on HSV gG. These type-specific proteins are easily differentiated based upon an additional HSV-2-specific domain that is not present in HSV-1. (Martins *et al.,* 2001; Morrow *et al.,* 2003; Summerton *et al.,* 2007)

Screening for HSV-2-specific antibodies would provide a diagnosis of HSV-2 infection without clinical symptoms (Johnston & Core 2016). The protein coding and genome structures of HSV-2 and HSV-1 are similar but can be differentiated serologically by use of the IgG specific type assays (Ades *et al.,* 1989; Brown *et al.,* 2007; Gupta *et al*., 2007).

At present there are three United States Food and Drug Administration (FDA) approved HSV IgG test kits for the detection of specific antibodies to HSV-2 on the market. Those kits are HerpeSelect HSV-2 ELISA (Focus Technologies), Kalon HSV-2 ELISA (Kalon Biologicals Ltd) and Biokit HSV-2 (Biokit) (Gamiel *et al*., 2008; Ngo *et al.,* 2008). HerpeSelect and Kalon enzyme-linked immunosorbent assays (ELISAs) showed high sensitivities (93 to 100 percent) and specificities (95 to 100 percent) compared to various "gold standards" for serum samples from western populations. (Ngo *et al*., 2008).

Polymerase Chain Reaction (PCR)

Molecular diagnosis of acute HSV can be accomplished via amplification and detection of specific viral genome targets. Early real-time PCR assays targeted highly conserved regions of the herpesvirus DNA polymerase in order to amplify both HSV-1 and HSV-2 (LeGoff, *et al.,* 2006; Bhullar, *et al.*, 2014) While sensitive and specific, this method was unable to differentiate HSV-1 from HSV-2 (Anderson, *et al*., 2014). An early assay capable of molecular typing of HSV utilized a LightCycler (Roche, Indianapolis, IN) for PCR combined with melting point analysis and fluorescence resonance energy transfer (FRET) probes (Koenig *et al.,* 2001). These methods have proven to be more sensitive than viral culture and also effectively differentiate HSV-1 and HSV-2 without additional steps (Anderson, *et al*., 2014; Arshad *et al*., 2019). The PCR technique is currently accepted as gold standard in many laboratories (Wagenlehner *et al.,* 2016).

Viral culture

Viral culture of HSV has been a gold standard for laboratory diagnosis of HSV for the past two decades. Using the swabs from the genital lesions, the virus can be grown on tissue culture, usually within 5 days, that is then detected using immunofluorescence assays or by enzyme immunoassay. The limitation with this method is that it lacks sensitivity as more viruses are usually obtained from patients with primary infection (80%) but less from patients with recurrent infections (20- 50%) or patients whose lesions have begun to heal (LeGoff, *et al*., 2014).

2.13.9 Treatment and Prevention of HSV-2

2.13.9.1 Standard HSV-2 antiviral therapy

Acyclovir, valacyclovir, and famciclovir are available for standard antiviral treatment of genital herpes. All drugs are acyclic nucleoside analogs, (Jiang, Feng, Lin & Guo, 2016) and their specific antiviral activity is based on one key enzyme of HSV-1 and HSV-2, the thymidine kinas convert the antiviral compounds to monophosphates and triphosphates which may be incorporated into the growing DNA chain as "false" substrate, leading to an inhibition of viral DNA synthesis. In the case of acyclovir/valacyclovir, this is caused by the absence of the hydroxy group in 3′ position essential for further linking. Other nucleoside analogs such as penciclovir arising from famciclovir can be incorporated into the growing DNA chain. (Workowski *et al*., 2015; CDC, 2018)

2.13.10 Determinants of HSV-2 Infection

Herpes Simplex Virus 2 serology virus is a biological marker of risky sexual conduct (Obasi *et al.,* 1999). In a prevalence study in Zimbabwe male plant workers, marital status, STD history, older age, and higher income were associated with HSV-2 infection (McFarland *et al*., 1999). Low income among women was associated with increased HSV-2 prevalence in a study conducted in the United States (Breining *et al.,* 1990). Other studies have found that HSV-2 is associated with age, HIV serostatus, perception of partner circumcision and STI status, lack of education, number of sexual partners, and ethnicity (Hunter *et al.,* 1994; Chawla *et al.,* 2008; Kramer *et al.,* 2008; Ng'ayo *et al.,* 2008).

2.13.11 Role of HSV-2 infection in HIV-1 acquisition and transmission

Herpes Simplex Virus type 2 is a key co-factor in global HIV pandemic (Weiss *et al*., 2001; Barbour *et al*., 2007; Rebbapragada *et al*., 2007; Watson-Jones et al., 2007; Bollen *et al*., 2008). Nearly 80-95% of people infected with HIV in Africa are HSV-2 seropositive (Weiss *et al*., 2001; Rebbapragada *et al*., 2007). A strong relationship has been cited between HSV-2 infection and HIV incidence while the risk of HSV-2 infections increases acquisition and transmission of HIV by two or three times when

co-infected with HSV-2 (Rebbapragada *et al.*, 2007; Watson-Jones *et al.*, 2007). Despite epidemiological evidence of a strong link between HIV-1 and HSV-2, clinical trials were not very successful in assessing whether arresting HSV-2 infection would reduce the acquisition of HIV (Watson-Jones, 2007; Baeten *et al.*, 2008; Celum *et al.*, 2008; Gray & Wawer, 2008; Tan *et al*., 2013; Rajagopal *et al*.,2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was conducted in selected beaches along Lake Victoria in Kisumu County, Kenya. The lake has a shoreline of 4,828 km with islands constituting 3.7% of this length and is divided among three countries: Kenya (6% or 4,100 km2), Uganda (45% or 31,000 km2) and Tanzania (49% or 33,700 km2) (Prado *et al*., 1991). There are about 32 beaches within a 70 km radius in this area on the shores of Lake Victoria, Kisumu, Kenya (Kwena *et al.*, 2012). This study was conducted in four beaches, namely: Nyamware, Kichinjio, Dunga, and Kobudho selected based on population size, fishing activity and mobility as shown in the map in **Figure 3.1.**

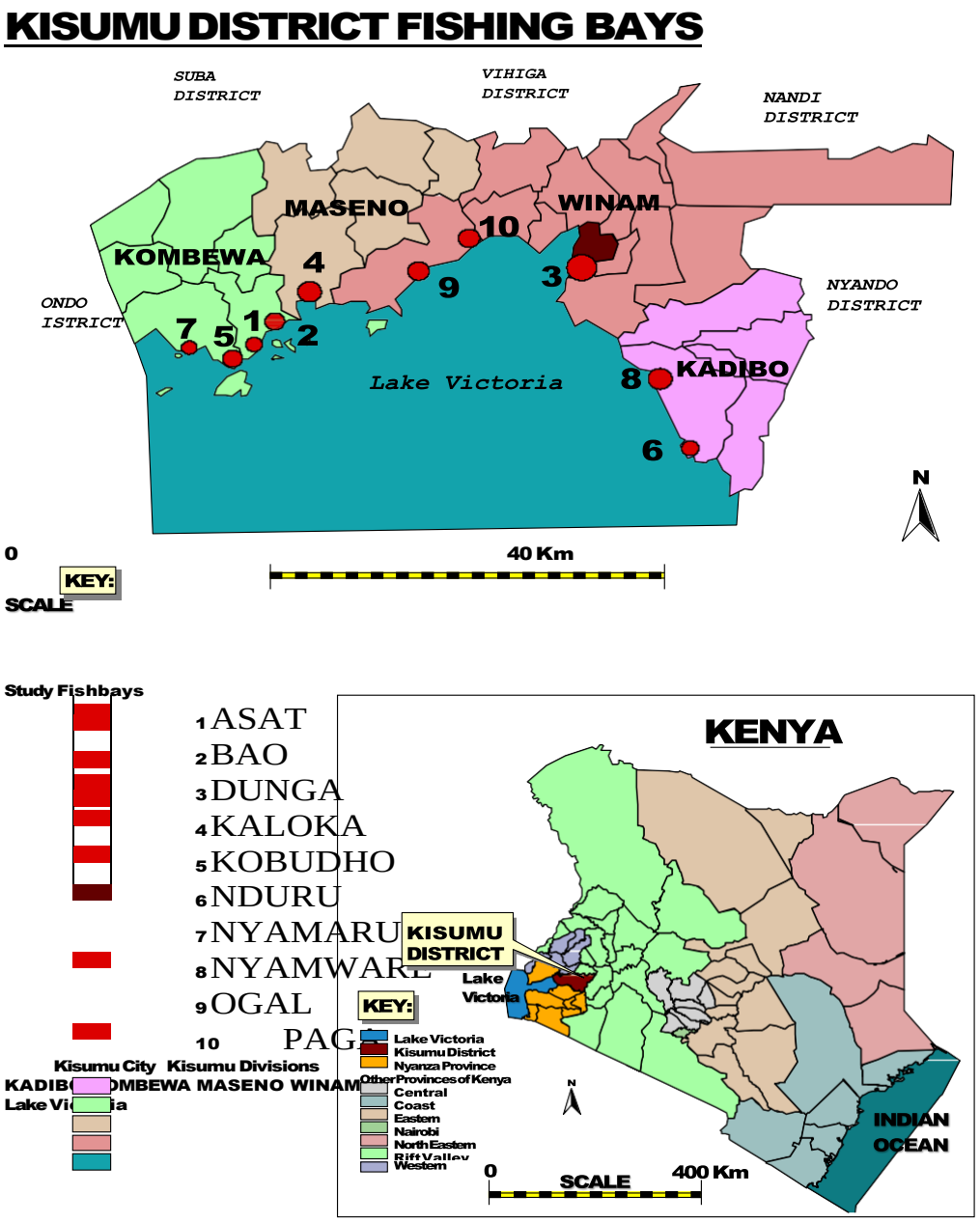


Figure 3.1: Map showing the study sites: Nyamware, Kichinjio, Dunga, and Kobudho along L. Victoria in Kisumu County, Kenya.

3.2 Study population

This study was conducted among fishermen working in Kisumu County along selected beaches namely: Dunga, Kichinjio, Kobudho and Nyamware as shown in **Figure 3.1**

3.3 Study design

This was a cross sectional study.

3.4 Eligibility criteria

3.4.1 Inclusion Criteria

The participants in this study were recruited if they met the following:

1. Adults aged 18 years or older
2. Working in the fishing industry for at least 3 months.
3. Living and working along the shores of Lake Victoria of Kisumu, Kenya
4. Ability and willing to give consent

3.4.2 Exclusion Criteria

1. Fishermen who had been on Antiretroviral Therapy (ART).
2. Fishermen who were sick and unable to participate.

3.5 Sample Size Determination

The minimum number of fishermen enrolled was determined using Lemeshow's formula (Lemeshow *et al*., 1990).

$$n = \left(\frac{z}{m}\right)^2 p(1-p)$$

Where,

- z is the critical value based on the desired confidence level (e.g., z = 1.96 for 95% confidence level);
- m is the margin of error or precision of the estimate (0.05).
- p is the proportion of fishermen co-infected with HSV-2/HIV in Kisumu, Kenya. This is valued at 19.6% (Ng'ayo *et al*., 2008).

Thus = 1.96^2 X 0.196X 0.804 / 0.05^2 = 242.1 =242

To account for lost to follow-up and missing data 3% 241 (8 participants) of the minimum sample size was added. Therefore, a minimum of 249 fishermen meeting the recruitment criteria were consented and enrolled 2 into the study and all were tested for both HIV and HSV-2.

3.6 Sampling design

The mapping of the beaches and fishermen in Kisumu along Lake Victoria by Kwena *et al*., 2012) was adopted in our study. Each beach had an association of fishermen known as beach management unit (BMU) which keeps the record or list of number crew members on the boat and the list of the registered boats. In this study out of the 18 beaches already mapped, only 4 beaches were selected purely based on the close proximity to Kisumu. In this regards the following beaches were visited for participant's recruitment namely: Dunga, Kichinjio, Kobudho and Nyamware. From each beach a sample of 62 fishermen meeting recruitment criteria were randomly identified, consented and enrolled into the study (Appendix 1 and 2) using Systematic sampling. The sampling interval was generated by dividing the total number of the fishermen in the beach by the expected sample size of fishermen from each beach. Example if the beach had a population total of 198 fishermen and in need of 62 fishermen. Then the interval was 198/62 = 3.

3.7 Demographic data collection

Once the participants were recruited and they signed the informed consent (Appendices 1and 2) and a face to face interview with study participants was carried out in private where a questionnaire was used (appendices 3 and 4) to capture demographic data and factors associated with transmission of HSV-2, HIV-1 and HSV-2/HIV coinfection.

3.8 HIV counseling and testing

The guidelines for HIV testing and counselling in Kenya were followed by involving a trained counselor (NASCOP, 2008). First, the participants were taught about the risks and benefits of the HIV and other STI testing (Appendices 5 and 6). They were taught about the importance of abstaining from sex until the right time, being faithful to one uninfected partner and using a condom during sexual encounters with a person of unknown health status. The counsellor explained the meaning of test results, taught how to minimise the chance of acquiring and spreading HIV and explained the risk of getting HIV.

3.9 Sample collection

Each of the 249 enrolled fishermen was invited to a collection centre at the beaches where about 5 ml whole blood was collected into EDTA tubes by a trained phlebotomist. The samples were given initials and coded numbers which were used to identify the participants. The blood samples were shipped to Nairobi in cool boxes to Molecular Virology Laboratory of the Department of Pediatrics and Child Health, University of Nairobi for laboratory analysis.

3.10 Laboratory analysis

Part of the whole blood (0.5ml) was used for HIV rapid testing using two parallel rapid assays (Alere Determine™ HIV-1/2 Ag/Ab Combo and Uni-Gold™ Recombigen® HIV Rapid Test Kits, Trinity Biotech) (NASCOP, 2015) and the rest (4.5ml) centrifuged at 1500 rpm for 10 minutes to collect plasma for HSV-2 ELISA and HIV genotyping. If not used immediately the plasma was stored at -80 °C until use.

3.10.1 HIV Testing

The HIV serology was carried out in accordance with local practice standards in Kenya, using two parallel rapid assays (Alere Determine™ HIV-1/2 Ag/Ab Combo and Uni-Gold™ Recombigen® HIV Rapid Test Kits, Trinity Biotech) (NASCOP, 2015). The two rapid diagnostic tests for HIV include immunochromatographic (lateral-flow tests) and immunofiltration (flow-through tests) formats that detect the presence of HIV-1/2 antibodies and/or HIV-1 p24 antigen. In brief, specimen (fingerstick/capillary whole blood, venous whole blood, serum, plasma, oral fluid) is added to the test device by a specimen transfer device or pipette. A reactive result is indicated by the appearance of a coloured band, line, spot or dot in the test region and in the control region, the latter which is used to indicate both successful addition of reagent or specimen. With this algorithm, two negative rapid HIV 1 assays at enrollment were defined as negative. Two positive rapid assays were defined as positive (WHO, 2015). ELISA resolved discordant or indeterminate rapid test results as described below.

An ELISA assay (Vironostika HIV Uni-Form II Ag/Ab) was employed to resolve discrepancies between the first two assays. Briefly, the Murex HIV Ag-Ab assay utilizes microtiter plates coated with HIV recombinant antigens and peptides from HIV-1 group M gp41 and polymerase (*pol*), HIV-1 group O gp41, and HIV-2 gp36 for the capture of antibodies and monoclonal antibodies for the capture of HIV antigen in test samples. In a three-step assay protocol, the sample (100 µl) is incubated for 60 min with antigen- and antibody-coated plates, followed by five buffer washes. In the next step, conjugate is added to the well and incubated for 30 minutes, followed by five buffer washes. The conjugate contains recombinant antigens and peptides from HIV-

1 group M gp41 and *pol*, HIV-1 group O gp41, HIV-2 gp36, and monoclonal antibodies to HIV-1 p24 conjugated with horseradish peroxidase. The amount of conjugate bound is determined by incubation with hydrogen peroxide–3,3′,5,5′-tetramethylbenzidine (TMB) substrate for 30 min, termination of incubation with H_2SO_4, and absorbance measurement at 450 nm. All incubations are carried out at 37°C and addition of each reagent is monitored by a color change that can be either visually observed or monitored by a spectrophotometer. The simultaneous detection of HIV p24 antigen and HIV antibody in a sample is achieved based on binding of the antigen and an antibody to the solid phase and is proportional to horseradish peroxidase activity. The cutoff is calculated as the mean of negative control values plus 0.15. The overall mean must be less than 0.150 for the run to be valid (Ly *et al*., 2019).

3.10.2 Herpes Simplex Virus- 2 serology

The HSV-2 serology was performed using Kalon HSV type 2 ELISA (Kalon Biological Ltd, Surrey, UK) Kalon HSV type 2 ELISA was performed according to the kit instructions as previously described (Ng'ayo *et al*., 2008). Briefly, the desired number of coated wells were selected and put into the well's holder. In each well, 200 µl Assay Diluent were dispensed. A volume of 10 µl of the calibrator, negative and positive controls and 10 of each sample was dispensed into their designated microwells. The Cut-off Calibrator was run in duplicate. The wells were mixed well by pumping the specimen and controls in and out of the micropipette five times. The plate was then sealed in the plastic bag and incubated at 37°C for 30 minutes. The microwells was then washed four times with diluted wash Buffer (1x) and residual washed solutions dried off by tapping on paper towel. The secondary Incubation involved dispensing 100 µl working strength tracer into microwell sealed in the plastic bag and incubated at 37°C for 30 minutes. The wells were then washed 4 times. Enzyme Incubation involved dispensing 100 µl working substrate solution and incubating the plate uncovered at 18° to 25°C for 30 minutes. The reaction was then stopped by dispensing 100 µl of the stop solution into each microwell. The assay was then read at the optical densities of 450 nm within 30 minutes of adding stop solution. The microstate plate reader was blanked on air or using the 620 nm reading. Score results with an optical density greater than cut-off were considered positive.

Score results with an optical density less than Cut-off were read as negative (Smith *et al*., 2008; LeGoff *et al*., 2008: Gamiel *et al*., 2008; Biraro *et al*., 2011).

3.10.3. Molecular assays for subtype determination

3.10.3.1 Extraction of RNA

Genotypic resistance testing from 59 plasma samples was evaluated using an in- house method using QIAamp viral RNA extraction kit, (Qiagen Inc., USA) according to manufacturer's instructions (QIAamp® Viral RNA Mini Handbook, 2014). Briefly, 560 µl of prepared Buffer AVL containing carrier RNA was pipetted into a 1.5 ml microcentrifuge tube. 140 µl of plasma samples was added into the tube and mixed by pulse-vortexing for 15seconds and incubated at room temperature (25°C) for 10 minutes. 560 µl of absolute ethanol was added to the sample, and mixed by pulse-vortexing for 15seconds. The 630 µl of the solution from step above was tranfered to the QIAamp Mini column and centrifuge at 6000 x *g* (8000 rpm) for 1 min. 500 µl of Buffer AW1 was then added and centrifuge at 6000 x *g* (8000 rpm) for 1 minnutesand the QIAamp Mini Column placed in a clean 2 ml collection tube. 500 µl of Buffer AW2 was added and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min. Carefully into QIAamp Mini column 60 µl of Buffer AVE equilibrated to room Temperature was added incubated at room temperature for 1 minnutes and centrifuge at 6000 x *g* (8000 rpm) for 1 minnutes into a clean tube and the collected RNA stored at -80^{O}C until ready for use (Monleau *et al.,* 2009; Barry *et al*., 2013; Burchard *et al*., 2014).

3.10.3.2 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Plasma-derived RNA samples were reverse transcribed using Qiagen OneStep RT-PCR kit (Qiagen, Maryland, USA). Briefly, each RT-PCR reaction included 1x PCR buffer that contained 1.5 mM MgCl2, 0.4 mM dNTPs, 0.6 µM forward primer (RT18:5′GGAAACCAAAAATGATAGGGGGAATTGGAGG3′ and reverse primer RT21 5′ CTGTATTTCTGCTATTAAGTCTTTTGATGGG 3′) (Lehman *et al.,* 2009), 1 µl enzyme mix, 5 µl of RNA template, 5 units RNAseOut and nuclease-free water in a final volume of 25 µl. Reverse transcription was done at 50^{o}C for 30 minutes followed immediately by PCR amplification cycles comprising denaturation at 95^{o}C

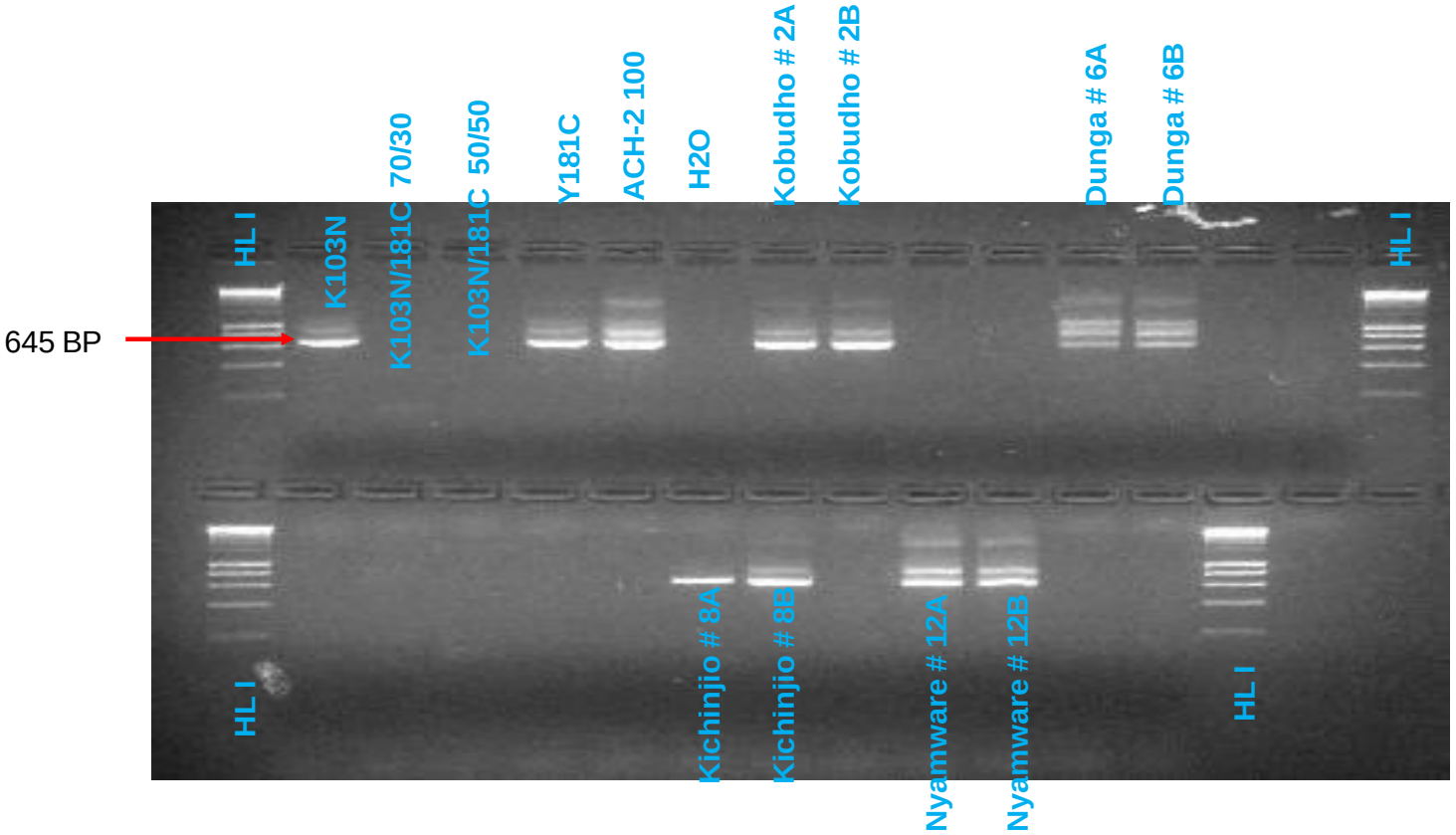
for 15 minutes and 38 PCR cycles of denaturation at 94^{o}C for 30 s; annealing for 5 s at 55^{o}C for polRT primers and extension at 72^{o}C for 1min. A final extension was done for 10 minutes at 72^{o}C.

3.10.3.3 Nested PCR amplification

A nested polymerase chain reaction (PCR) was performed using AmpliTaq Gold (Roche Molecular Systems, Branchburg, NJ). The first round of 25ul reaction mixture contained 5 µl H_2O, 12.5ul 2x reaction mix, 1ul primer RT18, 1 µl primer RT21, and 0.5µl SSIII RT/Platinum Taq and 5µl, the starting template for the nesting PCR was DNA from round 1 PCR for RNA Samples. Briefly, in the first round; HIV- 1 pol gene was amplified using primers (RT18:5′GGAAACCAAAAAT GATAGGGGGAATTGGAGG3′ and RT21 5′ CTGTATTTCTGCTATTAAGTCT TTTGATGGG 3′) (Lehman *et al*., 2009) which was achieved as follows: 1 cycle of 45°C for 1 minutes and 94°C for 2 minutes followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 2 min. The second-round amplification included 50ul of reaction mixture: 27.7ul Nuclease free H_2O, 5µl 10x buffer, 5 µl 25mM $MgCl_2$, 5 ul dNTP 8mM, 2.5ul primer RT1, 2.5 µl primer RT4, 0.3 AmpliTaq Gold and 2µl 1st round template. primers (RT1: 5′CCAAAAGTTAAACAATGGCCATTGACAGA 3′and RT4:5′ AGTTCATAACCCATCCAAAG 3′) which was achieved as follows 1 cycle of 94°C for 2 minutes and 30 cycles of 94°C for 30 s, 55°C for 30s, and 72°C for 1 min, with a final extension of 72°C for 10 minutes (Nyamache *et al*., 2012). Gel electrophoresis through Ethidium bromide staining and visualization was used to confirm the success in PCR amplification. The nested reverse transcriptase-polymerase chain reaction (RT-PCR) amplified a positive sample of 645 base pairs (Koigi *et al*., 2014).

3.10.3.4 Analysis of PCR amplicons by gel electrophoresis

After DNA amplification the PCR products were run on a 2% agarose gel and electrophoresed at a voltage of 100 for 20 minutes. Staining was done with ethidium bromide solution for 20 minutes and thereafter ultra violet transilluminator used to visualize the PCR products (Kiptoo *et al.,* 2013).



Results: The RT PCR for controls K103N and Y181C and the samples Kobudho #2A/B, Dunga #6A/B, Kichinjio #8A/B and Nyamware #12A/B amplified and were subsequently sequenced

Figure 3.2: The gel photo showing Nested PCR HIV-1 positive samples

3.10.3.5 PCR product clean-up for sequencing

The PCR positive samples were cleaned off excess primers and nucleotides in a single step using ExoSAP-IT™ PCR technology according to manufacturer's instruction (https://tools.thermofisher.com). Briefly for each positive PCR product visualized on the gel; 5ul of the PCR product was mixed with 2ul ExoSAP-IT reagent and held at 37ºC for 15 minutes followed by 80ºC for 15 minutes in a thermocycler. This cleaned PCR product was then directly sequenced (Lehman *et al*., 2009).

3.10.3.6 Bigdye sequencing reactions

Sequencing was done by dideoxynucleoside-based analysis using a Big Dye terminator kit (Applied Biosystems) and ABI Prism 3300 equipment (Applied Biosystems, Foster City, US). This was achieved in a 10 µl reaction comprising 5.5 µl DNA grade water, 2 µl 5x Sequencing buffer, 1 µl Big Dye, 0.5µl primers (either RT1 or RT4) RT 1: 5' CCAAAAGTTAAACAATGGCCATTGACAGA 3' or RT4: 5' AGTTCATAACCCAT CCAAAG3', and 1ul PCR product template. This was amplified as follows: 1 cycle of 94°C for 10 seconds and 25 cycles of 94°C for 10 s, 50°C for 5s, and 60°C for 4 minutes (Lehman *et al*., 2009).

3.10.3.7 Bigdye PCR product clean up

The bigdye PCR amplification PCR product was cleaned up using spin columns impregnated with Sephadex® G-50 (Sigma, US) according to the methods described by Lehman *et al*., (2009). Briefly dry sephadex G-50 was loaded into unused wells of 96-well MultiScreen HV plate using the Column Loader and 300ul of Milli-Q water added to each well with sephadex to swell the resin. This was followed by a 3 hours incubation at room temperature. Once the mini-columns were swollen in MultiScreen plates, were span to remove water out of the sephadex columns. All the 10ul bigdye PCR product were carefully loaded into the center of each sephadex containing columns/wells and centrifuged at 910g for 5 minutes to collect the PCR product onto a clean 96-well.

To denature the purified bigdye PCR product, 10ul of Hi-Di™ Formamide was added directly into samples using multichannel and pipette up and down to mix formamide with samples, incubated at 95 ºC for 2 minutes then placed on ice for 10 minutes. The 20ul of the denatured bigdye product was loaded into 96 sequencing well plate and directly sequenced using Big Dye technology on ABI 3300 (Applied Biosystems, Foster City, CA) (Lehman *et al*., 2009).

3.10.3.8 HIV-1 Subtypes and drug resistance interpretation

The sequences were then aligned and cleaned using *Sequencer* - sequence analysis software version 5.4.9 (Gene code Cooperation, MI, US). To determine the HIV subtypes and presence of HIV drug resistant mutations, the sequences were blasted in the Stanford University and International AIDS society-USA web site (http://hivdb.stanford.edu) as described by *(Johnson et al.,* 2008). Using the Stanford Genotypic Resistance Interpretation Algorithm, HIV drug resistance was defined as the occurrence of resistance mutations associated with impaired drug susceptibility.

3.11 Data management

All informed consent records, filled questionnaires and consent forms to participate in HIV testing were stored in a locked cabinet stationed in a secured room only accessible to the principal investigator. This research adhered to the STROBE guidelines for observational studies as outlined (von Elm *et al*., 2008). Statistical analysis was done using Stata version 13 (StataCorp. LP, College Station, USA). The distributions of the demographic characteristics were presented in terms of means (±SD) and frequency (%). The prevalence of HSV-2/HIV co-infection in the studied population was expressed using frequency and percentage. Bivariate and multivariate analyses were done using Poisson regression to evaluate factors that were associated with transmission of HIV, HSV-2 HIV/HSV-2 and TDR (at $P \leq 0.05$).

3.11 Ethical considerations

The study was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice (ICH-GCP) (Tobian *et al.,* 2009). The protocol and informed consent form were reviewed and approved by the Kenyatta National Hospital Ethical Review Committee (KNH-ERC/RR/707-P545/08/2015) prior to any protocol-related procedures being conducted (Appendix 7). Written informed consent was obtained from each participant prior to any protocol-specified procedures being conducted. To maintain confidentiality, initials and coded numbers were used to identify the participant's source documents, CRFs, and study reports. All study records were maintained in a secured location. Permission to conduct the study in the different beaches in Kisumu along Lake Victoria was sought from the Beach Management Units (BMU).

Participation in this study was completely voluntary. The results of the study were disseminated to the relevant health care providers including the county's ministry of health to help in management and treatment of those infected. The participants were also informed of the benefits of the study.

3.12 Quality Assurance

Standard operating procedures regarding face to face interviews were adopted, especially those pertaining to identification of participants, analysis and posting of results. The developed questionnaire was pretested in Nduru beach which is also one of the beaches the Kisumu CMR-FACES program has significant activities to assess its utility and the appropriateness of the questions. Adjustments were made where necessary. The researcher maintained the code of ethics governing research including maintaining recruitment and inclusion criteria. Eventually data was cleaned and counter- checked for errors before the actual analysis and results presentation.

CHAPTER FOUR

RESULTS

4.1Characteristics of the fishermen in Kisumu, Kenya.

A total of 249 fishermen were enrolled in the study. The characteristics of the fishermen are shown in **Table 4.1**. The mean age (SD) was 35.1 (7.8) years. The mean ages according to the infections were as follows: The HIV infected 34.7 (8.3) years, HSV-2 infected 35.6 (8.98) years and HIV-1/HSV-2 34.3 (7.49) years. Overall, the majority (170, 68.3%) of participants were married and 196 (78.7%) had other source of income apart from fishing only. Majority (232, 93.2%) had their age of sexual debut below 18 years, 214 (85.9%) were uncircumcised, while 105 (42.2%) had more than one sexual partner. For the fishermen who travelled away from their fishing beaches, 29 (11.6%) had at least one sexual act and 52 (20.9%) used condoms.

Table 4.1: Baseline characteristics of the fishermen in Kisumu, Kenya

Characteristics	Unit	N	%
Beach	Dunga	63	25.3
	Kichinjio	62	24.9
	Kobudho	62	24.9
	Nyamware	62	24.9
Age	Mean (SD) (Years)	35.1	7.8
	Range (Years)	40	26 to 66
	21-30	80	32.1
	31-40	127	51.0
	>41	42	16.9
Marital status	Single	71	28.5
	Married	170	68.3
	Divorced/Widowed	8	3.2
No of wives	1	160	64.3
	2	10	4.0
	N/A	79	31.7
Education Level	Primary	144	57.8
	Secondary	104	41.8
	Tertiary	1	.4
Income (Ksh)	<10000	223	89.6
	>10001	26	10.4
Age sex debut	Mean (SD) (Years)	15.1	2.6
	Range (Years)	19	7 to 26
	<18	232	93.2
	>18	17	6.8
Circumcised	Yes	35	14.1
	No	214	85.9
No of sexual partner	1	140	56.2
	> 1	105	42.2
	None	4	1.6
No travelled in past month	1	58	23.3
	>1	72	28.9
	None	119	47.8
Sexual acts during last travel	None	220	88.4
	At least once	29	11.6
Condom use last two act	Yes	52	20.9
	No	183	73.5
	N/A	14	5.6

N-Number; % - Percentage

4.2 Prevalence of HIV, HSV- 2 and HIV/HSV-2 mono and co-infections among fishermen in Kisumu, Kenya

Among the study participants, overall, 104 (41.8%) were HIV/HSV-2 negative, 86 (34.5%) were HSV-2 mono-positive, 11 (4.4%) were HIV-1 mono positive while 48 (19.3%) were HIV/HSV-2 co-infected. **Figure 4.1** shows the distribution of HIV, HSV-2 and HIV/HSV-2 mono and co-infections among fishermen.

