HUMAN IMMUNODEFICIENCY VIRUS AND SELECTED COINFECTIONS IN KENYA: AN INVESTIGATION ON PREVALENCE, GENOTYPES, CD4 DISTRIBUTION AND TESTING ALGORITHMS AMONG HIV INFECTED INDIVIDUALS

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Human Immunodeficiency Virus and Selected Coinfections in Kenya: An Investigation on Prevalence, Genotypes, CD4 Distribution and Testing Algorithms Among HIV Infected Individuals

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DECLARATION

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

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DEDICATION

I dedicate this thesis to those who, despite constraint; have made it their business, in small, bigger or larger ways to contribute towards the global fight on HIV and AIDS.

To them,

'When you see HIV around', run! In whatever direction, just run

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ART	Anti Retroviral Therapy
ARV	Antiretroviral Drug
CCC	Comprehensive Care Clinic
CCR5	Chemokine receptor number 5.
CD4	Cluster of Differention 4
cDNA	Complementary DNA
CXCR4	Chemokine receptor number 4
DNA pol	DNA Polymerase
DNA	Deoxy Ribonucleic Acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylene diamine tetra acetic acid
EID	Early Infant Diagnosis
Env	Envelope gene
Gag	Group specific antigen gene
HIV	Human Immuno deficiency syndrome
HIV 2	Human Immunodeficiency Virus type 2
HIV	Human Immunodeficiency Virus infection
HIV	Human Immunodeficiency Virus type 1
KAIS	Kenya AIDS Indicator Survey
KDHS	Kenya Demographic and Health Survey
KEMRI	Kenya Medical Research Institute
LMIC	Low and middle-income countries
LTR	Long terminal repeats
$MgCl_2$	Magnesium chloride
mM	milli molar
MOH	Ministry of Health

nef	Non Nucleoside reverse transcriptase inhibitor
NNTRI	Negative regulator factor
NRTI	Nucleoside reverse transcriptase inhibitor
PCR	Polymerase Chain Reaction
PI	Protease Inhibitors
PMTCT	Prevention of Mother-to-Child Transmission
Pol	polymerase gene
Rev	Regulator of virion protein expression
RNA	Ribonucleic acid
RPM	Revolutions per Minute
RT- PCR	Reverse Transcription Polymerase Chain Reaction
RT	Reverse Transcriptase
SPSS	Statistical package for the social sciences
Taq	Thermus aquaticus
Tat	Transactivator of transcription
UNAIDS	Joint United Nations Programme on HIV and AIDS
µl	Micro litre
µM	Micro mol
vif	Viral infectivity factor
vpr	Viral protein R
vpu	Viral protein U
WHO	World Health Organization

ABSTRACT

HIV and AIDS has been one of the greatest public health challenges of our times.Currently, this epidemic is being compounded by co-infections including other viral infections which share similar modes of transmission and complicated pathophysiologies. The aim of this study was to determine seroprevalence of selected HIV co-infections, identify circulating genotypes and CD4 distributon in a population of HIV infected. To inform diagnostic strategies on selected co-infections, utility of targeted test kits was also determined. Hepatitis B, Hepatitis C, Herpes simplex virus 2 and syphilis were tested in a population of 1829 HIV infected subjects from different parts of Kenya. ELISA and rapid test kits were used for serological testing while polymerase chain reaction, sequencing assays and molecular analysis tools were used to determine genotypes.The CD4 counts were determine using FacsCalibur. Data generated was analysed for serostatus, genotypes and CD4 countdistribution. Comparison of test methods was carried out to determine utility of available test kits. Seroprevalence of HBV was 29% (530), HCV 14 % (256), HSV2 47% (860) and syphilis 14% (256). In this study, 71% (1299) of the subjects (were seropositive with any of the four infections (HBV, HCV, HSV2 and syphilis) while 29% had none.Co-infections ranged from one to multiple infections with 27% of the population having 2 to 4 infections. Highest combination of infections was that of HIV, HBV and HSV (34%); HIV, HSV and syphilis (16%) and HIV, HCV and HSV (10%). Study population mean CD4 count was 382 cells/m^3, the mean difference between gender was 31.79yrs for female and 32.25yrs male. Distribution of CD4 count across population groups indicated that 34% of the study population had counts below 250 cells/m^3. Test algorithm for HBV indicated Determine kit as the best option for screening while for HCV SD Bioline kit had the best performance. Molecular analysis of the samples indicated HIV subtypes detected were A (70%), D (18%), G (7%), and C (5%). For HBV genotypes A (82%), C (9%), D (6%) and E (3%) were identified in the study population. Genotypes 1(86%) and 2b(14%) were detected for HCV. In conclusion; there was a higher burden of infections and molecular variants of co-infections among the HIV infected. It is recommended there is need to make testing for HBV.HCV, HSV2 and syphilis accesible in health facilities alongside that of HIV. Since 34% of individuals were categorized as having advanced HIV disease based on CD4 count, CD4 count monitoring services are necessary in this particular aspect.Monitoring of circulating genotypes is an important undertaking in order to determine trends and emergence of new ones.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Globally management and care for acquired immunodeficiency syndrome (AIDS) has greately improved. major breakthroughs and progress continue to be realized towards management of the HIV epidemic. Such developments including the emergence of diagnostic tests (Alvarez *et al.*, 2012) and effective antiretroviral treatment (ART) (WHO, 2011) both of which have greatly inproved access to HIV care and management.There is also New HIV prevention approaches based on awareness and behavior change.Availability of HIV testing and counseling, new biomedical HIV prevention modalities, such as voluntary medical male circumcision (VMMC) (WHO, 2007), pre-exposure and post-exposure prophylaxis (Hankins & Dybul, 2013), microbicides, (Abdool *et al.,* 2010), and early ART for prevention (Cohen *et al.*, 2011),are some of the major interventions that are helping to turn the tide on AIDS. With the ongoing and upcoming interventions, we are heading to a time when HIV & AIDS will be normalized and individual infected can live longer and quality life (Cohen *et al*., 2011; Kim, *et al.,* 2010).

Notwithstanding the- gains made so far in HIV and AIDS control, it is important to note that significant challenges continue to block the path to a cure for this epidemic (Deeks *et al.,* 2012;)and to create an efficacious vaccine (Kim*et al.,* 2010). It appears the HIV epidemic will remain with us for years to come. At the same time newer challenges continue to emerge especially among the infected population groups; Drug resistance, co-infections and structural challenges like poor responses due to hard economic times especially in under-developed and low and middle-income countries (LMIC).

Co-infection is importance in health because pathogen species can interact within the host. The net effect of this co-infection on human health is mostly negative since disease transmission and progression are enhanced. This also complicates management of such infections.

Co-infections' of HIV and other parasitic, bacterial or viral infections have been reported in a number of studies (Casado *et al.,* 2014; Mitsumoto *et al.,* ,2015; Hull *et al.*,2014; Huy *et al.,*2014; Bernardes *et al.,* ,2014). Notably, morbidity and mortality due to co-infection is momentous, and may be further enhanced in a population owing to the influence of host, viral and environmental factors.

A common feature of co-infections is the resultant viral/bacterial interactions and inter-relationships. HIV interacts and shares mode of transmission and risk factors with Hepatitis B virus (HBV) and Hepatitis C virus (HCV), Herpes Simplex Virus 2 (HSV2) and treponema pallidum. They are transmitted through sexual contact, blood transfusion and vertical transmission (Ikeako *et al.,* 2014).Sexually transmited infections, on the other hand, have bidirectional pathogenic relationship where for example; HIV can accelerate disease progression of other viral infections and vice versa (White *et al.*, 2006). Sexually transmitted co-infections pose considerable health threats to people living with HIV/ AIDS. Syphilis, for example, is related to both increased concentrations of HIV RNA in blood plasma and decreased CD4 cells (Buchacz *et al.,* 2004). Viral STI and genital ulcer diseases, particularly HSV-2, are also linked to increased concentrations of HIV in blood plasma and genital fluids (Duffus *et al.,* 2005).

Co-infections interactions also occur in immunological responses, leading to mixed reactions often with distracting or conflicted results. For example; high amounts of HBsAg have been shown to exhaust CD8+ and CD4+ T cells (Kondo *et al.,* 2013), while HIV infection directly increases HCV replication, leading to hepatic inflammation and release of profibrogenic cytokines. The result is increased hepatocyte apoptosis, microbial translocation from the gut, and eventually impairment of HCV-specific

immune responses. Virological interactions between HIV and HBV demonstrate the impact of HIV proteins on hepatocyte, where co-synthesized proteins of HIV and HBV viruses might compete for host machinery involved in virion secretion pathways and higher HBV DNA (Popov *et al.*, 2008).

Effect of viral interactions has also been shown in diagnostic approaches. Detection of HIV, HBV and HCV, HSV infections and diagnosis is mainly based on immunological assays since these infections elicit development of specific antibodies during infection.Currently, due to proximity in immune responses and targeted sample analyte, tests that combine detection for these viruses are available in the market. Interrelationship also occurs in therapeutic approaches as well. In HIV co-infected individuals, the design of therapeutic regimens, like HAART are recommended to minimize the risk of hepatotoxicity. Drugs like Lamivudine, Emtricitabine and Tenofovir are efficacious against both HBV and HIV (CDC, 2009). As a result, studies on co-infections are important to inform a range of interventions to improve care and management in HIV and AIDS.It on this basis that this study was conceptualized.

1.2 Statement of the problem

The control of HIV and AIDS over the years has been a product of rigorous research and management. However the public health problemofHIV co-infections has added complexity to treatment of HIV and AIDS. Surveillance data from a number of countries indicates upward trends in hepatitis and other viral and bacterial STIs co-existing with HIV. People with HIV who are co-infected with either HBV or HCV are at increased risk of serious, life-threatening complications. On the other hand, most STIs facilitate acquisition of HIV. Individuals living with HIV or those susceptible can benefit from testing for common co-infections.

Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) or HCV coinfection has been associated with higher morbidity and mortality and may impact significantly on

healthcare resource utilization. Gaps exist in prevalence estimates of HIV and HBV/HCV/HSV/Syphilis coinfection needed to inform policy decisions and design of public health interventions in Kenya. For example, its known from a number of national surveys including KAIS 2007;KAIS 2012,KDHS and sentinel surveillance carried out every four and two years respectively that HIV prevalence in Kenya has been declining from 14%, 2008 to 5.6%(KAIS 2012) and currently 4.9% (KENPHIA,2018).This is not the situation with HBV,HCV, syphilis or HSV whose prevalence is not clearly known.The prevalence given for any of these infections in Kenya is mainly estimates since no national surveys are carried out. The prevalence of HBV, HCV, HSV and syphilis co-infections is also not clearly known since testing for these infections is not routinely available in most health facilities.There is also scarce program based data since this infections are yet to be identified as program priority diseases

Since testing for infections is the mainstay of disease diagnosis and entry to various health services, access to testing is an important strategy in disease management. While testing for HIV has been implemented in health programs and is currently universally accessible, this is not the case for co-infections. Many testing platforms and test kits are available for HIV, hepatitis and other STIs including syphilis. However, unlike for HIV testing, there are no universal testing programs for most of other infections with potential for co-infection. Due to changing technological advancements testing strategies are dynamic and testing algorithms are usually developed, evaluated and approved for testing within a country. However performance of test algorithms and their utility has given adequate attention in Kenya. This calls for vigilance and monitoring of suitability of testing strategies available locally. Gaps in testing are likely to affect the quality of diagnostic services and subsequently disease management.