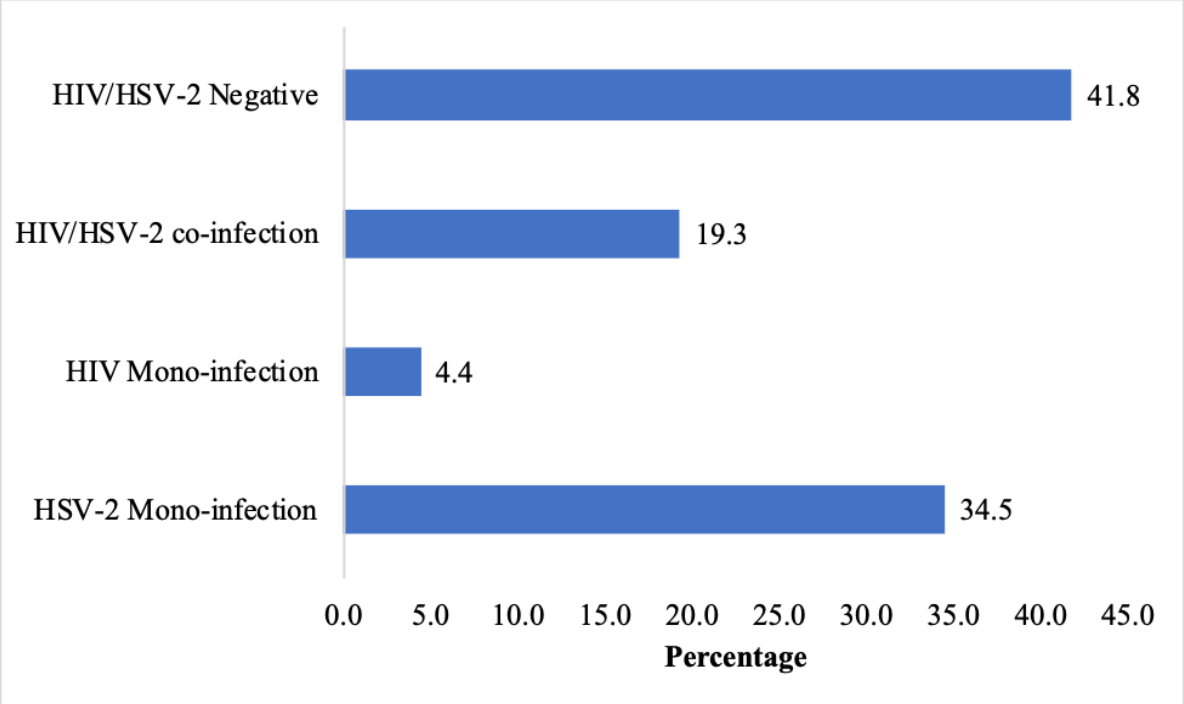


Figure 4.1: The distribution of HIV, HSV- 2 and HIV/HSV-2 mono and co-infections among fishermen working in beaches along the shores of L. Victoria

4.3 Distribution of HIV infections

4.3.1 Distribution of HIV, HSV- 2 and HIV/HSV-2 mono and co-infections across socio-Demographic characteristics of fishermen in Kisumu, Kenya.

The majority of those infected with HIV (20, 33.9%) were recruited from Dunga beach, 54 (40.3%) HSV-2 infected from Nyamware while 20 (41.7%) HIV-1/HSV-2 co- infected were recruited from Dunga. Distribution of mono and co-infection of HIV/HSV-2 varied across participant's age. Most of the HIV mono-infection (24, 40.7%) were common among those aged 31-40 years; HSV-2 infections (60, 44.8%) were common among those aged 31-40 years while HIV/HSV-2 co-infections were common among those aged 21-30 years (20, 41.7%). The majority of HIV infections (38, 64.4%), HVS-2 infections (90, 67.2%) and HIV/HSV-2 co-infections (29, 60.4%), were common among married fishermen. Majority of HIV infection (32, 54.2%) and HSV-2 infection (69, 51.5%) were common among fishermen with primary level of education while HIV/HSV-2 co-infections (26, 54.2%) were common among fishermen with secondary level of education. However, this difference in prevalences was not subjected to any statistical analysis thus significance of the difference cannot be scientifically ascertained. Majority of HIV infection 33 (55.9%), HSV-2 infection 93 (69.4%) and HIV/HSV-2 co-infection 25 (52.1%) was common among those men who were actual fishermen (went fishing in the lake). Further majority of HIV infection 53 (89.8%), HSV-2 infection 117 (87.3%) and HIV/HSV-2 co-infection 43 (89.6%) was common among fishermen who earned less than Ksh10, 000 (**Table 4.2**).

Table 4.2: Distribution of HIV, HSV- 2 and HIV/HSV-2 mono and co-infections across socio-Demographic attributes of fishermen in Kisumu, Kenya.

Variable	Total		HIV-1		HSV-2		HIV-1/HSV-2	
	N=249	%	N=59	%	N=134	%	N=48	%
HIV-1 status								
Positive	59	23.7						
Negative	190	76.3						
HSV-2 status								
Positive	134	**53.8**						
negative	115	46.2						
HIV/HSV status								
Positive	48	19.3						
negative	201	81.7						
Beach								
Dunga	63	25.3	20	35.9	39	29.1	20	41.7
Kichinjio	62	24.9	15	25.4	24	17.9	6	12.5
Kobudho	652	24.9	7	11.9	17	12.7	5	10.4
Nyamware	62	24.9	17	28.8	54	40.3	17	35.4
Age								
Mean (SD) (Years))	35.1	7.8	34.7	8.3	35.6	8.98	34.3	7.49
Range (Years)	40	26 to 66	36	26 to 66	40	26 to 66	31	26 to 66
21-30	80	32.1	24	40.7	46	34.3	20	41.7
31-40	127	51.0	24	10.7	60	44.8	19	39.6
41	42	16.9	11	18.6	28	20.9	9	18.8
Marital status								
Single	71	28.5	19	32,2	42	31.3	18	37.5
Married	170	68.3	38	64.4	90	67.2	29	60.4
No of wives								
1	160	64.3	35	59.3	83	61.9	26	54.2
2	10	4.0	3	5.1	7	5.2	3	6.3
Not application	79	31.7	21	35.6	44	32.8	19	39.6
Educational level								
Primary	144	57.8	32	54.2	69	51.5	22	45.8
Secondary	104	41.8	27	45.8	64	47.8	26	54.2
Tertiary	1	0.4	0	0	1	0.7	0	0
Other sources of income								
Fisher men	196	78.7	33	55.9	93	69.4	25	52.1
Fish trader	53	21.3	26	44.1	41	30.6	23	47.9
Income (Kshs)								
<10,000	223	89.6	53	89.8	117	87.3	43	89.6
>10,001	26	10.4	6	10.2	17	12.7	5	10.4

Key: No. number; % Percentage; SD standard deviation

4.3.2 Distribution of HIV, HSV- 2 and HIV/HSV-2 mono and co-infections across sexual and behavioral characteristics of fishermen in Kisumu, Kenya.

Participants mean age of sexual debut was 15.1 (SD± 2.6) years. The majority (91.8%) HSV-2 infected had their age of sexual debut below 18 years. (**Table 4.3**).

Table 4.3: Distribution of HIV, HSV- 2 and HIV/HSV-2 mono and co-infections across sexual and behavioral characteristics of fishermen in Kisumu, Kenya.

					Mono or co-infection			
	Total		**HIV-1**		**HSV-2**		**HIV-1/HSV-2**	
Variable	N = 249	%	n = 59	%	n = 134	%	n = 48	%
Age sex debut								
<18	232	93.2	59	100	123	91.8	48	100
>18	17	6.8	0	0	11	8.2	0	0
Circumcised								
Yes	35	14.1	12	20.3	25	18.7	10	20.8
No	214	85.9	47	79.7	109	81.3	38	79.2
No of sexual partner								
1	140	56.2	32	54.2	76	56.7	23	47.9
> 1	105	42.2	23	54.2	54	40.3	21	43.8
None	4	1.6	4	6.8	4	3	4	8.3
No travelled in past month								
1	58	23.3	14	23.7	34	25.4	13	27.1
>1	72	28.9	20	33.9	40	29.9	14	29.2
None	119	47.8	25	42.4	60	44.8	21	43.8
Sexual acts during last travel								
None	220	88.4	51	86.4	116	86.6	40	83.3
At least once	29	11.6	8	13.6	18	13.4	8	16.7
Sexual partner								
Girlfriend	124	49.8	28	47.5	71	53	22	45.8
Wife	5	2	2	35.1	4	3	3	6.3
Casual partner	106	42.6	21	35.6	48	35.8	17	35.4
Not applicable	14	5.6	7	11.9	11	8.2	6	12.5
Condom use last two act								
Yes	52	20.9	19	32.2	33	24.6	18	37.5
No	183	73.5	33	55.9	90	67.2	24	50
Not applicable	14	5.6	7	11.9	11	8.2	6	12.5

No - Number; % - Percentage; SD - Standard Deviation

4.4 HIV-1 subtypes among fishermen in Kisumu, Kenya.

Subtype analysis of the *pol* region showed that HIV-1 subtype A was most common (28/59, 47.5%) [78.6% in HIV-1/HSV-2 co-infection verses 21.4% in HIV-1 mono-infection], followed by subtype D (16/59, 27.1 %) [93.8% in HIV-1/HSV-2 co-infection verses 6.3% in HIV-1 mono-infection], subtype C (3/59, 5.1%) [66.7% in HIV-1/HSV-2 co-infection verses 33.3% in HIV-1 mono-infection], subtype B (3/59, 5.1%) [All in HIV-1/HSV-2 co-infection], subtype G (2/59, 3.4%) and CRF01_AE recombinants (7/59, 13.6%).

Among the 59 sequenced samples (HIV-1positive), majority of subtype A were found in Kichinjio beach (10/59, 16.95%), Dunga beach (9/59, 15.3%), Nyamware (7/59, 11.86%) and Kobudho (2/59, 3.4%). Dunga, Nyamware and Kobudho each had 1/59 (1.7%) subtype B. Majority of subtype D were found in Dunga beach (6/59, 10.2%), Nyamware (5/59, 8.5%), Kobudho (2/59, 3.4%) and Kichinjio (1/59, 1.7%). Subtype C were only found in Dunga and Kichinjio beaches with 2/59 (3.4) and 1/59 (1.7%) respectively. Kichinjio and Kobudho had 1/59 (1.7%) subtype G each. Possible CRFs were found among patients from Kichinjio (2/59, 3.4%), Nyamware (2/59, 3.4%), Dunga (2/59, 3.4%) and Kobudho (1/59, 1.7%) **(Table 4.4**).

Table 4.4: Distribution of HIV-1 Subtypes for HIV mono and HIV/HSV-2 co-infected fishermen along Nyamware, Kobudho, Dunga and Kichinjio beaches, Kisumu, Kenya.

HIV-1/HSV-2 Co-infection		HIV-1 Monoinfection	
Beach	**Subtype**	**Beach**	**Subtype**
1 Nyamware (17)	A	1 Kichinjio (9)	A
Age (31-51)	B D	Age (29-62)	C G
	CRF01_AE		*CRF01_AE*
2 Dunga (19)	A	2 Kobudho (2)	A
Age (26-43)	B	Age (32)	D
	C		
	D		
	CRF01_AE		
3 Kichinjio(6)	A		
Age (32-56)	D		
4 Kobudho(4)	A		
Age (30-57)	D		
	G		
	CRF01_AE		

4.5 Drug Resistance Mutations among fishermen in Kisumu, Kenya.

Twenty-three of 59 (38.9%) sequenced samples had drug resistance. Majority, 19/23 (82.6%) were among HIV/HSV-2 co-infected fishermen. Among the 59 HIV infected fishermen, 10 had NRTI resistance mutations with NAMS, M184V (80%) and K65R (10%) (About 90% found in the HIV/HSV-2 co-infected fishermen). Twenty-two (22) had NNRTI mutations; five (22.7%) had K103N, Y181C, and G190A each. Three (13.6%) fishermen had V179T, two fishermen had V901V while A98G, and Y188L mutations were also detected. About 81.8% of these NNRTI mutations were found in the HIV/HSV-2 co-infected fishermen (**Table 4.5**)

Table 4.5: HIV-1 Subtype and Drug Resistance Mutations (DRM) among fishermen in Kisumu, Kenya (Sequences available in gene bank Accession No. KX505314-KKX505372).

	HIV-1/HSV-2 co-infected				IV-1 Mono-infected		
AgeBeach	Subtype	NRTI	NNRTI	Age	Beach	Subtype	NRTI	NNRTI
31Dunga	A	*Susceptible*	**V179T**	29	Kichinjio	G	*Susceptible*	**V90I, K103N, F227FL**
47Kobudho	B	**K65KR**	**V106AV, F227FL, M230I**	62	Kichinjio	*CRF01_AE*	*Susceptible*	**Y181CY**
30Nyamware	D	**M184V**	**V106A**	57	Kichinjio	C	**M184V**	*Susceptible*
35Dunga	C	**M184V**	**K103KN**	29	Kichinjio	A	*Susceptible*	**G190A**
30Dunga	C	**M184V**	**K103KN**	37	Kichinjio	*CRF01_AE*	*Susceptible*	*Susceptible*
30Nyamware	A	*Susceptible*	**K103KN**	32	Kobudho	D	*Susceptible*	*Susceptible*
40Dunga	D	**M184V**	**A98G, Y181C, H221HY**	32	Kobudho	A	*Susceptible*	*Susceptible*
28Dunga	D	**M184V**	**Y188L**	36	Kichinjio	A	*Susceptible*	*Susceptible*
26Dunga	D	*Susceptible*	**G190AG**	33	Kichinjio	A	*Susceptible*	*Susceptible*
42Kichinjio	A	*Susceptible*	**K103N**	30	Kichinjio	A	*Susceptible*	*Susceptible*
38Dunga	*CRF01_AE*	*Susceptible*	**G190AG**	29	Kichinjio	A	*Susceptible*	*Susceptible*
28Dunga	*CRF01_AE*	**M184V**	**E138Q, G190A**					
33Nyamware	D	*Susceptible*	**V90IV**					
36Kichinjio	A	**M184V**	**G190A**					
37Nyamware	D	*Susceptible*	**V179DV**					
31Nyamware	D	**V75MV**	**Y181CY**					
30Nyamware	*CRF01_AE*	*Susceptible*	**V90IV, Y181FINY**					
33Kobudho	G	*Susceptible*	**Y181C, H221Y**					
35Nyamware	A	*Susceptible*	**V179T**					
32Dunga	A	*Susceptible*	*Susceptible*					
28Dunga	A	*Susceptible*	*Susceptible*					
41Dunga	A	*Susceptible*	*Susceptible*					
31Dunga	D	*Susceptible*	*Susceptible*					
30Kobudho	D	*Susceptible*	*Susceptible*					
30Dunga	B	*Susceptible*	*Susceptible*					
37Dunga	D	*Susceptible*	*Susceptible*					
37Dunga	A	*Susceptible*	*Susceptible*					
30Kobudho	A	*Susceptible*	*Susceptible*					
29Dunga	A	*Susceptible*	*Susceptible*					
41Dunga	A	*Susceptible*	*Susceptible*					
43Dunga	A	*Susceptible*	*Susceptible*					
38Nyamware	D	*Susceptible*	*Susceptible*					
35Dunga	D	*Susceptible*	*Susceptible*					
45Kichinjio	A	*Susceptible*	*Susceptible*					
28Dunga	A	*Susceptible*	*Susceptible*					
27Nyamware	A	*Susceptible*	*Susceptible*					
33Kichinjio	D	*Susceptible*	*Susceptible*					
28Nyamware	B	*Susceptible*	*Susceptible*					
28Nyamware	A	*Susceptible*	*Susceptible*					
28Nyamware	A	*Susceptible*	*Susceptible*					
32Kichinjio	A	*Susceptible*	*Susceptible*					
27Nyamware	A	*Susceptible*	*Susceptible*					
56Kichinjio	A	*Susceptible*	*Susceptible*					
26Nyamware	D	*Susceptible*	*Susceptible*					
51Nyamware	A	*Susceptible*	*Susceptible*					
27Nyamware	*CRF01_AE*	*Susceptible*	*Susceptible*					
32Nyamware	D	*Susceptible*	*Susceptible*					
57Kobudho	*CRF01_AE*	*Susceptible*	*Susceptible*					

NNRTI- Non-Nucleoside Reverse Transcriptase Inhibitors, NRTI- Nucleoside Reverse Transcriptase Inhibitors, *CRF01_AE*-Circulating Recombinant Form,

Age presented as absolute value. Subtypes are shown as single strain or as circulating recombinant. All detected NRTI and NNRT Drug Resistance Mutations (DRM) are listed for each participant. Susceptible strains based on the RT region are also listed per participant.

4.5.1 Factors associated with the prevalence of TDR among fishermen in Kisumu, Kenya.

In the regression model, HSV-2 co-infection was the only factor independently associated with TDR; OR 4.1 (95% CI 1.4 -11.9). **Table 4.6** summarizes the bivariate and multivariate analysis of factors associated with the prevalence of Drug Resistance. The sequence script of the prevalence is illustrated in **appendix IX.**

Table 4.6: Factors associated with Drug Resistance variant among the fishermen along Nyamware, Kobudho, Dunga, and Kichinjio beaches in Kisumu Kenya

Variable	Total	TDR infected fishermen		Bivariate	Multivariate
		n	%	OR (95% CI)	OR (95% CI)
HSV-2 infection					
Positive	134	19	14.2	**4.1(1.4-11.9)**	NS
Negative	115	4	3.5	1	
Beach					
Dunga	63	8	12.7	1.1(0.4-3.1)	
Kichinjio	62	6	9.7	0.9(0.3-2.6)	NS
Kobudho	62	2	3.2	0.3(0.1-1.4)	
Nyamware	62	7	11.3	1	
Age					
21-30	80	9	11.3	0.8(0.3-2.7)	
31-40	127	10	7.9	0.7(0.3-1.7)	NS
>41	42	4	9.5	1	
Marital status					
Single	71	5	7		
Married	170	18	10.6	ND	NS
Divorced/Widowed	8	0	0		
No of wives					
1	160	17	10.6	1.7(0.6-4.6)	
2	10	1	10	1.5(0.2-13.5)	NS
Not applicable	79	5	6.3	1	
Education Level					
Primary	144	8	5.6		
Secondary	104	15	14.4	ND	ND
Tertiary	1	1	100		
Other source of income					
Fisherman	196	15	7.7	1.9(0.8-4.7)	NS
Fish trader	53	8	15.1	1	
Income (Ksh)					
<10,000	223	21	9.4	1.2(0.3-5.2)	NS
>10,001	26	2	7.7	1	
Age sex debut					
<18	232	23	9.9	ND	ND
>18	17	0	0		
Circumcised					
Yes	35	5	14.3	1.7(0.6-4.6)	NS
No	214	18	8.4	1	
No of sexual partner					
1	140	16	11.4	0.5(0.06-3.4)	
> 1	105	6	5.7	0.2(0.03-1.9)	NS
None	4	1	25	1	
No travelled in past month					
1	58	5	8.6	1.8(0.3-9.9)	
>1	72	8	11.1	0.6(0.1-2.6)	NS
None	119	10	8.4	1	
Sexual acts during last travel					
At least once	29	3	10.3	0.9(0.3-2.9)	NS
None	220	20	9.1	1	
Sexual partner					
Girlfriend	124	13	10.5	0.7(0.2-3.3)	
Wife	5	0	0	ND	
Casual partner	106	8	7.5	0.5(0.1-2.4)	NS
Not applicable	14	2	14.3	1	
Condom use last two act					
Yes	52		13.5	1.7(0.7-4.1)	
No	183		7.7	1	NS

No - Number; % - Percentage; OR - Odds ratio; CI - confidence interval; NS - Not significant; ND - Not done

4.6 Factors associated with transmission of HIV, HSV-2 and co-infections among the fishermen along Nyamware, Kobudho, Dunga, and Kichinjio beaches in Kisumu Kenya.

Fishermen were more likely to be HIV mono-infected if they were HIV-1 positive OR 3.7 (95% CI 1.9 -7.2), worked as fishermen than fish trader OR 2.9 (95% CI 1.7 -4.8) and owned fishing net OR 1.7 (95% CI 1.1 -2.9). On the contrary fishermen from Kobudho beach OR 0.4 (95% CI 0.2 -0.9) were less likely to be HIV mono-infected as compared to those from Nyamware (**Table 4.7**).

HSV-2 infection was more common among HIV-1 positive fishermen OR 1.7 (95% CI 1.3 -2.6), those who worked as fishermen as compared to the fish traders OR 1.6 (95% CI 1.1 -2.3) and among those who owned fishing net OR 1.4 (95% CI 1.1 -2.1). Those fishermen from Kichinjio OR 0.4 (95% CI 0.3 -0.7) and Kobudho beach OR 0.3 (95% CI 0.2 -0.5) were less likely to be HSV-2 mono-infected as compared to those who worked in Nyamware.

HIV/HSV-2 co-infection was more common among fishermen who worked as fishermen as compared to fish traders OR 3.4 (95% CI 1.9 – 5.9) and among those who owned fishing net as compared to those did not own one OR 2.1 (95% CI 1.2 -3.6). Those fishermen from Kichinjio beach OR 0.4 (95% CI 0.2 -0.9) and Kobudho beach OR 0.3 (95% CI 0.1 -0.8) were less likely to be HIV/HSV-2 co-infected as compared to those from Nyamware.

Table 4.7: Socio-demographic factors associated with HIV, HSV-2 mono and co-infection among fishermen in Kisumu, Kenya.

Socio-demographic variables	Total	HIV-1 infection Bivariate OR (95% CI)	*P*	HSV-2 infection Bivariate OR (95% CI)	*P*	HIV/HSV-2 co-infection Bivariate OR (95% CI)	*P*
HSV-2 or HIV infection							
Positive	134	**3.7(1.9-7.2)**	**0.0001**	**1.7(1.3-2.6)**	**0.001**	NS	0.998
Negative	115	1		1			
Beach							
Dunga	63	1.1(0.6-2.2)	0.657	0.7(0.5-1.10	0.104	1.2(0.6-2.2)	0.657
Kichinjio	62	0.9(0.4-1.7)	0.724	**0.4(0.3-0.7)**	**0.001**	**0.4(0.2-0.9)**	**0.028**
Kobudho	62	**0.4(0.2-0.9)**	**0.048**	**0.3(0.2-0.5)**	**0.001**	**0.3(0.1-0.8)**	**0.016**
Nyamware	62	1	1	1	1	1	1
Age							
21-30	80	0.9(0.4-1.7)	0.709	1.2(0.7-1.8)	0.537	0.8(.4-1.9)	0.701
31-40	127	0.6(0.4-1.1)	0.109	0.8(0.6-1.2)	0.316	0.6(0.3-1.1)	0.109
>41	42	1	1	1	1	1	1
Marital status							
Single	71	0.8(0.3-2.7)	0.231	2.3(0.6-9.7)	0.180	2.1(0.3-15.2)	0.491
Married	170	0.7(0.3-1.7)	0.112	2.1(0.5-8.6)	0.235	1.4(0.2-10.1)	0.760
Divorced/Widowed	8	1	1	1	1	1	1
No of wives							
1	160	0.8(0.5-1.4)	0.480	0.9(0.6-1.3)	0.703	0.7(0.4-1.2)	0.194
2	10	1.1(0.3-3.8)	0.845	1.3(0.6-2.7)	0.574	1.2(0.3-4.2)	0.722
Not applicable	79	1	1	1	1	1	1
Education Level							
Primary	144		0.998	0.5(0.1-3.4)	0.480		0.998
Secondary	104	NS	0.989	0.6(0.2-4.4)	0.845	NS	0.998
Tertiary	1	1	1	1	1	1	1
Other source of income							
Fisherman	196	**2.9(1.7-4.8)**	**0.0001**	**1.6(1.1-2.3)**	**0.009**	**3.4(1.9-5.9)**	**0.0001**
Fish trader	53	1	1	1	1	1	1
Owns fishing boat							
Yes		1.4(0.8-2.5)	0.209	1.2(0.8-1.7)	0.447	1.3(0.7-2.5)	0.439
No		1	1	1	1	1	1
Owns fishing net							
Yes		**1.7(1.1-2.9)**	**0.047**	**1.4(1.1-2.1)**	**0.035**	**2.1(1.2-3.6)**	**0.018**
No		1	1	1	1	1	1
Income (Ksh)							
<10,000	223	1.1(0.4-2.3)	0.945	0.8(0.5-1.3)	0.396	1.1(0.4-2.6)	0.995
>10,001	26	1	1	1	1	1	1

Key: No - Number; % - Percentage; OR - Odds ratio; CI - confidence interval; NS - Not significant

HIV-1 infection was more common among fishermen who used condom in the last two sexual acts as compared to those who reported to never using it (OR 1.7, 95% CI 1.3 -3.1). Those fishermen who did not have sexual partner OR 0.4 (95% CI 0.1 -0.9), sexual partners less than 18 years OR 0.3 (95% CI 0.2 -0.8), more than 18 years OR 0.4 (95% CI 0.2 -0.9) and those who had sexual relationship with a casual partner OR 0.3 (95% CI 0.2 -0.9) were less likely to be HIV-1 mono-infected. None of sexual behavioral factors were found associated with HSV-2 mono-infection (**Table 4.8**).

The HIV/HSV-2 co-infection was more common among fishermen who used condoms as compared to those who did not use it (OR 2.2, 95% CI 1.3 – 4.1). On the other hand, fishermen who had one sexual partner OR 0.2 (95% CI 0.05 -0.5) or more than one sexual partner OR 0.2 (95% CI 0.06 -0.6), sexual partners less than 18 years OR 0.3 (95% CI 0.3 -0.8), more than 18 years OR 0.4 (95% CI 0.2 -0.9) and those who had sexual relationship with a casual partner OR 0.3 (95% CI 0.2 -0.9) were less likely to be HIV/HSV-2 co -infected as compared to those who reported the type of sexual partner as not applicable.

Table 4.8: Sexual behavioral factors associated with HIV, HSV-2 and HSV-2/HIV mono and co-infection among fishermen in Kisumu, Kenya.

Sexual behavioral	Total	HIV-1 infection Bivariate OR (95% CI)	*P*	HSV-2 infection Bivariate OR (95% CI)	*P*	HIV/HSV-2 co-infection Bivariate OR (95% CI)	*P*
Age sex debut							
<18	232	NS		0.8(0.4-1.5)	0.527	NS	0.988
>18	17	1	1	1	1	1	1
Circumcised							
Yes	35	1.5(0.8-2.9)	0.168	1.4(0.9-2.1)	0.127	1.6(0.8-3.2)	0.181
No	214	1	1	1	1	1	1
No of sexual partner							
1	140	**0.2(0.08-0.6)**	**0.005**	0.5(0.2-1.5)	0.234	**0.2(0.05-0.5)**	**0.001**
> 1	105	**0.2(0.07-0.6)**	**0.005**	0.5(0.2-1.4)	0.199	**0.2(0.06-0.6)**	**0.003**
None	4	1	1	1	1	1	1
Age of sexual partner							
<18	232	**0.3(0.2-0.8)**	**0.015**	0.6(0.3-1.2)	0.131	**0.3(0.3-0.8)**	**0.013**
>18	17	**0.4(0.2-0.9)**	**0.046**	0.7(0.4-1.2)	0.227	**0.4(0.2-0.9)**	**0.045**
Not stated		1	1	1	1	1	1
No travelled in past month							
1	58	1.8(0.3-9.9)	0.680	1.1(0.4-2.4)	0.983	1.2(0.4-4.1)	0.680
>1	72	0.6(0.1-2.6)	0.140	0.6(0.3-1.2)	0.170	0.4(0.9-1.10)	0.090
None	119	1	1	1	1	1	1
Sexual acts during last travel							
At least once	29	0.9(0.3-2.9)	0.647	0.8(0.5-1.4)	0.131	0.7(0.3-1.4)	0.282
None	220	1	1	1	1	1	1
Sexual partner							
Girlfriend	124	0.4(0.2-1.1)	0.060	0.7(0.4-1.3)	0.329	**0.4(0.2-0.9)**	**0.046**
Wife	5	1.2(0.3-4.6)	0.792	1.1(0.3-3.1)	0.975	1.4(0.4-5.5)	0.634
Casual partner	106	**0.3(0.2-0.9)**	**0.034**	0.5(0.3-1.1)	0.099	**0.4(0.2-0.9)**	**0.038**
Not applicable	14	1	1	1	1	1	1
Condom use last two act							
Yes	52	**1.7(1.1-3.1)**	**0.035**	1.2(0.8-1.8)	0.287	**2.2(1.3-4.1)**	**0.006**
No	183	1	1	1	1	1	1

No - Number; % - Percentage; OR - Odds ratio; CI - confidence interval; NS - Not significant

In multivariate analyses, HIV positive fishermen were more likely to be infected with HSV-2 OR 4.1 (95% CI 1.4 -11.9), worked as fishermen OR 2.8 (95% CI 1.7 -4.8) but were less likely to have more than 1 sexual partner OR 0.2, 95% CI 1.19 to 1.86) (**Table 4.9**).

The HSV-2 positive fishermen were more likely to be infected with HIV-1 OR 2.1 (95% CI 1.3 -2.6) and worked as fishermen than fish trader OR 1.6 (95% CI 1.1 -2.4). The HIV/HSV-2 positive fishermen were more likely to work as fishermen OR 3.1 (95% CI 1.7 -5.6) and having used condoms in the last two sexual acts OR 2.1 (95% CI 1.1 -3.8). On the contrary, these men were less likely to have one casual partner OR 0.3 (95% CI 0.1 -0.9) or more than 1 sexual partner OR 0.3, 95% CI 0.08 to 0.9) and that they had not travelled in the past one-month OR 0.3 (95% CI 0.09 -0.7).

Table 4.9: Socio-demographic and Sexual behavioral factors independently associated with HIV, HSV-2 and HSV-2/HIV mono and co-infection among fishermen working along Nyamware, Kobudho, Dunga, and Kichinjio beaches in Kisumu Kenya.

Variables	Total	HIV-1 infection Multivariate OR (95% CI)	*P*	HSV-2 infection Multivariate OR (95% CI)	*P*	HIV/HSV-2 co-infection Multivariate OR (95% CI)	*P*
HSV-2 infection							
Positive	134	**4.1(1.4-11.9)**	**0.0001**	**2.1(1.3-2.6)**	**0.0001**	NS	0.998
Negative	115	1	1	1	1	1	1
Occupation							
Fisherman	196	**2.8(1.7-4.8)**	**0.0001**	**1.6(1.1-2.4)**	**0.0001**	**3.1(1.7-5.6)**	**0.0001**
Fish trader	53	1	1	1	1	1	1
No of sexual partner							
1	140	0.5(0.06-3.4)	0.068		0.988	**0.3(0.1-0.9)**	**0.028**
>1	105	**0.2(0.09-0.9)**	**0.033**	NS	0.989	**0.3(0.08-0.9)**	**0.036**
None	4	1	1	1	1	1	1
No travelled in past month							
1	58		0.68		0.989	1.1(0.3-3.6)	0.901
>1	72	NS	0.14	NS	0.989	1	1
None	119	1	1	1	1	1	1
Condom use last two act							
Yes	52	**1.8(1.1-3.2)**	**0.035**	NS	0.127	**2.1(1.1-3.8)**	**0.023**
No	183	1	1	1	1	1	

No - Number; % - Percentage; OR - Odds ratio; CI - confidence interval; NS - Not significant

CHAPTER FIVE
DISCUSSION

5.1 Prevalence of HIV, HSV- 2 and HIV/HSV-2 mono and co-infections among fishermen along Nyamware, Kobudho, Dunga, and Kichinjio beaches in Kisumu Kenya.

The HIV frequency among the participants in this study was about four-fold the national prevalence of 5.9% (KAIS, 2012). Corroborating evidence from Kissling *et al*., 2005 found that the prevalence among fishing communities in ten low- to middle - income countries in Africa, Asia and Latin America was 4 to 14 times higher than that of the national average of adult population aged 15 to 49 years. Other epidemiological studies in Kenya and Uganda have also supported this assertion (Kwena *et al*., 2010; Asiki *et al*., 2011). Studies have demonstrated that many factors contribute to the vulnerability of fishing communities to HIV / AIDS including: socio-political and geographical marginalisation; high mobility, inaccessible health care; prevalence of other life-threatening diseases; daily financial revenue; and cultural practices, such as wife inheritance (Lubega *et al*, 2015).

Fishermen in Kisumu have been cited to engage in transaction- driven behavior like fish for money or money for fish (Bershteyn *et al*., 2018) as well as cultural practices associated with risky behavior (Njue *et al*., 2009). Women on those beaches reportedly exchange sex for fish or for money (Kwena *et al*., 2012). KAIS, 2012 report described the migratory nature of fishing and fishing activities as a risk for HIV infection.

The prevalence of HIV amongst fishermen in this study reflects a region which is highly burdened by HIV. The HIV burden in Nyanza is relatively greater than in other regions in Kenya at 14.9% compared to 8.8%, 4.6% and 0.8% for Nairobi, Eastern and North Eastern regions respectively (KAIS 2012). In this study many independent predictors for HIV acquisition were observed: inconsistent use of condoms with the last sexual partner,

multiple sexual partners and lack of male circumcision that were also observed by Oluoch *et al.* in 2011.

Similar studies among this population of fishermen in Kenya and Uganda depicted the same picture. Prevalences of 26% and 23.6% were reported in previous studies respectively (Ng'ayo *et al*., 2007; Kwena *et al*., 2010 and Ondondo *et al*., 2014). The same was also observed among the fishermen in Uganda where the prevalence was 22.4% (Kiwanika *et al*., 2015). A census among volunteers in the fishing communities in Uganda reported a prevalence of 28.8% (Asiki *et al.,* 2011). A Sentinel Surveillance study among Cambodian fishermen also reported a HIV seroprevalence of 16.1 percent (Samnang et al., 2004). In this study, adults aged between 21 and 30 years had a similar prevalence (34.3 percent) as those reported in a cohort of young adults (18 to 34 years) enrolled in an HIV incidence study in western Kenya which was at 31.5 percent. (Akinyi *et al*., 2017).

Notwithstanding, the HSV-2 infection burden was also high in this population. This is far much high than the regional and national prevalence which are estimated at 49.1% and 35.1% (KAIS, 2007). This is not surprising in a region where highest HIV prevalence was reported (Ng'ayo *et al.*, 2007; Kwena *et al*., 2010; Ondondo *et al.,* 2014). There is four to five times likelihood of shedding of HSV-2 from a HIV-positive individual which is likely to increase HSV transmission (Allen *et al*., 2004). In addition, the susceptibility of HSV-2 infection among immunocompromised HIV positive persons may be increased (Tassiopoulos *et al*., 2007). HIV infection exacerbates HSV-2 infection increasing the frequency and persistence of herpetic ulceration (Serwadda *et al.*, 2003).

Previous studies in this population also reported high HSV-2 disease burden, with prevalences of 56.3% and 63.9% (Ng'ayo *et al*., 2007; Ondondo *et al*, 2014). Seroprevalences varies across regions and sociodemographic factors. In South Africa among HIV negative cohort the prevalence was 65% (Daniels *et al*., 2016), while among persons aged 15-65 in Kampala, Uganda it was reported to be 58%. Lower prevalences

have been cited in various studies: In Pakistan prevalence of HSV2 was reported as 3.4% (Mil *et al*., 2010).

The high prevalence of HIV / HSV-2 coinfection reported in this study is corroborating evidence. HSV-2 and HIV have a synergistic relationship; HSV-2 infection increases HIV susceptibility and transmission, while HIV infection increases HSV-2 infection susceptibility and HSV-2 genital shedding (Paz-Bailey *et al.,* 2007; Okuku *et al*., 2011). In Other observational studies the association between HSV-2 and HIV incidence was strong. HSV-2 accounts for about 63% of new HIV infections (Watson- Jones *et al.*, 2007). HSV-2 seropositivity is associated with the acquisition of HIV and HIV infection exacerbates HSV-2 infection increasing the frequency and persistence of herpetic ulceration (Serwadda *et al.*, 2003).

Lower prevalences of coinfection with HIV and HSV-2 was reported in other settings, although not necessarily among fishermen: Three studies in India among HIV patients recorded an HSV-2 seropositivity of 47% in Kolkata (Chakraborty *et al.*, 2010), 49% in Andhra Pradesh (Anuradha *et al.,* 2008), 48.4 % in Delhi (Karad *et al.,* 2007) and 50% in Tamil Nadu (Jacob *et al*., 2015). Further higher rates of HIV/HSV-2 co-infection was observed elsewhere, for example, a prevalence of 55% in the United Kingdom (Hill *et al*., 2009), 87% in South Africa (Schaftenaa*et al.,* 2014) and 86% in Uganda (Nakubulwa *et al.*, 2009). Many studies have demonstrated a proportionate increase in HIV RNA both in plasma and genital tract with increase in genital herpes recurrence. The HIV virus is also more efficiently transmitted from genital sores (Allen *et al*., 2004). The rate of HSV-2 shedding among those with HIV infection increases upto five folds than in HIV-negative individuals, resulting in increased HSV transmission (Allen *et al*., 2004).

5.1.1 Prevalence of HIV subtypes among fishermen along Nyamware, Kobudho, Dunga, and Kichinjio beaches in Kisumu Kenya.

In this study, the overall distribution of HIV-1 subtypes was synonymous with earlier research patterns with predominance of HIV-1 subtype A (63%), followed by D (15%), C (3%) and G (1%). Recombinant variants constituting 18% with AD dominating at 15%, followed by AC (2%) and AG (1%) (Yang *et al.*, 2004). Previous studies suggested a consistent and continuous dominance of subtypes A and D in Western Kenya (Yang *et al*., 2004; Arroyo *et al.,* 2009; Micah *et al.,* 2011). It is in view of this that subtypes A and D were introduced to Kenya through Uganda (Gray *et al*., 2009).

Hypothetically, the upward scale of subtypes variation in Kenya could be due to ease in travel locally, human migration and increasing heterogeneity in demographics. High mobility and human migration observed in this region could be a contributory factor to the spread and intermixing of various subtypes. In our study, subtype D was the second most common subtype. This figure is slightly higher than those reported elsewhere in Kenya (Kageha *et al*., 2012). Kisumu borders Uganda where HIV-1 subtype D is more common than in Kenya (Gonzalo *et al*., 2015). Cross border interaction could be the the underlying factor to explain this high prevalence of subtype D. It was also reported the presence of subtype C in this population. This subtype is more common in South Africa (Lihana *et al.*, 2012).

Kenya is a neighbour to five countries with mutable distribution of HIV-1 subtypes. Subtypes C and AC are dominant in Somalia and Ethiopia, C and D in Sudan, while A and D are the most common subtypes in Uganda. Subtypes A and C are the major circulating strains in Tanzania (Arroyo *et al.,* 2004; Yang *et al.,* 2010; Mosha *et al*., 2011). Subtype B appears to be the most reported in Northern African countries like Algeria, Egypt, Morocco, and Tunisia. (Lihana *et al.*, 2012).

In regions where the HIV epidemic is the oldest, for example in sub - Saharan Africa, the highest genetic variation of HIV-1 has been found where HIV-1 subtypes and many CRFs are continuously reported (McCutchan, 2000).

5.1.2 Drug Resistance mutation among the fishermen along Nyamware, Kobudho, Dunga, and Kichinjio beaches in Kisumu Kenya.

Prevalence of Transmitted Drug Resistance (TDR) is high based on WHO surveillance criteria (Bennett *et al*., 2006). This is expected due to high burden of HIV and the significant ART treatment roll-out in over a decade. Long availability of ARVs in a community has been attributed to the emergence of TDR (Hamers *et al*., 2011). Large ARV area coverage increases the likelihood of appearance and dissemination of TDR (Baggaley *et al.,* 2006). Previous studies conducted on TDR in Kenya showed a varying prevalence ranging from 1.1% to 13.3% (Sigaloff *et al.,* 2012; Hassan *et al.,* 2013; Onsongo *et al.,* 2016). A prevalence of 6 percent TDR among fishermen along shores of Lake Victoria in Uganda was also cited (Nazziwa *et al*., 2013).

Resistance to both NNRTIs (K103N, Y181C, V106A and G190A) and NRTIs (M184V and K65KR) were associated with the mutations seen in this study. These mutant strains fail to respond to the ARVs used in Kenya as the standard first - line regime. Reports on NNRTI mutants show that some are relatively fit and can be transmitted over time. (Koval *et al*., 2006). Similar Kenya reports among ART naive persons indicated either NNRTI mutation alone (Hassan *et al.,* 2013) or NRTI and PI mutations (Budambula *et al*., 2015). The most common drug resistance mutations identified were K103N, thymidine analog mutations, M184V and Y181C / I in a multisite study in 6 sub-Saharan African States (Kenya, Nigeria, South Africa, Uganda, Zambia, Zimbabwe) (Hamers *et al.,* 2011). The levels of HIVDR seem to be on rise since the advent of ART in Kenya (Gupta *et al.,* 2011). This is a trend that is yet to be established everywhere (Manasa *et al.,* 2012).

The direct synergy between HSV-2 and HIV infection as established elsewhere could perhaps explain why most of the TDR mutations in this study was common among those who were coinfected (Baeten *et al*., 2011). During the HSV-2 reactivation phase, studies have consistently demonstrated cases of higher plasma HIV RNA that may increase the risk of vertical transmission and enhance progression to HIV disease. (Celum *et al.*, 2008; Watson Jones *et al*., 2008). Coinfection with HIV / HSV-2 has been shown to enhance both viruses ' clinical severity and infectiousness (Rebbapragada *et al.*, 2007). Whether the high prevalence of TDR in HVS-2 infected men is linked to the synergistic relationship between HIV and HVS-2 or TDR viral fitness is an area that requires more investigation.

5.1.3 Factors associated with transmission of HIV, HSV-2 and HSV-2/HIV co-infection among the fishermen along Nyamware, Kobudho, Dunga, and Kichinjio beaches in Kisumu Kenya

This Kisumu - based study found that HIV was high amongst young people (31-40 years) adults. This group comprises fishermen who are very active in daily activities such as fishing and frequent travels as well as sexual activeness. Low use of condoms and a broad sexual network can partially explain the increased prevalence of HIV among study participants. There has been report on inconsistent condom use and the perception that condom use during sex was undesirable despite knowing the risk of contracting HIV (NASCOP, 2015).

Several studies have identified similar risks of HIV infection, including unprotected sex with new sexual partners (Sander *et al*., 2013; Guy *et al.*, 2011), being single (Sander *et al*., 2013; Aaron *et al*., 2009), recent sexual intercourse with sex worker or casual partner (Sutcliffe *et al*., 2009; Guy *et al*., 2011), youth age (Seelay *et al*., 2012; Vandepitt *et al.*, 2013), had a high significant association with new HIV cases in Nyanza.

Most of the infection with HIV, HSV-2 and their co - infection was common among married fishermen. Being in a marriage relationship has been cited to be a risk to acquisition of HIV and other STI such as syphilis and Herpes-2 (Oluoch *et al.,* 2011). Widow inheritance is a common cultural practice around Lake Victoria. Some people believe that sexual intercourse is required between the widow and the inheritor, whereby it is deemed incomplete if there is no sexual contact regardless of whether the persons are HIV-infected or have unknown status (NASCOP, 2015).

High HSV-2 prevalence among older and married individuals was observed. This could be probably as result of the cumulative acquisition of HSV-2 virus with increasing age. The odds of marrying an infected partner in an HSV-2 high prevalent population are high hence it's likely to acquire HSV or HIV from the spouse. There is more frequent sexual contact in marriage with increased exposure to infection among individuals married to a HSV-2-positive which is also seen in HIV infection (Auvert *et al.,* 2001).

Antibodies to HSV-2 are produced on exposure to the virus (Arvin *et al.,* 2007). Depending on age at exposure, antibodies tend to persist in a lifetime with the virus undergoing latency and active stages (Ozouaki *et al.*, 2006; Bollen *et al.*, 2008; Sauerbrei, 2016). Higher circulating antibody levels could reflect a higher frequency or greater magnitude of viral reactivation and antigenic stimuli. HSV-2 seropositivity with a greater lifetime number of sexual partners and a greater number of past sexually transmitted diseases suggest that a higher HSV-2 antibody level could denote a profile of risky behaviors for HSV (Cowan *et al.,* 1994). The limitation of Herpes antibody testing is lack of the undisputed "gold standard" test for detecting HSV antibodies. There is data day-to-day variability and varying color intensity and the observation that inconsistent (Schmid *et al.,* 1999).

Low income was associated with HSV-2 infection. Majority of our participants with low income were most likely the crew of the boat engaged in physical fishing. Unlike the boat owners who earns much higher, they are involved in strenuous expeditions in the lake at

night with dangers of sinking due to strong winds and physical violence from other crew members, these factors stress them making them risk takers (Eleanor *et al*., 2012). Low household income has been associated disease progression among the people with HIV/AIDS. Results indicated that in subjects with daily expenses below US$ 1, disease progression was five times more likely than in subjects with expenses of more than US$ 5 per day (Gitahi-Kamau *et al*. 2015).

These findings confirm the importance of behavioral and biological reasons which simultaneously contribute to HIV transmission. This was seen among those participants who reported having had sex with their girlfriend or casual partner instead of their wife on the last trip. They were more likely to have been infected with HIV. Additionally, it is not surprising that the lack of condom use was a risk factor for HIV infection at the last sexual intercourse. High-risk behaviors promote STI transmission that directly increases HIV infectiousness and HIV susceptibility (Fleming *et al.,* 2009). Lack or inconsistent condoms and sex due to the use of alcohol were reported (Sales *et al*., 2012; Rem *et al.,* 2012; Woolf-king *et al.,* 2011).

High Presence of STI among these fishermen is an indicator of previous or recent exposure to risky sexual practice. Prior exposure to unsafe sexual activities poses a significant risk to HIV acquisition and transmission. There was stable HIV prevalence and a substantial decline in STI prevalence in Tamil Nadu in India following a comprehensive HIV prevention program that targeted safer sex among high - risk MSM in Tamil Nadu (MSM) (Subramanian *et al*., 2013).

In bivariate analysis, condom use was strongly associated with HIV-1 infection in the last two sexual acts. Respondents who reported having ever used condoms were more likely to have HIV infection. Condom use reduces the risk of HIV acquisition and transmission, and people with HIV who are aware of their HIV infection are more likely to use condoms (Crepaz *et al*., 2006; Brunell *et al*., 2008). The reported use of condoms in South Africa among HIV - positive men and women is higher than in the general population (Shisana *et al*., 2012; Vu *et al.,* 2012).

It was uncommon to find HIV in those who are perceived to be “safer” in this context, those who were abstaining from sex or had sexual partners less than eighteen years. Adoption and maintenance of healthier and safer behaviours like few sexual partners and abstinence is a risk reduction strategy for HIV infection. Reduced sexual risk and sexual partners can protect against HIV and STIs. Safe behavioral practices in systematic reviews have shown that comprehensive programs for sex education that include abstinence and information on risk reduction are more effective in reducing risk behaviors (Coyle *et al.,* 2016).

In multivariate analysis, the risky sexual behaviors were the predictors for HIV or HIV/HSV-2 coinfection. Being a fisherman exposes one to the risky nature of fishing industry ("Safety for fishermen", 2019). They encounter female fish traders along the beaches who engage in risky sexual activities along the beaches. (Camlin *et al.,* 2013). When their primary partners are away, men or women can engage with casual partners in transactional sex. HIV - infected individuals who know their status are most likely to use condom during sexual encounters with their sexual partners (Crepaz *et al.,* 2006).

Fishermen engage in a labor-intensive job of obtaining fish throughout the night or daytime. Fishing especially at night poses a big challenge to the fishermen due to risks of drowning and sudden change of weather. This creates a culture of risk taking. Men occasionally find places to sleep at the beach where alcohol is available (Elenor *et al.,* 2012). Cases of inconsistent condom use after alcohol intoxication has been reported elsewhere (Tumwesigye *et al.,* 2012; Seelay *et al.,* 2012).

The fact that they had not travelled for the last one month could be because they live with their families locally. High-risk sexual behavior is often associated with STI / HIV (Gorbach *et al*., 2005; Wardlow, 2007). This study revealed a significant proportion of married people are being exposed to HIV through unprotected sex with their HIV- infected spouse, and other sexual partners especially among men. There is more frequent sexual contact in marriage with increased exposure to infection among individuals married to a

HSV-2-positive which is also seen in HIV infection (Auvert *et al*., 2001). Concurrent sexual partners increase the spread of HIV because at a given point in time it increases the size of individual sexual networks in a population (Neal, 2015). Fishermen reported having more than one partner which exposes them to HIV and HSV-2. However, several studies have mixed results on the association between HIV and concurrent partnership. In a survey in four Sub-Saharan African cites, showed a lower HIV prevalence in areas with highest rates of concurrent partnerships (Lagarde *et al.,* 2001). The relationship between the prevalence of HIV and polygyny was negative in two other studies, one ecological study (Reniers and Watkins, 2010) and another on individual level study (Kasamba *et al.,* 2011).

HSV-2 infection was associated with HIV infection independently. Further evidence that HIV and HSV-2 infections are predictive of one another is provided by the close association observed between HSV-2 and HIV among these fishermen. A synergestic relationship was established between HIV and HSV-2 whereby HSV-2 infection increases HIV susceptibility and transmission while HIV infection potentiate HSV-2 infection and genital shedding. (Paz-Bailey *et al.,* 2007; Okuku et *al.,* 2011).

CHAPTER SIX

CONCLUSION AND RECOMENDATIONS

6.1 Conclusions

1. The prevalence of HIV-1 was 59/249 (23.7%), 134/249 (53.8%) were HSV-2 positive, 48/249 (19.3%) were HIV/HSV-2 co-infected.

   HIV-1 subtype A was most common 28/59 (47.5%) (78.6% in HIV-1/HSV-2 co- infection verses 21.4% in HIV-1 mono-infection), followed by subtype D 16/59 (27.1%) (93.8% in HIV-1/HSV-2 co-infection verses 6.3% in HIV-1 mono- infection), subtype C 3/59 (5.1%) [66.7% in HIV-1/HSV-2 co-infection verses 33.3% in HIV-1 mono-infection], subtype B 3/59 (5.1%) [All in HIV-1/HSV-2 co-infection], subtype G 2/59 (3.4%) and possible unique recombinants 7/59 (11.9%).

   Twenty-three of 59 (38.98%) sequenced samples had Drug Resistance mutations. The mutations seen in this study were associated with resistance to both NNRTIs (K103N, Y181C, V106A and G190A) and NRTI (M184V (80%) and K65KR (10%).

   Low condom use, coupled with multiple sexual partnerships, predisposes fishermen to HSV-2 infections. Working as a fisherman and owning of fishing net were strongly associated with HIV-1, HSV-2 and HIV-1/HSV-2 coinfection. Number of sex partners, use of condoms, sexual debut age, and being married were strong predictors of HIV and HSV-2 infection. The HSV-2 infection was associated with HIV serostatus including the drug resistant strains.

6.2 Recommendations

There is a compelling need for improved STI services and targeted behavioral interventions in addition; proper use of ARVs should be promoted with a focus on BMU officials.

Monitoring strategies are required to screen populations systemically (especially the high risk) in Kenya in order to monitor the existing HIV subtypes and to generate drug resistance data.

There is need to carry a larger study to establish whether the high prevalence of TDR in HVS-2 infected men is linked to the synergistic relationship between HIV and HSV-2 or TDR viral fitness.

REFERENCES

Abecasis AB, Vandamme AM, Lemey P. (2009). Quantifying differences in the tempo of human immunodeficiency virus type 1 subtype evolution. *Journal of Virology, 83,* 12917.

Abu-Raddad, L., Magaret, A., Celum, C., Wald, A., Longini, I., Self, S., & Corey, L. (2008). Genital Herpes Has Played a More Important Role than Any Other Sexually Transmitted Infection in Driving HIV Prevalence in Africa. *Plos ONE*, *3*(5), e2230.

Abram, M., Ferris, A., Das, K., Quinones, O., Shao, W., Tuske, S. Gregory, A., Eddy, A., Hughes, H., & Doms, W. (2014). Mutations in HIV-1 Reverse Transcriptase Affect the Errors Made in a Single Cycle of Viral Replication. *Journal Of Virology*, *88*(13), 7589-7601.

Abram, M., Ferris, A., Shao, W., Alvord, W., & Hughes, S. (2010). Nature, Position, and Frequency of Mutations Made in a Single Cycle of HIV-1 Replication. *Journal of Virology*, *84*(19), 9864-9878.

Ackerman, M.E., Dugast, A. S. &Alter, G. (2012). Emerging concepts on the role of innate immunity in the prevention and control of HIV infection. *Annual review of medicine*, 63, 113-130.

Ades, A. E., Peckham, C. S., Dale, G. E., Best, J. M., and Jeansson, S. (1989). Prevalence of antibodies to herpes simplex virus types 1 and 2 in pregnant women, and estimated rates of infection, *J Epidemiol Community Health 4*3, 53- 60.

Akinyi, B., Odhiambo, C., Otieno, F., Inzaule, S., Oswago, S., Kerubo, E., Richard Ndivo & Zeh, C. (2017). Prevalence, incidence and correlates of HSV-2

infection in an HIV incidence adolescent and adult cohort study in western Kenya. *PloS one*, *12*(6), e0178907.

Alcantara, L. C. J., Cassol, S., Libin, P., Deforche, K., Pybus, O. G., Van Ranst, M., Galvão-Castro, B., Vandamme, A.M. & De Oliveira, T. (2009). A standardized framework for accurate, high-throughput genotyping of recombinant and non-recombinant viral sequences. *Nucleic acids research*, *37*(suppl_2), W634-W642.

Allen PS, Das S. (2004). Prevalence of HSV-1/HSV-2 antibodies in HIV seropositive patients in Coventry, United Kingdom. Sex Transm Infect, 80:77.

Ali, M. A., & Abbas, A. (2016). Analysis of reporter proteins GUS and DsRed driven under the control of CaMV35S promoter in syncytia induced by beet cyst nematode Heterodera schachtii in Arabidopsis roots. *Advancements in Life Sciences*, *3*(3), 89-96.

Ali, S., Mann-Nüttel, R., Schulze, A., Richter, L., Alferink, J., & Scheu, S. (2019). Sources of Type I Interferons in Infectious Immunity: Plasmacytoid Dendritic Cells Not Always in the Driver's Seat. *Frontiers In Immunology*, *10*.

Allison EH and Seeley JA. (2004). HIV and AIDS among fisher folk: a threat to responsible fisheries? *Fish and Fisheries.* 5. 3: 215–234.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research*, *25*(17), 3389-3402.

Anaedobe, C., & Ajani, T. (2019). Co-infection of herpes simplex virus Type 2 and HIV infections among pregnant women in Ibadan, Nigeria. *Journal Of Global Infectious Diseases*, *11*(1), 19.

Antinone, S. E., & Smith, G. A. (2010). Retrograde axon transport of herpes simplex virus and pseudorabies virus: a live-cell comparative analysis. *Journal of virology*, *84*(3), 1504-1512.

Antiretroviral drugs used in the treatment of HIV infection. (2019). Retrieved 19 September 2019, from https://www.fda.gov/patients/hiv-treatment/antiretroviral-drugs-used-treatment-hiv-infection.

Anuradha K, Singh HM, Gopal KVT, Rao GRR, Ramani TV, Padmaja J. (2008). Herpes simplex virus 2 infection: a risk factor for HIV infection in heterosexuals. *Indian Journal of Dermatology, Venereology* and Leprology, 74:230–3.

Anzivino E, Fioriti D, Mischitelli M, Bellizzi A, Barucca V, Chiarini F, Pietropaolo F (2009). Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. *Virology Journal*., (6):40.

Apetrei, C., Robertson, D. L. & Marx, P. A. (2004). The history of SIVS and AIDS: epidemiology, phylogeny and biology of isolates from naturally SIV infected non-human primates (NHP) in Africa. *Front Biosci* 9, 225-254.

Apetrei, C., Robertson, D. L. & Marx, P. A. (2004). The history of SIVS and AIDS: epidemiology, phylogeny and biology of isolates from naturally SIV infected non-human primates (NHP) in Africa. *Frontiers in Bioscience*, *9*(1-3), 225.

Arroyo, M. A., Hoelscher, M., Sanders-Buell, E., Herbinger, K. H., Samky, E., Maboko, L., Hoffmann, O., Robb M.R., Birx D.B. & McCutchan, F. E. (2004). HIV type 1 subtypes among blood donors in the Mbeya region of southwest Tanzania. *AIDS research and human retroviruses*, *20*(8), 895-901.

Arroyo, M. A., Sateren, W. B., Foglia, G., Kibaya, R., Langat, L., Wasunna, M. & McCutchan, F. E. (2009). HIV Type 1 Genetic Diversity among Tea Plantation Workers in Kericho, Kenya. *AIDS Res and Human Retro, 25*(11), 1061-1064.

Arvin, A. (2007). *Human herpesviruses*. Cambridge: Cambridge University Press. Arvin, A. (2007). Safety for fishermen. (2019).

Arvin, A., Campadelli-Fiume, G., Mocarski, E., Moore, P. S., Roizman, B., Whitley, R., & Yamanishi, K. (Eds.). (2007). *Human herpesviruses: biology, therapy, and immunoprophylaxis*. Cambridge University Press.

Arts, E. & Hazuda, D. (2012). HIV-1 Antiretroviral Drug Therapy. *Cold Spring Harbor Perspectives in Medicine, 2*(4),

Asiki, G., Mpendo, J., Abaasa, A., Agaba, C., Nanvubya, A., Nielsen, L., Seelay, J., Kaleebu, P., Grosskurth, H., & Kamali, A. (2011). HIV and syphilis prevalence and associated risk factors among fishing communities of Lake Victoria, Uganda. *Sexually transmitted infections*, sti-2010.

Attia, S., Egger, M., Müller, M., Zwahlen, M., & Low, N. (2009). Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. *AIDS, 23*(11), 1397-1404.

Augenbraun M, Feldman J, Chirgwin K, Zenilman J, Clarke L, DeHovitz J, Landesman,S. and Minkoff H. (1995). Increased genital shedding of herpes simplex virus type 2 in HIV-seropositive women. Ann Int Med, 123:845–7.

Auvert, B., Buvé, A., Ferry, B., Caraël, M., Morison, L., Lagarde, E., Robinson, L., Kahindo, M., Chege, J., Laourau, M., Akam, E. & Musonda, R. (2001). Ecological and individual level analysis of risk factors for HIV infection in four urban populations in sub-Saharan Africa with different levels of HIV infection. *Aids, 15*, S15-S30.

Baeten JM, Chohan B, Lavreys L, Chohan V, McClelland RS, Certain L, Mandaliya K, Jaoko W, Julie O (2007). HIV-1 subtype D infection is associated with faster disease progression than subtype A in spite of similar plasma HIV-1 loads. J Infect Dis, 195:1177.

Baeten, J., Kahle, E., Lingappa, J., Coombs, R., Delany-Moretlwe, S., Nakku-Joloba, E. Mugo, N.R., Wald, A., Corey, L., Donnell, D. and Campbell, M.S (2011). Genital HIV-1 RNA Predicts Risk of Heterosexual HIV-1 Transmission. *Science Translational Medicine*, *3*(77), 7ra29-77ra29.

Baeten, J., McClelland, R., Corey, L., Overbaugh, J., Lavreys, L., …& Kreiss, J. (2004). Vitamin A Supplementation and Genital Shedding of Herpes Simplex Virus among HIV-1–Infected Women: A Randomized Clinical Trial. *The Journal of Infectious Diseases*, *189*(8), 1466-1471.

Baeten, J., Strick, L., Lucchetti, A., Whittington, W., Sanchez, J., Coombs, R. … & Celum, C. (2008). Herpes Simplex Virus (HSV)–Suppressive Therapy Decreases Plasma and Genital HIV-1 Levels in HSV-2/HIV-1 Coinfected Women: A Randomized, Placebo-Controlled, Cross-Over Trial. *The Journal of Infectious Diseases*, *198*(12), 1804-1808.

Baggaley, R. F., Garnett, G. P., & Ferguson, N. M. (2006). Modeling the impact of antiretroviral use in resource-poor settings. *PLoS Medicine, 3*(4), e124.

Bailey, R. C., Moses, S., Parker, C. B., Agot, K., Maclean, I., Krieger, J. N., …& Ndinya-Achola, J. O. (2007). Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *The lancet*, *369*(9562), 643-656.

Bannister WP, Ruiz L, Loveday C, Vella S, Zilmer K, Kjoer J, Knysz B, …& Lundgren JD. (2006). HIV-1 subtypes and response to combination antiretroviral therapy in Europe. *Antiviral Therapy*, 11,707-715.

Barbour, J. D., Sauer, M. M., Sharp, E. R., Garrison, K. E., Long, B. R., Tomiyama, H.,

… & Kallas, E. G. (2007) HIV-1/HSV-2 co-infected adults in early HIV-1 infection have elevated CD4+ T cell counts, *PLoS ONE* 2, e1080.

Barnabas RV, Webb EL, Weiss HA, Wasserheit JN (2011). The role of coinfections in HIV epidemic trajectory and positive prevention: a systematic review and meta-analysis. *AIDS*, 25(13):1559-1573 Barnabas, R., Webb, E., Weiss, H., & Wasserheit, J. (2011). The role of coinfections in HIV epidemic trajectory and positive prevention. *AIDS*, *25*(13), 1559-1573.

Barry, O., Powell, J., Renner, L., Bonney, E. Y., Prin, M., Ampofo, W., ... & Paintsil, E. (2013). Effectiveness of first-line antiretroviral therapy and correlates of longitudinal changes in CD4 and viral load among HIV-infected children in Ghana. *BMC infectious diseases*, *13*(1), 476.

Beck, E. C., Birkett, M., Armbruster, B., & Mustanski, B. (2015). A data-driven simulation of HIV spread among young men who have sex with men: the role of age and race mixing, and STIs. *Journal of acquired immune deficiency syndromes (1999)*, *70*(2), 186.

Bennett, D. E., Myatt, M., Bertagnolio, S., Sutherland, D., & Gilks, C. F. (2008). Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antiviral therapy*, *13*, 25.

Bennett, D. E., Camacho, R. J., Otelea, D., Kuritzkes, D. R., Fleury, H., Kiuchi, M. Walid, H.,& Vandamme, A. M. (2009). Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. *PloS one*, *4*(3), e4724.

Benedetti, J., Corey, L., & Ashley, R. (1994). Recurrence rates in genital herpes after symptomatic first-episode infection. *Annals of internal medicine*, *121*(11), 847-854.

Berger, E., Murphy, P., & Farber, J. (1999). Chemokine Receptors as HIV-1 Coreceptors: Roles in Viral Entry, Tropism, and Disease. *Annual Review of Immunology*, *17*(1), 657-700.

Biraro, S., Mayaud, P., Morrow, R. A., Grosskurth, H., & Weiss, H. A. (2011). Performance of commercial herpes simplex virus type-2 antibody tests using serum samples from Sub-Saharan Africa: a systematic review and meta-analysis. *Sexually transmitted diseases*, *38*(2), 140-147.

Blood, G. A. C. (2016). Human immunodeficiency virus (HIV). *Transfusion Medicine and Hemotherapy*, *43*(3), 203.

Boerma, J., Gregson, S., Nyamukapa, C., and Urassa, M. (2003). Understanding the Uneven Spread of HIV within Africa. *Sexually Transmitted Diseases*, *30*(10), 779-787.

Bollen, L., Whitehead, S., Mock, P., Leelawiwat, W., Asavapiriyanont, S., Chalermchockchareonkit, A.….& Shaffer, N. (2008). Maternal herpes simplex virus type 2 coinfection increases the risk of perinatal HIV transmission: possibility to further decrease transmission? *AIDS*, *22*(10), 1169-1176.

Breinig, M., Kingsley, L., Armstrong, J., Freeman, D., & Ho, M. (1990). Epidemiology of Genital Herpes in Pittsburgh: Serologic, Sexual, and Racial Correlates of Apparent and Inapparent Herpes Simplex Infections. *Journal of Infectious*

Diseases, *162*(2), 299-305.

Brennan CA, Bodelle P, Coffey R, Harris B, Holzmayer V, Luk, K.C., ….& Schochetman, G., (2006). HIV global surveillance: foundation for retroviral discovery and assay development. *J Med Virol* 78 Suppl 1: S24–29

Brown, E., Gardella, C., Malm, G., Prober, C., Forsgren, M., Krantz, E. …. & Corey, L. (2007). Effect of maternal herpes simplex virus (HSV) serostatus and HSV type on risk of neonatal herpes. *Acta Obstetricia Et Gynecologica Scandinavica*, *86*(5), 523-529.

Brugha, R., Keersmaekers, K., Renton, A., & Meheus, A. (1997). Genital herpes infection: a review. *International journal of epidemiology*, *26*(4), 698-709.

Byrne, C., Gantt, S., & Coombs, D. (2018). Effects of spatiotemporal HSV-2 lesion dynamics and antiviral treatment on the risk of HIV-1 acquisition. *PLOS Computational Biology*, *14*(4), e1006129.

Budambula, V., Musumba, F. O., Webale, M. K., Kahiga, T. M., Ongecha-Owuor, F., Kiarie, J. N., & Were, T. (2015). HIV-1 protease inhibitor drug resistance in Kenyan antiretroviral treatment-naive and-experienced injection drug users and non-drug users. *AIDS research and therapy*, *12*(1), 27

Buonaguro L, Tornesello ML, Buonaguro FM (2007). Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. J Virol, 81:10209

Buch, A., Müller, O., Ivanova, L., Döhner, K., Bialy, D., Bosse, J. B. Pohlmann, A., …. & Koltzenburg, M. (2017). Inner tegument proteins of Herpes Simplex Virus are sufficient for intracellular capsid motility in neurons but not for axonal targeting. *PLoS pathogens*, *13*(12), e1006813.

Burchard, P. R., Tayoun, A. N. A., Scherer, A., & Tsongalis, G. J. (2014). A rapid RT-PCR assay for the detection of HIV-1 in human plasma specimens. *Experimental and molecular pathology*, *97*(1), 111-115.

Bush LM, Talledo-Thais K, Casal-Fernandez A, Perez MT (2011). Resistant herpes simplex virus infection and HIV: a potential diagnostic and therapeutic dilemma. *Lab Medicine*, 42:452–7.

Caldeira, T. D. M., Goncalves, C. V., Oliveira, G. R. D., Fonseca, T. V. D., Goncalves, R., Amaral, C. T. D., Vanusa P. H & Martinez, A. M. B. D. (2013). Prevalence of herpes simplex virus type 2 and risk factors associated with this infection in women in southern Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, *55*(5), 315-321.

Camacho RJ, Vandamme AM (2007). Antiretroviral resistance in different HIV-1 subtypes: impact on therapy outcomes and resistance testing interpretation. Curr Opin HIV AIDS, 2:123.

Cameron DW, D'Costa, L., Maitha, G., Cheang, M., Piot, P., Simonsen, J.N., …. & Plummer, F. *(*1989). Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men. *Lancet*; 2 8660:403-7.

Camlin, C. S., Kwena, Z. A., & Dworkin, S. L. (2013). Jaboya vs. jakambi: Status, negotiation, and HIV risks among female migrants in the "sex for fish" economy in Nyanza Province, Kenya. *AIDS education and prevention*, *25*(3), 216-231.

Carpenter, L. M., Kamali, A., Ruberantwari, A., Malamba, S. S., & Whitworth, J. A. (1999). Rates of HIV-1 transmission within marriage in rural Uganda in relation to the HIV sero-status of the partners. *Aids*, *13*(9), 1083-1089.

Celum, C., Wald, A., Lingappa, J.R., Magaret, A.S., Wang, R.S., Mugo, N., …. & Bukusi, E. (2010) Acyclovir and transmission of HIV-1 from persons who are infected with HIV-1 and HSV-2. *New England Journal of Medicine,* 362:427– 39.

Celum, C. L. (2004). The interaction between herpes simplex virus and human immunodeficiency virus. *Herpes: the journal of the IHMF*, *11*, 36A-45A.

Celum, C., Wald, A., Hughes, J., Sanchez, J., Reid, S., Delany-Moretlwe, S…..& Buchbinder, S. (2008). Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. *The Lancet*, *371*(9630), 2109-2119.

Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. Genital HSV infections; Retrieved from: http://www.cdc.gov/ std/tg2018/herpes.htm.

Centers for Diseases Control and Kenya Ministry of Health. (2013). *The Cost of Comprehensive HIV Treatment in Kenya. Report of a Cost Study of HIV Treatment Programs in Kenya.* (p. XI). Atlanta, GA (USA) and Nairobi, Kenya.

Chakraborty, N., Bhattacharyya, S., De C, Mukherjee, A., Bhattacharya, D., Santra, S., Sarkar, N., …. & ChakrabartiS ‘2010). Incidence of multiple herpes virus infection in HIV seropositive patients, a big concern for eastern Indian scenario. *Virol J*, 7:147–53.

Challa, S., Woelfel, M., Guildford, M., Moquin, D., & Chan, F., (2010). Viral cell death inhibitor MC159 enhances innate immunity against vaccinia virus infection. *Journal of virology*, 84(20), 10467-10476.

Chawla, R., Bhalla, P., Bhalla, K., Singh, M., & Garg, S. (2008). Community-based study on seroprevalence of herpes simplex virus type 2 infection in New Delhi. *Indian Journal of Medical Microbiology*, *26*(1), 34.