Laboratory testing and treatment monitoring for most infections is dependent on immune responses.Particularly, in HIV and co-infection, CD4 count had been one among the laboratory tests usually requested for. Currently, viral load has replaced CD4 count as the main HIV treatment monitoring parameter. However, CD4 count is still applicable in

treatment decisions especially on changing prophylaxis where CD4 counts below 200 cells/mm^3 is an important consideration.Despite, there is lack of sufficient population based data on CD4 immunological parameter locally to inform such decisions.In addition, lack of CD4 profile in a population of HIV infected is a gap, especially since this can be utilized generally for benchmarking responses to interventions.Testing and monitoring of viral genotypes and testing strategies is important to guide diagnostics, vaccination and disease management.

It is therefore important to address the data gaps existing on co-infections of HIV, genotypes for co-infections, testing strategies and immunological responses including CD4 profile.

1.3 Justification of the study

Human Immunodeficiency virus infection has been a priority infectious disease globally. However trends over the last few years indicate that other infections, increasingly being reported also require much attention. Such infections as Hepatis B and Hepatitis C, Herpes and syphilis especially as co-infections of HIV, are currently becoming important. Yet data on co-infections among the HIV infected is minimal to inform diagnostic strategies and management interventions in Kenya.Health systems failure to prioritize or effectively include co-infections in policy, funding and in-country implementation plans is likely a threat to public healthIt is important that priority infections be handled collectively for multiple gains in control and prevention. This seems to be the preferred case for hepatitis B and hepatitis C, syphilis and herpes among other infections that are currently driving the global HIV co-infections. Results obtained from this study are likely to inform this important policy direction.

HIV infections, hepatis B and C, HSV2 and treponema pallidum share similar modes of transmission. Despite this, services for these infections still largely exist as separate, vertical programs. The gaps that exists in this case is lack of determination of suitable

tests and data to inform testing strategies.Testing for such infections, if integrated could effectively support early interventions and prevention. Genaration of data on testing strategies for co-infections therefore is an important goal.

Availability of CD4 counts data in a population of HIV infected is important for evaluation of interventions. In the wake of increasing prevalence of co-infections, mapping of CD4 variability could provide insight into immunological trends among the HIV and co- infected individuals. Relationship between CD4 cell count and age, sex, and infection categories is also an important consideration in multiple and dual infectionssince $CD4^{+}$ cell dynamics are required in evaluating wellness and response to care and treatment. Availability of such data is important among other measurement like viral load for evaluating and determining success of various disease interventions including those of HIV.World Health Orgainsation recommends CD4 counts as an important determinant in advanced HIV disease.Its also an important parameter for changing of prophylaxis treatment for opoortunistic infections (WHO, 2017).

Genotypes determination on HIV, HBV and HCV is required in order to identify circulating genotypes.Genotypes are important in disease progression and management.Treatment for HCV for example,is mainly genotype dependent,genotypes in HIV are likely to form recombinants that complicate treatment and enhance transmissions since they become more fit and escape immune surveillance and therefore data on genotypes is informative.Since treponema pallidum,the causitive agent of syphilis share similar modes of transmission with HIV, HBV,HCV and HSV2 and specifically enhances HIV transmission,its an important co-infections and was included in this study.

Research evidence is required to influence integration of particular infectious disease services especially HIV and AIDS and related co-infections. This study aimed at generating such evidence for possible policy development towards implementation of integrated health services in HIV and related co-infections. Findings of this study can

therefore provide a policy plank for country based investments in integration of HIV and related coinfections services.

1.4. Research Questions

1. What is the sero-prevalence of HBV, HCV and herpes simplex virus 2 infections in a population of HIV infected in Kenya?
2. What is the sero-prevalence of *treponema pallidum* infections in a population of HIV infected in Kenya
3. What is the distribution of CD4 count among the HIV infected population?
4. What is the relative performance of testing strategies for HIV co-infections in Kenya?
5. What are the circulating genotypes of HIV, HBV and HCV in a population of HIV infected?

1.5. Hypothesis

1. There is no difference in sero-prevalence of HBV, HCV and herpes simplex virus 2 infections in a population of HIV infected in Kenya.
2. The sero-prevalence of *treponema pallidum* infections in a population of HIV infected in Kenya is zero.
3. There is no difference in distribution of CD4 count among the HIV infected population from different study sites.
4. There is no difference in performance of testing strategies for HIV co-infections in Kenya.

5. The circulating genotypes of HIV, HBV and HCV in a population of HIV infected are the same.

1.6 Study objectives

1.6.1 General objective

To determine sero-prevalence of HBV, HCV, HSV2 and syphilis co-infections, genotypes, CD4 distribution and testing algorithms among the HIV infected in Kenya.

1.6.2 Specific Objectives

1. To determine the prevalence of co-infections; HBV, HCV, HSVand syphilis in a population of HIV infected.
2. To describe distribution of CD4 cell counts in a population of HIV infected as an important parameter in advanced HIV disease.
3. To determine the performance of testing strategies for HIV, HBV& HCV in Kenya.
4. To determine circulating HIV subtypes, HBV and HCV genotype among a population of HIV infected in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Transmission of HIV, HBV, HCV, HSV and syphilis

Human Immunodeficiency Virus, Hepatitis B virus, Hepatitis C virus, Herpes simplex virus 2 are viral infections while syphilis is a bacterial infection that share common risk factors and routes of transmission. They are transmitted through sexual contact, blood transfusion, and vertical transmission (Ikeako *et al.,* 2014).

Due to shared routes of infection, HIV-infected persons are at times coinfected with other sexually transmitted infections (STIs). A bidirectional relationship between HIV and several STIs, including HSV-2, hepatitis B and C viruses and syphilis, is likely. HIV-1 may affect the clinical presentation, treatment outcome, and progression of STIs, such as HSV-2, HBV and HCV viruses (Chun *et al*., 2013)). Likewise, the presence of an STI may increase both genital and plasma HIV-1 RNA levels, enhancing the transmissibility of HIV-1, with important public health implications.The interrelationships of HIV and co-infections presents in a number of ways including; transmission, bidirectional pathogenic relationship, recombination, immunological responses in, virological interaction, diagnostic and therapeutic approaches to co-infections.

2.2 Transmission of Human Immunodeficiency virus

More than 80% of adults infected with HIV-1 become infected through the exposure of mucosal surfaces to the virus; the remaining 20% are infected by percutaneous or intravenous inoculations. The risk of infection associated with different exposure routes varies (Hladik *et al.,* 2008), but no matter the transmission route, the timing of the

appearance of viral and host markers of infection is generally uniform and follows an orderly pattern (Weiss *et al.,* 2010). Immediately after exposure and transmission, as HIV-1 is replicating in the mucosa, submucosa, and draining lymphoreticular tissues (Weiss *et al.,* 2010), the virus cannot be detected in plasma; this so-called eclipse phase generally lasts 7 to 21 days (Lee *et al.,* 2009). Given the varied routes of viral transmission including cervicovaginal, penile, rectal, oral, percutaneous, intravenous, in utero and the distinctly different histologic features of these tissues several cell types are candidates for early infection. The preponderance of evidence implicates CD4 T cells and Langerhans' cells as the first targets of the virus, but other dendritic cells may play an important accessory role. However, recent observations of mucosally transmitted strains of HIV-1 reveal that monocyte-derived macrophages are generally poor targets for infection as compared with CD4 T cells. (Salazar-Gonzalez, 2009 & Li *et al.,* 2010).

Regardless of the route of viral transmission and the cells first infected, within a few days, viral replication converges on the lymphoreticular system of the gastrointestinal tract (gut-associated lymphoid tissue)(Salazar-Gonzalez ,2009). In this tissue, in both humans and macaques, the phenotype of most productively infected cells appears to be the resting CD4 T cell lacking activation markers and expressing low levels of the chemokine receptor CCR5(Lee *et al.*,2009). Many of these cells express $\alpha_4\beta_7$ integrin receptors and type 17 helper T (Th17)–cell surface markers (Richman *et al.,* 2009). Since these receptors are also detected on T cells harvested from the genital mucosa, they may play an important role in HIV acquisition(Lee *et al.*,2009) The rapid expansion of HIV-1, first in gut-associated lymphoid tissue and then systemically(Richman *et al.*,2009), along with a sharp rise in plasma levels of viral RNA, is clinically important because of the coincident irreversible destruction of reservoirs of helper T cells and the establishment of viral latency (defined as the silent integration of HIV-1 DNA into the genomes of resting T cells, an effect that has stymied curative treatment efforts (Ribeiro *et al.,* 2010).

HIV transmission is largely related to sexual risk behaviors and injecting drug use. Individuals with recent human immunodeficiency virus type 1 (HIV-1) acquisition are likely to be a major source for - new infections because they have a high level of plasma virus, and the circulating virions possess unique properties that are highly suited for transmission. Various studies suggest that 5 to 50% of new HIV-1 infections are acquired from newly infected subjects (Abu-Raddad *et al.,* 2008; Lewis *et al.,* 2008).

Transmission rates are sharply elevated during the first few months of HIV infection (Hollingsworth & Anderson., 2008), likely due to increased viral concentrations and founder virus transmission advantages that facilitate transmission at a lower inoculum (Carlson *et al.,* 2014).The strongest predictor of HIV sexual transmission is plasma viral load. As plasma viral load increases, the risk of transmission also increases.

Transmission of HIV-1 from mother to child can take place during pregnancy, labor, and delivery as well as after birth via breastfeeding especially in mixed feeding. The risk of transmission varies at different stages with the risk during pregnancy ranging from 5–10%, 10–20% during labor and delivery, and 10–20% through mixed infant feeding. It is estimated that in the absence of any intervention to prevent mother-to-child transmission (MTCT) ranges from 15–45%. This rate can be reduced to levels below 5% with effective interventions (WHO, 2011).

2.3 Transmission of Herpes simplex virus

Following skin–skin or skin–mucosa contact, HSV enters epithelial cells in the mid-to-basal epidermis, where it replicates and spreads to adjacent cells. HSV is then transported via sensory neuronal axons to the neuronal cell bodies of dorsal root ganglia (Hofstetter, *et al.,* 2014), where it again replicates and spreads to neighbouring neurons. Thereafter, virus either establishes latent infection or is transported back to mucocutaneous sites where it is released and replicates, potentially leading to cell lysis

and the characteristic vesicular and/or ulcerative lesions of primary genital herpes (Hofstetter, *et al.,* 2014).