Chen, W., Zhan, P., De Clercq, E., & Liu, X. (2012). Recent progress in small molecule CCR5 antagonists as potential HIV-1 entry inhibitors. *Current pharmaceutical design*, 18(1), 100-112.

Chew, T., Taylor, K., & Mossman, K. (2009). Innate and Adaptive Immune Responses to Herpes Simplex Virus. *Viruses*, *1*(3), 979-1002.

Chiu, I., Yaniv, A., Dahlberg, J., Gazit, A., Skuntz, S., Tronick, S., & Aaronson, S. (1985). Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. *Nature*, *317*(6035), 366-368.

Chohan, V., Baeten, J., Benk, S., Graham, S., Lavreys, L., Mandaliya, K & McClelland, R., (2009). A prospective study of risk factors for herpes simplex virus type 2 acquisition among high-risk HIV-1 seronegative women in Kenya. *Sexually transmitted infections*, 85(7), 489-492.

Chotiyaputta, W., Hongthanakorn, C., Oberhelman, K., Fontana, R. J., Licari, T., & Lok, A. S. F. (2012). Adherence to nucleos (t) ide analogues for chronic hepatitis B in clinical practice and correlation with virological breakthroughs. *Journal of viral hepatitis*, *19*(3), 205-212.

Chung MH, Beck IA, Dross S, Tapia K, Kiarie JN, Richardson BA, …& Frenkel LM. (2014) Oligonucleotide ligation assay detects HIV drug resistance associated with virologic failure among antiretroviral-naive adults in Kenya. *Journal ofe Acquired Immune Deficiency Syndrome*. 67:246–253.

Coffin, J., & Swanstrom, R. (2013). HIV pathogenesis: dynamics and genetics of viral and infected cells. *Cold Spring Harbor perspectives in medicine*, *3*(1), a012526.

Corey, L., and Handsfield, H. H. (2000). Genital herpes and public health: addressing a populations global problem, *Jama* 283, 791-794.

Corey, L., Wald, A., Celum, C., & Quinn, T. (2004). The Effects of Herpes Simplex Virus-2 on HIV-1 Acquisition and Transmission: A Review of Two Overlapping Epidemics. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *35*(5), 435-445.

Coyle, K., Basen-Engquist, K., Kirby, D., Parcel, G., Banspach, S., Collins, J., Baumler, E., … & Harrist, R. (2016). Safer choices: reducing teen pregnancy, HIV, and STDs. *Public health reports*.

Craigie, R., & Bushman, F. (2012). HIV DNA Integration. *Cold Spring Harbor Perspectives In Medicine*, *2*(7), a006890-a006890.

Crepaz, N., Hart, T. A., & Marks, G. (2004). Highly active antiretroviral therapy and sexual risk behavior: a meta-analytic review. *Jama*, *292*(2), 224-236.

Crepaz, N., Lyles, C. M., Wolitski, R. J., Passin, W. F., Rama, S. M., Herbst, J. H., … & Robert, M (2006). Do prevention interventions reduce HIV risk behaviours among people living with HIV? A meta-analytic review of controlled trials. *Aids*, *20*(2), 143-157.

Crepaz, N., Lyles, C. M., Wolitski, R. J., Passin, W. F., Rama, S. M., Herbst, J. H., …& Rinke de Wit, T. F. (2012). High prevalence of transmitted antiretroviral drug resistance among newly HIV type 1 diagnosed adults in Mombasa, Kenya. *AIDS research and human retroviruses*, *28*(9), 1033-1037.

Cuadros, D., Crowley, P., Augustine, B., Stewart, S., & García-Ramos, G. (2011). Effect of variable transmission rate on the dynamics of HIV in sub-Saharan Africa. *BMC Infectious Disease, 11*(1), 216.

Cunningham AL, Donaghy H, Harman AN, Kim M, Turville SG (2010). Manipulation of dendritic cell function by viruses. *Current opinion in microbiology, 13*(4): 524–529.

Dai, X., & Zhou, Z. H. (2018). Structure of the herpes simplex virus 1 capsid with associated tegument protein complexes. *Science*, *360*(6384), eaao7298.

Damond, F., Worobey, M., Campa, P., Farfara, I., Colin, G., Matheron, S., Brun- Vézinet, F., Robertson, D.L. & Simon, F. (2004). Identification of a highly divergent HIV type 2 and proposal for a change in HIV type 2 classification. *AIDS research and human retroviruses, 20*(6), pp.666-672.

Daniels, B., Wand, H., & Ramjee, G. (2016). Prevalence of Herpes Simplex Virus 2 (HSV-2) infection and associated risk factors in a cohort of HIV negative women in Durban, South Africa. *BMC research notes*, *9*(1), 510.

Daud, S., Shahzad, S., Shafique, M., Bhinder, M. A., Niaz, M., Naeem, *Naee A., Zia* ur Rehman, Husnain T (2014). Optimization and validation of PCR protocol for three hypervariable regions (HVI, HVII and HVIII) in human mitochondrial DNA. *Advancements in Life Sciences*, *1*(3), 165-170.

David, W., Robert, M. &HIV/AIDS Prevention Research Synthesis (PRS) Team. (2006). Do prevention interventions reduce HIV risk behaviours among people living with HIV? A meta-analytic review of controlled trials. *Aids*, *20*(2), 143-157.

Davison, A., Eberle, R., Ehlers, B., Hayward, G., McGeoch, D., Minson, A., Pellett, P., Roizman, B., Studdert, M. & Thiry, E., (2008). The order Herpesvirales. *Archives of Virology*, *154*(1), 171-177.

Davison, A. J. (2010). Herpesvirus systematics. *Veterinary. Microbiology.* 143, 52–69.

De Béthune, M. P. (2010). Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989–2009). *Antiviral research*, *85*(1), 75-90.

Desai, M., Dikshit, R. and Iyer, G. (2012). Antiretroviral drugs: Critical issues and recent advances. *Indian Journal of Pharmacology*, 44(3), p.288.

De Walque, D., Nakiyingi-Miiro, J. S., Busingye, J., & Whitworth, J. A. (2005). Changing association between schooling levels and HIV-1 infection over 11 years in a rural population cohort in south-west Uganda. *Tropical medicine & international health*, *10*(10), 993-1001.

Dickerson, M., Johnstone, J., Delea, T., White, A., & Andrews, E. (1996). The Causal Role for Genital Ulcer Disease as a Risk Factor for Transmission of Human Immunodeficiency Virus. *Sexually Transmitted Diseases*, *23*(5), 429-440.

Doekes, H. M., Fraser, C., & Lythgoe, K. A. (2017). Effect of the latent reservoir on the evolution of HIV at the within-and between-host levels. *PLoS computational biology*, *13*(1), e1005228.

Duriux-Smith, A., TW, E. C., & Goodman, J. T. (1992). Comparison of female to male and male to female transmission of HIV in 563 stable couples. *Bmj*, *304*, 809.

Dungan, J. (2010). Genital Herpes Simplex Virus Type 1 in Women: Detection in Cervicovaginal Specimens from Shaw, G. M., & Hunter, E. (2012).

EACIR Home. (2018). *Repository.eac.int*. Retrieved 20 March 2018, from

http://repository.eac.int

Eaton, D. K., Kann, L., Kinchen, S., Ross, J., Hawkins, J., Harris, W. A., …. & Lim, C. (2006). Youth risk behavior surveillance—United States, 2005. *Journal of school health*, *76*(7), 353-372.

Erb, P., Battegay, M., Zimmerli, W., Rickenbach, M., & Egger, M. (2000). Effect of antiretroviral therapy on viral load, CD4 cell count, and progression to acquired immunodeficiency syndrome in a community human immunodeficiency virus–infected cohort. *Archives of internal medicine*, *160*(8), 1134-1140.

Fanales-Belasio, E., Raimondo, M., Suligoi, B., & Buttò, S. (2010). HIV virology and pathogenetic mechanisms of infection: a brief overview. *Annali Dell'Istituto Superiore Di Sanità*, *46*(1), 5-14

Fauci A.S., & Lane H.C. (1997). Human immunodeficiency virus (HIV) disease: AIDS and related disorders. *Harrison's principles of internal medicine*, pp. 1791–1856. McGraw-Hill, New York.

Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, & Longo DL. (1998). Harrison´s Principles of Internal Medicine. 14th ed. Singapore; McGraw-Hill.

Faust, T., Binning, J., Gross, J., & Frankel, A. (2017). Making Sense of Multifunctional Proteins: Human Immunodeficiency Virus Type 1 Accessory and Regulatory Proteins and Connections to Transcription. *Annual Review of Virology*, *4*(1), 241-260.

Fleming, D., & Wasserheit, J. (1999). From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sexually Transmitted Infections*, *75*(1), 3-17.

Fleming, T., & Richardson, B. (2004). Some Design Issues in Trials of Microbicides for the Prevention of HIV Infection. *The Journal of Infectious Diseases*, *190*(4), 666-674.

Fox, Z., Phillips, A., Cohen, C., Neuhaus, J., Baxter, J., Emery, S., Hirschel, B., Hullsiek,H.K, Stephan, C., Lundgren, J. & SMART Study Group. (2008). Viral re-suppression and detection of drug resistance following interruption of a suppressive NNRTI-based regimen. *AIDS (London, England)*, *22*(17), 2279.

Freeman, E. E., Weiss, H. A., Glynn, J. R., Cross, P. L., Whitworth, J. A., & Hayes, R. J. (2006). Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *Aids*, *20*(1), 73-83.

Fujita, M. (2013). Study on Molecular Function of Proteins of Human Immunodeficiency Virus. *YAKUGAKU ZASSHI*, *133*(10), 1103-1111.

Galdiero, S., Falanga, A., Tarallo, R., Russo, L., Galdiero, E., Cantisani. , Morelli, G. & Galdiero, M. (2013). Peptide inhibitors against herpes simplex virus infections. *Journal Of Peptide Science*, *19*(3), 148-158.

Galvin, S. R., & Cohen, M. S. (2004). The role of sexually transmitted diseases in HIV transmission. *Nature Reviews Microbiology*, *2*(1), 33.

Gamiel, J. L., Tobian, A. A., Laeyendecker, O. B., Reynolds, S. J., Morrow, R. A., Serwadda, D., ….& Quinn, T. C. (2008) Improved performance of enzyme- linked immunosorbent assays and the effect of human immunodeficiency virus coinfection on the serologic detection of herpes simplex virus type 2 in Rakai, Uganda, *Clinal Vaccine Immunology 15*, 888-890.

Gan, S., & Patel, K. (2013). Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay. *Journal Of Investigative Dermatology*, *133*(9), 1-3.

Gao, F., Bailes, E., Robertson, D.L., Chen, Y., Rodenburg, C.M., Michael, S.F., …& Sharp, P.M. (1999). Origin of HIV-1 in the chimpanzee Pan Troglodytes troglodytes. *Nature*, *397*(6718), 436.

Gao, F., Yue, L. & White, A. T. (1992). Human infection by genetically diverse SIVSM-related HIV-2 in West Africa. *Nature 358,* 495-499.

Garcia, M., dos Ramos Farias, M., Ávila, M., & Rabinovich, R. (2011). Presence of p24-Antigen Associated to Erythrocyte in HIV-Positive Individuals Even in Patients with Undetectable Plasma Viral Load. *Plos ONE*, *6*(1), e14544.

Gardner, E. M., Hullsiek, K. H., Telzak, E. E., Sharma, S., Peng, G., Burman, W. J., Macarthur, R.D., Chesney, M. & Terry Beirn Community Programs for Clinical Research on AIDS (CPCRA. (2010). Antiretroviral medication adherence and class-specific resistance in a large prospective clinical trial. *AIDS (London, England)*, *24*(3), 395.

Garg H, Mohl J, Joshi A (2012).HIV-1 induced bystander apoptosis. *Viruses, 4*(11): 3020–43.

Gaschen, B., Taylor, J., Yusim, K., Foley, B., Gao, F., Lang, D., … & Korber, B. (2002). Diversity considerations in HIV-1 vaccine selection. Science, 296:2354.

Gaschen, B., Taylor, J., Yusim, K., Foley, B., Gao, F., Lang, D., …& Korber, B. (2002).

Diversity considerations in HIV-1 vaccine selection. *Science*, *296*, 2354-2360.

Geretti AM, Harrison L, Green H, Sabin C, Hill T, Fearnhill E, Pillay D, & Dunn D: (2009). On behalf of the UK Collaborative Group on HIV Drug Resistance and the UK Collaborative HIV Cohort Study: Effect of HIV-1 Subtype on Virologic and

Immunologic Response to Starting Highly Active Antiretroviral Therapy. *Clinical Infectious Disease, 48,* 1296–1305.

United Nations Programme on HIV & AIDS (UNAIDS) UNAIDS (2019). *fact sheet on the global AIDS update.* Retrieved from: http://www.unaids.org.

Gorbach, P. M., Drumright, L. N., & Holmes, K. K. (2005). Discord, discordance, and concurrency: comparing individual and partnership-level analyses of new partnerships of young adults at risk of sexually transmitted infections. *Sexually transmitted diseases*, *32*(1), 7-12.

Götte, M. (2012). The distinct contributions of fitness and genetic barrier to the development of antiviral drug resistance. *Current opinion in virology*, *2*(5), 644-650.

Gray, R. H., & Wawer, M. J. (2008). Reassessing the hypothesis on STI control for HIV prevention, *Lancet, 371,* 2064-2065.

Gray, R. R., Tatem, A. J., Lamers, S., Hou, W., Laeyendecker, O., Serwadda, D. & Goodenow, M. M. (2009). Spatial phylodynamics of HIV-1 epidemic emergence in east Africa. *AIDS (London, England)*, *23*(14), F9.

Gregson, S., Waddell, H., & Chandiwana, S. (2001). School education and HIV control in sub-Saharan Africa: from discord to harmony? *Journal of International Development: The Journal of the Development Studies Association*, *13*(4), 467-485

Grinde, B. (2013). Herpesviruses: latency and reactivation–viral strategies and host response. *Journal of oral microbiology*, *5*(1), 22766.285

Gupta, R. K., Jordan, M. R., Sultan, B. J., Hill, A., Davis, D. H., Gregson, J., …. & Bertagnolio, S. (2012). Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *The Lancet*, *380*(9849), 1250-1258.

Gupta HA, Haley T, Hamers RL, Pillay D (2011). Transmitted drug resistance in low- and middle-income settings-a meta regression analysis. XX International Drug Resistance Workshop Mexico, Los Cabos, pp: 7-11.

Gupta, R., Warren, T., and Wald, A. (2007). Genital herpes. *The Lancet, 370*, 2127- 2137.

Guy, R.J., Spelman, T., Stoove, M., El-Hayek, C., Goller, J., Fairley, C.K … & Hellard,

M.E. (2011) Risk factors for HIV Seroconversion in Men Who Have Sex with Men in Victoria, Australia: Results from a Sentinel Surveillance System. *Sexual Health*, *8*, 319-329.

Gynecological Practices in the United States. *Yearbook Of Obstetrics, Gynecology And Women's Health*, *2010*, 303-304.

Haase, A. (1999). Population Biology of HIV-1 Infection: Viral and CD4 + T Cell Demographics and Dynamics in Lymphatic Tissues. *Annual Review of Immunology*, *17*(1), 625-656.

Hahn, B. H., Shaw, G. M., De Cock, K. M. & Sharp, P. M. (2000). AIDS as a Zoonosis: Scientific and public health implications. *Science 287*, 607-614.

Hamers, R.L., Wallis, C.L., Kityo, C., Siwale, M., Mandaliya, K., Conradie, F., …. & Nankya, I. (2011). HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. *The Lancet infectious diseases*, *11*(10), 750-759.

Hamers, R. L., Sigaloff, K. C., Kityo, C., Mugyenyi, P., & de Wit, T. F. R. (2013). Emerging HIV-1 drug resistance after roll-out of antiretroviral therapy in sub-Saharan Africa. *Current Opinion in HIV and AIDS*, *8*(1), 19-26.

Hargreaves, J. R., Morison, L. A., Kim, J. C., Bonell, C. P., Porter, J. D. H., Watts, C., Busza, j., Phetla, G.& Pronyk, P. M. (2008). The association between school attendance, HIV infection and sexual behaviour among young people in rural South Africa. *Journal of Epidemiology & Community Health*, *62*(2), 113-119.

Haqqani, A. A., & Tilton, J. C. (2013). Entry inhibitors and their use in the treatment of HIV-1 infection. *Antiviral research*, *98*(2), 158-170.

Hassan, A.S., Mwaringa, S.M., Obonyo, C.A., Nabwera, H.M., Sanders, E.J., Rinke de

…. & Berkley, J.A., (2013). Low prevalence of transmitted HIV type 1 drug resistance among antiretroviral-naive adults in a rural HIV clinic in Kenya. *AIDS research and human retroviruses*, *29*(1), 129-135.

Havlir DV, Richman DD. (1996). Viral dynamics of HIV: implications for drug development and therapeutic strategies. *Annal of Internal Medicine*. *124*, 984- 994

Hemelaar J, Gouws E, Ghys PD, Osmanov S (2011). WHO-UNAIDS Network for HIV Isolation and Characterisation: Global trends in molecular epidemiology of HIV-1 during 2000–2007. *AIDS*, 25:679

Harness, J. M., Kadar, M., & DeLuca, N. A. (2014). Transcription of the herpes simplex virus, type 1 genome during productive and quiescent infection of neuronal and non-neuronal cells. *Journal of virology*, JVI-00516.

Hecht, F., Wellman, R., Busch, M., Pilcher, C., Norris, P., & Margolick…. & Holte, S. (2011). Identifying the Early Post-HIV Antibody Seroconversion Period. *The*

Journal of Infectious Diseases, 204(4), 526-533.

Hemelaar, J. (2012). The origin and diversity of the HIV-1 pandemic. *Trends In Molecular Medicine*, *18*(3), 182-192.

Heng, M. C., Heng, S. Y., & Allen, S. G. (1994). Co-infection and synergy of human immunodeficiency virus-1 and herpes simplex virus-1. *The Lancet*, *343*(8892), 255-258

Hill, C., McKinney, E., Lowndes, C.M., Munro, H., Murphy, G., Parry, J.V. & Gill,O.N. (2009). Epidemiology of herpes simplex virus types 2 and 1 amongst men who have sex with men attending sexual health clinics in England and Wales:implications for HIV prevention and management. *Eurosurveillance*, *14*(47), 19418.

Hinkley, T., Martins, J., Chappey, C., Haddad, M., Stawiski, E., Whitcomb, J.M., Petropoulos, C.J. & Bonhoeffer, S. (2011). A systems analysis of mutational effects in HIV-1 protease and reverse transcriptase. *Nature genetics*, *43*(5), 487.

Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H. & Johnson, P. R. (1989). An African primate lentivirus (SIVsm) closely related to HIV-2. *Journal Acquired Immune Deficiency Syndrome 35*, 435-445.

Hirsch, V., Olmsted, R., Murphey-Corb, M., Purcell, R., &Johnson, P. (1989). An African primate lentivirus (SIVsm closely related to HIV-2. *Nature*, *339*(6223), 389-392.

Holguín A, Aracil B, Álvarez A, Barros C, & Soriano1 V. (2001). Prevalence of Human Immunodeficiency Virus Type 1 (HIV-1) Non-B Subtypes in Foreigners Living in Madrid, Spain, and Comparison of the Performances of the AMPLICOR HIV-

1 MONITOR Version 1.0 and the New Automated Version 1.5. *Journal of Clinical Microbiology. 39,* 1850–1854

Hope-Rapp, E., Anyfantakis, V., Fouéré, S., Bonhomme, P., Louison, J., & de Marsac,

T. (2010). Etiology of Genital Ulcer Disease. A Prospective Study of 278 Cases Seen in an STD Clinic in Paris. *Sexually Transmitted Diseases*, *37*(3), 153-158.

Huang, X., Liu, X., Chen, J., Bao, Y., Hou, J., Lu, X. Xia, W., …. & Wu, H. (2018).

Evaluation of Blood-Based Antibody Rapid Testing for HIV Early Therapy: A Meta-Analysis of the Evidence. *Frontiers In Immunology*, *9*.

Hu, W. S., & Hughes, S. H. (2012). HIV-1 reverse transcription. *Cold Spring Harbor perspectives in medicine*, a006882.

Information on HIV/AIDS Treatment, Prevention and Research. (n.d.). Retrieved from https://aidsinfo.nih.gov/

Jacob SM., Gopal T, Kanagasabai S, Durairaj A, Sushi KM., & Arumugam G. (2015). Herpes Simplex Virus 2 infection in HIV-seropositive individuals in Tamil Nadu, India. *International Journal Medicine Science Public Health, 4,* 404-407.

Jahn A, Floyd S, & Crampin AC, (2008). Population-level effect of HIV on adult mortality and early evidence of reversal after introduction of antiretroviral therapy in Malawi. *The Lancet*. 10; *371*(9624):1603–1611.

Jean, S., Kay, M., Song, L. B., Levine, M. A., & Knipe, D. M. (2001). Herpes Simplex Virus 1 ICP27 Is Required for Transcription of Two Viral Late (g2) Genes in Infected Cells, *Virology 283*, 273-284.

Jiang, Y., Feng, H., Lin, Y., & Guo, X. (2016). New strategies against drug resistance to herpes simplex virus. *International Journal of Oral Science*, *8*(1), 1-6.

Johnson, V.A., Brun-Vézinet, F., Clotet, B., Gunthard, H.F., Kuritzkes, D.R., Pillay, D., Schapiro, J.M. & Richman, D.D. (2009). Update of the drug resistance mutations in HIV-1: December 2009. *Top HIV Med, 17*(5), 138-145.

Johnson, V. A., Calvez, V., Günthard, H. F., Paredes, R., Pillay, D., Shafer, R., Wensing, A.M. & Richman, D. D. (2010). 2011 update of the drug resistance mutations in HIV-1. *HIV Med, 18,* 156-163.

John-Stewart, G., Nduati, R., Rousseau, C., Mbori-Ngacha, D., Richardson, B., Rainwater, S., Panteleeff, D., & Overbaugh, J., (2005). Subtype C Is Associated with Increased Vaginal Shedding of HIV-1. *Journal Infectious Disiease*, 192, 492–496.

Kageha, S., Lihana, R.W., Okoth, V., Mwau, M., Okoth, F.A., Songok E.M., Ngaira

J.M. & Khamadi S.A. (2012). HIV types 1 subtype surveillance in central Kenya.

AIDS Research and Human Retroviruses, *28*(2), 228-31.

KAIS. (2013) National AIDS & STI Control Programme, (2012) Kenya AIDS Indicator Survey, Official Report, *Official KAIS report*.

Kalish, M. L., Wolfe, N. D., Ndongmo, C. B., McNicholl, J., Robbins, K. E., Aidoo, M., Fonjungo, P. N., & Djoko, C. F. (2005). Central African hunters exposed to simian immunodeficiency virus. *Emerging Infectious Disiease*, *11*, 1928-1930.

Gitahi–Kamau, N. T., Kiarie, J. N., Mutai, K. K., Gatumia, B. W., Gatongi, P. M., & Lakati, A. (2015). Socio-economic determinants of disease progression among HIV infected adults in Kenya. *BMC public health*, *15*(1), 733.

Karad AB, Khade SL. 9 (2013). Seroepidemiological study of herpes simplex virus type 2 infection in HIV positive patients, Delhi, India, 2007. *International Journal Medicine Public Health* 2013; *3*, 168–72.

Kasamba, I., Sully, E., Weiss, H. A., Baisley, K., & Maher, D. (2011). Extra-spousal partnerships in a community in rural Uganda with high HIV prevalence: a cross-sectional population-based study using linked spousal data. *Journal of acquired immune deficiency syndromes (1999)*, *58*(1), 108.

Keele, B. F., Van Heuverswyn, F., Li, Y., Bailes, E., Takehisa, J., Santiago, M. L.…. & Wain, L. V. (2006). Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. *Science, 313,* 523-526.

Khodai, T., Chappell, D., Christy, C., Cockle, P., Eyles, J., Hammond, DGore, K., …& Bright, H. (2011). Single and Combination Herpes Simplex Virus Type 2 Glycoprotein Vaccines Adjuvanted with CpG Oligodeoxynucleotides or Monophosphoryl Lipid A Exhibit Differential Immunity That Is Not Correlated to Protection in Animal Models. *Clinical and Vaccine Immunology*, *18*(10), 1702-1709.

Khoja S, Ojwang P, Khan S, Okinda N, Harania R, et al. (2008). Genetic Analysis of HIV-1 Subtypes in Nairobi, Kenya. *PLoS ONE* 3(9): e3191.

Kenya AIDS indicator survey: final report. NASCOP; Nairobi: 2007. 2009.

National AIDS/STI Control Programme (NASCOP). Kenya AIDS Response Progress Report 2014. Progress towards Zero.

Kenyon, C., Colebunders, R., & Hens, N. (2013). Determinants of generalized herpes simplex virus-2 epidemics: the role of sexual partner concurrency. *International journal of STD & AIDS*, *24*(5), 375-382.

Kiptoo, M., Brooks, J., Lihana, R. W., Sandstrom, P., Ng'ang'a, Z., Kinyua, J., &Songok, E. M. (2013). HIV-1 drug resistance-associated mutations amongHIV- 1 infected drug-naïve antenatal clinic attendees in rural Kenya. *BMC Infectious Diseases, 13,* 517.

Kirunga, C. T., & Ntozi, J. P. (1997). Socio-economic determinants of HIV serostatus: a study of Rakai District, Uganda. *Health Transition Review*, 175-188.

Kissling, E., Allison, E., Seeley, J., Russell, S., Bachmann, M., Musgrave, S., & Heck,S. (2005). Fisherfolk are among groups most at risk of HIV: cross-country analysis of prevalence and numbers infected. *AIDS*, *19*(17), 1939-1946.

Kiwanuka, N., Laeyendecker, O., Robb, M., Kigozi, G., Arroyo, M., McCutchan, F., Eller, L.A., Eller, M., Makumbi, F., Birx, D. and Wabwire-Mangen, F. (2008). Effect of human immunodeficiency virus Type 1 (HIV-1) subtype on disease progression in persons from Rakai, Uganda, with incident HIV-1 infection. *The Journal of infectious diseases*, *197*(5), 707-13.

Koito, A., & Ikeda, T. (2013). Intrisic immunity against retrotransposons by APOBEC cytidine deaminases. *Frontiers in microbiology*, 4, 28.

Kouyos, R.D., von Wyl, V., Hinkley, T., Petropoulos, C.J., Haddad, M., Whitcomb, J.M., Böni, J., Yerly, S., Cellerai, C., Klimkait, T. and Günthard, H.F. (2011). Assessing predicted HIV-1 replicative capacity in a clinical setting. *PLoS pathogens*, *7*(11).

Koval, C. E., Dykes, C., Wang, J., & Demeter, L. M. (2006). Relative replication fitness of efavirenz-resistant mutants of HIV-1: correlation with frequency during clinical therapy and evidence of compensation for the reduced fitness of K103N+ L100I by the nucleoside resistance mutation L74V. *Virology*, *353*(1), 184-192.

Kozal, M. J. (2009). Drug-resistant human immunodefiency virus. *Clinical Microbiology and Infection*, *15*, 69-73.

Koyuncu, O. O., Hogue, I. B., & Enquist, L. W. (2013). Virus infections in the nervous system. *Cell host & microbe*, *13*(4), 379-393.

Kramer, M. A., Uitenbroek, D. G., Ujcic-Voortman, J. K., Pfrommer, C., Spaargaren, J., Coutinho, R. A., and Dukers-Muijrers, N. H. (2008) Ethnic differences in HSV1 and HSV2 seroprevalence in Amsterdam, the Netherlands, *Euro Surveill* 13.

Kuiken C, Thakallapalli R, Esklid A, de Ronde A (2000). Genetic analysis reveals epidemiologic patterns in the spread of human immunodeficiency virus. *Am J Epidemiol* 1(2), 814–822.

Kumar, V. (2012). *Robbins Basic Patholog*. (9th ed) p.47.

Kwena, Z. A., Bukusi, E., Omondi, E., Ng'Ayo, M., & Holmes, K. K. (2012). Transactional sex in the fishing communities along Lake Victoria, Kenya: a catalyst for the spread of HIV. *African Journal of AIDS Research*, *11*(1), 9-15.

Kwena, Z. A., Cohen, C. R., Sang, N. M., Ng'Ayo, M. O., Ochieng, J. H., & Bukusi, E.

A. (2010). Fishermen as a suitable population for HIV intervention trials. *AIDS research and treatment*, *2010*.

Laeyendecker, O., Henson, C., Gray, R.H., Nguyen, R.H.N., Horne, B.J., Wawer, M.J., Serwadda, D., Kiwanuka, N., Morrow, R.A., Hogrefe, W. and Quinn, T.C. (2004). Performance of a commercial, type-specific enzyme-linked immunosorbent assay for detection of herpes simplex virus type 2-specific antibodies in Ugandans. *Journal of clinical microbiology*, *42*(4), 1794-1796.

Lafeuillade, A., & Stevenson, M. (2011). The search for a cure for persistent HIV reservoirs. *AIDS Review*, *13*(2), 63-66.

Lafferty, W., Downey, L., Celum, C., & Wald, A. (2000). Herpes Simplex Virus Type 1 as a Cause of Genital Herpes: Impact on Surveillance and Prevention. *The Journal of Infectious Diseases*, *181*(4), 1454-1457.

Lake Victoria Basin Commission. (2010). HIV sero-behavioural study among fishing communities in Lake Victoria, Kenya.

Langer, S., & Sauter, D. (2017). Unusual Fusion Proteins of HIV-1. *Frontiers In Microbiology*, *7*.

Larder, B. A., & Kemp, S. D. (1989). Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). *Science, 246*(4934), 1155- 1158.

Larder, B. A., Darby, G., & Richman, D. D. (1989). HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science, 243*(4899), 1731-1735.

Lagarde, E., Auvert, B., Caraël, M., Laourou, M., Ferry, B., Akam, E. Tom, S., Linda, M., Bertrand, M., Chege, J., Mbuve, A. & N'doye, I. (2001). Concurrent sexual partnerships and HIV prevalence in five urban communities of sub-Saharan Africa. *Aids*, *15*(7), 877-884.

Larson, B., Bii, M., Henly-Thomas, S., McCoy, K., Sawe, F., Shaffer, D., & Rosen, S. (2013). ART treatment costs and retention in care in Kenya: a cohort study in three rural outpatient clinics. *Journal of the International AIDS Society*, *16*(1), 18026.

Lau, K., & Wong, J. (2013). Current trends of HIV recombination worldwide. *Infectious Disease Reports*, *5*(1S), 4.

Legoff, J., Bouhlal, H., Gresenguet, G., Weiss, H., Khonde, N., & Hocini, H. Desire, N., Si-Mohamed, A., de Dieu Longo, J., Chemin, C., Frost, E., Pepin, J., Malkin, JE., Mayaud, P., Belec, L. (2006). Real-Time PCR Quantification of Genital Shedding of Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV) in Women Coinfected with HSV and HIV. *Journal Of Clinical Microbiology*, *44*(2), 423-432.

LeGoff J, Bouhlal H, Lecerf M, Klein C, Hocini H, Si-Mohamed A, Muggeridge M, and Bélec L. (2007). HSV-2- and HIV-1- permissive cell lines co-infected by HSV-2 and HIV-1 co-replicate HSV-2 and HIV-1 without production of HSV-2/HIV-1 pseudotype particles. *Virology Journal,* 4:2

LeGoff, J., Mayaud, P., Gresenguet, G., Weiss, H.A., Nzambi, K., Frost, E., Pepin, J., Belec, L. and ANRS 12-12 Study Group. (2008). Performance of HerpeSelect and Kalon assays in detection of antibodies to herpes simplex virus type 2. *Journal of clinical microbiology*, *46*(6), 1914-1918.

LeGoff, J., Péré, H., & Bélec, L. (2014). Diagnosis of genital herpes simplex virus infection in the clinical laboratory. *Virology Journal*, *11*(1).

Lehman, D., Chung, M., Mabuka, J., John-Stewart, G., Kiarie, J., Kinuthia, J., & Overbaugh, J. (2009). Lower Risk of Resistance After Short-Course HAART Compared with Zidovudine/Single-Dose Nevirapine Used for Prevention of HIV-1 Mother-to-Child Transmission. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *51*(5), 522-529.

Lemeshow, S., Hosmer, D. K., Klar, J. & Lwanga, S. K. (1990). World Health Organization. Adequacy of samples size in health studies. Available at www.tbrieder.org/publications/books_english/lemeshow_samplesize.pdf (accessed February 2015).

Li, G., & De Clercq, E. (2016). HIV Genome-Wide Protein Associations: a Review of 30 Years of Research. *Microbiology And Molecular Biology Reviews*, *80*(3), 679-731.

Li, G., Piampongsant, S., Faria, N., Voet, A., Pineda-Peña, A., & Khouri, R. (2015). An integrated map of HIV genome-wide variation from a population perspective. *Retrovirology*, *12*(1). Li, Y., Learn, G. H., Beasley, T.M. & other authors (2009). Increased mortality and AIDS-like immunopathology in wild chimpanzees

infected with SIVcpz. *Nature* 460, 515-51

Lien, S., &Lowman, H. B. (2003). Therapeutic peptides. *Trends in biotechnology*, *21*(12), 556-562.

Lihana, R. W., Ssemwanga, D., Abimiku, A. L., & Ndembi, N. (2012). Update on HIV-1 diversity in Africa: a decade in review. *AIDS Review*, *14*(2), 83-100.

Lihana, R. W., Khamadi, S. A., Lubano, K., Lwembe, R., Kiptoo, M. K., Lagat, N., Ichimura, H. (2009). HIV type 1 subtype diversity and drug resistance among

HIV type 1-infected Kenyan patients initiating antiretroviral therapy. *AIDS research and human retroviruses*, *25*(12), 1211-1217.

ihana, R., Khamadi, S., Kiptoo, M., Kinyua, J., Lagat, N., Magoma, G., Mwau, M., Makokha, E., Onyango, V., Osman, S. and Okoth, F. (2006). HIV type 1 subtypes among STI patients in Nairobi: a genotypic study based on partial pol gene sequencing. *AIDS Research & Human Retroviruses*, *22*(11), 1172-1177.

Liu, T. F., & Shafer, R. W. (2006). Web resources for HIV type 1 genotypic-resistance test interpretation. *Clinical infectious diseases*, *42*(11), 1608-1618.

Looker KJ, Garnett GP, Schmid GP. (2008). An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. *Bull World Health Organ*, *86*(10):805–12.

Looker, K., Magaret, A., May, M., Turner, K., Vickerman, P., Gottlieb, S. and Newman, L., (2015). Global and Regional Estimates of Prevalent and Incident Herpes Simplex Virus Type 1 Infections in 2012. *PLOS ONE*, *10*(10).

Looker, K., Elmes, J., Gottlieb, S., Schiffer, J., Vickerman, P., Turner, K. and Boily, M. (2017). Effect of HSV-2 infection on subsequent HIV acquisition: an updated systematic review and meta-analysis. *The Lancet Infectious Diseases*, *17*(12),

pp.1303-1316.

Lubega, M., Nakyaanjo, N., Nansubuga, S., Hiire, E., Kigozi, G., Nakigozi, G., Lutalo, T., Nalugoda, F. Serwadda, D., Gray, R., Wawer, M., Kennedy, C. and Reynold,

J. (2015). Risk denial and socio-economic factors related to high HIV transmission in a fishing community in Rakai, Uganda: A qualitative study. *PLOS one*, *10*(8), e0132740.

Lwembe, R., Ochieng, W., Panikulam, A., Mongoina, C. O., Palakudy, T., Koizumi, Y., & IchimLwembe, R., Ochieng, W., Panikulam, A., Mongoina, C. O., Palakudy,

T., Koizumi, Y., & Ichimura, H. (2007). Anti-retroviral drug resistance- associated mutations among non-subtype B HIV-1-infected Kenyan children with treatment failure. *Journal Medical Virology, 79*(7), 865-872.

Vonura, H. (2007). Anti-retroviral drug resistance-associated mutations among non-subtype B HIV-1-infected Kenyan children with treatment failure. *Journal Medical Virology, 79*(7), 865-872.

Masciotra, S., Yang, C., Pieniazek, D., Thomas, C., Owen, S., McClure, H., & Lal, R. (2002). Detection of Simian Immunodeficiency Virus in Diverse Species and of Human Immunodeficiency Virus Type 2 by Using Consensus Primers within the pol Region. *Journal Of Clinical Microbiology*, *40*(9), 3167-3171.

Mafigiri, R., Matovu, J.K., Makumbi, F.E., Ndyanabo, A., Nabukalu, D., Sakor, M., Kigozi, G., Nalugoda, F. and Wanyenze, R.K., (2017). HIV prevalence and uptake of HIV/AIDS services among youths (15–24 Years) in fishing and neighboring communities of Kasensero, Rakai District, South Western Uganda. *BMC public health*, *17*(1), 251.

Malamba, S., Morgan, D., Clayton, T., Mayanja, B., Okongo, M., & Whitworth, J. (1999). The prognostic value of the World Health Organisation staging system for HIV infection and disease in rural Uganda. *AIDS*, *13*(18), 2555-2562.

Manasa, J., Katzenstein, D., Cassol, S., Newell, M. L., & de Oliveira, for the Southern Africa Treatment and Resistance Network, T. (2012). Primary drug resistance in South Africa: data from 10 years of surveys. *AIDS research and human retroviruses*, *28*(6), 558-565.

Martin, J. C., Hitchcock, M. J., De Clercq, E., & Prusoff, W. H. (2010). Early nucleoside reverse transcriptase inhibitors for the treatment of HIV: a brief history of stavudine (D4T) and its comparison with other dideoxynucleosides. *Antiviral research*, *85*(1), 34-38.

Marx, P. A. (2005). Unsolved questions over the origin of HIV and AIDS. *ASM News*, *71*(1), 15-20.

Mbopi-Kéou, F., Grésenguet, G., Mayaud, P., Weiss, H., Gopal, R., & Matta, M. (2000). Interactions between Herpes Simplex Virus Type 2 and Human Immunodeficiency Virus Type 1 Infection in African Women: Opportunities for Intervention. *The Journal Of Infectious Diseases*, *182*(4), 1090-1096.

Mbopi-Kéou, F.X., Grésenguet, G., Mayaud, P., Weiss, H.A., Gopal, R., Matta, M.,

Paul, J.L., Brown, D.W., Hayes, R.J., Mabey, D.C. and Bélec, L. (2000). Interactions between herpes simplex virus type 2 and human immunodeficiency virus type 1 infection in African women: opportunities for intervention. *The journal of infectious diseases*, *182*(4), 1090-1096.

McCutchan F. (2006). Global epidemiology of HIV. *J Med Virol*. *78*(S1), S7–S12

McCutchan FE (2000). Understanding the genetic diversity of HIV-1. Aids *14*(Suppl 3):

S31–44.

McFarland, W., Gwanzura, L., Bassett, M.T., Machekano, R., Latif, A.S., Ley, C., Parsonnet, J., Burke, R.L. and Katzenstein, D. (1999). Prevalence and incidence of herpes simplex virus type 2 infection among male Zimbabwean factory workers. *The Journal of infectious diseases*, *180*(5), 1459-1465.

McMichael, A.J., Borrow, P., Tomaras, G.D., Goonetilleke, N., & Haynes, B.F. (2010). The immune response during acute HIV-1 infection: clues for vaccine development. *Nature Reviews Immunology*, *10*(1) 11.

McGeoch, D. J., Rixon, F. J., & Davison, A. J. (2006). Topics in herpesvirus genomics and evolution. *Virus research*, *117*(1), 90-104.

Melikyan, G. (2008). Common principles and intermediates of viral protein-mediated fusion: the HIV-1 paradigm. *Retrovirology*, *5*(1), 111.

Mertz, G. (1992). Risk Factors for the Sexual Transmission of Genital Herpes. *Annals of Internal Medicine*, *116*(3), 197.

Métifiot, M., Marchand, C., & Pommier, Y. (2013). HIV integrase inhibitors: 20-year landmark and challenges. *Advanced Pharmacology*, *67*, 75-105.

Micah, O., Mbithi, J., Oyugi, F., Laten, A., Anzala, O., & Engelbrecht, S. (2011). Molecular Characterization of HIV Type 1 Among HIV-Infected Respondents in a Cohort Being Prepared for HIV Phase III Vaccine Clinical Trials, Western Kenya *AIDS Research and Human Retroviruses, 27*(3), 257-264.

Mindel, A., Taylor, J., Tideman, R. L., Seifert, C., Berry, G., Wagner, K., Page, J., Marks, C., Trudinger, B., and Cunningham, A. (2000). Neonatal herpes prevention: a minor public health problem in some communities. S*exually transmitted infections 76*(4), 287-291.

Mir, A. M., Wajid, A., Reichenbach, L., Khan, M., & Masood, I. (2010). Herpes Simplex Virus-2 infection amongst urban male population in Pakistan. *JPMA- Journal of the Pakistan Medical Association*, *60*(11), 918.

Mitchell, E., Stewart, G., Bajzik, O., Ferret, M., Bentsen, C., & Shriver, M. (2013). Performance comparison of the 4th generation Bio-Rad Laboratories GS HIV Combo Ag/Ab EIA on the EVOLIS™ automated system versus Abbott ARCHITECT HIV Ag/Ab Combo, Ortho Anti-HIV 1+2 EIA on Vitros ECi and Siemens HIV-1/O/2 enhanced on Advia Centaur. *Journal of Clinical Virology*, *58*, e79-e84.

Modjarrad, K., & Vermund, S. (2010). Effect of treating co-infections on HIV-1 viral load: a systematic review. *The Lancet Infectious Diseases*, *10*(7), 455-463.

Monleau, M., Montavon, C., Laurent, C., Segondy, M., Montes, B., Delaporte, E., François Boillot, F. & Peeters, M. (2009). Evaluation of different RNA extraction methods and storage conditions of dried plasma or blood spots for human immunodeficiency virus type 1 RNA quantification and PCR amplification for drug resistance testing. *Journal of clinical microbiology*, *47*(4), 1107-1118.

Mourez, T., Simon, F., & Plantier, J. (2013). Non-M Variants of Human Immunodeficiency Virus Type 1. *Clinical Microbiology Reviews*, *26*(3), 448-461.

Mosha, F., Urassa, W., Aboud, S., Lyamuya, E., Sandstrom, E., Bredell, H., & Williamson, C. (2011). Prevalence of genotypic resistance to antiretroviral drugs in treatment-naive youths infected with diverse HIV type 1 subtypes and recombinant forms in Dar es Salaam, Tanzania. *AIDS research and human retroviruses*, *27*(4), 377-382.

Mühlbacher, A., Schennach, H., van Helden, J., Hebell, T., Pantaleo, G., & Bürgisser, P. (2012). Performance evaluation of a new fourth-generation HIV combination antigen–antibody assay. *Medical Microbiology And Immunology*, *202*(1), 77-86.

Munthali, C., Taegtmeyer, M., Garner, P., Lalloo, D., Squire, S., & Corbett, E. (2014). Diagnostic accuracy of the WHO clinical staging system for defining eligibility for ART in sub-Saharan Africa: a systematic review and meta-analysis. *Journal of the International AIDS Society*, *17*(1), 18932.

Muriaux, D., & Darlix, J. (2010). Properties and functions of the nucleocapsid protein in virus assembly. *RNA Biology*, *7*(6), 744-753.

Nagot, N., Ouedraogo, A., Defer, M. C., Vallo, R., Mayaud, P., & Van de Perre, P. (2007). Association between bacterial vaginosis and Herpes simplex virus type-2 infection: implications for HIV acquisition studies. *Sexually transmitted infections*, *83*(5), 365-368.

Nahmias, A.J., Weiss, J., Yao, X., Lee, F., Kodsi, R., Schanfield, M., Matthews, T., Bolognesi, D., Durack, D., Motulsky, A. and Kanki, P. (1986). Evidence for human infection with an HTLV III/LAV-like virus in Central Africa, 1959. *The Lancet*, *327*(8492), 1279-1280.

Nakubulwa, S., Mirembe, F.M., Kaye, D.K., Kaddu-Mulindwa D. (2009). Association between HSV 2 and HIV serostatus in pregnant women of known HIV serostatus attending Mulago Hospital antenatal clinic, Kampala, Uganda. *Journal Infections Developing Countries*, 3(10):803–6.

National AIDS Control Council (NACC). Kenya HIV Estimates Report 2018 (2018). p.1–34.

National AIDS/STI Control Programme (NASCOP), (2015). The Kenya HIV testing services guidelines. Retrieved from https://aidsfree.usaid.gov/sites/default/files/

hts_policy_kenya_2015.pdf

National AIDS/STI Control Programme (NASCOP), (2016). Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya.

Nazziwa, J., Njai, H.F., Ndembi, N., Birungi, J., Lyagoba, F., Gershim, A., Nakiyingi-Miiro, J., Nielsen, L., Mpendo, J., Nanvubya, A. and Debont, J. (2013). HIV type1 transmitted drug resistance and evidence of transmission clusters among recently infected antiretroviral-naive individuals from Ugandan fishing communities of Lake Victoria. *AIDS research and human retroviruses, 29*(5), 788-795.

Neal, Zachary P. (2015). Handbook of Applied System Science. New York: Routledge.

Ng'ayo MO, Friedrich D, Holmes K, Bukusi E, Morrow RA. (2010). Performance of HSV-2 type specific serological tests in sera from fishermen in Kisumu district, Kenya. *Journal of Virological Methods.* 163(2) 276–281.

Ng'ayo, M., Bukusi, E., A Morrow, R., Rowhani-Rahbar, A., Obare, B., Friedrich, D., & Holmes, K. (2007). Sexual and demographic determinants for herpes simplex virus type 2 among fishermen along Lake Victoria, Kenya. *Sexually Transmitted Infections, 84*(2), 140-142.

Ng'ayo, M., Bukusi, E., Rowhani-Rahbar, A., Koutsky, L., Feng, Q., Kwena, Z., & Holmes, K. (2008). Epidemiology of human papillomavirus infection among fishermen along Lake Victoria Shore in the Kisumu District, Kenya. *Sexually Transmitted Infections, 84*(1), 62-66.

Ngo, T. D., Laeyendecker, O., La, H., Hogrefe, W., Morrow, R. A., and Quinn, T.C. (2008) Use of commercial enzyme immunoassays to detect antibodies to the herpes simplex virus type 2 glycoprotein G in a low-risk population in Hanoi, Vietnam, *Clinical Vaccine Immunology, 15*(2) 382-384.

Ngo, T., Laeyendecker, O., Morrow, R., Lai, S., & Quinn, T. (2008). Comparison of Three Commercial Immunoassays for Detection of Herpes Simplex Virus Type 2 Antibodies in Commercial Sex Workers in Yunnan Province, China. *Clinical and Vaccine Immunology*, *15*(8), 1301-1303.

NIAID: National Institute of Allergy and Infectious Diseases / Leading research to understanding, treat, and prevent infectious, immunologic, and allergic diseases. (2019). Retrieved from https://www.niaid.nih.gov/

Nicoll, M. P., Proença, J. T., & Efstathiou, S. (2012). The molecular basis of herpes simplex virus latency. *FEMS microbiology reviews*, *36*(3), 684-705.

Njue, C., Voeten, H. A., & Remes, P. (2009). Disco funerals, a risk situation for HIV infection among youth in Kisumu, Kenya. *AIDS (London, England)*, *23*(4), 505.

Nickle DC, Rolland M, Jensen MA, Pond SL, Deng W, Seligman M, Heckerman D, Mullins JI, Jojic N (2007). Coping with viral diversity in HIV vaccine design. *PLoS Computational Biology*, *3*, e75.

Nicolosi, A., Leite, M. L. C., Musicco, M., Arici, C., Gavazzeni, G., & Lazzarin, A. (1994). The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. *Epidemiology*, 570-575.

Obasi, A., Mosha, F., Quigley, M., Sekirassa, Z., Gibbs, T., Munguti, K., Todd J, Grosskurth, H., Mayaud, P., Changalucha, J., Mabey, D., Hayes, R., & Brown,

D. (1999). Antibody to herpes simplex virus type 2 as a marker of sexual risk behavior in rural Tanzania. *The Journal of infectious diseases*, *179*(1), 16-24.

Odongkara, K., Abila, R., & Onyango, P. (2005). Distribution of economic benefits from the fisheries of Lake Victoria. In *Proceedings of the Lake Victoria stakeholders*

conference: meeting the challenges to sustainability of the fisheries resources.

Okoye, A. A., & Picker, L. J. (2013). CD 4+ T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunological reviews*, *254*(1), 54-64.

Okuku, H. S., Sanders, E. J., Nyiro, J., Ngetsa, C., Ohuma, E., McClelland, R. S., Price,

M.A & Graham, S. M. (2011). Factors associated with herpes simplex virus type 2 incidence in a cohort of human immunodeficiency virus type 1-seronegative Kenyan men and women reporting high-risk sexual behavior. *Sexually transmitted diseases*, *38*(9), 837-844.

Oluoch, T., Mohammed, I., Bunnell, R., Kaiser, R., Kim, A. A., Gichangi, A., Mwangi, M., Dadabhai, S., Marum, L., Orago, A. & Mermin, J. (2011). Correlates of HIV infection among sexually active adults in Kenya: a national population-based survey. *The open AIDS journal*, *5*, 125.

Ondondo, R. O., Mpoke, S., Kiptoo, M., & Bukusi, E. (2014). Incidence and prevalence of herpes simplex virus type-2 infections among fishermen in Kisumu, Kenya. *International Journal of Infectious Diseases*, *21*, 422.

Onsongo, S., Abidi, S.H., Khamadi, S., Shah, R., Kageha, S., Ojwang, P., Ali, S. and Okinda, N. (2016). Prevalence of transmitted drug resistance mutations in HIV- 1-infected drug-naive patients from urban and suburban regions of Kenya. *AIDS research and human retroviruses*, *32*(3), 220-225.

Onywera, H., Maman, D., Inzaule, S., Auma, E., Were, K., Fredrick, H., Owiti, P., Opollo, V., Etard, J.F., Mukui, I. and Kim, A.A. (2017). Surveillance of HIV-1 pol transmitted drug resistance in acutely and recently infected antiretroviral drug-naïve persons in rural western Kenya. *PloS one*, *12*(2).

Otecko, N., Inzaule, S., Odhiambo, C., Otieno, G., Opollo, V., Morwabe, A., Were, K., Ndiege, K., Otieno, F., Kim, A.A. and Zeh, C. (2016). Viral and Host Characteristics of Recent and Established HIV-1 Infections in Kisumu based on a Multiassay Approach. *Scientific Reports*, *6*, 37964.

Over, M., & Piot, P. (1991). *HIV infection and sexually transmitted diseases*. Population, Health and Nutrition Division, Population and Human Resources Department, World Bank.

Ozouaki, F., Ndjoyi-Mbiguino, A., Legoff, J., Onas, I. N., Kendjo, E., Si-Mohamed, A., Mbopi-Keou, F. X., Malkin, J. E., and Belec, L. (2006) Genital shedding of herpes simplex virus type 2 in childbearing-aged and pregnant women living in Gabon, *International journal of STD & AIDS*, *17*, 124-127.

Palma AC, Covens K, Snoeck J, Vandamme A-M, Camacho RJ, Van Laethem K. (2012). HIV-1 protease mutation 82M contributes to phenotypic resistance to protease inhibitors in subtype G. *Journal Antimicrobiology Chemotherapy* 2012, *67*, 1075–1079.

Pavie, J., Rachline, A., Loze, B., Niedbalski, L., Delaugerre, C., & Laforgerie, E. (2010). Sensitivity of Five Rapid HIV Tests on Oral Fluid or Finger-Stick Whole Blood: A Real-Time Comparison in a Healthcare Setting. *Plos ONE*, *5*(7), e11581.

Pasternak, A., Lukashov, V., & Berkhout, B. (2013). Cell-associated HIV RNA: a dynamic biomarker of viral persistence. *Retrovirology*, *10*(1).

Patiño-Galindo, J., & González-Candelas, F. (2017). The substitution rate of HIV-1 subtypes: Zachary, D., Mwenge, L., Muyoyeta, M., Shanaube, K., Schaap, A., & Bond, V. (2012). Field comparison of OraQuick® ADVANCE Rapid HIV-1/2 antibody test and two blood-based rapid HIV antibody tests in Zambia. *BMC Infectious Diseases*, *12*(1).

Patiño-Galindo, J., & González-Candelas, F. (2017). The substitution rate of HIV-1 subtypes: a genomic approach. *Virus Evolution*, *3*(2).

Paz-Bailey, G., Ramaswamy, M., Hawkes, S., & Geretti, A. (2006). Herpes simplex virus type 2: epidemiology and management options in developing countries. *Sexually Transmitted Infections*, *83*(1), 16-22.

Peeters, M., Toure-Kane, C., & Nkengasong, J. (2003). Genetic diversity of HIV in Africa. *AIDS*, *17*(18), 2547-2560.

Pinzone, M. R., Cacopardo, B., Condorelli, F., Rosa, M. D., & Nunnari, G. (2013). Sirtuin-1 and HIV-1: an overview. *Current drug targets*, *14*(6), 648-652

Pironti, A., Pfeifer, N., Walter, H., Jensen, B., Zazzi, M., & Gomes, P. (2017). Using drug exposure for predicting drug resistance – A data-driven genotypic interpretation tool. *PLOS ONE*, *12*(4), e0174992.

Pomerantz, R. J., & Horn, D. L. (2003). Twenty years of therapy for HIV-1 infection. *Nature medicine*, *9*(7), 867.

Prado J, Beare RJ, Mbuga JS, Oluka LE. (1991). A catalogue of fishing methods and gear used in Lake Victoria. UNDP/FAO

Rajagopal, S., Magaret, A., Mugo, N., & Wald, A. (2014, July). Incidence of herpes simplex virus type 2 infections in Africa: a systematic review. *Open forum infectious diseases*, *1*(2), ofu043

Regional Project for Inland Fisheries Development (IFIP), FAO RAF/87/099-TD/19/91 (En). Rome, Food and Agricultural Organization.

Price, M. A., Wallis, C. L., Lakhi, S., Karita, E., Kamali, A., Anzala, O.,Sanders, E.J.,Bekker, G.L, Twesigye, R., Hunter, E., Kaleebu, P., Kayitenkore, K., Allen, S., Ruzagira, E., Mwangome, M. Mutua, G. Amornkul, P.N., Gwynn Stevens,G.,

Pond, L.K& Kaleebu, P. (2011). Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. *AIDS research and human retroviruses*, *27*(1), 5-12.

Preston, B., Poiesz, B., & Loeb, L. (1988). Fidelity of HIV-1 reverse transcriptase. *Science*, *242*(4882), 1168-1171.

Price, M.A., Wallis, C.L., Lakhi, S., Karita, E., Kamali, A., Anzala, O., Sanders, E.J., Bekker, L.G., Twesigye, R., Hunter, E. and Kaleebu, P. (2011). Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. *AIDS research and human retroviruses*, *27*(1), 5-12.

Rebbapragada, A., Wachihi, C., Pettengell, C., Sunderji, S., Huibner, S., Jaoko, W., Ball, B., Fowke, K., Mazzulli, T., Plummer, F. A., and Kaul, R. (2007). Negative mucosal synergy between Herpes simplex type 2 and HIV in the female genital tract, *Aids*, *21*(5) 589-598.

Rehm, J., Shield, K. D., Joharchi, N., & Shuper, P. A. (2012). Alcohol consumption and the intention to engage in unprotected sex: Systematic review and meta-analysis of experimental studies. *Addiction*, *107*(1), 51-59.

Reniers, G., & Watkins, S. (2010). Polygyny and the spread of HIV in Sub Saharan Africa: a case of benign concurrency. *AIDS (London, England)*, *24*(2), 299.

Renjifo, B., Gilbert, P., Chaplin, B., Msamanga, G., Mwakagile, D., Fawzi, W., Essex, M., Vitamin, T. and HIV Study Group. (2004). Preferential in-utero transmission of HIV-1 subtype C as compared to HIV-1 subtype A or D. *Aids*, *18*(12), 1629-1636.

Roizman, B., and P. E. Pellet. (2001). The family *Herpesviridae*: a brief introduction, p. 2381-2397. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology. Lippincott Williams & Wilkins, Philadelphia, Pa.

Roizman, B., Knipe, D. M., & Whitley, R. J. (2001). Fields virology. *Herpesviridae: A Brief Introduction*, *2*, 2381-2398.

Rong, L., Feng, Z., & Perelson, A. S. (2007). Emergence of HIV-1 drug resistance during antiretroviral treatment. *Bulletin of Mathematical Biology*, *69*(6), 2027- 2060.

Roth, W. (2019). History and Future of Nucleic Acid Amplification Technology Blood Donor Testing. *Transfusion Medicine and Hemotherapy*, *46*(2), 67-75.

Saathoff, E., Pritsch, M., Geldmacher, C., Koehler, R.N., Maboko, L., Maganga, L., Geis, S., McCutchan, F.E., Kijak, G.H., Kim, J.H. and Arroyo, M.A. (2010). Viral and host factors associated with the HIV-1 viral load setpoint in adults from Mbeya Region, Tanzania. *Journal of acquired immune deficiency syndromes (1999)*, *54*(3), 324.

Safety for fishermen. (2019). Retrieved from http://www.fao.org/fishery/safety-for-fishermen/en/

Sales, J.M, L Brown, J., T Vissman, A., & J DiClemente, R. (2012). The association between alcohol use and sexual risk behaviors among African American women across three developmental periods: a review. *Current drug abuse reviews*, *5*(2), 117-128.

Salomon, J. A., Hogan, D. R., Stover, J., Stanecki, K. A., Walker, N., Ghys, P. D.and Schwartländer, B. (2005). Integrating HIV Prevention and Treatment: From Slogans to Impact. *PLoS Medicine*, *2*(1).

Samnang, P., Leng, H., Kim, A., Canchola, A., Moss, A., Mandel, J. and Page-Shafer,K. (2004). HIV prevalence and risk factors among fishermen in Sihanouk Ville, Cambodia. *International Journal of STD & AIDS*, *15*(7), pp.479-483.

Sauerbrei, A. (2016). Herpes Genitalis: Diagnosis, Treatment and Prevention. *Geburtshilfe and Frauenheilkunde, 76*(12), 1310-1317.

Saunders, K.O., Ward-Caviness, C., Schutte, R.J., Freel, S.A., Overman, R.G., Thielman, N.M & Tomaras, G.D (2011). Secretion of MIP-1β and MIP-1α by $CD8^+$ T-lymphocytes correlates with HIV-1 inhibition independent of coreceptor usage. *Cellular immunology*, *266*(2), 154-164.

Sharma, V., Mobeen, F., and Prakash, T. (2016). Comparative genomics of herpesviridae family to look for potential signatures of human infecting strains. *International Journal Genomics* 2016:9543274.

Sharp, P., & Hahn, B. (2011). Origins of HIV and the AIDS Pandemic. *Cold Spring Harbor Perspectives in Medicine*, *1*(1).a006841-a006841.

Sharp, P., & Hahn, B. (2011). Origins of HIV and the AIDS Pandemic. *Cold Spring Harbor Perspectives In Medicine*, *1*(1), a006841-a006841.

Shaw, G.M., & Hunter, E. (2012). HIV transmission. *Cold Spring Harbor perspectives in medicine*, a006965.

Shaw, G. M., & Hunter, E. (2012). HIV transmission. *Cold Spring Harbor perspectives in medicine*, *2*(11), a006965.

Schaftenaar, E., Verjans, G. M., Getu, S., McIntyre, J. A., Struthers, H. E., Osterhaus, A. D., & Peters, R. P. (2014). High seroprevalence of human herpesviruses in HIV-infected individuals attending primary healthcare facilities in rural South Africa. *PLoS One*, *9*(6), e99243.

Schechter M. (2002). HIV vaccine evaluation center in Rio de Janeiro, Brazil, *Vaccine. 20*(15), 1909–1911.

Schiffer JT, Corey L. (2009). New concepts in understanding genital herpes. *Current Infectious Disease Report, 11*(6):457–64.

Schiffer, J. and Corey, L. (2013). Rapid host immune response and viral dynamics in herpes simplex virus-2 infection. *Nature Medicine*, *19*(3), 280-288.

Schmit, J., Cogniaux, J., Hermans, P., Vaeck, C. V., Sprecher, S., Remoortel, B., Witvrouw M., Balzarini, J., Desmyter, J., Erik De Clercq and Vandamme, A. (1996). Multiple Drug Resistance to Nucleoside Analogues and Nonnucleoside Reverse Transcriptase Inhibitors in an Efficiently Replicating Human Immunodeficiency Virus Type 1 Patient Strain. *Journal of Infectious Diseases*, *174*(5), 962-968.

Schüpbach, J., Böni, J., Flepp, M., Tomasik, Z., Joller, H., & Opravil, M. (2001). Antiretroviral treatment monitoring with an improved HIV-1 p24 antigen test: an inexpensive alternative to tests for viral RNA. *Journal Of Medical Virology*, *65*(2), 225-232.

Schuster, P., Boscheinen, J., Tennert, K., & Schmidt, B. (2011). The Role of Plasmacytoid Dendritic Cells in Innate and Adaptive Immune Responses against Alpha Herpes Virus Infections. *Advances in Virology*, *2011*, 1-12.

Seeley, J.A & Allison, E.A. (2005). HIV/AIDS in fishing communities: challenges to delivering antiretroviral therapy to vulnerable groups. *AIDS Care, 17*(6), 688– 697

Seeley, J., Nakiyingi-Miiro, J., Kamali, A., Mpendo, J., Asiki, G., Abaasa, A., Jan, D., Leslie, R.N & Kaleebu, P. (2012). High HIV incidence and socio-behavioral risk patterns in fishing communities on the shores of Lake Victoria, Uganda. *Sexually transmitted diseases*, *39*(6), 433-439.

Serwadda, D., Gray, R.H., Sewankambo, N.K., Wabwire-Mangen, F., Chen, M.Z., Quinn, T.C., Lutalo, T., Kiwanuka, N., Kigozi, G., Nalugoda, F. and Meehan,

M.P. (2003). Human immunodeficiency virus acquisition associated with genital ulcer disease and herpes simplex virus type 2 infection: a nested case-control study in Rakai, Uganda. *The Journal of infectious diseases*, *188*(10), 1492-1497.

Shafer, R. W., Levee, D. J., Winters, M. A., Richmond, K. L., Huang, D., & Merigan, T.

C. (1997). Comparison of QIAamp HCV kit spin columns, silica beads, and phenol-chloroform for recovering human immunodeficiency virus type 1 RNA from plasma. *Journal of clinical microbiology*, *35*(2), 520-522.

Sharp, P., & Hahn, B. (2011). Origins of HIV and the AIDS Pandemic. *Cold Spring Harbor Perspectives In Medicine*, *1*(1), a006841-a006841.

Shetty, S. B., Divakar, D. D., Dalati, M. H. N., Vellappally, S., Anil, S., Alshehry, M. A., Felemban, B., Mamdouh, A.S, & Alshahrani, O. A. (2016). AIDS awareness: Indispensible prerequisite among fishermen population. *Osong public health and research perspectives*, *7*(5), 327-333.

Shisana, O., Rehle, T., Simbayi, L. C., Zuma, K., Jooste, S., Zungu, N., Labadarios, D. & Onoya, D. (2014). South African national HIV prevalence, incidence and behaviour survey, 2012.

Sigaloff, K.C., Mandaliya, K., Hamers, R.L., Otieno, F., Jao, I.M., Lyagoba, F., Magambo, B., Kapaata, A., Ndembi, N. and Rinke de Wit, T.F. (2012). High prevalence of transmitted antiretroviral drug resistance among newly HIV type 1 diagnosed adults in Mombasa, Kenya. *AIDS research and human retroviruses*, *28*(9), 1033-1037.

Silvestri, G., Sodora, D. L., Koup, R. A., Paiardini, M., O'Neil, S. P., McClure, H. M., Staprans, S. I. & Feinberg, M. B. (2003). Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. *Immunity*, *18*(3), 441-452.

Simon, V., Ho, D., & Abdool Karim, Q. (2006). HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *The Lancet*, *368*(9534), 489-504.

Smith, J., Bailey, R., Westreich, D., Maclean, I., Agot, K., Ndinya-Achola, J., Hogrefe, W., Morrow, R. and Moses, S. (2008). Herpes simplex virus type 2 antibody detection performance in Kisumu, Kenya, using the Herpeselect ELISA, Kalon ELISA, Western blot and inhibition testing. *Sexually Transmitted Infections*, *85*(2), 92-96.

Smyth, R. P., Davenport, M. P., & Mak, J. (2012). The origin of genetic diversity in HIV-1. *Virus research*, *169*(2), 415-429.

Sonigo, P., Alizon, M., Staskus, K., Klatzmann, D., Cole, S., Danos, O., Retzel, E., Tiollais, P., Haase, A. and Wain-Hobson, S., (1985). Nucleotide sequence of the visna lentivirus: relationship to the AIDS virus. *Cell*, *42*(1), 369-382.

Spira, S. (2003). Impact of clade diversity on HIV-1 virulence, antiretroviral drug sensitivity and drug resistance. *Journal of Antimicrobial Chemotherapy*, *51*(2), 229-240.

Stanberry, L., Spruance, S., Cunningham, A., Bernstein, D., Mindel, A., & Sacks, S. et al. (2002). Glycoprotein-D–Adjuvant Vaccine to Prevent Genital Herpes. *New England Journal of Medicine*, *347*(21), 1652-1661.

Stanford University. The REGA HIV-1 Subtyping Tool. HIV drug resistance database. Accessed on 6th June 2016.

Stanford University, Stanford HIV drug resistance database. (2006). HIVdb program: Genotypic resistance interpretation algorithm.

Steckbeck, J. B., Kuhlmann, A.-S., & Montelaro, R. C. (2013). C-terminal tail of human immunodeficiency virus gp41: Functionally rich and structurally enigmatic. *J General Virology, 94*(1), 1-19.

Stephenson, J. (2006). HIV and Circumscision. *JAMA.* 2006; 296(7):759.

Straface, G., Selmin, A., Zanardo, V., De Santis, M., Ercoli, A., & Scambia, G. (2012). Herpes simplex virus infection in pregnancy. *Infectious diseases in obstetrics and gynecology*, *2012*.

Straus, S., Wald, A., Kost, R., McKenzie, R., Langenberg, A., & Hohman, P. (1997). Immunotherapy of Recurrent Genital Herpes with Recombinant Herpes Simplex

Virus Type 2 Glycoproteins D and B: Results of a Placebo-Controlled Vaccine Trial. *The Journal Of Infectious Diseases*, *176*(5), 1129-1134.

Subramanian, T., Ramakrishnan, L., Aridoss, S., Goswami, P., Kanguswami, B., Shajan, M., Adhikary, R., Purushothaman, G.K.C., Ramamoorthy, S.K., Chinnaswamy,

E. and Veeramani, I.B. (2013). Increasing condom use and declining STI prevalence in high-risk MSM and TGs: evaluation of a large-scale prevention program in Tamil Nadu, India. *BMC Public Health*, *13*(1), 857.

Sutcliffe, C.G., Aramrattana, A., Sherman, S.G., Sirirojn, B., German, D., Wongworapat, K., Quan, V.M., Keawvichit, R. and Celentano, D.D. (2009). Incidence of HIV and sexually transmitted infections and risk factors for acquisition among young methamphetamine users in northern Thailand. *Sexually transmitted diseases*, *36*(5), 284.

Tan, D., Murphy, K., Shah, P., & Walmsley, S. (2013). Herpes simplex virus type 2 and HIV disease progression: a systematic review of observational studies. *BMC Infectious Diseases, 13*(1)

Tang, M., & Shafer, R. (2012). HIV-1 Antiretroviral Resistance. *Drugs*, *72*(9), e1-e25.

Tassiopoulos, K.K., Seage III, G., Sam, N., Kiwelu, I., Shao, J., Ao, T.T., Essex, M., Coplan, P., Rosenberg, Z., Hughes, M. and Kapiga, S. (2007). Predictors of herpes simplex virus type 2 prevalence and incidence among bar and hotel workers in Moshi, Tanzania. *The Journal of infectious diseases*, *195*(4), 493-501.

Thellman, N. M., & Triezenberg, S. J. (2017). Herpes simplex virus establishment, maintenance, and reactivation: in vitro modeling of latency. *Pathogens*, *6*(3), 28.

Tindall, B., & Cooper, D. (1991). Primary HIV infection. *Aids*, *5*(1), 1-14.

Thermo Fisher Scientific. (n.d.). Retrieved January17, 2018, from https://www.thermofisher.com/

Tobian, A., Ssempijja, V., Kigozi, G., Oliver, A., Serwadda, D., Makumbi, F., Nalugoda F., Iga, B., Reynolds, S., Wawer, M., Quinns, T., and Gray, R. (2009). Incident HIV and herpes simplex virus type 2 infection among men in Rakai, Uganda. *AIDS*, *23*(12), 1589-1594.

Tognarelli, E., Palomino, T., Corrales, N., Bueno, S., Kalergis, A., & González, P. (2019). Herpes Simplex Virus Evasion of Early Host Antiviral Responses. *Frontiers In Cellular and Infection Microbiology*, *9*.