Studies have shown that HSV infection increases HIV-1 viral load (Duffus *et al.,* 2005). HSV-2 entry receptors are constitutively expressed on immune cells, thereby allowing the uptake of HSV-2 (Karasneh *et al.,* 2011). Using real-time polymerase chain reaction, Sartorie *et al*.(2011) reported that HSV-2 upregulated expression of CCR5, a coreceptor essential for HIV entry in macrophages. As most of the primary HIV infections have been attributed to CCR5 tropic viruses (Margolis & Shattock), this study provided one of the possible mechanisms by which HSV-2 increases the chances of acquiring HIV. Langerhan cells (LCs) prevent HIV-1 transmission via the C-type lectin, langerin, which targets the virus for degradation, thereby reducing HIV-1 transmission to T cells. De Jong *et al., 2010* showed that HSV-2 increases susceptibility of LCs to HIV-1 by downregulating langerin expression. Also, the HSV released by infected LCs compete with HIV-1 for langerin binding. This allows HIV-1 to infect LCs efficiently and transmit infection to T cells (de Jong *et al.,* 2010).

Three biological mechanisms contribute to increased HIV-1 incidence among HSV-2 seropositive individuals: physical disruption of the epithelial surface by HSV-2, recruitment and persistence of inflammatory cells in the genital tract during HSV-2 reactivation at mucosal surfaces, and increase in plasma HIV-1 RNA with HSV-2 co-infection. Macro and micro HSV-2 ulceration creates a portal of entry for HIV-1 (Celum *et al.,* 2004). Further, HSV-2 reactivation increases the recruitment and persistence of inflammatory cells targeted by HIV-1 (Celum *et al.,* 2004; Zhu *et al.,* 2009). Recent studies have demonstrated an increase and persistence of HIV-1 target cells (CCR-5-expressing CD4 T cells and CD-SIGN-expressing dendritic cells) that are present after ulcer healing in normal skin, are minimally affected by antiviral therapy, and increase susceptibility for HIV-1 (Zhu *et al.,*2009).

2.4 Transmission of Hepatitis B virus

Hepatitis B virus (HBV) is transmitted via infectious blood and body fluids and can survive on environmental surfaces for at least 7 days (Michaela *et al.,* 2015). The HBV Incubation period for range from 1 to 6 months with an average of 2 to 3 months. Often, viral hepatitis is referred to as a silent epidemic, because many infected patients have no symptoms, are not diagnosed and are unaware of their infectious status (US HHS, 2011). This increases the risk for the spread of the disease and the occurrence of outbreaks.

The most common risk factors for acquiring HBV among adults are injection drug use and sexual contact, particularly with multiple sexual partners and men who have sex with men. Even though risk factors are similar with HIV, HBV is more efficiently transmitted..Percutaneous exposure to infected blood carries a 30% risk of HBV transmission compared with a 0.3% risk of HIV transmission (Stephen & Marion, 2007).

The modes of HBV transmission at an early age include perinatal transmission, vertical transmission from an infected mother to her infant, and horizontal transmission from an infected household contact to the child (Muhammad et *al.,* 2013).

2.5 Transmission of Hepatitis C virus

Hepatitis C infection is bloodborne and prevalent among intravenous drug users (IVDU) (Nelson *et al.,*2011) and men who have sex with men (MSM), particularly those with HIV co-infection (Yaphe *et al.,*2012; Hagan *et al.,* 2014) and their contacts. African and Asian countries are thought to have the highest burden of infection, but current prevalence estimates are variable (Barth *et al.,* 2010).

Transmission of HIV and HCV can occur through percutaneous exposure to blood, sexual intercourse, and from a mother to her infant, but the efficiency of transmission varies for each virus. HCV is ∼10 times more infectious than HIV through percutaneous blood exposures (Jennifer *et al.,* 2014). HIV-infected persons who inject drugs represent

the majority of HCV/HIV co-infection. More specifically, HCV co-infection rates among HIV-infected individuals who use injection drugs often exceed 90%, as demonstrated in studies in Europe and Asia (Quan *et al.*, 2009).

Studies have revealed that transmission of HIV through sexual intercourse and from a mother to her infant is more efficient than for HCV transmission, although the efficiency of HCV transmission might increase in the presence of co-infection. For example, in a women and infants transmission study, the risk of HCV infection was 3.2-fold greater in HIV-1-infected infants compared with HIV-1-uninfected (17.1% versus 5.4%) (Jennifer *et al.*, 2014).

Hepatitis C virus infection is strongly associated with health inequities; in low- and middle-income countries, infection with HCV is most commonly associated with unsafe injection practices and procedures such as renal dialysis and unscreened blood transfusions (Candotti *et al.*, 2001). Between 8 and 12 billion injections are administered yearly around the world and 50% of these are considered to be unsafe (mainly in sub-Saharan Africa and Asia) (Kane *et al.*, 1999).

2.6 Transmission of Syphilis

Most cases of syphilis are transmitted by sexual contact (vaginal, anogenital and orogenital), but it can also be spread congenitally (in utero or less commonly during passage through the birth canal) (De Santis *et al.*, 2012). Rare cases of acquisition through blood products and organ donation have also been reported (Owusu-Ofori *et al.*, 2011; Perkins *et al.*, 2010) as have cases resulting from occupational and other exposures (Oh *et al.*,2008; Zhou *et al*.,2009; Chiu & Tsai,2012; Fanfair *et al.*, 2014;).

The probability of syphilis transmission within a sexual partnership depends on the frequency of sex, type of sexual contact (penile-vaginal, penile-anal or penile-oral), the stage of syphilis in the source, susceptibility of the partner and use of condoms (Gray *et al.,* 2011).

Despite its preventable nature, congenital syphilis remains common in many parts of the world. Most cases of syphilis transmission during pregnancy are thought to occur in *utero* transplacentally, although transmission during birth is possible (Shafii *et al.,* 2008). Syphilis transmission has occurred via blood transfusion, organ transplant and occupational exposure (Raguse *et al.,* 2012). In addition, the transmission of syphilis via human bite in both sexual and non-sexual circumstances has been reported (Chiu *et al.,* 2012), as well as transmission via mouth-to-mouth feeding of infants with pre-chewed food from infected relatives (Fanfair *et al.,* 2014).

2.7 Pathobiology: Bidirectional pathogenic relationship between HIV and co-infections

STIs have a bidirectional pathological relationship with HIV. HIV can accelerate disease progression of other viral infections and vice versa (White *et al.,* 2006). Sexually transmitted co-infections pose considerable health threats to people living with HIV/ AIDS. Syphilis, for example, is related to both increased concentrations of HIV RNA in blood plasma and decreased CD4 cells (Buchacz *et al.,* 2004). Viral STI and genital ulcer diseases, particularly HSV-2, are also linked to increased concentrations of HIV in blood plasma and genital fluids (Duffus *et al.,* 2005). When individuals are immune compromised, co-occurring STI are more difficult to treat and symptomatic periods may linger. Local inflammation of the genital tract caused by viral and non-viral STI promotes HIV shedding, therefore increasing HIV infectiousness (Cohen *et al.,* 1997). HIV concentrations in semen and vaginal fluids are directly associated with the number of leucocytes migrating to the genital tract. A dose relationship exists between leucocyte concentrations, a marker for inflammatory processes, and HIV viral shedding. Various

STI differ in their impact on HIV shedding, and the same STI can have different effects on HIV concentrations in men and women. Overall, the greater the inflammatory response, the greater the impact on HIV infectiousness (Johnson *et al.*, 2008). Therefore, the STI with the greatest impact on HIV shedding are those that produce genital ulcers and urethral/vaginal discharge, especially syphilis, HSV-2, among others.

The increase in mortalities and morbidities from liver diseases amongst HIV patients is in part due to co-infection with hepatitis B and C viruses (HBV and HCV) as these viruses promote liver fibrosis by increasing intra-hepatic apoptosis (Iser *et al.,* 2011; Chiekulie *et al.*, 2013). Huma immunodeficiency virus depletion of gastrointestinal tract associated CD4 lymphocytes also makes the gastrointestinal mucosa more permeable thereby causing microbial translocation and liberation of endotoxins such as lipopolysaccharide (LPS) which is pro-inflammatory and pro-fibrotic on the liver (Balagopal *et al.,*2008; Crane *et al.,*2012).

2.8 Bidirectional pathogenic relationship between HBV and HIV

The spectrum of clinical manifestations of HBV infection varies in both acute and chronic disease. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis. During the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Extrahepatic manifestations can also occur with both acute and chronic infection (Kowdley *et al.,* 2012**)**

Globally,the consequences of hepatitis B virus (HBV) and human immunodeficiency virus (HIV) co-infection on progression of severe liver diseases is a serious public health issue. HIV alone, infects nearly 40 million individuals leading to about 5% cases of mortality while HBV circulates in > 400 million persons causing about one million deaths, annually worldwide (Thio,2009; Sun,2014) HBV co-infection persists in almost 25% of HIV-infected adults ,with approximately 50-90% of them acquiring it at birth or

in early age(Yim,2006). The spectrum of HIV-induced liver diseases includes hepatitis, alcohol-associated steatohepatitis, non-alcoholic steatohepatitis, endothelialitis, necrosis and granulomatosis (Bongiovanni *et al.,* 2007). Moreover, HIV co-infection leads to further complications in liver diseases compared to HBV mono-infection (Thio *et al.,* 2002). It is found that in HIV co-infected individuals, liver-related mortality is over 17 times higher than those infected with HBValone (Sun,2014).

2.9 Bidirectional pathogenic relationship between HCV and HIV

Hepatitis C virus causes both acute and chronic infection. Acute HCV infection is defined as the presence of HCV within six months of exposure to and infection with HCV. It is usually clinically silent, and is only very rarely associated with life-threatening disease. Spontaneous clearance of acute HCV infection occurs within six months of infection in 15–45% of infected individuals in the absence of treatment. Almost all the remaining 55–85% of persons will harbour HCV for the rest of their lives (if not treated) and are considered to have chronic HCV infection.Left untreated, chronic HCV infection can cause liver cirrhosis, liver failure and hepatocellular carcinoma (HCC).

Chronic HCV infection in HIV infected individuals differs from that in individuals without HIV infection in several ways. Coinfected individuals have a higher HCV viral load (Daar, 2001) and thereby have higher transmission rates. Coinfection is also associated with more rapid progression of fibrosis (Benhamou *et al.,* 1999), cirrhosis (Graham *et al.,* 2001), end-stage liver disease (ESLD) and hepatocellular carcinoma (Ragni *et al.,* 2001; Martin *et al.,*2004; Sulkowski *et al.,*2007; Limketkai *et al.,*2012; Kirk *et al.,*2013;). Recently, Kirk *et al.,* (2013) reported that even after an adjustment for HCV viral load (VL) levels, HBV chronicity, sex, race and alcohol use, HIV-HCV coinfection was associated with liver fibrosis as advanced as those without HIV who were 10 years older.

2.10 Bidirectional pathogenic relationship between HSV-2 and HIV

HSV-2 infection increases the risk of HIV acquisition by approximately three-fold (Freeman *et al.,* 2006), and the increase in risk is even greater in those with newly-acquired (incident) HSV-2 infection (Reynolds *et al.,* 2003; Brown *et al.,* 2007). Among those with HIV, HSV-2 infection increases genital shedding of HIV (Todd *et al.,* 2013), increases transmissibility of HIV up to five-fold through genital ulcers (Gray *et al.,* 2001), and may accelerate HIV disease progression (Lingappa *et al.,* 2010). In turn, HIV infection increases HSV-2 shedding frequency and quantity (Augenbraun *et al.,* 1995; Schacker *et al.,* 1998). The high burden of HSV-2 infection is thought to have contributed substantially to HIV prevalence in a number of settings, most notably in sub-Saharan Africa (Wald, 2002; Freeman *et al.,* 2006), although it is difficult to control for shared sexual risk driving both epidemics (Kenyon *et al.,* 2013). Daily suppressive antiviral therapy against HSV-2 has been shown to reduce symptomatic recurrences and asymptomatic HSV shedding; however, in clinical trials, suppressive therapy did not reduce the excess risk of HIV acquisition or transmission due to HSV-2 nor fully suppress HSV-2 shedding in one study (Kenyon *et al.*, 2013).

2.11 Bidirectional pathogenic relationship between syphilis and HIV

Sexually transmitted co-infections increase HIV infectiousness through local inflammatory processes. The prevalence of STI among people living with HIV/AIDS has implications for containing the spread of HIV in general and the effectiveness of HIV treatments for prevention. Syphilis is related to both increased concentrations of HIV RNA in blood plasma and decreased CD4 cells. (Buchacz *et al.,* 2004). STIs have a bidirectional pathogenic relationship with HIV; HIV can accelerate disease progression of other viral infections and vice versa (White *et al.,* 2006). When individuals are immune compromised, co-occurring STIs are more difficult to treat and symptomatic periods may linger. In addition, local inflammation of the genital tract caused by viral and non-viral STI promotes HIV shedding increasing HIV infectiousness (White *et al.,*

2006).Importantly, HIV-infected patients may present with multiple chancres that are deeper and slower to resolve than the solitary chancre typically seen in HIV-uninfected patients (Wade *et al.,* 2005). It is also evident that primary and secondary syphilis overlap more often in patients with HIV infection than in those without (Rompalo *et al.,* 2001a & b). In fact, one quarter of coinfected patients present with concurrent primary and secondary disease (Karp *et al.,* 2009).

HIV-infected patients may progress to tertiary syphilis more rapidly than HIV-uninfected, resulting in earlier onset of cardiovascular and neurologic sequelae (Maharajan and Kumaar, 2005). Optic neuritis, uveitis, and other ocular manifestations of syphilis are common among HIV-infected patients but not among HIV-uninfected patients. This atypical presentation of syphilis is clinically important: One study showed that 85% of HIV-infected patients with ocular syphilis had concomitant neurosyphilis (Joseph *et al.,* 2011).

2.12 Recombination and HIV co-infections

Recombination is a general molecular process that generates new combinations of genetic material (Leach, 1996). Viral recombination occurs when two viruses of different parental strains superinfect the same cell and interact during replication to generate progeny whose genomes consist of genetic segments obtained from both parental strains. Recombination has been shown to participate in the evolution of several alphaherpes viruses (Thiry *et al.,* 2005) as well as betaherpes viruses (Haberland *et al.,* 1999) and gammaherpes viruses. The underlying mechanisms are poorly understood but are associated with DNA replication and different cell factors. Several studies have shown that homologous recombination occurs frequently under experimental conditions (Schynts *et al.,* 2003; Meurens *et al.,* 2004;**)**.Recombination has also been shown experimentally for several varicelloviruses, VZV, pseudorabies virus, bovine herpesvirus-1, and feline herpesvirus (Henderson *et al.,* 1990; Schynts *et al.,* 2003**)**. Furthermore, recombinants between bovine herpesvirus-1 mutants after coinoculation of

calves by the natural route of infection have been demonstrated recently **(**Schynts *et al.,* 2003)**.** Also, wild-type recombinants have recently been described forHSV-1 (Bowden *et al.,* 2004**;** Norberg *et al.,* 2004**)**, VZV (Norberg *et al.,* 2006), and pseudorabies virus.

2.12.1 HIV Recombination

One of the major characteristics of HIV-1 is its high genetic variability and extensive heterogeneity. This characteristic is due to its molecular traits, which allows it to vary, recombine, and diversify at a high frequency. As such, it generates complex molecular forms, termed recombinants, which evade the human immune system and so survive. There is no sequence constraint to the recombination pattern as it appears to occur at inter-group (between groups M and O), as well as inter and intra-subtype within group M. Rapid emergence and active global transmission of HIV-1 recombinants, known as circulating recombinant forms (CRFs) and unique recombinant forms (URFs). HIV-1 possesses high recombination rates, due to the highly recombinogenic reverse transcriptase (RT) enzyme (Katherine *et al.,* 2013). RT has a high error rate and low binding affinity, which are necessary for strand transfers of reverse transcription.Co-circulation of different subtypes and CRFs result in the continuing emergence of new intersubtype recombinants or M/O recombinants (Yamaguchi *et al.,* 2004)

2.12.2 HBV recombination

Recombination is a very important mechanism for HBV genome evolution and variation. Novel variants generated by recombination events between different hepatitis B virus (HBV) genotypes have been increasingly documented worldwide, and the role of recombination in the evolutionary history of HBV is of significant research interest (Kramvis *et al.,* 2005; Yang *et al.,* 2006; Ye *et al.,* 2010). B/C recombinants are prevalent in Southeast Asia and E.ast Asia, but not in Japan (Sugauchi *et al.,* 2002). Other inter-genotypic recombinants, such as A/D (Owiredu *et al.,* 2001), A/E (Kurbanov *et al.,* 2005), C/D (Wang *et al.,* 2005) and G/C (Suwannakarn *et al.,* 2005), have also

been identified in different geographical regions. In fact, the generation of the proposed "genotype I" was also associated with a triple recombination event among genotypes A, C and G (Huy *et al.,* 2008).

2.12.3 Recombination in Hepatitis C Virus

Regarding HCV, recombination has been reported both inter and intragenotypic in populations in different geographic locations. Some earlier reports described some HCV strains from Honduras in which the study of partial sequences from different regions of the viral genome resulted in HCV discordant genotype, providing first evidence for the possible existence of HCV recombination (Yun *et al.,* 1996). However it was not until 2002 that the first convincing report of an intergenotypic HCV recombinant strain was published by Kalinina *et al.,* (2002) in Saint Petersburg (Russia). These authors described six different natural HCV strains that belonged to different subtypes, 2k and 1b. They found that the 5' untranslated region and the core coding region belonged to subtype 2k, whereas the NS5B region corresponded to subtype 1b (Kalinina *et al.,* 2002). The reported recombinant was cautiously designated RF1_2k/1b, in agreement with the nomenclature used for human immunodeficiency virus (HIV) recombinants (Kalinina *et al.,*2002). This same recombinant strain has since then been isolated in other countries, like Ireland(Moreau *et al.,*2006), Uzbekistan(Kurbanov *et al.,*2008), Cyprus(Demetriou *et al.,*2011), France(Morel *et al.,*2010) and Estonia(Tallo *et al.,*2007)

At least ten other different intergenotypic recombinant forms (RFs) of HCV have been described and are totally or partially characterized recombinant forms between genotypes 2 and 6 described in Vietnam(Noppornpanth *et al.,*2006) and Taiwan(Lee *et al.,*2010), between genotypes 2 and 5 described in France(Legrand *et al.,*2007), between genotypes 3 and 1 in Taiwan(Lee *et al.,*2010) and China(Du *et al.,*2012), and between genotypes 2 and 1 reported in Japan, the United States and the Philippines (Kageyama *et al.,*2006; Bhattacharya *et al.,*2011; Yokoyama *et al.,*2011; Yokoyama *et al.,*2011; Hoshino *et al.,*2012;). Considering that recombination may influence vaccine

development, virus control programs, patient management as well as antiviral therapies, it is important to determine the extent to which this mechanism plays a role in HCV evolution (Hoshino *et al.,* 2012).

2.12.4 Recombination in Herpes Simplex Virus

Although limited data on HSV genotypes is available. There is evidence of recombination reported either as a gene function or as an interaction between two strains of viruses existing in a host cell. Phylogenetic analysis has revealed a divergence into at least two genogroups arbitrarily designated A and B (Norberg *et al.,* 2007). , a new genetic variant of HSV-2 (named HSV-2v) characterized by a much higher degree of variability has been described (Burrel *et al.,* 2015). These findings suggest that HSV-2v may have acquired genomic segments from chimpanzee alphaherpesvirus (ChHV) by recombination. This new HSV-2 variant from west/central Africa highlights the possible occurrence of recombination between human and simian herpesviruses under natural conditions, potentially presenting greater challenges for the future. Norberg *et al.* (2011) has described recombinants for HSV-1 and VZV, suggesting recombination is a prominent feature of the evolution of HSV-2 as well.

2.12.5 Recombination in Syphilis

Pathogenic treponemes, similar to syphilis-causing *Treponema pallidum* subspecies include *T. pallidum ssp. pertenue, T. pallidum ssp*. *endemicum and Treponema carateum*, which cause yaws, bejel and pinta, respectively. Genetic analyses of these pathogens reveal striking similarity among these bacteria. Pathogenic treponemes of this group differ in two principal etiopathogenic parameters including host range and degree of invasivity; with TPA being the most invasive (Smajs *et al.,* 2012). Genomic studies in syphilis are still developing and little information is available on recombinations among the various strains of T *pallidum*.

2.13 Immunological responses in co-infections

Immune responses to viral infection can be divided into innate responses, mediated by complement, phagocytes and natural killer (NK) cells), and adaptive responses, mediated by B lymphocytes, CD4+ T lymphocytes, CD8+ T lymphocytes, and antigen-presenting cells such as dendritic cells and macrophages.

2.13.1 Immune Responses in HIV /HBV infection

In HIV-infected patients, the CD4-independant tissue tropism, including liver and its impact on hepatopathogenesis has been described (Parvez *et al.*, 2013). HIV infection requires interaction of the viral envelop protein (gp^{120}) with cell surface receptor, CD4, aided by chemokine co-receptors, CCR5 and CXCR4 (Dalgleish *et al.,* 1984; Berger *et al.,* 1999). While some HIV strains also require both of these co-receptors, few rare laboratory-adapted isolates are shown to utilize alternative co-receptors like, CCR1, CCR2b, CCR8, CXCR6, GPR1 and GPR15/Bob, *in vitro*(Berger *et al.*,1998). Ample of reports has demonstrated cross-tissue tropism of HIV capable of infecting a variety of non-lymphoid tissues *in vivo* (Cao, 1990).