Tooley L. (2019). Detecting HIV earlier: Advances in HIV testing. Retrieved from https://www.catie.ca/en/pif/august-2010/detecting-hiv-earlier-advances-hiv-testing

Tongo, M., Dorfman, J., & Martin, D. (2015). High Degree of HIV-1 Group M (HIV- 1M) Genetic Diversity within Circulating Recombinant Forms: Insight into the Early Events of HIV-1M Evolution. *Journal Of Virology*, *90*(5), 2221-2229.

Tronstein, E., Johnston, C., Huang, M. L., Selke, S., Magaret, A., Warren, T, Corey, L & Wald, A. (2011). Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection. *Jama*, *305*(14), 1441-1449.

Truong, N., Smith, J., Sandgren, K., & Cunningham, A. (2019). Mechanisms of Immune Control of Mucosal HSV Infection: A Guide to Rational Vaccine Design. *Frontiers In Immunology*, *10*.

Turner, B. G., & Summers, M. F. (1999). Structural biology of HIV1. *Journal of molecular biology*, *285*(1), 1-32.

Tumwesigye, N. M., Atuyambe, L., Wanyenze, R. K., Kibira, S. P., Li, Q., Wabwire-Mangen, F., & Wagner, G. (2012). Alcohol consumption and risky sexual behaviour in the fishing communities: evidence from two fish landing sites on Lake Victoria in Uganda. *BMC public health*, *12*(1), 1069.

United Nations, Development and Harmonization of Environmental Laws Volume 1: 1999. Report on the Legal and Institutional Issues in the Lake Victoria Basin, United Nations, 1999, page 17.

United States, Centers for Diseases Control and Kenya Ministry of Health. (2013). *The Cost of Comprehensive HIV Treatment in Kenya. Report of a Cost Study of HIV Treatment Programs in Kenya*. Nairobi. Retrieved from http://nacc.or.ke

UNAIDS Data 2021. Retrieved from https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf

Valere, K., Rapista, A., Eugenin, E., Lu, W., & Chang, T. L. (2015). Human alpha-defensin HNP1 increases HIV traversal of the epithelial barrier: a potential role in STI-mediated enhancement of HIV transmission. *Viral immunology*, *28*(10), 609-615.

Vallari, A., Holzmayer, V., Harris, B., Yamaguchi, J., Ngansop, C., Makamche, F., Mbanya, D., Kaptué, L., Ndembi, N., Gürtler, L. and Devare, S. (2011). Confirmation of putative HIV-1 group P in Cameroon. *Journal of virology*, *85*(3), 1403-1407.

Vanable, P. A., Ostrow, D. G., McKirnan, D. J., Taywaditep, K. J., & Hope, B. A. (2000). Impact of combination therapies on HIV risk perceptions and sexual risk among HIV-positive and HIV-negative gay and bisexual men. *Health psychology*, *19*(2), 134.

Van der Snoek, E. M., de Wit, J. B., Götz, H. M., Mulder, P. G., Neumann, M. H., & van der Meijden, W. I. (2006). Incidence of sexually transmitted diseases and HIV infection in men who have sex with men related to knowledge, perceived susceptibility, and perceived severity of sexually transmitted diseases and HIV infection: Dutch MSM–cohort study. *Sexually transmitted diseases*, *33*(3), 193-198.

Vandepitte, J., Weiss, H.A., Bukenya, J., Nakubulwa, S., Mayanja, Y., Matovu, G., Kyakuwa, N., Hughes, P., Hayes, R. and Grosskurth, H. (2013). Alcohol Use, Mycoplasma genitaliumand Other STIs Associated with HIV Incidence among Women at High Risk in Kampala, Uganda. *Journal of Acquired Immune Deficiency Syndromes*, *62*, 119- 126.

Van Dyck, E., Buvé, A., Weiss, H.A., Glynn, J.R., Brown, D.W., De Deken, B., Parry, J. and Hayes, R.J. (2004). Performance of commercially available enzyme immunoassays for detection of antibodies against herpes simplex virus type 2 in African populations. *Journal of clinical microbiology*, *42*(7), 2961-2965.

Van Lint, A., Ayers, M., Brooks, A. G., Coles, R. M., Heath, W. R., & Carbone, F. R. (2004). Herpes simplex virus-specific CD8+ T cells can clear established lytic infections from skin and nerves and can partially limit the early spread of virus

after cutaneous inoculation. *The Journal of Immunology*, *172*(1), 392-397.

Van Lint, A., Ayers, M., Brooks, A., Coles, R., Heath, W., & Carbone, F. (2003). Herpes Simplex Virus-Specific CD8+ T Cells Can Clear Established Lytic Infections from Skin and Nerves and Can Partially Limit the Early Spread of Virus after Cutaneous Inoculation. *The Journal of Immunology*, *172*(1), 392-397.

Vernazza, P., Gilliam, B., Flepp, M., Dyer, J., Frank, A., Fiscus, S., Cohen, M. and Eron, J. (1997). Effect of antiviral treatment on the shedding of HIV-1 in semen. *Aids*, *11*(10), 1249-1254.

Vidya Vijayan, K., Karthigeyan, K., Tripathi, S., & Hanna, L. (2017). Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. Frontiers In Immunology, 8.

Visseaux, B., Damond, F., Matheron, S., Descamps, D., & Charpentier, C. (2016). Hiv-2 molecular epidemiology. *Infection, Genetics and Evolution*, *46*, 233-240.

Von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., & Vandenbroucke, J. P. (2007). STROBE Initiative et al (2008). The Strengthening the Reporting of Observational Studies in Epidemiology Njue, C., Voeten, H. A., & Remes, P. (2009). Disco funerals, a risk situation for HIV infection among youth in Kisumu, Kenya. *AIDS (London, England)*, *23*(4), 505.

Vreeman, R., and Scanlon, M. (2013). Current strategies for improving access and adherence to antiretroviral therapies in resource-limited settings. *HIV/AIDS - Research and Palliative Care, 1*.

Vu, L., Andrinopoulos, K., Mathews, C., Chopra, M., Kendall, C., & Eisele, T. P. (2012). Disclosure of HIV status to sex partners among HIV-infected men and women in Cape Town, South Africa. *AIDS and Behavior*, *16*(1), 132-138.

Wagenlehner, F. M., Brockmeyer, N. H., Discher, T., Friese, K., & Wichelhaus, T. A. (2016). The presentation, diagnosis, and treatment of sexually transmitted infections. *Deutsches Ärzteblatt International*, *113*(1-2), 11.

Wald, A. (2004). Herpes simplex virus type 2 transmission: risk factors and virus shedding. *Herpes: the journal of the IHMF*, *11*, 130A-137A.

Wald, A., & Link, K. (2002). Risk of Human Immunodeficiency Virus Infection in Herpes Simplex Virus Type 2–Seropositive Persons: A Meta-analysis. *The Journal of Infectious Diseases*, *185*(1), 45-52.

Wald, A., Zeh, J., Selke, S., Warren, T., Ryncarz, A.J., Ashley, R., Krieger, J.N. and Corey, L. (2000). Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. *New England Journal of Medicine*, *342*(12), 844-850.

Wang, J., Yuan, S., Zhu, D., Tang, H., Wang, N., Chen, W., Gao, Q., Li, Y., Wang, J., Liu, H., Zhang, X., Rao, Z. and Wang, X. (2018). Structure of the herpes simplex virus type 2 C-capsid with capsid-vertex-specific component. *Nature Communications*, *9*(1).

Wardlow, H. (2002). Passenger-women: changing gender relations in the Tari Basin. *Papua New Guinea Medical Journal*, *45*(1/2), 142-146.

Wardlow, H. (2007). Men's extramarital sexuality in rural Papua New Guinea. *American Journal of Public Health, 97*(6), 1006-1014.

Wasserheit, J. (1992). Epidemiological Synergy. *Sexually Transmitted Diseases*, *19*(2), 61-77.

Watson-Jones, D., Weiss, H.A., Rusizoka, M., Baisley, K., Mugeye, K., Changalucha, J., Everett, D., Balira, R., Knight, L., Ross, D. and Hayes, R.J. (2008). Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. *New England Journal of Medicine*, *358*(15), 1560-1571.

Watson-Jones, D., Weiss, H.A., Rusizoka, M., Baisley, K., Mugeye, K., Changalucha, J., Everett, D., Balira, R., Knight, L., Ross, D. and Hayes, R.J. (2007). Risk factors for herpes simplex virus type 2 and HIV among women at high risk in northwestern Tanzania: preparing for an HSV-2 intervention trial. *Journal of acquired immune deficiency syndromes (1999)*, *46*(5), 631.

Weiss, H.A., Buve, A., Robinson, N.J., Van Dyck, E., Kahindo, M., Anagonou, S., Musonda, R., Zekeng, L., Morison, L., Carael, M. and Laga, M. (2001). The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations. *Aids*, *15*, S97-S108.

Wensing, A. M., Calvez, V., Günthard, H. F., Johnson, V. A., Paredes, R., Pillay, D., & Richman, D. D. (2017). 2017 Update of the Drug Resistance Mutations in HIV-1 Topics in antiviral medicine, 24(4), 132-133.

Weiss, R. A. & Wrangham, R. W. (1999). From pandemic to pandemic. *Nature 397*, 385-386.

World Health Organization. (2005). *Interim WHO clinical staging of HVI/AIDS and HIV/AIDS case definitions for surveillance: African Region* (No. WHO/HIV/2005.02). Geneva: World Health Organization.

Wilson, S.S., Wiens, M.E. and Smith, J.G. (2013). Antiviral mechanisms of human defensins. *Journal of molecular biology*, *425*(24), 4965-4980.

Wilen, C.B., Tilton, J.C. & Doms, R.W. (2012). Molecular mechanisms of HIV entry. *Advance Experimental Medicine Biology*. *726*, 223–242.

Wilson, T. E., Gore, M. E., Greenblatt, R., Cohen, M., Minkoff, H., Silver, S., & Gange,

S. J. (2004). Changes in sexual behavior among HIV-infected women after initiation of HAART. *American Journal of Public Health*, *94*(7), 1141-1146.

Wittkop L, Gunthard HF, de Wolf F, *et al*. (2011). Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): A European multicohort study. *Lancet Infectious Disease*. *11*(5), 363–371.

Woolf-King, S. E., & Maisto, S. A. (2011). Alcohol use and high-risk sexual behavior in Sub-Saharan Africa: a narrative review. *Archives of sexual behavior*, *40*(1), 17-42.

Workowski, K. A., & Bolan, G. A. (2015). Sexually transmitted diseases treatment guidelines, 2015. *MMWR. Recommendations and reports: Morbidity and mortality weekly report. Recommendations and reports*, *64*(RR-03), 1.

Wu L. (2011). The role of monocyte-lineage cells in human immunodeficiency virus persistence: mechanisms and progress, 6(3), 129-132.

Xiumin, C., Qingting, Y., Zhibin, L., Jiping, Y., Liran, X., & Huijin, G. (2015). Asymptomatic stage of human immunodeficiency virus infection is the optimal timing for its management with Chinese Medicine. *Journal of Traditional Chinese Medicine*, *35*(2), 244-248.

Xu, F., Markowitz, L. E., Gottlieb, S. L., and Berman, S. M. (2007). Seroprevalence of herpes simplex virus types 1 and 2 in pregnant women in the United States, *American journal of obstetrics and gynecology 196*(1), 41-46.

Yang, C., Li, M., Shi, Y. P., Winter, J., Van Eijk, A. M., Ayisi, J., Hu D.J, Steketee, R., Nahlen B.L. & Lal, R. B. (2004). Genetic diversity and high proportion of intersubtype recombinants among HIV type 1-infected pregnant women in Kisumu, western Kenya. *AIDS research and human retroviruses*, *20*(5), 565-574.

Yuan, S., Wang, J., Zhu, D., Wang, N., Gao, Q., Chen, W., Tang, H., Wang, J., Zhang, X., Liu, H., & Rao, Z. (2018). Cryo-EM structure of a herpesvirus capsid at 3.1 Å. *Science*, *360*(6384).

Yu, X., & He, S. (2016). The interplay between human herpes simplex virus infections and the apoptosis and necroptosis cell death pathways. *Virology journal*, *13*(1), 77.

Zhou, Y., Liang, S., Wang, Q., Gong, Y., Nie, S., Nan, L., Yang, A., Liao, Q., Song, X. and Jiang, Q. (2014). The geographic distribution patterns of HIV-, HCV- and co-infections among drug users in a national methadone maintenance treatment program in Southwest China. *BMC Infectious Diseases*, *14*(1).

Zhu, T., Korber, B. T., Nahmias, A. J., Hooper, E., Sharp, P. M. & Ho, D. D. (1998). An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. *Nature, 391*, 594-59

Zulfiqar, H., Javed, A., Sumbal, Afroze, B., Ali, Q., & Akbar, K. (2017). HIV Diagnosis and Treatment through Advanced Technologies. *Frontiers in Public Health*, *5*.

APPENDICES

Appendix I: Informed Consent Form (English)

My name is Victor Mburu Macharia. I am conducting a study entitled "HSV-2 prevalence and HIV diversity among co-infected fishermen along Lake Victoria in Kisumu County"

Description: Herpes simplex virus type 2 (HSV-2) is a major infection that has been shown to increase sexual transmission and acquisition of HIV-1. I am therefore asking you to participate in a research study which seeks to find out the prevalence of HSV- 2and HIV diversity among co-infected fishermen along Lake Victoria in Kisumu County. We will also find out which factors contributes to the HSV-2/HIV co-infection. If you agree to participate, we will consent you and conduct a face-to-face interview to assess this issue to help us answer these questions.

Sample collection

If you agree to participate, about 5 ml single draw whole blood sample will be collected from you into EDTA tubes by venipuncture by a trained phlebotomist.

HIV/HSV-2 testing

HIV testing and counseling will be carried out according to local standards of practice in Kenya, using two parallel rapid assays (Abbot Determine and Trinity Biotech Uni-Gold™), with a third ELISA (Vironostica) test for resolving discrepancies between the first two methods. Two negative rapid HIV 1 assays at enrollment will be defined as negative for HIV while two positive rapid assays will be defined as HIV seropositive. Discordant or indeterminate rapid assay results will be resolved by ELISA assay. HSV-2 Kalon ELISA which will be used is considered most sensitive and specific than any other serological test.

Risks: One potential risk of being in the study is that you might feel a little discomfort at the time we will be taking your blood sample. Further, knowing your HIV status might be distressful to you. We will however, use a trained phlebotomist to collect your blood samples. While, before testing your blood samples, a trained counselor will engage with you on this issue, answer all your questions.

Benefits: There is no monetary benefit for your participation in this study. The benefit which may reasonably be expected to result from this study includes; (i) knowing your HIV and HSV-2 status, (ii) contributing to the efforts to accurately minimize the spread of HIV (iii)If you are sick refer you to groups providing management for HIV and HSV-

b) Your decision whether or not to participate in this study will not affect your current fishing activity or deny you any heath benefit from health providers. The educational material to be handed will be obtained from NASCOP and will include those educating on ABC and HIV in general.

Time involvement: This interview will take about 30 minutes of your time.

Data storage Data gathered by questionnaires will be securely stored in lockable cabinets located in the research center and only accessible to the research personnel. The biological samples will be stored in -80°C freezers located in research center to ensure their viability for further confirmatory. All the data will be destroyed three years after the study.

Sample shipment and storage: The collected samples will be transported to Molecular Virology Laboratory of the Department of Pediatrics and Child Health, University of Nairobi and stored in -80°C until the analysis is done. After the analysis, the samples will be stored at -80°c for three years upon completion of the study.

Subject's rights: If you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participating at any time without penalty. You

have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact - anonymously if you wish – Director, Kenyatta National Hospital/university of Nairobi - Ethics & Research Committee

P.O Box 19676- 00202; Telephone: +254-20-2725698; Cell phone: (+254)-735274288 / I have read or have had the document read to me. I have discussed the information with study staff YES___NO___

I agree to participate in this research study: YES,_____NO_____

I agree to have my blood collected/stored and analyzed for study assays: YES, NO_____

My questions have been answered. My decision whether or not to take part in the study is voluntary. If I decide to join the study, I may withdraw at any time. By signing this form, I do not give up any rights that I have as a research participant.

Participant name _____ Date___

Participant signature/ Thumb print _____

_____ _____

Study Staff Study Staff Signature Date

Appendix II: Fomu Ya Makubaliano Ya Kushiriki Katika Utafiti (Swahili Version)

Jina langu ni Victor Mburu Macharia. Tutafanya utafiti kuhusu kiwango cha maabukizi ya UKIMWI na "Herpes 2" na aina ya virusi vya UKIMWI vinavyopatikana kati ya wavuvi walioambukizwa maradhi ya ukimwi eneo la ziwa Victoria Kaunti ya Kisumu.

Maelezo: Virusi vya "Herpes simplex virus type 2 (HSV-2)" huchangia katika uenezaji na kupata maradhi ya ukimwi katika tendo la kujamiiana. Tunakuomba ushiriki katika utafiti huu ili utusaidie kubainisha kiwango cha wanaoishi na virusi vya "Herpes" na UKIMWI kati ya wavuvi wa enoe hili. Tutachunguza pia mambo yanayochangia katika uambukizi wa maradhi haya. Ukikumbali kushiriki katika utafiti huu, tutakudodosa kwa maswali na tukutoe damu kidogo ya kupima ili kufanikisha utafiti huu.

Kutolewa damu: Ukikubali kushiriki katika utafiti wetu, utatolewa mililita tano za damu kwenye mshipa na mhudumu wa afya aliyehitimu**.**

Upimaji wa UKIMWI/HSV-2: Upimaji utafanywa kulingana na mpangilio unaofwata Kenya. Tutatumia njia mbili za haraka (Abbot Determine and Trinity Biotech Uni-Gold), na jia ya "ELISA" (VIRONOSTICA) kutatatua mshkili unaoweza kutokana na vipimi vya kwanza viwili. Kama vipimi vya kwanza havitaonyesha ugonjwa wa ukimwi basi mshiliki haugui ukimwi. Kama vipimi vyote vitaonyesha kuwa damu ina virusi vya ukimwi basi mshiriki atakuwa anaugua ukimwi. Kama vipimi vitatoa majibu yasiofanana basi hili litatatuliwa kwa kutumia jia ya tatu ya "ELISA." Upimaji wa tego utatumia njia ya Kalon ambayo imedhibitishwa kuwa njia mwafaka kabika katika upimaji wa tego.

Madhara: Hatutegemei utapata madhara yoyote kutokana na utafiti huu. Hata hivyo, unaweza hisi uchungu kiasi ukitolewa damu. Pia kujua hali yako ya maambikizo ya UKIMWI inaweza kufanya uhisi uoga kidogo. Tutamhusisha

mhudumu wa afya aliyehitimu kukutoa damu. Kabla ya kukutoa damu utapata mshauri mwingine ambaye atajadili nawe juu ya utafiti huu kama ukiridhia kujibu maswali kuhusu utafiti.

Faida: Hautalipwa pesa zozote kushiriki katika utafiti huu, hata hivyo utapata faida kama vile: (i) Kujua kama umeambukizwa maradhi ya UKIMWI ama "Herpes" (ii) kujua mikakati ya kupunguza maambukizi na uenezaji wa maradhi haya (iii) Kama utapatikana na maradhi haya tutakutuma kwa wahudumu wanaohusika na kuyakabili na kuyatibu maradhi haya. Kukubali kushiriki ama kutoshiriki hutapoteza stahili zako za kupata huduma za kiafya ama kuadhiri biashara yako ya uvuvi. Utapewa vitabu vitakavyokuelimisha jinsi ya kujikinga na kuepukana na maradhi ya ukimwi.

Muda wa udadisi: Udadisi utachukuwa takribani dakika thelathini hivi.

Utunzaji wa takwimu: takwimu kutokana na udadisi zitatunzwa katika sehemu ya siri inayofungwa katika maabara yetu.Majibu yote utakayotoa yatakuwa siri na hayataonyeshwa kwa wengine zaidi ya wafanyakazi wa utafiti huu tu. Damu ya uchunguzi itahifadhiwa katika barafu chini ya -80°C. Takwimu zitahifadhiwa tu kwa miaka mitatu tu baaada ya utafiti.

Uhifadhi na ubebaji wa damu: Damu itakayotolewa itapelekwa kwenye maabara ya "Molecular Virology Laboratory of the Department of Pediatrics and Child Health, University of Nairobi" na kuhifadhiwa kwenye barafu chini ya -80°C. Baada ya utafiti kukamilika tutahifadhi damu iliyobaki kwenye barafu chini ya -80°c kwa miaka mitatu.

Haki ya kujitoa au vinginevyo: Ushiriki katika utafiti huu ni wa hiari. Unaweza kusitisha mahojiano wakati wowote endapo utaona ni vyema kufanya hivyo na hakutakuwa na athari zozote. Hata hivyo, kama utashiriki utatusaidia sana katika utafiti huu kwani taarifa utakazotoa zitasaidia sana katika kupanga mikakati ya kupunguza maambukizi ya Ukimwi. Majibu yote utakayotoa yatakuwa siri na

hayataonyeshwa kwa wengine zaidi ya wafanyakazi wa utafiti huu. Taarifa utakazotoa zitatumika kwa lengo la utafiti tu na sio sababu nyingine yeyote

Kama una maswali katika utafiti huu kuhusu haki yako, unaweza kuwasiliana na idara ya maadili ya hospitali ya Kenyatta kwa**:** Director, Kenyatta National Hospital/university of Nairobi - Ethics & Research Committee.

P.O Box 19676- 00202; Telephone: +254-20-2725698; Cell phone: (+254)-735274288 / 0721665077; E-mail: uonknh_erc@uonbi.ac.ke

Nimesoma au nimesomewa fomu hii, nimeelezwa yote na mtafiti mkuu katika utafiti huu.NDIO……. LA……

Nakubali kushiriki katika utafiti huu: NDIO……. LA……

Nakubali kutolewa damu, ihifadhiwe na kufanyiwa utafiti NDIO……
LA…….

Nimeelezwa juu ya nini kitafanyika, faida, hatari, na haki zangu katika utafiti. Katika matumizi ya habari za utafiti utambulisho wangu hautajitokeza. Natambua kuwa naweza kujiondoa muda wowote. Natambua kuwa kwa kusaini katika fomu hii hakuniondelei haki yangu ya kimsingi, bali ni kuonyesha kuwa nafahamu juu ya utafiti ambapo nakubali kushiriki kwa hiari.

Jina la mshiriki……sahihi/dole gumba……Tarehe…….

Jina la mdodosaji……sahihi……Tarehe……

Appendix III: Face to Face Interview Questionnaire

DEMOGRAPHIC INFORMATION

1. Date of visit (day/month/year) _____/___/____
2. Date of birth (day/month/year) _____/___/____
3. State your nationality ___
4. What is your marital status?

a. Single (never married), b. Married c. Divorced/Separated d. Widowed

e. Cohabiting f. Other (specify)

5.If married, how many wives? _

6.Are there times you live alone away from your wife/wives or your regular partner(s)?

c) Yes b. No c. Decline
d) What is your level of education? (_____)
e) What is your main occupation?

f) Fisherman, b. Fish agent (buys fish in small quantities and sells in large quantities). C. Other (specify)

_____

g) How much do you earn from your occupation per month?

_____

h) Do you have your own fishing boat?

i) Yes b. No c. Decline
j) Do you have your own fishing net?

A) Yes b. No c. Decline

k) Where do you currently live?
l) Rented house b. Ancestral home c. A friend/relative's house d. Other (specify) ____
m) If you are currently living in a rented house or friend/relative's house, how many times have you visited your ancestral village in the:

Last month? _____

Last six months? _____

Last year? _____

n) For the last six months, in what town or beach were you:

Working_____ Living _____

o) How many times in the last six months have you traveled and spent away from your current house? _____
p) The last time you travelled who accompanied you?

q) No one b. Wife c. Relative d. Friend e. Workmate f. Girlfriend g. Other (specify) __
r) Where had you traveled to? _____
s) What was the reason for your travel?
t) Work b. Visit c. Funeral d. Cultural function e. Leisure f. Workshop g. religious function h. Other (specify) _____
u) Where did you sleep during this travel?

v) Relative's house b. Friend's house c. Hotel d. Lodging e. Bar or night club f. Other (specify) ___
w) Did you take alcohol during this travel? A) Yes b. No c. Decline

x) Did you take any hard drugs (e.g. bhang, cocaine, brown sugar) during this travel?

A) Yes b. No c. Decline

y) How many times did you have sex during this travel? ___

z) With whom did you have sex during this travel?

A) Wife b. Girlfriend c. casual partner/commercial sex partner

Consider the most recent sexual partners

aa) When did this sexual experience occur? _____

bb) What type of sex did you have?

A) Oral sex b. Penetrative sex c. non-penetrative sex d. Anal sex e. Other (specify) ___

cc) How old was this person? _____years

dd) Who was this person to you?

A) Wife b. Girlfriend c. casual partner/commercial sex partner d. Other (specify)_

ee) Did you drink alcohol before this sexual encounter?

A) Yes b. No c. Decline

ff) Which of the following drugs did you use before this sexual encounter?

gg) beer b. others (specify)… c. Decline

hh) Did you put on a condom before you started having sex?

A) Yes b. No c. Decline

After sexual encounter, did you give or promise to give anything to your partner (money, favor, and gift)? A) Yes b. No c. Decline

ii) Have you ever heard of HIV?

A) Yes b. No c. Decline

jj) Have you heard of HSV-2? A) Yes b. No c. Decline

kk) Have you taken either HIV or HSV-2 test before?

HIV test: (a) Yes b) No c) Declined

Herpes Simplex Virus-2 test: (a)Yes b. No c. Declined

35. Have you ever taken Anti-retroviral drugs?

a) Yes b. No

36 Do you think taking either HIV/HSV-2 test changes your sexual behavior?

a) Yes b. No c. Decline

37 Does your HIV status influence your condom use? A) Yes b. No c. Decline

Appendix IV: Kidodosi – Mahojiano Ya Uso Kwa Uso TAARIFA ZA KIDEMOGRAFIA

1. Tarehe ya kutembelea (siku/mwezi/mwaka) _____/_____/_____
2. Tarehe ya kuzaliwa (siku/mwezi/mwaka) _____/_____/_____
3. Taja nchi unayotoka
4. Je, umeoa?

   a. Sijaoa/sijaolewa b. Nimeoa c Nimetarakiwa d. Mjane e. Kinyumba

5. Je, kama umeoa, una wake wangapi? (___)
6. Je, kuna wakati huwa unaishi mbali na mkewe/wakewe ama mpenzi wako wa karibu?

   a. Ndio b. La c. kukataa kujibu

7. Je, umefikia kiwango kipi cha elimu?

A) Shule ya msingi b. Shule ya upili c. Chuo cha shahada d. Chuo kikuu

8. Kazi yako kuu ni nini?

a) uvuvi (b) wakala samaki (unanunuzi wa samaki kiasi kidogo kisha unauza kwa wingi)

9. Unapata mapato kiasi gani kwa mwezi? ( )
10. Je, una boti yako binafsi ya uvuvi? (a)Ndio (b) La (c) sitaki kujibu
11. Je, una neti yako binafsi ya kuvulia samaki?

    a) Ndio (b) La (c) sitaki kujibu

12. Je, kwa sasa unaishi wapi?

a) Nyumba ya kukodi (b) boma ya mababu zangu (c) Nyumba ya rafiki/jamaa (d) zingine(taja)

13. Kama unaishi nyumba ya kukodi, ya rafiki ama ya jamaa, umetembelea nyumbani ulikozaliwa mara ngapi?

a) Mwezi uliopita?
b) Kila mwezi katika miezi sita iliyopita
c) Mwaka jana

14. Je, kwa miezi sita iliyopita ulikuwa unafanya kazi mji au ufuo upi? uliishi wapi wakati huu? ()
15. Je, kwa muda wa miezi sita iliyopita, kwa mara ngapi umeenda safarini na kukaa mbali na nyumbani kwako?()
16. Je, ulipokuwa safarini, uliandamana na nani?

a) Hakuna b) mkewe c) jamaa d) rafiki e) mfanyikazi mwenzangu f) mpenzio g) mwingine (taja) (.....)

17. Ulikuwa umesafiri hadi wapi?()
18. Je, sababu ya kusafiri ilikuwa ipi?

a) Kikazi b) matembezi c) matanga d) sherehe ya kitamaduni e) kujiburudisha f) mafunzo g) sababu za kidini h) nyingine (taja)

19. Je, ulipokuwa safarini ulilala wapi?

a) Kwa jamaa yangu b) kwa rafiki c) hotelini d) lojini e) kilabu f) kwingine

(taja)

20. Je, ulitumia pombe ulipokuwa safarini?

a) Ndio b) La c) sijibu

21. Je ulitumia dawa za kulevya ukiwa safarini?

a) Ndio b) La c) sijibu

22. Je, kama ulifanya kitendo cha kujamiiana ulipokuwa safarini, ilikuwa mara ngapi? (…)

23. Je ulifanya kitendo cha ngono na nani?

a) Mke b) mpenzi c) kahaba

Fikiria wapenzi wako wa hivi karibuni

24. Lini mwisho mlifanya kitendo cha kujamiiana? ( )
25. Je, mlifanya kitendo hiki kivipi?

a) Ngono ya mdomo b) Ngono ya tupu ya mbele c) kuguzana mwili kwa mwili d) Ngono ya tupu ya nyuma d) Zingine (taja) ()

26. Huyo mliyekuwa naye alikuwa wa umri upi? miaka
27. Je, mna uhusiano upi?

a) Mke b) mpenzi c) kahaba d) mwingine (taja)

28. Je, ulikunywa pombe kabla ya kujamiiana? a. Ndio b) La c) Sijibu
29. Je, ulitumia dawa za kulevya zipi kabla ya kujamiiana?

a) Pombe b) bangi c. Hakuna d. Sijibu

30. Je, ulitumia mpira ya kondomu kabla ya kujamiiana?

a) Ndio b) La c) Sijibu

31. Je, baada ya kujamiiana, ulimwahidi pesa ama zawadi mpenzio?

a) Ndio b) La c) Sijibu

32. Je unajua virusi vya ukosefu wa kinga mwilini (UKIMWI)?

a) Ndio b) La c) Sijibu

33. Je, unajua virusi vya tego?

a) Ndio b) La c) Sijibu

34. Je, umewahi pimwa UKIMWI ama tego?

UKIMWI: (a) Ndio b) La c) Sijibu Tego: (a) Ndio b) La c) Sijibu

35. Je, umewahi kunywa dawa za kupunguza makali ya UKIMWI (ARVs)?

(a) Ndio b) La

36 Je, unadhani kupimwa maradhi haya mawili kunabadilisha mienedo yako ya kiuhusiano ya mapenzi?

A) Ndio b) La c) Sijibu

37 Je, kufahamu/kutofahamu hali yako ya UKIMWI kunashawishi utumizi/utotumizi wako wa mipira ya kondomu?

a) Ndio b) La c) Sijibu

Appendix V: HIV Antibody Test Consent Form (English)

My name is Victor Mburu Macharia. I am conducting a study entitled of study "HSV-2 prevalence and HIV diversity among HSV-2/HIV co-infected active fishermen along Lake Victoria in Kisumu County"

Introduction

A virus called HIV (Human Immunodeficiency Virus) causes the disease AIDS (acquired immunodeficiency syndrome). Anyone with HIV can spread it to others. It is spread through unsafe sex, sharing needles, or donating blood or other tissues. Infected mothers can also spread HIV to their babies. The test for HIV detects the body's reaction to the virus (antibody). It does not detect the virus itself. The decision to be tested for antibody to the virus that causes AIDS is voluntary; you are not required to have the test. This test is being done for a research study. You should know the advantages and disadvantages of testing before you decide to take the test. Please read this consent form with care so that you can make an informed choice about having the blood test.

What the test means

If you test POSITIVE, you have the HIV virus. That means you can pass it to others. The test cannot tell how long a person has been infected. It does not mean that you have AIDS, which is the most advanced stage of HIV infection. If the test is NEGATIVE, you probably do not have the HIV virus. It could take up to six months after infection for the test to turn positive.

Procedures

This is what will happen if you decide to have the test. First, you will meet with a counselor who will give you more information about the risks and benefits of the test. The

counselor will also talk to you about the importance of abstaining from sex until the right time, being faithful to one uninfected partner and using a condom each time you have sex with a person you do not know her health status. He / She will explain the meaning of test results, teach you how to reduce the chance of spreading HIV and explain the dangers of getting HIV. Approximately 5mls of blood will be taken from your vein using a sterile needle for the antibody test. Your blood will be tested in the laboratory and be able to provide you with results the following day. When you learn the test results, you will also be counseled in order to increase your understanding of HIV transmission, to reduce your risks of getting or transmitting sexually transmitted diseases, and how to notify your sexual partner(s) if your test result is positive.

Benefits of being tested

The benefits of being tested are very personal. If you are worried about AIDS, you might feel better if you have a negative test. Sometimes knowing that the test is positive can relieve stress. You may want to know your test result before you have sex with a partner. In some cases, test results may help diagnose a medical problem or help you make decisions about your future or on health care.

Risks of being tested

Learning test results may cause you and your partner severe stress, anxiety and depression. This may result into blaming each other and even cause separation or divorce. Other people learning about your HIV status may lead to discrimination in travel, work and insurance. You might be tempted to have unsafe sex if the result is negative. This would increase your risk of getting HIV. If the results of the test get into the wrong hands, prejudice, discrimination, risk to employment, travel restrictions, and other adverse effects could result. There may be other risks and stresses of being tested that we don't know about now. You may get a bruise where the needle enters the vein and there is a small risk of infection. You may feel some pain as the needle enters your vein.

Information about confidentiality

Your HIV status will be held in the strictest confidence, and no identifying information of any kind will be released to any other person or agency without your specific permission in writing. We will not publish or discuss in public anything that could identify you.

Subject's rights: If you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participating at any time without penalty. You have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact – anonymously if you wish – Director, Kenyatta National Hospital/university of Nairobi - Ethics & Research Committee

P.O Box 19676- 00202

Telephone: +254-20-2725698 ; Cell phone: (+254)-735274288 / 0721665077
E-mail: uonknh_erc@uonbi.ac.ke

I have read or have had the document read to me. I have discussed the information with study staff YES_____NO_____

I agree to participate in this research study: YES_____NO_____

I agree to have my blood collected/stored and analyzed for study assays: YES____NO

_____

My questions have been answered. My decision whether or not to take part in the study is voluntary. If I decide to join the study, I may withdraw at any time. By signing this form, I do not give up any rights that I have as a research participant.