Further, HIV RNA, proviral-deoxyribonucleic acid (DNA) and capsid antigen (p^{24}) have been detected in liver sinusoidal endothelial cells, Kupffer cells, portal mononuclear inflammatory cells as well as hepatocytes of infected patients (Cao ,1992). Iser *et al.,* (2010) have demonstrated the indispensability of CCR5 and CXCR4 in HIV entry into multiple liver cell lines. The activation of HBV-specific $CD8^+$ cells, crucial in controlling HBV replication as well as the liver pathogenesis (Chang *et al.,* 2009), is shown to be clearly impaired during HIV secondary infection. This might partially explain the tendency of progression of acute hepatitis B towards chronicity in HIV co-infection cases (Thimme *et al.,* 2003). In co-infected patients with lower ALT levels compared to HBV mono-infection, the progression of cirrhosis and HCC is suggested to be related to lower $CD4^+$ cell counts (Clifford *et al.,* 2008).

For HBV, The age of infection largely dictates the type and level of the immune response to acute HBV infection and the subsequent natural history of CHB.

HBV interferes with the adaptive immune response through multiple mechanisms. Early functional virus-specific CD4+ and CD8+ T-cell responses are attenuated, coinciding with a surge in the immunosuppressive cytokine IL-10, while type I IFN, IL-15, and IFN-λ1 are not induced (Dunn *et al.,* 2009). Patients who resolve HBV infection have superior HBV specific CD4+ and CD8+ T cell frequency and function when compared to patients who become chronically infected (Bertoletti *et al.,* 2009). CHB has been associated with an impaired ability of CD8+ T cells to lyse, proliferate, and produce certain cytokines (TNF-α and IL-2), as well as with a reduced ability to produce IFN-γ (Bertoletti *et al.,* 2009).High amounts of HBsAg have been shown to exhaust CD8+ and CD4+ T cells (Kondo *et al.,* 2013).

2.13.2 Immune Responses in HIV/HCV infection

Although HIV infection significantly impairs cellular immunity by $CD4^+$ T cell depletion, it has become clear that the increased liver inflammation and fibrosis seen in HIV/HCV co-infection is due to a combination of impaired cellular immunity with direct effects on the gastrointestinal tract and the liver. *In vitro* exposure to HIV (or its envelope protein gp120) increases HCV replication in hepatocytes 2-3 fold. This increased replication can be blocked by antibodies to the HIV co-receptors CCR5 and CXCR4, suggesting that although HIV does not directly infect hepatocytes, it mediates its effects indirectly through these co-receptors. Similarly, exposure of hepatic stellate cells (HSCs) to HIV *in vitro* leads to increased production of specific cytokines. HIV can directly infect HSCs leading to increased collagen and monocyte chemoattractant (MCP-1) production **(**Tuyama *et al.,* 2010).

Although HIV infection causes chronic immune activation, the immune response to specific pathogens including HCV, is dysfunctional, presumably related to depletion of

$CD4^+$ T cells. In chronic HCV infection, co-infected patients have reduced HCV-specific lymphoproliferative responses, and those with higher $CD4^+$ T cell counts tend to have more robust responses. Natural killer (NK) cells are stimulated by $CD4^+$ T-cells in an IL-2 dependent fashion and can modulate liver fibrosis by killing activated HSCs. (Jennifer *et al.,* 2014). These data indicate that HIV infection directly increases HCV replication, augments HCV-induced hepatic inflammation and the release of profibrogenic cytokines, increases hepatocyte apoptosis, increases microbial translocation from the gut, and leads to an impairment of HCV-specific immune responses. These combined effects might explain the increased liver inflammation and fibrosis seen in HIV/HCV co-infection.The spontaneous HCV resolution rate is considered to be lower in HIV infected individuals than in HCV infected individuals without HIV, with estimates varying between 5and 25% (Villano *et al.,* 1999).

Individuals who resolve HCV infection have a broader and more sustained CD4 and CD8 T-cell response (Burke *et al.,* 2010)) than individuals with chronic HCV. The detection of fully functional HCV-specific CD4 T lymphocytes during acute infection is associated with subsequent HCV resolution (Rehermann *et al.,* 2005). Further, a temporal association between the detection of HCV-specific CD8 T-cell responses indicates that CD8 T-cells are also important in HCV resolution. Hence, both CD4 and CD8 T-cell responses are required to achieve HCV resolution. The importance of CD4 T lymphocytes in resolution is further underscored by the lower resolution rate found with HIV coinfection, which may in part be due to CD4 T lymphocyte depletion Burke *et al.,* 2010.

CD4 countdisparity between absolute and Percentages has been reported with high frequency in individuals with fibrosis, cirrhosis and ESLD (Claassen *et al.,* 2012; Hull *et al.,* 2014;) and thus, the consideration of CD4 measurements in individuals with HCV may be appropriate.

2.13.3 Immune responses in HIV/ HSV infection

During HIV and HSV infections, deficits in host innate or cellular immunity results in more frequent clinical disease, which can be persistent or disseminated. Indeed, persistent HSV-2 is a common presentation of advanced HIV-1 infection; low CD4 counts and high viral load are associated with increased frequency of HSV-2 shedding (McClelland *et al.,* 2002; Posavad *et al.,* 2004). Use of antiretroviral therapy with subsequent increases in CD4 cells does not completely eliminate the effect of HIV-1 infection on HSV-2 shedding and GUD (Posavad *et al.,* 2004; Ameli *et al.,* 2006).

HSV-2 up-regulates HIV-1 replication at the cellular level through different pathways including transactivation of the HIV-1 long terminal repeat by HSV-1-infected cell protein (ICP)0, ICP4, ICP27 and US11 gene products and cytokine release and antigen presentation from HSV-2 infected cells (Albrecht *et al.,*1989; Golden *et al.,*1992; Palu *et al.,*2009). This up-regulation increases HIV-1 viral load, the primary determinant of HIV-1 transmission (Quinn *et al.,* 2000; Baeten *et al.,* 2011). Plasma HIV-1 RNA levels in HIV-1/HSV-2 co-infected individuals pre-ART are 0.20–0.55 $\log_{10}$ copies/ml higher than those without HSV-2 co-infection (Mole *et al.,* 1997; Serwadda *et al.,* 2003; Gray *et al.,* 2004; Duffus *et al.,* 2005), which may translate to more rapid progression to AIDS and/or increased HIV-1 infectiousness for sexual partners (Mellors *et al.,* 1995; Quinn *et al.,* 2000; Schacker *et al.,* 2002).

2.13.4 Immune responses in syphilis

The particular manifestation of *Treponema pallidum* infection depends upon time, site, and the immune status of the infected individual. Time (duration of infection) relates to the designation of the stages of syphilis as primary, secondary, and tertiary disease (Salazar *et al.,* 2007). These clinical stages, in turn, reflect the interaction of the infectious agent with the host, and the effects of the immune response on the infection. Site refers to whether the lesions are located in the skin or mucous membranes (example

the mouth), or internally. Many questions remain unanswered about the biological characteristics of the spirochete—most importantly, why *T. pallidum* can persist for extended periods in tissues, despite evoking vigorous cellular and humoral immune responses (Radolf and lukehart, 2006).

Since *T.Pallidum* has extraordinarily low density of integral outer membrane proteins (OMPs) (Cox *et al.,* 2010; Anand *et al.,* 2012) and the limited antibody responses they elicit in humans (Desrosiers *et al.,* 2011), anti-treponemal antibodies alone are unlikely to be sufficient to control bacterial replication and prevent further dissemination.

Venereal syphilis is well recognized as a risk factor for the transmission of HIV (Salazar *et al.,* 2007). Evidence has suggested that the epidemiological synergy between syphilis and HIV infection is due not only to the association of shared high-risk behaviors or the breakdown of the epithelial barrier associated with genital ulcers but also to the ability of *T. pallidum* and its constituent lipoproteins to activate immune cells that are likely to be present in syphilitic genital lesions (Radolf and lukehart, 2006; Salazar *et al.*, 2007). Syphilis and HIV could interact at the cellular level in tissues and blood. Resident and recruited mDCs and pDCs in skin have been shown to express high levels of 2 key HIV coreceptors, CCR5 and DC-SIGN.Activated DCs, which express high levels of CCR5 and DC-SIGN, could become infected or more effectively capture HIV in tissue via gp-120, respectively, and then migrate to regional lymph nodes where they may transmit the virus to $CD4^+$ naive T cells (Salazar *et al.*, 2007). Likewise, the abundance of $CCR5^+$ $CD4^+$ T cells in tissues could facilitate the direct infection by HIV without needing DCs or macrophages (CDC, 2002). Additionally, increased expression of CCR5 and DC-SIGN in circulating DCs and the striking shift of $CD4^+$ T cells expressing CCR5 within the memory ($CD45RO^+$) immunophenotype has the potential to generate an environment in PB that is highly conducive to HIV transmission (Buchacz *et al.,*2004). $CD4^+$ T cell activation, may also contribute to a more rapid $CD4^+$ T cell decline, as has been seen in the context of other HIV coinfections (Buchacz *et al.,* 2004).

2.14 Virological interactions in HIV/ HBV infections

The spectrum of HIV-induced liver diseases includes hepatitis, alcohol-associated teatohepatitis, non-alcoholic steatohepatitis, endothelialitis, necrosis and granulomatosis (Bongiovanni *et al*.,2007) It is found that in HIV co-infected individuals, liver-related mortality is over 17 times higher than those infected with HBValone (Sun *et al*.,2014).

HBV-DNA can be directly integrated into the host DNA but, unlike what happens for retroviruses, integration has no role in the viral replicative cycle since it involves only fragments of the viral genome. Integration is possibly a cofactor related to the development of HCC (Pollicino *et al*., 2004; Neuveut *et al*., 2010). Moreover, HBV may exert its direct pro-oncogenic role also through the production of proteins - such as X and truncated preS-S proteins - with potential transforming properties (Zoulim *et al*., 2005; Pollicino *et al*., 2011).

HIV infection requires interaction of the viral envelop protein (gp120) with cell surface receptor, CD4, aided by chemokine co-receptors, CCR5 and CXCR4. A number of reports have demonstrated cross-tissue tropism of HIV capable of infecting a variety of non-lymphoid tissues in vivo. Further, HIV RNA, proviral-deoxyribonucleic acid (DNA) and capsid antigen (p24) have been detected in liver sinusoidal endothelial cells, Kupffer cells, portal mononuclear inflammatory cells as well as hepatocytes of infected patients (Mohmed, 2015). The CCR5 and CXCR4 co-receptors of hepatocytes as well as stellate cells are reported to involve in fibrogenic developments (Hong *et al*., 2009). Very recently, the viral gp120 has been demonstrated to modulate hepatic chemokine receptors as well as stellate cell biology, and linked to liver fibrogenesis (Svegliati *et al*., 2010; Bruno *et al*., 2010). In the co-infection, the co-synthesized proteins of the two viruses might compete for host machinery involved in virion secretion pathways (Popov *et al*., 2008).Though several studies have demonstrated impact of HIV proteins on hepatocyte biology (Svegliati *et al*., 2010),only limited data is available on viral interactions between HBV and HIV proteins.

2.15 Virological interactions in HIV/ HCV infections

Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) cause considerable global health problems. Coinfection with HCV is frequent in HIV infected individuals, because the viruses share modes of transmission (Kim and Chung, 2009; Chen *et al.*, 2014). While HCV is mainly a hepatotropic virus, HIV infects a variety of immune cells, such as CD4+ T lymphocytes and monocytes/macrophages. However, several studies showed that HCV replicates outside the liver (Sengupta *et al.*, 2013), while HIV may infect hepatocytes and Hepatic Stellate Cells (HSCs) as well (Kong *et al.*, 2012). The disease course of HIV-1 infection is associated with a profound dysregulation of the immune system. During HIV/HCV coinfection, the immune dysregulation is more severe, leading to lower rates of spontaneous control of HCV infection as well as to a faster progression of liver disease (Ansari *et al.*, 2014).

HCV and HIV evade the host immune response through intricate processes including signaling interference, effector modulation, and continuing viral genetic variations (Park *et al.*, 2014). HIV infection produces effects on hepatocytes and HSCs; indeed, both cells express key HIV coreceptors; the interaction of HIV gp 120 with C–C chemokine receptor type 5 (CCR5) and C–X–C chemokine receptor type 4 (CXCR4) activates specific cell signaling in the liver (Peng *et al.*, 2015).

For both the viruses therefore, HIV infection is associated with a profound dysregulation of the immune system; during HIV/HCV coinfection, this immune dysregulation is more severe, leading to lower rates of spontaneous control of HCV replication and to a faster progression of liver disease (Maria *et al.*, 2015); cross-talk among HCV and HIV proteins modulates fibrogenic/inflammatory mediators, immune system response, and replication of both viruses (Maria *et al.*, 2015).

Characterization of viral protein interactions and their effects both on replication of these viruses and on liver function at a cellular level significantly improve understanding of HIV/HCV pathogenesis (Park *et al.,* 2014; Peng *et al.,* 2015; Maria *et al.*, 2015).

2.16 Virological interactions in HBV/HCV infections

From both the biological and clinical points of view, a crucial question is whether HBV and HCV may interfere in the life cycle of each other in cases of co-infection. *In vitro* studies performed since the early 90s demonstrated that the HCV "core" protein strongly inhibit HBV replication (Shih *et al.,* 1993; Shih *et al.,* 1995; Schüttler *et al.,* 2002; Chen *et al.,* 2003).

The HBV and HCV virological patterns have also been investigated in clinical studies. Most of these studies were cross-sectional evaluation of the viral load of the two viruses at a single time point, showing an apparent dominant role of the HCV (high HCV RNA and low HBV DNA levels) in the majority of the cases. Other reportssuggest a reciprocal interference or even a dominant effect of HBV and ethnic factors have also been proposed to influence the dominant role of one virus on the other (Nguyen *et al.,* 2011; Vermehren *et al.,* 2011).

In the middle of the last decade, an Italian multicenter study longitudinally examined a large series of HBsAg and anti-HCV antibody positive patients and showed that a wide and complex spectrum of virological profiles may occur in cases of coinfection (Raimondo *et al.,* 2006). About one third of the cases presented broad changes over time of the amount of circulating HBV DNA or - less frequently - HCV RNA, thus revealing alternate phases of activity of one or both viruses. In this context, one should consider that the typical anti-HBe positive chronic hepatitis B is often characterized by phases of low levels of HBV replication interspersed with episodes of viral reactivation(Ikeda *et al.,*2000), and many HBsAg/anti-HCV cases are anti-HBe positive. Similarly, also HCV

- although infrequently - may show alternating phases of active and suppressed replication also in cases of single infection (Ikeda *et al.,* 2000).

On virological interactions between the two viruses and particularly of the inhibitory effect of HCV on HBV, anecdotal reports concerning co-infected patients treated with interferon (IFN) therapy for the productive HCV infection had showed the reactivation of the previously, apparently suppressed HBV once a favorable response to therapy had been achieved as shown by the permanent disappearance of serum HCV RNA (Alberti *et al.,* 1995). Therefore, curing the HCV infection would produce the loss of the suppression on HBV that may reactivate (Raimondo *et al.,* 2008).

2.17 Virological interactions in syphilis infection

Syphilis represents the second most common cause of GUD among HIV-1-infected persons, with documented increasing rates of syphilis among HIV-1-infected persons over the past decade, especially among MSM (Zetola and Klausner 2007**;** Heffelfinger *et al.,* 2007**;** Su *et al.,* 2011). Multiple studies have confirmed that syphilis infection is associated with an increased risk of acquiring and transmitting HIV-1 (Zhang *et al.,* 2007; Patterson *et al.,* 2008), with mechanisms including the disruption of the mucosa and the influx of CCR5+ cells. Studies on the impact of HIV-1 infection on syphilis, have shown that it adversely affects the serologic response to syphilis treatment, especially at lower CD4 cell counts <200 cells/μL, but that combination antiretroviral therapy (CART) reduces the failure rate (Ghanem *et al.,* 2008; Knaute *et al.,* 2012).

Several studies have evaluated the impact of syphilis on the natural history of HIV-1 infection. Overall, syphilis increases the risk of HIV-1 transmission and leads to transient increases in HIV-1 RNA plasma levels and decreases in CD4 counts among a subset of patients regardless of the receipt of ART (Chun *et al.,* 2013). Treatment of syphilis leads to resolution of the viral load and CD4 cell changes with no apparent long-term impact on HIV-1 progression (Buchacz *et al.,* 2004**)**.

2.18 Diagnostic Approaches for HIV, HBV, HCV, HSV2 and syphilis

Since, Human immunodeficiency virus (HIV), Hepatitis B (HBV) and Hepatitis C (HCV) viruses are blood borne pathogens. Therefore, there is need for testing of markers for HIV1 and 2, Hepatitis B, Hepatitis C and other sexually transmitted diseases (STD). Detection of HIV, HBV, HCV, HSV infections and diagnosis is mainly based on immunological assays with ELISA and rapid tests being most common and widespread (Khan *et al.,* 2010).This is also the case with HSV and syphilis.

2.18.1 Point-of-care and rapid tests

Rapid diagnostic tests (RDTs) (CDC, 2010) are antigen-based tests developed for rapid diagnosis of virus and bacterial infections in POC settings. These tests use monoclonal antibodies that target the viral nucleoprotein and employ either enzyme immunoassay or immunochromatographic (lateral flow) techniques (Chuck *et al.,* 2008; Owusu *et al.,* 2011). Available in dipstick, cassette, or card formats, RIDTs can be completed in less than 30 min, with the results observed visually based on a color change or other optical signals. Due to simplicity in their use and the speed of obtaining assay results, RIDTs are commonly used for the diagnosis of many infections (Bristow *et al.,* 2014; Hess *et al.,* 2014 and Bristow *et al.,* 2015).

Points of care (POC) tests are both simple and capable of providing rapid results to guide clinical decisions during the same encounter (Tucker *et al.,* 2013; In recent years, a number of rapid POC tests, which can deployed in a variety of settings and provide results without specialized laboratory equipment, have been developed for syphilis (Tucker *et al.,* 2010; Kay *et al.,* 2014; Gaydos *et al.,* 2014).These include single tests, combination and multiplex tests that simultaneously test for antibodies and antibodies to other infections such as HIV and hepatitis C. The majority of these tests rely on lateral flow technology. Several are commercially available internationally (Mishra *et al.,* 2011; Binnicker *et al.,* 2012 and Gratrix *et al.,* 2012).

2.18.2 Enzyme Linked Immunoabsorant Assay

ELISA-based tests are available in either a solid phase plate or a paper strip format. ELISA-based tests were first introduced for disease diagnoses in the early 1990s and have consistently demonstrated high sensitivity and specificity. Several FDA-approved ELISA-based tests are available for the diagnosis of several bacterial and viral infections (Khan *et al.,* 2010).

2.18.3 Nucleic Acid Testing

The development of the polymerase chain reaction (PCR) technique by Kary Mullis in 1983 greatly impacted the field of infectious disease diagnosis. Nucleic Acid Test assays are based on PCR and detect virus-specific DNA or RNA sequences/ genetic material rather than viral antigens or antibodies (EASL, 2012).These tests are far more sensitive compared with the antigen-based serological tests, and can detect viruses much earlier in clinical samples. A variety of different NATs are currently available and used for diagnosis of viral infections in humans (Sai *et al.,* 2016). Viruses that do not proliferate in conventional cell culture but cause illnesses with great morbidity and mortality (including; HIV, HCV, HBV,) can now be detected with clinically meaningful results (Sai *et al.,* 2016).

Owing to their low specimen quantity requirement, superior sensitivity and specificity, and dramatically faster turnaround time, NAATs are currently accepted as the gold standard for clinical virology (Domiati *et al*., 2006).

2.19 Virology of HIV

The human immunodeficiency viruses 1 and 2 (HIV-1, HIV-2) originated from the simian immunodeficiency viruses (SIVs) of primates. Thus, HIV-1 and HIV-2 each had a zoonotic origin but now spread directly from human to human. HIV-1 was first isolated in 1983 and HIV-2 in 1986 and they represent two different epidemics. The SIV

of chimpanzees (SIVcpz) gave rise to HIV-1 in humans, and the SIV of the sooty mangabey monkey (SIVsm) to HIV-2 in humans (Weiss *et al.,* 2004) It is still uncertain exactly how the transmission of these SIVs to humans occurred, but it may have been during the hunting and preparation of these primates for food, by the indigenous people of these areas in Central and Western Africa, where these primate species live(Kalish *et al.,* 2005) Studies using molecular clock evolutionary assumptions have suggested that the ancestor virus for HIV-1 appeared in around 1931. (Korber *et al.,* 2000) and that of HIV-2 in around 1940. (Lemey *et al.,* 2003). After this initial transmission event, it is likely individuals infected with these primate SIVs then transmitted the human form of the viruses (HIV-1, HIV-2) to other people in their communities, from where it spread, world-wide.

2.19.1 Classification of HIV

The two human immunodeficiency viruses, HIV-1 and HIV-2, are members of the family ofRetroviruses, in the genus of Lentiviruses. Many different strains of HIV-1 exists and have been separated into major (M), new (N),outlier (O) and (P) groups (Figure 2.1), which may represent separate zoonotic transfers from chimpanzees. Groups N and O are mainly confined to West and Central Africa (Gabon and Cameroon), though cases of Group O have been found world-wide due to international travel, after contact with infected individuals from these areas.