_____	_____	_____
Participant Name	**Participant Signature/ Thumb print**	**Date**
_____	_____	_____
Study Staff	**Study Staff Signature**	**Date**

Appendix VI: Fomu Ya Makubaliano Ya Kushiriki Kupima Virusi Vya Ukimwi

Jina langu ni Victor Mburu Macharia. Tunafanya utafiti kuhusu kiwango cha maabukizi ya UKIMWI na “Herpes 2” na aina ya virusi vya UKIMWI vinavyopatikana kati ya wavuvi walioambukizwa maradhi ya ukimwi katika ziwa Victoria kaunti ya Kisumu.

Utangulizi

Virusi vya ukimwi husababisha UKIMWI. Kila mtu anaweza eneza ukimwi kupitia tendo la kujamiiana, kutumia sindano moja pamoja, ama ukipewa damu ama viungo vingine vya mwili hospitalini. Wanawake walioambukizwa wanaweza aidha kuambukiza wanao. Upimaji wa ukimwi hupima jinsi mwili unavyokabiliana na virusi vya ukimwi, haupimi virusi vyenyewe. Kupimwa ni hiari yako. Kwa sasa tutakupima ili tufanye utafiti wetu. Tafadhari soma fomu hii ili ujue umuhimu wako wa kukumbali kupimwa.

Majibu baada ya kupimwa

Upimaji wetu utaonyesha kama una virusi vya UKIMWI ama huna. Ukiwa na maradhi unaweza ambukiza wengine. Kama huna virusi haimaanishi una ukosefu wa kinga mwilini (AIDS). Kama huna maradhi, labda huna virusi hata hivyo, ni vizuri kupimwa baada ya miezi sita kuhakikisha hunavirusi kwani huchukua kama miezi sita mwili wako kuonyesha kama una virusi.

Utaratibu

Ukikubali kupimwa utapata mshauri atakayekupa nasaha kuhusu upimaji, kujikinga ama kuishi na virusi vya ukimwi. Baadaye tutakutoa damu mililita tano ya kupima. Matokeo utayapata baada ya siku moja ambapo utapata ushauri mwingine jinsi ya kujikinga, kuishi na ukimwi ama jinsi ya kumwelezea mwenzi wako kama utapatikana na virusi.

Faida ya kupimwa

Faida ya kupimwa ni yako binafsi. Majibu yanaweza kukuondolea hofu kama umeambukizwa virusi vya UKIMWI au la. pia, majibu yanaweza kuonyesha maradhi yanayokusumbua mwilini na jinsi ya kujipanga maisha ya baadaye kiafya.

Madhara ya kupimwa

Kujua hali yako inaweza kukutia hofu ama kuogopa. Hii inawezafanya mke na mume kuachana, unyanyapaa kazini ama ukisafiri. Kama huna maradhi unaweza pata ari ya kujihusisha na vitendo vya kujamiiana na unaweza kuambukizwa UKIMWI. Utakapotolewa damu unawezahisi uchungu kiasi.

Usiri: Matokeo ya uchunguzi wa hali yako yatatunzwa kwa usiri na yatatumika kwa shughuli za utafiti tu. Utambulisho wako hautawekwa bayana ila kwa mjibu wa sheria ama baada ya idhini yako kwa kuandika. Jina lako halitaonekana mahala popote katika dodoso, na kumbukumbu zote zitahifadhiwa kwa siri.

Haki ya kujitoa au vinginevyo: Ushiriki katika utafiti huu ni wa hiari. Unaweza kusitisha mahojiano wakati wowote endapo utaona ni vyema kufanya hivyo. Hata hivyo, Utambulisho wako hautawekwa bayana ila kwa mujibu wa sheria ama baada ya idhini yako kwa kuandika. Jina langu halitaonekana mahala popote katika dodoso, kumbukumbu zote zitahifadhiwa kwa siri Kama una maswali katika utafiti huu kuhusu haki yako, unaweza kuwasiliana na idara ya maadili ya hospitali ya Kenyatta kwa :Director, Kenyatta National Hospital/university Of Nairobi - Ethics & Research Committee, P.O Box 19676- 00202 Telephone: +254-20-2725698;Cell phone: (+254)-735274288 / 0721665077
;E-mail: uonknh_erc@uonbi.ac.ke

Nimesoma au nimesomewa fomu hii, nimeelezwa yote na mtafiti mkuu katika utafiti huu. NDIO……. LA……

Nakubali kushiriki katika utafiti huu: NDIO……..LA……

Nakubali kutolewa damu, ihifadhiwe na kufanyiwa utafiti NDIO…… LA…….

Nimeelezwa juu ya nini kitafanyika, faida, hatari, na haki zangu katika utafiti. Katika matumizi ya habari za utafiti utambulisho wangu hautajitokeza. Natambua kuwa naweza kujiondoa muda wowote. Natambua kuwa kwa kusaini katika fomu hii hakuniondelei haki yangu ya kimsingi, bali ni kuonyesha kuwa nafahamu juu ya utafiti ambapo nakubali kushiriki kwa hiari.

Jina la mshiriki… ..... **sahihi/dole gumba……Tarehe…….**

Jina la mdodosaji……sahihi……Tarehe……

Appendix VII: Ethical Approval Certificate

UNIVERSITY OF NAIROBI
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P O BOX 19676 Code 00202
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KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/404

5th October 2015

Victor Mburu Macharia
TM303/2126/2014
JKUAT

Dear Victor

Research Proposal: "HSV-2 Prevalence and HIV Diversity among co-infected fishermen along Lake Victoria in Kisumu county" (P545/08/2015)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 5th October 2015 – 4th October 2016.

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) 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*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
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**

"Protect to Discover"

Appendix VIII: Publication/ Manuscript

Research Article

iMedPub Journals
http://www.imedpub.com/

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Transmitted HIV-1 Drug resistance and the Role of Herpes Simplex Virus-2 Co-infection among Fishermen along the Shores of Lake Victoria, Kisumu, Kenya

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Abstract

Introduction: Herpes simplex virus type 2 (HSV-2) infection has been associated with a 3-fold risk of HIV-1 acquisition. The prevalence of HIV-1 and HSV-2 in the fishing communities along the shores of Lake Victoria in Kisumu have been reported to be high. This may contribute to the growing HIV epidemic in Kenya including the spread of transmitted drug resistance (TDR). We report data on the association of HSV2/HIV-1 co-infection and TDR in this antiretroviral (ARV)-naïve population.

Methods: Blood samples were obtained from 249 consenting fishermen from 5 beaches and a detailed sociodemographic questionnaire was administered. Blood samples were analyzed for HIV-1/HSV2 co-infection. The HSV-2 serology was performed using Kalon HSV type 2 enzyme-linked immunosorbent assay (ELISA). The HIV-1 counselling and serology were carried out according to local standards of practice in Kenya, using two parallel rapid assays (Alere Determine HIV-1/2 and Trinity Biotech Uni-Gold), with a third ELISA-Vironostika HIV Uni-Form II Ag/Ab

resistance; K103N, G190A and Y181C mutations each. In the regression model, HIV/HSV-2 co-infection was independently associated with TDR [OR 4.1 (95% CI 1.4 to 11.9)].

Conclusion: The level of TDR to NNRTIs in these ARV-naive fishermen was significantly high especially among those co-infected with HSV-2. HSV-2 infection may increase the risk of TDR in this population.

Keywords: HIV; AIDS; HIV-1; Drug resistance

Introduction

Fishing communities along the shore of Lake Victoria in Kenya comprise young, highly migratory men who spend long periods away from their families and local communities and engage in high risk sexual behaviour [1-3]. This community has one of the highest prevalence of sexually transmitted infection (STI) and HIV in East Africa [4]. In Kenya, higher HIV prevalence 25.6% and 19.6% HSV-2/HIV co-infection was reported among fishermen

Appendix IX: Nucleotide Sequence and Gene bank Accession Number

The gene sequences determined in this study were deposited in Gene Bank under accession numbers KX505314-KX505372 as follows:

PID	SEQUENCE
1	CAATGGCCATTGACAGMAGAAAAAATAAAAGCATTAACAGAAATTTGCACAGAT ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCTATAAAGAAAAAAGACAGTACTAAATGGAGGAAATTAGTAGATT TCAGGGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGGATACC GCATCCAGCGGGCTTAAAAAAGAAGAAATCAGTAACAGTACTAGATGTGGGGGA CGCATATTTTTCAGTTCCTTTAGATGAAGGCTTTAGGAAATATACTGCGTTCACCA TACCTAGTATAAACAATGAGACACCAGGAATCAGATATCAGTATAATGTGCTCCC ACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGYATGACAAAAATCTT AGAGCCCTTCAGATCAAAAAATCCAGAAATAACTATTTATCAATACATGGATGAC TTGTATGTAGGATCTGATTTAGAAATAGGGCAACATAGAGCAAAAATAGAGGAG CTAAGAGAACATCTATTARGGTGGGGATTAACCACACCAGATAAGAAACATCAG AAAGAACCCCCGTTTCTTTGGATGGGTTATGAACTA
2	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTATG GAAATGGAGAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAT ACACCAATATTTGCAATAAAGAAAAAGGATAGCACTAAATGGAGGAAATTAGTA GATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAGCTAGGAA TACCGCATCCAGCGGGTCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGG GGGACGCATATTTTTCAGTTCCTTTACATGAAGGCTTTARAAAATATACTGCATTC ACCATACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGC TTCCACAGGGATGGAAAGGATCACCATCAATATTCCAGAGTAGCATGATAAAAAT TTTAGAACCTTTCAGATCAAAAAATCCAGAAATAATTATCTATCAATATATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGAGCAACATCGAGCAAAAATAGAAG AGTTGAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAAAAGCATCA GAAAGAACCTCCATTCCTTTGGATGGGWTATGAACTA
3	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTATG GAAATGGAGAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAT ACACCAATATTTGCAATAAAGAAAAAGGATAGCACTAAATGGAGGAAATTAGTA GATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAGCTAGGAA TACCGCATCCAGCGGGTCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGG GGGACGCATATTTTTCAGTTCCTTTACATGAAGGCTTTAGAAAATATACTGCATTC ACCATACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGC TTCCACAGGGATGGAAAGGATCACCATCGATATTCCAGAGTAGCATGATAAAAAT TTTAGAACCTTTCAGATCAAAAAATCCAGAAATAATTATCTATCAATATATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGAGCAACATCGAGCAAAAATAGAAG AGTTGAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAAAAGCATCA GAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTA
4	GTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGT ACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGACCTGAAAATCCATAC AATACTCCAATATTTGCTATAAAGAAAAAGGACAGCACTAAATGGAGAAAATTA GTAGATTTCAGAGAGCTCAATAAAAGAACTCAAGATTTTTGGGAAGTTCAATTAG GAATACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTGGATG TGGGGGATGCATATTTTTCAGTACCTTTAGATGAAAGCTTTAGAAAATATACTGCA TTCACCATACCTAGTACAAACAATGAGACACCAGGAATCAGGTATCAGTACAATG TGCTTCCACAGGGATGGAAAGGGTCACCAGCAATATTCCAGAGTAGCATGACAAA

	AATCTTAGAGCCCTTTAGATCAAAAAATCCAGAAATAGTTATCTACCAATACATG GATGACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAGCAAAAATAG AAGAATTAAGAGCTCATCTGTTGAGCTGGGGATTTACTACCCCMGACAAAAAACA TCAGAAAGAACCGCCATTTCTTTGGATGGGTTATGAAC
5	AGAAATTTGTACAGATATGGAAAAGGAAGGAAAACTATCAAGGATTGGGCCTGA AAATCCATATAACACTCCAATATTTGCTATAAAGAAAAAAGACAGTACCAAGTGG AGAAAATTAGTAGATTTCAGGGAACTTAATAAGAGAACTCAAGATTTCTGGGAAG TTCAATTAGGAATACCACACCCGGCAGGGCTAAAAAAAGAAAAAATCAGTAACAG TACTGGATGTGGGTGATGCCTATTTTTCAGTTCCCTTATGTGAAGAGTTTARAAAA TATACTGCATTTACCATACCTAGTATAAACAATGAGACACCAGGAATTAGATATC AGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAG CATGACAAAAATCTTAGAACCCTTTAGAGAACAAAATCCAGAAATAGTTATCTAT CAATACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAG CAAAAATAGAGGAACTAAGAGAACATCTATTGAGGTGGGGATTTACCACACCAG ATAAAAAACATCAGAAAGAACCTCCATTTCTTTGGATGGGTTATGAACTAAA
6	CAATGGCCATTGACAGAAGAAAAAATAAAMGCATTAACAGAAATCTGTACAGAA ATGGAGGAAGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCGATAAAGAAAAAAGATAGCACTAAATGGAGGAAATTAGTAGAT TTTAGAGAGCTTAATAAAAGAACTCAAGACTTTTGGGAGGTTCAATTAGGAATAC CGCATCCAGCAGGTTTAAAAAAGAAMAAATCAGTAACAGTACTAGATGTGGGGG ACGCATATTTTTCAGTTCCTTTAGATGAAGGCTTTAGAAAGTATACAGCGTTCACC ATACCTAGTACAAACAATGAGACACCAGGAATCAGGTATCAGTACAATGTGCTTC CACAGGGATGGAAAGGATCACCGGCAATATTCCAAAGTAGCATGACAAAAATCT TAGAGCCCTTTAGAATAAAAAATCCAGACATAATTATCTATCAATACATGGATGA CTTATATGTAGGATCTGATTTAGAGATAGGGCAGCATAGAGCAAAAATAGAAGA GTTGAGAGCTCACCTATTGAGCTGGGGATTCACTACACCAGACAAAAAGCATCAG AAAGAACCTCCATTCCTTTGGATGGGTTATGAACTA
7	AAACAATGGCCATTGACAGAAGAAAAGATAAAAGCATTGACAGAAATTTGTACA GACATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAAT ACTCCAATATTTGCTATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAG ATTTCAGAGAGCTTAATAAAAGAACTCAAGACTTCTGGGAAGTTCAACTAGGAAT ACCACATCCTGCAGGGCTAAAGAAGAAAAAGTCAGTAACAGTACTAGATGTGGG TGATGCATATTTTTCAGTTCCCTTATATGAAGATTTTAGAAAATATACCGCATTCA CCATACCTAGTATAAACAATGAGACACCAGGAATTAGATATCAGTACAATGTGCT TCCACAGGGATGGAAAGGATCGCCGGCAATATTCCAAAGTAGCATGACAAAAAT CTTAGAACCTTTTAGAAAACGAAATCCAGAAGTGGTTATCTATCAATACATGGAT GATTTGTATGTAGGGTCTGACTTAGAGATAGGGCAGCATAGAATAAAAATAGAGG AATTAAGGGAACACCTATTGAAGTGGGGATTTACCACACCAGACAAAAAGCATC AGAAAGAACCTCCATTTCTTTGGATGG
8	CAATGGCCATTGACAGAAGAAAAAATAAAGGCATTGATAGAAATTTGTACAGAG ATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAGAATCCATACAATACT CCAGTATTTGCCATAAAAAAGAARGACAGTACWAAGTGGAGAAAATTAGTAGAT TTCAGGGAACTCAATAAAAGRACCCARGACTTTTGGGAAGTTCAATTAGGRATAC CACACCCAGCAGGGTTAAAARAGAAAAAATCAGTGACAGTACTAGATGTGGGGG ATGCRTATTTTTCAGTWCCTTTAGATGAAAGCTTCAGGAAATATACTGCATTCACC ATACCAAGTATAAACAATGAGACACCAGGAATCAGRTATCAGTACAATGTGCTTC CACAAGGATGGAAAGGATCACCAGCAATATTCCAAGCTAGCATGACAAAAATYC TGGAACCTTTTAGGAAACAAAATCCAGAAATGATTATCTATCAATACATGGATGA TTTGTATGTAGGATCTGACTTAGAAATAGGGCAACATAGAGCAAAAATAGAGAA ATTAAGGGAACACCTGTTRAAGTGGGGGTTTACTACACCAGACAAAAAGCATCAG AAAGAACCTCCATTCCTTTGGATGGGTTATGAACTA
9	AATGGCCATTGACRGAAGAAAAAATAAAGGCATTGATAGAAATTTGTACAGAGA TGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAGAATCCATACAATACTC

	CAGTATTTGCCATAAAAARGAAAGACAGTACTAAGTGGAGAAAATTAGTAGATTT CAGGGAACTCAATAAAAGAACCCAAGACTTTTGGGAAGTTCAATTAGGRATACCA CACCCAGCAGGGTTAAAARAGAAAAAATCAGYGACAGTACTAGATGTGGGGGAT GCRTATTTTTCAGTWCCTTTAGATGAAAGCTTCAGGAAATATACTGCATTYACCAT ACCRAGTRTAAACAATGAGACACCAGGAATCAGRTATCAGTACAATGTGCTTCCA CAAGGATGGAAAGGATCACCRGCAATATTCCAAGCTAGCATGACAAAAATYCTG GAACCTTTTAGGAAACAAAATCCAGAAATGATTATCTATCAATACATGGATGATT TGTATGTAGGATCTGACTTAGAAATAGGGCAACATAGAGCAAAAATAGAGRAATT AAGGGAACACCTGTTAAAGTGGGGGTTTACTACACCAGACAAAAAGCATCAGAA AGAACCTCCAYTCCTTTGGATTGGTTAT
10	AACAATGGCCATTGACAGAAGAAAAAATAAAGGCATTGATAGAAATTTGTACAG AGATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAGAATCCATACAATA CTCCAGTATTTGCCATAAAAAAGAAAGACAGTACTAAGTGGAGAAAATTAGTAG ATTTCAGGGAACTCAATAAAAGAACCCAAGACTTTTGGGAAGTTCAATTAGGGAT ACCACACCCAGCAGGGTTAAAAGAGAAAAAATCAGCGACAGTACTAGATGTGGG GGATGCGTATTTTTCAGTACCTTTAGATGAAAGCTTCAGGAAATATACTGCATTCA CCATACCAAGTATAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGCT TCCACAAGGATGGAAAGGATCACCAGCAATATTCCAAGCTAGCATGAAAAAAAT TCTGGAACCTTTTAGGAAACAAAATCCAGAAATGATTATCTATCAATACGTGGAT GATTTGTATGTAGGATCTGACTTAGAAATAGGGCAACATAGAGCAAAAATAGAG GAATTAAGGGGACACCTGTTGAAGTGGGGGTTTACTACACCAGACAAAAAGCATC AGAAAGAACCTCCATTCCTTTGGATGGGTTATGAAC
11	CAGAAGARAAAATAAAAGCATTAACAGCAATTTGTGAAGARATGGAGAAGGAAG GAAAAATTACAAAAATTGGGCCTGAAAATCCATATAACACTCCAGTATTTGCCAT AAAAAAGAAGGACAGTACTAAGTGGAGAAAATTAGTAGATTTCAGGGAACTCAA TAAAAGAACTCAAGACTTTTGGGAAGTTCAATTAGGGATACCACACCCAGCAGGT TTAAAGAAAAACAAATCAGTGACAGTACTAGATGTGGGGGATGCATATTTTTCAG TCCCTTTAGATGAAAATTTCAGGAAGTATACAGCATTCACCATACCTAGTAYAAA CAATGAGAMACCAGGGATTAGATATCAATACAATGTGCTTCCACAGGGATGGAA AGGATCACCAGCAATATTCCAAAGTAGTATGACAAAAATCTTAGAACCCTTTAGG GCACAAAATCCAGAAATGGTTATCTATCAATATGTGGATGATTTGTATGTAGGGT CTGACTTAGAAATAGGGCAACATAGAGCAAAAATAGAGGAGTTGAGAAACCATC TATTGAAGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGAACCCCCATT TCTTTGGATGGGTTATGAACT
12	AATGGCCATTGACAGAAGAGAAAATAAAAGCAATAACAGCAATTTGTGAAGAAA TGGAGAAGGAAGGAAAAATTACAAAAATTGGGCCTGAAAATCCATATAACACTC CAGTATTTGCCATAAAAAAGAAGGACAGTACTAAGTGGAGAAAATTAGTAGATTT CAGGGAACTCAATAAAAGAACTCARGASTTTTGGGAAGTTCAATTAGGGATACCA CACCCAGCAGGTTTAAAGAAAAACAAATCAGTGACAGTACTAGATGTGGGGGAT GCATATTTTTCAGTCCCTTTAGATGAAAATTTCAGGAAGTATACAGCATTCACCAT ACCTAGTATAAACAATGAGAAACCAGGGATTAGATATCAATACAATGTGCTTCCA CAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGTATGACAAAAATCTTAR AACCCTTTAGGGCACAAAATCCAGAAATGGTTATCTATCAATATGTGGATGATTT GTATGTAGGGTCTGACTTAGAAATAGGGCAACATAGAGCAAAAATAGAGGAGTT GAGAAACCATCTATTGAAGTGGGGATTTACCACACCAGACAAAAAACATCAGAA AGAACCCCCATTTCTTTGGATGGGTTATGAAC
13	CAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTATGGAA ATGGAGAAAGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCCATAAAGAAAAAGGACAGTACAAAATGGAGAAAATTGGTAGAT TTCAGAGAACTTAACAAGAGAACGCAAGATTTCTGGGAAGTTCAATTAGGAATAC CGCATCCTGCAGGGCTAAAAAARAARAAATCAGTAACAGTACTGGATGTGGGTG ATGCATATTTTTCAGTTCCCTTATATGAARATTTTARGAAGTATACTGCATTCACC ATACCCAGTATAAACAATGAGACACCAGGAATTAGATATCAGTACAATGTACTTC

	CACAGGGATGGAAAGGATCACCGGCAATATTCCAAAGTAGTATGACAAAAATCTT AGAACCCTTTAGGAAGAAAAATCCAGAAATGGTCATCTATCAATACATGGATGAT TTGTATGTAGGATCTGACTTAGAAATAGGACAGCATAGAACAAAAATAGAAGAA TTAAGGGAACATTTATTGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGA AAGAACCTCCATTTCTTTGGATGGGTTATGAACTA
14	GACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAGACATGGAAAGGGA AGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAATACTCCAATATTTGCC ATAAARAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAGATTTCAGAGAACTT AATAAGAGAACTCAAGACTTTTGGGAAGTTCAGCTAGGAATACCACATCCTGGAG GGCTAAAAAAGAAGAAATCAGTAACAGTATTGGATGTGGGTGATGCATATTTTTC AGTTCCCTTATATGAAGAATTTAGAAAATATACTGCATTCACCATACCTAGTACAA ACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAAGGATGGAA AGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAGAACCTTTTAGA AAACAAAATCCAGAAATGGTTATCTGTCAATACGTGGATGATTTGTATGTAGGAT CTGACTTAGAACTAGGGCAGCATAGAATAAAAATARAAAAATTAAGAGAACACC TGTTAAAGTGGGGATTTACCACACCAGACAAAAAAYATCAGAAAGAACCTCCATT TCTTTGGATGGGTTATGAACT
15	AACAATGGCCATTGACAGAAGAGAAAATAAAAGCATTAACAGAAATTTGTACAG AAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATA CTCCAATATTTGCAATAAAGAAAAAAGATAGCACTAAATGGAGAAAATTAGTAG ATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGGAT ACCGCATCCAGCGGGCTTAAAAAAGAAAAAATCAGTAACAGTGCTAGATGTGGG GGACGCATATTTCTCAGTTCCTTTACATGAAAGTTTCAGGAAGTATACTGCGTTCA CCATACCTAGTATAAACAATGAGACACCAGGAATCAGGTATCAGTACAATGTGCT TCCACAGGGATGGAAGGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAAT CTTAGAGCCCTTTAGATCAAAAAATCCAGAAATAGTTATCTATCAATACATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAGCAAAAATAGAA GAGTTAAGARCTCATCTATTGAGCTGGGGGTTTACTACACCAGACAAAAAGCATC AGAAAGAACCTCCATTCCTTTGGATGGGTTATGAA
16	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTGCA GATATGGAAAAAGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAC ACCCCAATATTTGCAATAAAGAAAAAAGATAGCACTAAATGGAGGAAATTAGTA GATTTCAGAGAGCTCAATAAGAGAACACAAGACTTCTGGGAAGTTCAATTAGGAA TACCACATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGG GGGATGCATATTTTTCAGTTCCTTTACATGAGGACTTTAGAAAGTATACTGCATTC ACCATACCTAGTACAAACAATGAGACACCGGGAATCAGATACCAGTACAATGTGC TTCCACAAGGATGGAAAGGATCACCAGCAATATTCCAGAGCAGCATGACAAAGA TCTTAGAGCCCTTTAGATCAAAAAATCCACAAATAATCATCTACCAATACATGGA TGACTTGTATGTAGGATCTGATTTAGAAATAGGCCAGCATAGAGCAAAAATAGAA GAGCTGAGAGCTCATCTATTGAGCTGGGGGTTTACTACACCAGACAAAAAGCATC AGAAAGAACCTCCATTCCTTTGGATGGGG
17	CAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTAATGAG ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAGAATCCATACAATACT CCAATATTTGCTATAAAGAAAAAAGACAGCACTAAATGGAGAAAATTAGTAGATT TTAGAGAGCTCAATAAAAGAACTCAAGACTTTTGGGAAGTTCAATTGGGAATACC GCATCCTGCAGGTTTAAAAAAGAAAAAATCAGTAACAGTATTAGATGTGGGGGA CGCCTATTTTTCAGTTCCCTTAGATGAAAGCTTTAGAAAGTATACTGCATTCACCA TACCTAGTATAAACAATGAGACACCAGGGATCAGATATCAGTACAATGTGCTTCC ACAGGGATGGAAAGGATCACCGGCAATATTCCAGGCTAGCATGACAAAAATATT AGAACCCTTTAGATCAAAAAATCCAGAAATAGTTATCTATCAATACATGGATGAC TTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAATAAAAGTAGAGGAGT TGAGAGATCATCTATTGAAGTGGGGATTTACTACACCAGACAAAAAGCATCAAAA AGAACCTCCATTTCTTTGGATGGGTTATGAACTA

18	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTAAT GAGATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAGAATCCATACAAT ACTCCAATATTTGCTATAAAGAAAAAAGACAGCACTAAATGGAGAAAATTAGTA GATTTTAGAGAGCTCAATAAAAGAACTCAAGACTTTTGGGAAGTTCAATTGGGAA TACCGCATCCTGCAGGTTTAAAAAAGAAAAAATCAGTAACAGTATTAGATGTGGG GGACGCCTATTTTTCAGTTCCCTTAGATGAAAGCTTTAGAAAGTATACTGCATTCA CCATACCTAGTATAAACAATGAGACACCAGGGATCAGATATCAGTACAATGTGCT TCCACAGGGATGGAAAGGATCACCGGCAATATTCCAGGCTAGCATGACAAAAAT ATTAGAACCCTTTAGATCAAAAAATCCAGAAATAGTTATCTATCAATACATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAATAAAAGTAGAGG AGTTGAGAGATCATCTATTGAAGTGGGGATTTACTACACCAGACAAAAAGCATCA AAAAGAACCTCCATTTCTTTGGATGGGTTATGAACTA
19	GCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTCTAGAAATGGA GAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAACACTCCAGT GTTTGCTATAAAGAAAAAAGATAGCACTAAATGGAGAAAATTAGTAGATTTTAGA GAACTCAATAAGAGAACTCAAGACTTCTGGGAAGTTCAGTTAGGAATACCACATC CAGCAGGATTAAAAAAGAAAAAATCAGTAACAGTATTAGATGTGGGGGACGCAT ATTTTTCCGTTCCCTTAGATAAAGAATTTAGAAAATATACTGCATTCACCATACCT AGTATAAACAATGAGACACCAGGAATTAGATATCAGTACAATGTGCTCCCACAGG GATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTTAGAGC CCTTTAGAAAACAAAATCCAGAAATGGTTATCTATCAATACGTGGATGATTTGCTT GTAGGATCTGACTTAGAAATAGGGCAGCATAGAGCAAAAATAGAGGAGTTAAGA GAACATCTATTGAAATGGGGATTTACCACACCAGATAAAAAACATCAAAAAGAA CCTCCATTTCTTTGGATGGGTT
20	AACAATGGCCATTGACAGAGGAAAAAATAAAAGCATTAACAGAAATCTGTACAG AAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATA CTCCAATATTTGCAATAAAGAAAAAAGATAGCACTAAGTGGAGAAAATTAGTAG ACTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAGTTAGGAAT ACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTGCTAGATGTGGG GGATGCATATTTTTCAGTTCCTTTAGATAAAGAGTTCAGAAAATATACTGCATTCA CCATACCTAGTACAAACAATGAGACACCAGGAATCAGGTATCAGTACAATGTGCT TCCACAGGGATGGAAAGGATCACCGGCAATATTCCAGAATAGCATGCTAAAAATT TTAGAGCCCTTTAGATCAAAGAATCCAGAAATAATTATCTATCAATACATGGATG ACTTGTATGTAGGATCTGATTTAGAAATAGAGCAGCATAGATCAAAAGTAGAAGA GTTGAGAGCTCATCTATTGAGATGGGGACTAACTACACCAGACAAAAAGCATCAG AAAGAACCTCCATTCCTTTGGATGGGWTATGARCTA
21	ACAATGGCCATTGACAGAAGAAAAAATAAAAGCACTAACAGAAATTTGTATGGA AATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAATAC TCCAATATTTGCCATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAGAT TTCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAGGTTCAACTAGGAATAC CACATCCTGCAGGGCTAAAAAARAAAAAATCAGTAACAGTACTGGATGTGGGTG ATGCATATTTTTCAGTTCCATTGTATGAAGACTTTAGAAAATATACCGCATTCACC ATACCTAGTATAAACAATGAGACACCAGGAATTAGATATCAGTACAATGTGCTTC CACAAGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGTATGACAAAAATCC TAGAACCTTTTAGAAGAAAAAATCCAGAAATAGTTATCTATCAATACATGGATGA TTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAATAAAAATAGAGGAA TTAAGGGAACACCTATTGAAGTGGGGATTTACCACACCAGACAAAAAGCATCAG AAAGAACCCCCATTTCTTTGGATGGGTTATGAACTA

22	ACAATGGCCATTGACAGAAGAAAAAATAAAAGCACTAACAGAAATTTGTATGGA AATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAATAC TCCAATATTTGCCATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAGAT TTCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAGGTTCAACTAGGAATAC CACATCCTGCAGGGCTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGTG

	ATGCATATTTTTCAGTTCCCTTGTATGAAGACTTTAGAAAATATACCGCATTCACC ATACCTAGTATAAACAATGAGACACCAGGAATTAGATATCAGTACAATGTGCTTC CACAAGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGTATGACAAAAATCC TAGAACCTTTTAGAAGAAAAAATCCAGAAATAGTTATCTATCAATACATGGATGA TTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAATAAAAATAGAGGAA TTAAGGGAACACCTATTGAAGTGGGGATTTACCACACCAGACAAAAAGCATCAG AAAGAACCCCCATTTCTTTGGATGGGTTATGAAC
23	TTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCACTAACAGACATTTGTA ATGAAATGGAAAAGGAAGGGAAAATTTCAAAGATTGGGCCTGAAAATCCATACA ATACCCCAATATTTGCCATAAAGAAAAAGGACAGTACTAAGTGGAGAAAATTAGT AGATTTCAGAGAGCTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAACTAGGA ATACCACATCCTGCAGGGCTAAAGAAGAAAAAATCAGTAACAGTACTGGATGTG GGTGATGCATATTTTTCAGTTCCCTTGGATGAAGACTTTAGAAAATATACTGCATT CACCATACCTAGTATAAACAATGAGACACCAGGAATTAGATATCAGTACAATGTG CTGCCACAAGGATGGAAAGGATCACCGGCAATATTTCAAAGTAGCATGACAAAA ATCTTAGAGCCTTTTAGAAAACAAAATCCAGAAATGGTTATCTATCAATATATGG ATGATTTGTATGTAGSATCTGACTTAGAAATAGGGCAGCATAGAATAAAAATAGA GGAATTAAGGGAACACCTATTGAAGTGGGGATTTACCACACCAGACAAAAAGCA TCAGAAAGAACCCCCATTTCTTTGGATGGGTTATGAACT
24	AACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAG AAATGGAAAAGGAGGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATA CTCCAATATTTGCGATCAAGAAAAAAGATAGCACTAAATGGAGGAAATTAGTAG ACTTCAGAGAGCTCAATAAAAGAACACAAGATTTTTGGGAAGTKCAATTAGGGAT ACCACATCCAGCRGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGG GGACGCATATTTTTCAGTCCCCTTAGATAAAGACTTTAGAAAATATACTGCATTCA CCATACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAATACAATGTGCT TCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAAT CTTAGAGCCTTTTAGATTAAAGAATCCAGAAATAATTATCTATCAATACATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAGAG GAGTTAAGAGCCCATCTATTGAGCTGGGGGTTTACTACACCAGACAAAAAGCATC AGAAAGAACCTCCATTCCTTTGGATGGGWTATGA
25	AAACAATGGCCATTGACAGAAGAAAAAATAAGAGCATTAMCAGAAATTTGTACA GAAATGGAAAAGGAAGGAAAAATTTCRAAAATTGGGCCAGAAAATCCATACAAT ACTCCAATATTTGCTATAAAGAAAAAAGACAGCACTAAATGGAGAAAATTAGTA GATTTCAGAGAGCTTAATAAAAGAACTCAAGATTTTTGGGAAGTTCAATTAGGAA TACCGCACCCAGCGGGCCTAAAAAAGAAYAAATCAGTAACAGTACTAGATGTGG GGGACGCATATTTTTCRGTTCCCTTAGATGAAAGYTTTAGAAAGTATACTGCGTTC ACCATACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGC TTCCACAGGGATGGAAAGGYTCACCATCAATATTCCAGAGTAGCATGACAAAAAT CTTAGARCCCTTTAGAGCAAAAAATCCAGAAATAATTATCTATCAATACATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAARTAGAAG ARTTGAGAGCTCATCTATTGAGCTGGGGATTTACTACCCCAGACAAAAARCATCA GAAAGAACCGCCATTTCTTTGGATGGGTTATGAACTA

26	AAACAATGGCCATTGACAGAAGAAAAAATAAGAGCATTAACAGAAATTTGTACA GAAATGGAAAAGGAAGGAAAAATTTCGAAAATTGGGCCAGAAAATCCATACAAT ACTCCAATATTTGCTATAAAGAAAAAAGACAGCACTAAATGGAGAAAATTAGTA GATTTCAGAGAGCTTAATAAAAGAACTCAAGATTTTTGGGAAGTTCAATTAGGAA TACCGCACCCAGCGGGCCTAAAAAAGAATAAATCAGTAACAGTACTAGATGTGG GGGACGCATATTTTTCGGTTCCCTTAGATGAAAGTTTTAGAAAGTATACTGCGTTC ACCATACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGC TTCCACAGGGATGGAAAGGCTCACCATCAATATTCCAGAGTAGCATGACAAAAAT CTTAGAACCCTTTAGAGCAAAAAATCCAGAAATAATTATCTATCAATACGTGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAGAA