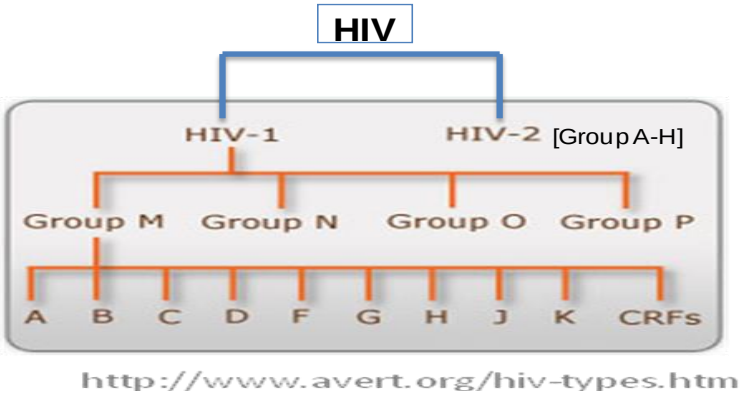

Figure 2.1: HIV family tree showing types of HIV groups and subtypes

The HIV strains in Group M are the ones mainly responsible for the HIV/AIDS pandemic and they are so diverse that they have been subclassified into subtypes (or clades) A-K. This huge diversity of HIV-1 is important when diagnostic testing, treatment and monitoring are applied as the results may differ between different subtypes or clades. The diversity of HIV-2 is much less, but subtypes A-H have been proposed. (Damond 2004)

2.19.2 Structure and replication of HIV

The human immunodeficiency viruses are approximately 100 nm in diameter. HIV has a lipid envelope embedded with transmembrane glycoprotein gp41 to which the surface glycoprotein gp120 is attached (Figure 2.2). These two viral proteins are responsible for attachment to the host cell and are encoded by the *env* gene of the viral RNA genome.

Beneath the envelope, is the matrix protein p17 and the core proteins p24, all encoded by the viral *gag* gene. Within the viral core, lies 2 copies of the ~10 kilobase (kb) positive-sense, viral RNA genome (it has a diploid RNA genome), together with the protease, integrase and reverse transcriptase enzymes.These three enzymes are encoded by the viral *pol* gene.

There are several other proteins coded for by both HIV-1 and HIV-2, with various regulatory or immuno-modulatory functions, including *vif* (viral infectivity protein), *vpr* (viral protein R), *tat* (transactivator of transcription), *rev*(regulator of viral protein expression) and *nef* (negative regulatory factor). An additional protein found in HIV-1 but not HIV-2 is *vpu* (viral protein U). Similarly, *vpx* (viral protein X) is found in HIV-2 and not HIV-1(Cleghorn et al., 2005).

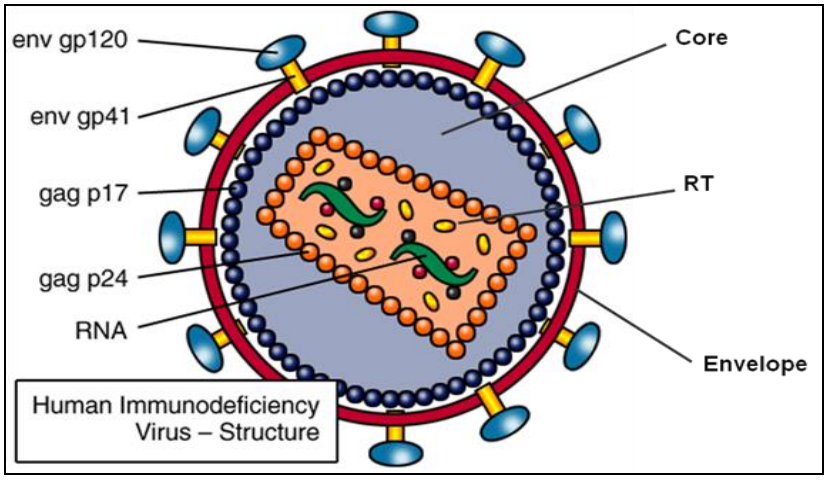

Figure 2.2: HIV structure showing structural components.

[Source : http://clipart-library.com/clipart/nrcLeybT8.htm]

2.19.3 HIV replication

The main attachment receptor for HIV is the CD4 molecule that is present on the CD4 positive T (helper) lymphocyte, macrophages, and microglial cells. The viral gp120 binds initially to this CD4 molecule, which then triggers a conformational change in the host-cell envelope that allows binding of the co-receptor (either CCR5 or CXCR4) which is required for fusion between virus envelope and cell membrane (Figure 2.3).

Macrophages carry the CCR5 co-receptor; hence HIV strains requiring the CCR5 co-receptor for entry are also referred to as 'macrophage-tropic' although they also infect

lymphocytes (Cleghorn *et al.*,2005**)** These HIV strains are also known, phenotypically, as R5 or non-syncytium inducing (NSI) strains as they do not form syncytia (cell-fusion) when cultured with CD4 lymphocytes *in vitro*.

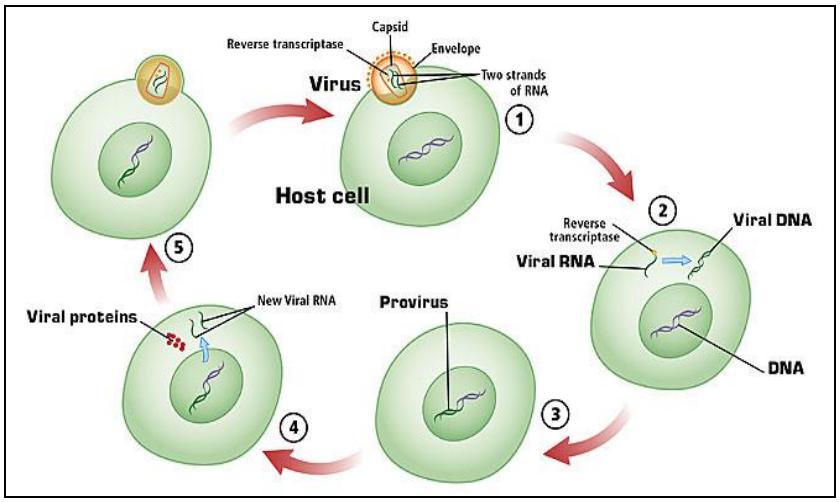

Figure 2.3: HIV Replication cycle

[Source:https://www.learner.org/courses/biology/archive/image]

Primary HIV-1 infections tend to involve this R5 NSI macrophage-tropic phenotype. Uncommonly, individuals may have a homozygous deletion mutation in the CCR5 gene (CCR5Δ32) resulting in the absence of the CCR5 molecule on their macrophages. Therefore, these individuals cannot be infected by this R5 phenotype. (Weiss *et al.*, 2004; Cleghorn *et al.*, 2005; Hladik *et al.*, 2005). The 'lymphotrophic' HIV strains use CXCR4 as the co-receptor. These viruses are also known as X4 viruses and do produce syncytia (i.e. are phenotypically syncytium-inducing, SI) when cultured *in vitro* with CD4 lymphocytes. X4 viruses tend to appear later in about 50% of HIV-1 subtype B-infected individuals, but seldom with other subtypes, as they progress to AIDS (Weiss *et al.*, 2004). So far, CXCR4 deficient individuals have not been found. This attachment and fusion process allows the HIV viral core to enter the host-cell.

All retroviruses encode a reverse transcriptase enzyme that transcribes its viral RNA into double-stranded DNA (dsDNA), which is then integrated, via the action of the integrase enzyme into the host-cell genome.

The viral integrated dsDNA or 'provirus' then acts as a template for viral genomic and messenger RNA transcription by the host cell's nucleic acid replicating machinery. Recombination between these two RNA strands during viral replication, coupled with the extremely error-prone action of the RT enzyme, give rise to the extreme genetic diversity of HIV **(**Weiss *et al.*, 2004; Cleghorn *et al.*, 2005).

Integration of the linear provirus dsDNA (Figure 2.4) into the genome of the host-cell establishes an infection that lasts for the lifespan of the cell, and all its progeny, which usually means life-long infection for the organism, in this case the human host.

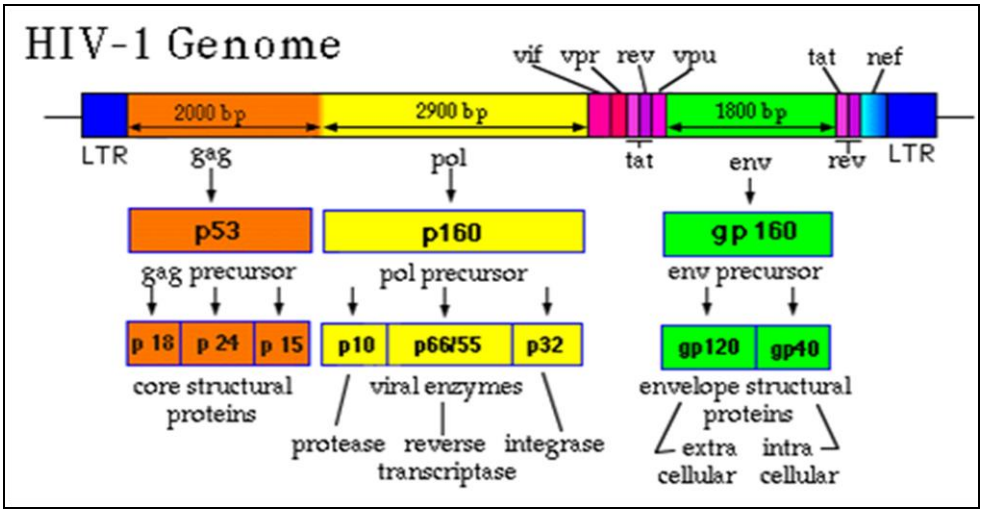

Figure 2.4: HIV Genome.

[Source: https://www.onlinebiologynotes.com/ genome/]

Viral replication occurs along with cellular replication and is enhanced by various factors, including coinfection with other organisms, the presence of inflammatory cytokines and cellular activation. During cellular replication, the provirus is transcribed by the host-cell RNA polymerase II enzyme, and the viral messenger RNA (vmRNA) and genomic RNA, are carried with the cellular mRNAs, to be translated into proteins. This vmRNA codes for a *gag-pol* precursor polypeptide that is ultimately cleaved by the viral-encoded protease enzyme to produce the *gag* and *pol* viral proteins. In addition, the vmRNA is also spliced to produce other vmRNAs coding for the viral proteins *tat, rev, vif, vpr, vpu* (for HIV-1), as well as the *env* precursor polypeptide. Ultimately, the *env* precursor polypeptide is cleaved by cellular (not viral) proteases, producing the envelope glycoproteins gp41 and gp120 (Figure 2.4). These viral proteins, together with the replicated diploid viral genomic RNA, are assembled and enveloped by budding through the host-cell membrane, producing complete HIV virions (Cleghorn *et al.*, 2005).