	GAATTGAGAGCTCATCTATTGAGCTGGGGATTTACTACCCCAGACAAAAAGCATC AGAAAGAACCGCCATTTCTTTGGATGGGTTATGAACTAA
27	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACA GAGATGGAAAAGGAAGGGAAAATTTCAAAAATTGGACCTGAAAATCCATACAAT ACTCCAATATTTGCTATAAAGAAAAAAGATAGCACTAAATGGAGAAAATTAGTAG ATTTCAGAGAGCTCAATAAGAGAACTCAGGACTTCTGGGAAGTTCAATTAGGAAT ACCACACCCAGCAGGTTTAAAAAAGAAGAAATCGGTAACAGTACTAGATGTGGG GGATGCATATTTTTCAGTTCCTTTAGATGAAAGCTTTAGAAAGTATACTGCATTCA CCATACCTAGTACAAACAATGAGACACCAGGAGTCAGGTATCAATATAATGTGCT TCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAAT CTTAGAGCCCTATAGATCAAAAAATCCAGAAATAATTATTTATCAATACATGGAT GATTTGTATGTAGCATCTGATTTAGAAATAGGACAACATAGAGCAAAAATAGAGG AGCTGAGAGCTCATCTATTAAGTTGGGGGTTTACTACACCAGACAAAAAGCATCA GAAAGAACCCCCATTTCTTTGGATGG
28	TGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAGAGATG GAAAAGGAAGGGAAAATTTCAAAAATTGGACCTGAAAATCCATACAATACTCCA ATATTTGCTATAAAGAAAAAAGATAGCACTAAATGGAGAAAATTAGTAGATTTCA GAGAGCTCAATAAGAGAACTCAGGACTTCTGGGAAGTTCAATTAGGAATACCACA CCCAGCAGGTTTAAAAAAGAAGAAATCGGTAACAGTACTAGATGTGGGGGATGC ATATTTTTCAGTTCCTTTAGATGAAAGCTTTAGAAAGTATACTGCATTCACCATAC CTAGTACAAACAATCAGACACCAGGAGTCAGGTATCAATATAATGTGCTTCCACA GGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAATCTTAGA GCCCTATAGATCAAAAAATCCAGAAATAATTATTTATCAATACGTGGATGATTTG TATGTAGCATCTGATTTAGAAATAGGACAACATAGAGCAAAAATAGAGGAGCTG AGAGCTCATCTATTAAGTTGGGGGTTTACTACACCAGACAAAAAGCATCARAAAG AACCCCCATTTCTTTGGATGGGTTATGAACT
29	AACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTAAWG ARATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATATAATA CTCCAATATTTGCMATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAG ATTTCAGAGAACTWAATAARAGAACTCAAGACTTYTGGGAARTTCAATTAGGAAT ACCRCATCCTGCAGGGCTAAAAAAYAAAAARTCAGTAACAGTACTRGATGTGGG AGATGCATATTTTTCAGTTCCCTTAYRTGAAGATTTTAGAAAGTATACTGCATTTA CCATACCTAGTRYAAAYAATGAGACACCAGGGRTTAGATATCAGTACAATGTGCT TCCACAGGGATGGAAAGGRTCACCAGCAATATTYCAAAGTAGCATGACAAAAAT CTTAGAACCTTTTAGAAAACAAAATCCAGARGTGGTTATCTATCAATACATGGAT GATTTGTATGTAGGATCTGACTTAGAAATAGGKMARCATAGAACAAAAATAGAG GAATTAAGGGAACAYCTATTAARGTGGGGRTTTACCACACCAGACAAAAAACATC AGAAAGAGCCTCCATTTCTTTGGATGGGTTATGAACTA

30	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTGAA GAGATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAT ACTCCAGTGTTTGCTATAAAGAAAAAGGATAGCACTAAATGGAGAAAATTAGTAG ATTTTAGAGAGCTCAATAAAAGAACACAGGACTTCTGGGAAGTTCAATTAGGAAT ACCCCATCCTGCAGGTTTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGGG GATGCCTATTTTTCAGTTCCTTTAGATAAAGATTTTAGAAAGTATACTGCATTCAC CATACCTAGTATAAACAATGAGACACCAGGAACCAGGTATCAGTACAATGTGCTT CCACAAGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGGCAAAAATC TTAGAGCCCTTTAGATCACAAAATCCAGGAATAATTATTTATCAATACGTGGATG ACTTGTATGTAGCATCTGATTTAGAAATAGGGCAGCATAGAACAAAAGTAGAAGA ATTGAGAGCTCATCTATTGAGTTGGGGATTTACTACACCAGACAAAAAACATCAG AAAGAACCTCSATTTCTTTGGATGGGTTATGAACTA
31	GTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGT ACAGAMATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATAC AATACTCCAATATTTGCCATAAAGAARAAAGACAGTACTAAGTGGAGAAAGTTAG

	TAGATTTCAGAGAACTCAATAAAAGAACTCAAGACTTTTGGGAAGTCCAATTAGG GATACCACACCCAGCAGGGTTGAAAAAGAAAAAATCAGTGACAGTATTGGATGT GGGGGATGCATATTTTTCAGTTCCTTTAGATGAAGACTTCAGAAAATATACTGCAT TTACAATACCTAGTATAAACAATGAAACACCAGGAATTAGATATCAGTATAATGT GCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAAAGCAGCATGACAAA AATTTTAGAGCCCTTTAGGGCGCAAAACCCAGAAATAGWTATCTATCAATACATG GATGACTTGTATGTAGGATCTGACTTAGAAATAGGGCAACATAGAGCAAAAATAG AGGAGTTAAGGGAACATCTGTTGARGTGGGGGTTTACCACACCAGATAAGAAAC ATCAGAAAGAACCTCCATTTCTWTGGATGGGTTATGAACTA
32	CAATGGCCATTGACAGAAGAAAAAATAAAAGCACTAACAGAAATTTGTAAAGAA ATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAGAATCCATACAATACT CCAATATTTGCCATAAGAAAGAAAGACAGTACTAAGTGGAGAAAATTARTGGATT TCAGGGAACTCAATAAAAGAACCCAAGACTTTTGGGAAGTTCAATTAGGGATACC ACACCCAGCAGGGTTAAAAAAGAAAAAATCAGTGACAGTACTAGATGTGGGAGA TGCATATTTTTCAGTTCCTTTAGATGAAGGCTTCAGAAAATATACTGCATTCACCA TACCTAGTAKRAAYAATGAGACACCAGGAATTAGATATCAGTACAATGTGCTTCC GCAAGGATGGAAAGGATCACCGGCAATATTCCAAAGTAGCATGACAAAAATCTT AGAACCCTTTAGGAAACAAAATCCAGAAATGGTTATCTRTCAATACATGGATGAT TTGTATGTAGGATCTGACTTAGAAATAGGGCAACATAGAATAAAAATAGGAGAGT TAAGGGAACACCTATTGAAGTGGGGATTTACTACACCAGACAAAAAGCATCAGA AAGAACCTCCATTCCTTTGGATGG
33	AAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAATATTTGCAATA AAGAAAAAAGATAGCACTAAATGGAGAAAATTAGTAGATTTCAGAGAGCTCAAT AAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGGATACCGCATCCAGCGGGCC TAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGGGGACGCCTATTTTTCAGT TCCTTTAGATGAAAACTTTAGAAAATATACTGCATTCACCATACCTAGTATAAATA ATGAAACACCAGGAATAAGGTATCAGTACAATGTGCTTCCACAGGGATGGAAAG GATCACCAGCAATATTCCAGAGTAGTATGACAAAAATCTTAGAGCCCTTTAGAAC AAAAAATCCAGAAATARTTATCTATCAATACATGGATGACTTGTATGTAGGATCT GATTTAGAGATAGGGCAGCATAGAGCAAAAATAGAAGAACTAAGAGCTCATCTG TTGAGCTGGGGATTTACYACACCAGACAAAAAGCATCAGAAAGAACCCCCATTCC TTTGGATGG

34	AACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAG ATATGGAAAAGGAGGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAATA CTCCAATATTTGCTATAAAGAAAAAGGACAGTACTAAATGGAGAAAATTAGTAGA TTTCAGAGAACTTAATAAAAGGACTCAAGACTTTTGGGAAGTTCAATTAGGAATA CCACACCCAGCAGGGTTAAAAAAGAAAAAATCAGTGACAGTACTGGATGTGGGT GATGCATATTTTTCAGTTCCTTTAGATAAAGACTTTAGAAAGTATACCGCATTCAC CATACCTAGTATAAACAATGAAACACCAGGAATTAGATATCAGTACAATGTGCTC CCACAGGGATGGAAAGGATCACCGGCAATATTCCAAAGTAGCATGACAAAAATC TTAGAGCCCTTTAGAAAGAAAAATCCAGAAATAGTTATTTATCAGTACATGGATG ATTTATATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAAG AATTAAGAGAGCATCTTTTAAGGTGGGGATTTACCACACCAGACAAAAAACATCA GAAGGAACCTCCATTTCTTTGGATGGGTTATGAACTA
35	GATGGAAAAGGAAGGAAAGATTTCAAAAATAGGGCCTGAAAATCCATACAATAC TCCAGTATTTGCTATAAAGAAAAAAGATAGCACTAAATGGAGAAAATTAGTAGAT TTTAGAGAACTCAATAAAAGAACTCAAGACTTTTGGGAAGTCCAACTAGGGATAC CACACCCAGCAGGTTTAAAGAAGAAAAAATCAGTGACAGTACTGGATGTGGGGG ATGCATATTTTTCAGTCCCTTTAGATGAAAACTTCAGGAAATACACTGCATTCACA ATACCTAGTATAAACAATGAAACACCAGGGATTAGATATCAATACAATGTGCTTC CACAGGGGTGGAAAGGGTCACCAGCAATATTCCAGAGTAGCATGACAAAAATCT TAGAGCCCTTTAGATCAAAAAATCCAGAAATAATTATCTATCAATACATGGATGA CTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAGTAGAGGA
	GTTGAGGGCTCATCTATTGAGGTGGGGATTTACTACACCAGACAAAAAACATCAG AAAGAACCTCCATTTCTTTGGATGGGTTATGAACTA
36	AAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAACACTCCATTA TTTGCTATAAARAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAG AACTTAATAAGAGAACTCAGGACTTCTGGGAAGTTCAATTAGGAATACCGCATCC AGCAGGTTTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGGGGACGCATA TTTTTCAGTTCCTTTACATGAAGACTTTARAAAGTATACTGCCTTCACCATACCTA GTACAAACAATGAGACACCAGGAGTCAGGTATCAGTACAATGTGCTCCCACAAG GATGGAAAGGATCACCAGCGATATTCCAGAGTAGCATGACAAAAATCTTAGAAC CCTTTAGAACAAAAAACCCAGAAATAGTTATCTACCAATACATGGATGACTTGTA TGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAGCTGAG AGCTCATCTATTGAGATGGGGGCTCACTACACCAGACAAAAAGCATCAGAAAGA ACCTCCATTTCTTTGGATGGGTTATGAACT
37	AACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAG AAATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATA CTCCAATATTTGCAATAAAGAAAAAAGATAGTACCAAATGGAGGAAATTAGTAG ATTTCAGAGAACTTAATAAAAGAACACAAGACTTTTGGGAARTTCAATTAGGAAT ACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGG AGATGCATATTTTTCAGTTCCTTTAGATGAAAGTTTTAGAAAATACACTGCATTCA CCATACCTAGTACAAACAATGAGACACCAGGAGTCAGATATCAGTACAATGTGCT TCCACAGGGATGGAAAGGATCTCCGGCAATATTCCAGAGTAGCATGACAAAAATC TTAGAGCCATTTAGATCAAAAAATCCAGAAATAATTATCWWTCAATACATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGGGCAACATAGAACAAAAATAGAA GAGTTAAGAGCTCATCTACTGAGCTGGGGATTTACTACACCAGACAAAAAACATC AGAAAGAACCTCCATTCCTTTGGATGGGTTATGAAC

38	AACAATGGCCATTGACAGAAGAGAAAATAAAAGCATTAACAGAAATTTGTGGAG AAATGGAAAAGGAAGGAAAAATTTCAAAGATTGGGCCTGAAAATCCATACAATA CTCCAATATTTGCAATAAAGAAAAAGGATAGCACTAAATGGAGGAAATTAGTAG ATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAGGTTCAATTAGGAAT ACCACATCCAGCAGGCCTAAAAAAGAAGAAATCAGTAACAGTACTGGATGTGGG GGATGCATATTTTTCAGTGCCTTTAGATAAGGACTTCAGAAAATATACTGCATTCA CCATACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGCT TCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAAT CTTAGAGCCCTTTAGATCAAAAAATCCAGACATAGTGATCTATCAATACATGGAT GACTTGTATGTAGGATCTGATCTAGAAATAGGGCAGCATAGAACAAAAATAGAA GAGTTGAGAGCTCATCTATTGAACTGGGGATTTACTACACCAGACAAAAAGCATC AGAAAGAACCCCCATTCCTTTGGATGGGTTATGAACTA
39	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACA GATATGGAAAAGGAAGGAAAGATTTCAAAAATTGGGCCTGAAAATCCATATAAT ACTCCAATATTTGCAATAAAGAAAAAAGATAGTACCAAATGGAGAAAATTGGTA GATTTCAGAGAGCTCAACAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGAA TACCGCATCCAGCGGGCTTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGG GGGATGCATATTTTTCAGTTCCTTTAGATGAAAACTTTAGAAAATACACCGCGTTC ACCATACCGAGTATAAACAATGAGACACCAGGAGTCAGATATCAGTACAATGTGC TTCCACAGGGATGGAAAGGATCACCAGCAATATTTCAGAGTAGCATGACAAAAAT CTTAGAGCCCTTTAGATCAAAAAATCCAGAAATAATTATCTATCAATACATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAGAA GAGTTGAGGGCTCATCTATTGAGCTGGGGGTTCACTACACCAGACAAAAAGCATC AGAAAGAACCTCCATTTCTTTGGATGG
40	CAATGGCCATTGACAGAAGAGAAAATAAAAGCTTTAATAGAAATTTGTACAGAA ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCCATAAAGAAAAAGGACAGTACTAAGTGGAGAAAACTAGTAGAT TTCAGAGAGCTCAATAAAAGAACTCAAGATTTCTGGGAAGTCCAATTAGGGATAC

	CTCACCCCGCGGGTCTAAAGAAGAAAAAATCAGTAACAGTACTAGATGTGGGGG ATGCATATTTCTCAGTTCCCTTAGATGAAAACTTTAGAAAGTATACAGCATTCACT ATACCTAGTGTAAATAATGAGACACCAGGGATTAGATACCAGTACAATGTGCTGC CTCAGGGATGGAAAGGATCACCAGCAATTTTTCAGAGTAGTATGACAAAAATCCT AGAGCCCTTTAGAAGAGAAAATCCAGAAATGGTAATTTGCCAATATATGGATGAT TTATATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAGCAAAAATAGAAGAAT TAAGAAAACATCTATTGAATTGGGGATTTACCACACCAGATAAAAAATATCAGAA AGAACCCCCATTCCTTTGGATGG
41	CAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTATAGAG ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAGAATCCATACAATACT CCAATATTTGCTATAAAGAAAAAAGACAGCACTAAATGGAGGAAACTAGTAGAT TTTAGAGAGCTCAATAAAAGAACACAAGACTTCTGGGAAGTTCAATTAGGGATAC CGCATCCAGCGGGACTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGGG ACGCATATTTTTCAGTTCCTTTACATAAAGACTTTAGAAAATATACTGCATTCACC ATACCTAGTACAAACAATGAGACACCAGGAATCAGGTATCAGTACAATGTGCTTC CACAGGGATGGAAAGGATCACCGGCAATTTTCCAGAGTAGCATGACAAAAATCTT AGAGCCCTTTAGATCAAATAATCCAGAAATAGTTATCTATCAATACATGGATGAC TTGTATGTAGGATCTGATTTAGAACTAGGGCAGCATAGAGCAAAAATAGAAGAGT TGAGGGCGCATTTATTGAGCTGGGGATTAACTACCCCAGACAAAAAGCATCAGAA AGAGCCGCCATTTCTTTGGATGG

42	ACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTGACAGAAATTTGTACAGA GATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATACAATAC TCCAATATTTGCAATAAAGAAAAAAGATAGTACTAAATGGAGGAAATTAGTAGA CTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGGATA CCGCATCCAGCGGGCCTAAAAAAGAAGAAATCAGTAACAGTACTAGATGTGGGG GACGCATATTTTTCAGTTCCTTTAGATGTAGACTTTAGAAAGTATACTGCGTTCAC CATACCTAGTACAAACAATGAGACACCAGGAATAAGGTATCAGTACAATGTGCTT CCACAGGGATGGAAAGGATCACCGGCAATATTCCAGAGTAGCATGACAAAAATC TTAGAGCCCTTTAGATCAAAAAATCCAGAAATAATTATCTATCAATACATGGATG ACTTGTATGTAGGATCTGATTTAGAAATAGGTCAGCATAGAGCAAAAGTAGAGGA GTTGAGAGCTCATCTATTGAGTTGGGGGTTTACTACACCAGATAAAAAACATCAG AAAGAACCTCCATTTCTTTGGATGGGTTATGAACTAC
43	AACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAG AAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAGAATCCATACAATA CCCCAATATTTGCTATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTGG ATTTTAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAAT ACCACATCCTGCAGGATTAAAAAAGAAAAATTCAGTAACAGTACTGGATGTGGGT GATGCATATTTTTCAGTTCCCTTAGATGAAGACTTTAGAAAATATACCGCATTCAC TATACCTAGTATAAATAACGAGACACCAGGAGTTAGATATCAGTACAACGTGCTT CCACAAGGATGGAAAGGGTCACCATCAATATTTCAAAGTAGCATGACAAAGATCT TAGAACCTTTTAGAAAACAAAATCCAGAAATAGTTATCTATCAATACATGGATGA TTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGA ATTAAGGGGACACCTATTGAAGTGGGGATTCACCACACCAGACAAAAAGCATCA GAAAGAACCTCCATTTCTTTGGATGGGTTATGAACTA
44	AGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTG TACGGAAATGGAAAAGGAGGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATA CAATACTCCAATATTTGCGATAAAGAAAAAGGATAGCACTAAATGGAGGAAATT AGTAGATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTA GGGATACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTGGAT GTGGGGGACGCATACTTTTCAGTTCCTTTACATAAGGACTTTAGAAAGTATACTGC GTTCACCATACCTAGTACCAACAATGAGACACCAGGAATCAGATATCAGTACAAT GTACTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAA AAATCTTAGAGCCCTTTAGATCAAAAAATCCAGAAATAATCATCTATCAATACAT

	GGATGATTTGTATGTAGGATCTGATTTAGAAATAGGGCAACATAGAGCAAAAATA GAAGAGTTGAGAGCTCATCTCTTGAGCTGGGGATTTACTACCCCAGACAAAAAGC ATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTA
45	CAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTTTAGAGATGGAAAAGGAGG GAAAGATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAATATTTGCAAT AAAGAAAAAGGATAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAGCTCAA TAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGGATACCGCATCCAGCGGGC CTAAAAAAGAAGAAATCAGTAACAGTACTAGATGTGGGGGATGCATATTTTTCAG TTCCTTTACATGAAGACTTTAGAAAGTATACTGCATTCACCATACCTAGCACAAAC AATGAGACACCAGGAATCAGATATCAGTAYAATGTGCTTCCACAAGGATGGAAA GGATCACCAGCAATATTCCAGAGTAGCATGACAAAAATCTTAGAGCCCTTTAGGT TAAAAAATCCAGAAATAATTATCTATCAATACATGGATGACTTGTATGTAGGATC TGATTTAGAGATAGGGCAGCATAGRACAAAAATAGAAGAGTTGAGGGCTCATCT ATTGAGCTGGGGATTTACTACACCAGACAAAAAGCATCAGAAAGAACCTCCATTC CTTTGGATGGGTTATGAACTA

46	CAATGGCCATTGACAGAGGAAAAAATAAAAGCATTAACAGAAATTTGTACAGAA ATGGAAAAGGAAGGAAAAATTTCAAGAATTGGGCCTGAAAATCCATATAATACT CCAATATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGGAAATTAGTGGATT TCAGAGAGCTCAATAAAAGAACTCAAGATTTTTGGGAAGTTCAATTAGGAATACC GCATCCAGCGGGCTTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGGGA CGCATATTTTTCAGTTCCCTTAGATGAAGGCTTTAGGAAGTATACGGCGTTCACCA TACCTAGTACAAACAATGAGACACCAGGGATCAGGTATCAGTACAATGTGCTTCC ACAGGGATGGAAAGGGTCTCCAGCAATATTCCAGAGTAGTATGACAAAAATCTTA GAGCCCTTTAGGCTAAAAAATCCAGAAGTAACTATCTATCAATACATGGATGACT TATATGTAGGGTCTGATTTAGAAATAGGGCAGCATAGAACAAAAGTAGAGGAGTT GAGAGATCATCTATTGAGCTGGGGATTAACTACACCAGACAAAAAGCATCAGAA AGAACCTCCATTTCTTTGGATGG
47	CAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTATAGAT ATGGAAAAGGAAGGAAARATTTCAAGAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCCATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAGATT TCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGAATACC ACACCCTGCAGGGCTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGTGA TGCATATTTTTCAGTTCCCCTATATGAAGATTTTAGAAAATATACTGCATTCACCA TACCTAGTACAAACAATGAGACACCAGGAATCAGGTATCAGTACAATGTGCTTCC ACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAATCTT AGAGCCCTATAGATTAAAAAATCCAGAAATAATTATCTATCAATACATGGATGAC TTGTATGTGGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAGAAGAGT TGAGAGCTCATCTATTGAGCTGGGGATTTACCACACCAGACAAAAAGCATCAAAA AGAACCTCCATTTCTTTGGATGG TCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGA AATTTGTACAGAAATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAA TCCATACAATACTCCAATATTTGCAATAAAGAAGAAAGATAGCACTAAATGGAGG AAACTAGTAGATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTC AATTAGGAATACCACATCCAGCAGGCCTGAAGAAGAAAAAATCAGTAACAGTAC TAGATGTGGGGGATGCATATTTTTCAGTTCCTCTAGATGAAAGCTTTAGAAAGTAT ACTGCATTCACCATACCTAGTAGAAACAATGAGACACCAGGAATCAGGTATCAGT ACAATGTGCTTCCACAAGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCAT GACAAAAATCTTAGATCCCTTTAGATCAAAAAATCCAGAAATAGTTATCTATCAA TACATGGATGACTTGTATGTAGGATCTGATTTAGAAATAGAGCAGCACAGAACAA AAATAGAAGAATTAAGAGCTCATCTATTGAGCTGGGGATTCACTACACCAGACAA AAAGCATCAGAAAGAACCTCCATTCCTTTGGATGGG
49	TATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATAC

	TCCAATATTTGCAATAAAGAAAAAAGATAGCACTAAGTGGAGGAAATTAGTAGA TTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTCCAATTAGGAATA CCGCATCCAGCGGGCCTAAAAAARAAAAAATCAGTAACAGTACTTGATGTGGGG GATGCATATTTTTCAGTTCCTTTATATGAAGACTTTAGAAAATACACAGCATTCAC CATACCTAGTAYAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGCTT CCACAGGGATGGAAAGGGTCACCAGCAATATTCCAGCATAGCATGACAAAAATTT TAGAGCCCTTTAGATTAAAAAATCCAGAAATAATTATCTATCAATACATGGATGA CTTGTATGTAGGATCTGATTTAGAAATAGGACAGCATAGAACAAAAATAGAAGA GTTAAGAGCTCATTTATTGAGCTGGGGATTTACTACACCAGACAAGAAGCATCAG AAAGAACCTCCATTCCTTTGGATGGGTTATGAACTA

50	AAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAATATTTGCCATA AAGAAAAAAGACAGTACTAAGTGGAGAAAACTAGTAGATTTTAGAGAGCTCAAT AAAAGAACTCAAGATTTCTGGGAGATCCAATTAGGAATACCCCATCCCGCAGGTT TAAAAAAGAAYAAATCAGTCACAGTACTAGATGTGGGGGATGCATATTTTTCAGT CCCCTTAGATAAAGATTTTAGAAAATATACAGCATTCACTATACCTAGTGCAAAT AATGAGACACCAGGAGTTAGATAYCAGTACAATGTGCTGCCACAGGGATGGAAA GGATCACCAGCAATCTTTCAGGCTAGCATGACAAAAATTTTAGAGCCYTTTAGAA MAGARAATCCAGACATAGTGATCTACCAATATATGGATGATTTATATGTAGGATC WGACYTAGAAATAGGGCARCATAGAGCAAAAATAGAGGAATTAAGAGAACATCT ATTGAGATGGGGATTTACCACACCAGATAAAAAACATCAGAAAGAACCTCCATT MCAATGGATGGGATATGAGCTCCATCCTGACAAATGGACGGTACAGCCTATACA GCTGCCAGAAAAAGAAAGCTGGACTGTCAATGATATACAAAAGTTAGTGGGAAA ACTAAATTGGGCAAGTCAGATTTATGCA
51	CAATGGCCATTGACAGAAGAAAAAATAAAAGCACTAACAGAAATTTGTACAGAA ATGGAAAAGGAAGGAAAAATMTCAAGAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCCATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAGATT TCAGRGAACTTAATAARAGGACTCAAGACTTCTGGGAAGTTCAACTAGGAATACC ACATCCAGCAGGGTTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGKGA TGCATATTTTTCAGTTCCCTTATATGAAGACTTTAGAAAGTATACTGCATTCACCA TACCTAGTATAAACAATGAGACACCAGGAATTAGATATCAATACAATGTGCTTCC ACAAGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAAAATCTT AGAACCTTTTAGACAACAAAATCCAGAAATAGTTGTCTATCAATACATGGATGAT TTGTAYGTAGGATCTGATTTAGACATAGGGCAGCACAGAAYAAAAATAGAGGAA TTAAGGGAACACCTCTTGAAGTGGGGATTTACCACRCCAGATAAAAAGCATCAGA AAGAACCCCCATTTCTTTGGATGGGTTATGAACTA
52	ACAATGGCCATTGACAGAAGAAAAAATAAAGGCATTAACAGAAATTTGTACAGA GATGGAAAAGGAAGGAAAAATTACAAAAATTGGGCCTGAAAATCCATACAATAC TCCAATATTTGCAATAAAGAAAAAGGATAGCACTAAATGGAGAAAATTAGTAGA TTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTCCAGTTAGGAATA CCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGGG GATGCATATTTTTCAATTCCTTTAGATGAAAACTTTAGAAAGTATACAGCATTCAC TATACCTAGTACAAACAATGAGACACCAGGAGTTAGATATCAGTATAATGTGCTT CCACAAGGATGGAAAGGGTCACCGGCAATATTCCAGTATAGCATGACAAAAATC CTGGAGCCCTTTAGATTAAAAAATCCAGAAATAGTTATCTATCAATACATGGATG ACTTGTATGTAGGATCTGATTTAGAAATAGGACAGCATAGAACAAAAATAGAAG AGTTAAGAGCTCATCTGTTGAGCTGGGGCTTGACTACACCAGACAAAAAGCATCA GAAAGAGCCTCCATTTCTTTGGATGG
53	AGTTAAACAATGGCCATTGACAGAAGAGAAAATAAAGGCATTAACAGAAATTTG TAAAGAGATGGAAAAGGAAGGAAAAATTACAAAAATTGGGCCTGAAAATCCATA CAATACTCCAATATTTGCAATAAAGAAAAAGGATAGCACTAAATGGAGAAAATT AGTAGATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTCCAGTTA GGAATACCGCATCCAGCGGGCCTAAAAAARAAAAAATCAGTAACAGTACTAGAT GTGGGGGATGCATATTTTTCAATTCCTTTAGATGAAAACTTTAGAAAGTATACAGC

	ATTCACTATACCTAGTACAAACAATGAGACACCAGGAGTTAGATATCAGTATAAT GTGCTTCCACAAGGATGGAAAGGGTCACCGGCAATATTCCAGTATAGCATGACAA AAATCCTGGAGCCCTTTAGATTAAAAAATCCAGAAATAGTTATCTATCAATACAT GGATGATTTGTATGTAGGATCTGATTTAGAAATAGGACAGCATAGAACAAAAATA GAAGAGTTAAGAGCTCATCTGTTGAGCTGGGGCTTGACTACACCAGACAAAAAGC ATCAGAAAGAGCCTCCATTTCTTTGGATGGG
54	CAATGGCCATTGACAGAAGAAAAGATAAAAGCATTAACAGAAATTTGTACAGAG ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCTATAAAGAAAAAAGACAGCACTAAGTGGAGAAAATTAGTAGATT TCAGAGAGCTCAATAAAAGAACTCAAGACTTTTGGGAAGTTCAATTAGGAATACC GCACCCAGCGGGCTTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGGGGA TGCATATTTTTCAATTCCCTTAGATGAAGACTTTAGAAAGTATACTGCGTTCACCA TACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGCTTCC ACAGGGATGGAAAGGATCACCGGCAATATTCCAGGCTAGCATGACAAAAATCTT AGAGCCCTTTAGATCAAAAAATCCAGAATTAATTATTTATCAATACATGGATGAC TTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAGAAGAGT TAAGAGCTCATCTATTGAGCTGGGGACTTACTACACCAGACAAAAAGCATCAGAA AGAACCTCCATTTCTTTGGATGG
55	AAACAATGGCCATTGACAGAAGAAAAAATAGAGGCATTAACAGAAATTTGCAAA GAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAT ACTCCAGTATTTGCTATAAAGAAGAAAGATAGCACTAAATGGAGAAAGTTAGTAG ATTTTAGAGAGCTAAATAAAAGAACTCAGGACTTCTGGGAAGTTCAGTTAGGAAT ACCGCATCCCGCAGGTTTAAAAAARAAAAAATCAGTAACAGTACTAGATGTGGG AGATGCATATTTTTCAGTTCCTTTAGATGAAAACTTTAGAAAGTATACTGCATTCA CCATACCTAGTATAAACAATGAGACACCAGGAATCAGGTATCAGTACAATGTGCT TCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAAT CTTAGAGCCCTTTAGGTCACAGAATCCAGAAATAATTATCTRTCAATACATGGAT GACTTGTATGTAGGATCTGATTTGGAAATAGGGCAGCATAGAACAAAAGTAGAG GAGTTGAGAGCTCATCTATTGAGATGGGGATTTACTACACCAGACAAAAAGCATC AAAAAGAACCTCCATTTCTTTGGATGGGTTATGAACTA
56	CAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAGAA ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCAATAAAGAAAAAAGATAGCACTAAATGGAGAAAATTAGTAGAT TTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGAATAC CGCATCCAGCGGGCCTGAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGGGG ACGCATATTTTTCAGTTCCTTTAGATGAAGGCTTTAGAAAATATACTGCATTCACC ATACCAAGCAYAAACAATGAGACACCAGGAATCAGATATCAGTATAATGTGCTTC CACAGGGATGGAAAGGGTCGCCGGCGATATTCCAGGCTAGCATGACAAAAATCTT AGAGCCCTTTAGAGCAAAAAATCCAGAAATAATTATCTATCAATATATGGATGAC TTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAGCAAAAATAGAAGAGT TAAGAGCTCACCTATTGAGCTGGGGATTTACTACACCAGACAAAAAACATCAGAA AGAACCTCCATTCCTTTGGATGGGWTATGARCTA
57	AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTATG GAAATGGAGAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAT ACACCAATATTTGCAATAAAGAAAAAGGATAGCACTAAATGGAGGAAATTAGTA GATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAGCTAGGAA TACCGCATCCAGCGGGTCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGG GGGACGCATATTTTTCAGTTCCTTTACATGAAGGCTTTAGAAAATATACTGCATTC ACCATACCTAGTACAAACAATGAGACACCAGGAATCAGATATCAGTACAATGTGC TTCCACAGGGATGGAAAGGATCACCATCGATATTCCAGAGTAGCATGATAAAAAT TTTAGAACCTTTCAGATCAAAAAATCCAGAAATAATTATCTATCAATATATGGAT GACTTGTATGTAGGATCTGATTTAGAAATAGAGCAACATCGAGCAAAAATAGAAG AGTTGAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAAAAGCATCA

	GAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTA
58	AGTTAAACAATGGCCATTGACAGAAGAGAAAATAAAGGCATTAACAGAAATTTG TAAAGAGATGGAAAAGGAAGGAAAAATTACAAAAATTGGGCCTGAAAATCCATA CAATACTCCAATATTTGCAATAAAGAAAAAGGATAGCACTAAATGGAGAAAATT AGTAGATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTCCAGTTA GGAATACCGCATCCAGCGGGCCTAAAAAAARAAAAAATCAGTAACAGTACTAGAT GTGGGGGATGCATATTTTTCAATTCCTTTAGATGAAAACTTTAGAAAGTATACAGC ATTCACTATACCTAGTACAAACAATGAGACACCAGGAGTTAGATATCAGTATAAT GTGCTTCCACAAGGATGGAAAGGGTCACCGGCAATATTCCAGTATAGCATGACAA AAATCCTGGAGCCCTTTAGATTAAAAAATCCAGAAATAGTTATCTATCAATACAT GGATGATTTGTATGTAGGATCTGATTTAGAAATAGGACAGCATAGAACAAAAATA GAAGAGTTAAGAGCTCATCTGTTGAGCTGGGGCTTGACTACACCAGACAAAAAGC ATCAGAAAGAGCCTCCATTTCTTTGGATGGG
59	CAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTATAGAT ATGGAAAAGGAAGGAAARATTTCAAGAATTGGGCCTGAAAATCCATACAATACT CCAATATTTGCCATAAAGAAAAAAGACAGTACTAAGTGGAGAAAATTAGTAGATT TCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGAATACC ACACCCTGCAGGGCTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGGGTGA TGCATATTTTTCAGTTCCCCTATATGAAGATTTTAGAAAATATACTGCATTCACCA TACCTAGTACAAACAATGAGACACCAGGAATCAGGTATCAGTACAATGTGCTTCC ACAGGGATGGAAAGGATCACCAGCAATATTCCAGAGTAGCATGACAAAAATCTT AGAGCCCTATAGATTAAAAAATCCAGAAATAATTATCTATCAATACATGGATGAC TTGTATGTGGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAGAAGAGT TGAGAGCTCATCTATTGAGCTGGGGATTTACCACACCAGACAAAAAGCATCAAAA AGAACCTCCATTTCTTTGGATGG