2.20. Overview of genetic diversity and epidemiology of HIV

The viral diversity of HIV-1 is mainly due to an RNA replication error rate of about $1{:}10^4$ bases.This has given rise to a complex classification system for the M group of HIV-1 viruses. Within the M group, there are multiple subtypes, currently: A, B, C, D, F, G, H, J, K and CRFs (circulating recombinant forms). Subtypes E and I are missing from this list because the viruses that were initially classified as E and I have since been reclassified as CRF01_AE and CRF04_cpx. This latter virus, originally named subtype I, is in fact a mixture of subtypes A, G, H and K, though the term subtype I is no longer used (Gao *et al.*, 1998; Noble *et al.*, 2006). It was first identified in Cyprus and Greece in the early 1990s and has spread within the Mediterranean region (Noble *et al.*, 2006; Requejo *et al.*, 2006).

Recombinant forms may be formed when two HIV-1 viruses of different subtypes infect the same cell allowing their genomes to mix during replication to create a new hybrid virus (occasionally referred to as 'viral sex') (Burke *et al.*, 1997; Noble *et al.*, 2006).

Many such hybrid viruses do not survive long, but if they manage to infect more than one person, they are termed CRFs, e.g. CRF01_AE is a mixture of subtypes A and E. In this particular example, there has been no pure E forms of the virus yet found, so the terminology here is not technically correct. However, it has been so widely used that the name has remained, as changing it may lead to confusion (Noble *et al.,* 2006; Requejo *et al.,* 2006).

2.20.1 HIV-1 subtypes

Like the HIV groups M, N and O, the different subtypes and CRFs have some distinct geographical distributions. Subtype A has been responsible for 80% of the HIV infections in Western Africa and 30% in Eastern Africa. In Europe, it has been spreading out from countries of the former Soviet Union since 1995, particularly from Russia and the Ukraine (Bobkov *et al.,* 2004; Noble *et al.,* 2006; Requejo *et al.,* 2006).

Subtype B is the most common subtype in Europe, the Americas, Japan and Australia. This is still the case, although other subtypes are increasing in prevalence, accounting for at least 25% of new infections in Europe. Subtype B has also been the predominant subtype in some Asian countries, including Korea, India and Singapore (Requejo *et al.,* 2006). Subtype B viruses seem to be more efficiently transmitted via homosexual contact and intravenous drug use (via blood contact), whereas subtypes C and CRF01_AE tend to drive heterosexual epidemics (Essex *et al.,* 1996; Bhoopat *et al.,* 2001; Noble *et al.,* 2006).This has however not yet been conclusively proven (Dittmar *et al.,* 1997; Pope *et al.,* 1997; Noble *et al.,* 2006).

Subtype C is mainly found in southern and eastern Africa, India and Nepal. It has now caused the world's worst epidemics (being the main subtype in these highly populated, developing countries, with poor healthcare infrastructures), accounting for about 60% of all HIV infections, worldwide, but mainly in east Africa and south Asia. It has also been reported from Malaysia and southwest China, possibly due to links with India. Recently,

subtype C has been causing more infections in southern Africa (Botswana, Zimbabwe, Malawi, Zambia, Namibia and South Africa). In Scotland, subtype C infections have been increasing since 2000, probably due to imported infections from these African and Asian countries (Requejo *et al.,* 2006). A study of sex workers from Senegal found that women infected with subtypes C, D or G were more likely to develop AIDS within 5 years of infection than those infected with subtype A (Kanki *et al.,*1999; Noble *et al.,* 2006).

Subtype D has caused 5-40% of HIV infections in east and central Africa, where it has been con-circulating with subtype A. At least one study from Uganda has shown that individuals infected with subtype D or CRFs including subtype D tended to develop AIDS and died sooner than those infected with subtype A (Noble *et al.,* 2006; Laeyendecker *et al.,* 2006) Different subtypes may affect the efficiency of vertical transmission from mother-to-child though many studies disagree with each other over these findings (Tranchat *et al.,* 1999 ; Murray *et al.,* 2000; Blackard *et al.,* 2001; Yang *et al.,* 2003; Tapia *et al.,* 2003 ; Renjifo *et al.,* 2004 ; Martinez *et al.,* 2006).

Subtype F has been found in central Africa, South America and Eastern Europe (particularly Romania). Subtypes G and CRF_A/G have been reported from western and eastern Africa and central Europe. Subtypes A, G, H, J and K have been found to be prevalent in Burkina Faso, Mali, Nigeria, Ivory Coast, Gabon and Democratic Republic of Congo, from where they have spread to southern Europe and Asia(Noble *et al.,* 2006; Requejo *et al.,* 2006).

2.20.2 HIV-1 circulating recombinant forms (CRFs)

There is about 20 CRFs that have been currently described (Requejo *et al.,* 2006). The formal requirement for a CRF is the existence of at least 3 epidemiologically independent complete genome sequences that share the same recombinant structure and form a monophyletic cluster in all regions of the genome. Alternatively, it should

produce two complete genome sequences with partial sequences of a third strain that cluster with complete genome sequences, sharing identical breakpoints (Noble *et al.*, 2006).

Monophyletic groups (also known as 'clades') are groups containing species which are more closely related (in terms of sequence homology) to each other than to any species outside of this group. CRF03_AB (meaning the 3rd occurrence of this CRF in a person, combining subtypes A and B) has been reported from Kaliningrad, Russia in an HIV epidemic involving IDUs in the mid 1990s (Fultz *et al.*, 2004). This virus has since spread all over Eastern Europe.

Further analysis showed that the parent subtypes A and B viruses probably came from Ukrainian IDUs. CRF02_AG was first found in Ibadan, Nigeria, and became epidemic predominantly in west and west central Africa, where it represents 50-70% of the circulating strains. From Africa, this virus has been imported into Europe where it is now spreading throughout France, Belgium, Italy and the UK. UK seems to be a melting pot for CRF HIV strains, as multiple CRFs have been reported from there, including: CRF01_AE, CRF14_BG, CRF03_AB, CRF05_DF, CRF06_cpx, CRF11_cpx and CRF02_AG (Requejo *et al.*, 2006).

CRF07_BC and CRF08_BC have been found in China, probably originating from a recombination between the subtype C virus imported from India and the endemic subtype B virus in China. However, CRF01_AE has been responsible for the explosive epidemic in Southeast Asia in Thailand, Vietnam, Cambodia, Myanmar, China and Taiwan. In some countries, such as Vietnam and Thailand, the majority of these infections have been found IDUs (Requejo *et al.*, 2006).

Interestingly, the CRF01_AE virus seems to have originated from several countries in central Africa (Central African Republic, Cameroon and the Democratic Republic of Congo) where it has been reported at low frequency only (Noble *et al.*, 2006). Exactly

why this same CRF has caused the rapidly spreading epidemic in Southeast Asia requires further investigation. CRF12_BF has been reported from Argentina, Uruguay and Brazil, mainly in the heterosexual population and vertically-infected children.

CRF06_cpx has been reported from Burkina Faso and Mali and is composed of fragments from subtypes A, G, J and K. CRF09_cpx has been found in Senegal and consists of fragments from subtypes A, C and D (Fultz *et al.,* 2004). Similarly, CRF11_cpx is composed of fragments from subtypes A, G and J, as well as CRF01_AE, and has been observed in Cameroon and the Central African Republic. CRF13_cpx was recently discovered in Cameroon and is a mosaic of subtypes A, G, J and CRF01_AE, but with a different subtype J from that in CRF11_cpx, which was found in the Democratic Republic of Congo. CRF18_cpx and CRF19_cpx have also been recently described, which include multiple segments from African subtypes A, D, G and H viruses (Requejo *et al.,* 2006).

2.20.3 Other HIV-1 recombinations

Unique recombinant forms (URFs) are viruses for which there is only one sequence available, and which do not resemble any previously described CRFs (Gross *et al.,* 2004). They most often occur in regions where several different CRFs are co-circulating, and have been reported from the UK, Thailand (CRF01_AE/B), Georgia, USA CRF01_AE/B), Malaysia (CRF01_AE/B), Cameroon (CRF02_AG/A), Estonia (CRF06_cpx/A), Switzerland (CRF11_cpx/B) and most recently, Cuba (CRF18_cpx/CRF19_cpx). Many of these recombinations (particularly the CRF01_AE/B viruses) seem to have been produced in individuals who contracted HIV through heterosexual and IDU exposure (Noble *et al.,* 2006).

Studies have subsequently shown that HIV-1 subtypes and CRFs have segregated amongst populations with different risk behaviours, such as homosexual and heterosexual men, IDUs and male and females. It has been suggested that at the

beginning of the HIV epidemic in the 1980s, only pure HIV-1 subtypes were prevalent, but later in the 1990s, the number of CRFs has increased (Gross *et al.,* 2004). However, when some of the later classified HIV subtypes were compared to older viruses from the Democratic Republic of Congo, there was evidence of recombination in the 1980s. Hence, at least some of these earlier recombinant forms were classified as pure subtypes which were exported from Africa to other parts of the world where they became endemic (Requejo *et al.,* 2006).

Intra-subtypes describe the variation in a specific region of the HIV-1 genome coding for the envelope protein *gp120*. This region contains the determinants for co-receptor affinity and cell tropism, immune evasion and the preferential use of the CCR5 co-receptor (Noble *et al.,* 2006). Recombination between HIV-1 groups has also been reported between HIV-1 M/O groups in patients from Cameroon, where these other HIV-1 groups are prevalent.

Dual infections of HIV-1 and HIV-2 have also been reported from Central Africa regions where both virus types co-circulate, but it is less likely for recombinants of HIV-1/ HIV-2 to appear because they maybe too divergent - though it is difficult to say that this is impossible. Hence, dual infection can be simultaneous or sequential 'superinfection' of an individual with two HIV-1 subtypes, CRFs or any combination of subtypes and CRFs (Gross *et al.,* 2004; Fultz *et al.,* 2004; Requejo *et al.,* 2006).

2.20.4 Implications of HIV-1 diversity

The huge diversity of HIV-1 viruses means that diagnostic assays used to monitor the HIV-1 epidemic, as well as vaccine development for universal protection against HIV-1 infection becomes very problematic. Although many screening tests can detect the known subtypes of HIV-1 as well as HIV-2(Phillips *et al.,* 2000; CDC, 2001, 2006), local screening programmes may not be designed to detect outlier strains. New recombinant strains may differ in fitness, transmissibility and virulence, as well as

responses to therapy. A continuously evolving classification scheme for such HIV variants is essential to maintain the research and development of new ways to track, diagnose, monitor and treat this ever-mutating virus.

2.21 Coinfections of HIV

While co-infections of HIV are diverse, HBV, HCV, HSV and syphilis are some of the most important infections.Their epidemiology, biological characteristics and associated morbidities in the era of HIV have generated enormous public health interest.

2.21.1 Hepatitis B Virus

Hepatitis B virus (HBV) is a DNA virus and humans are the only known natural host. Hepatitis B virus (HBV) causes acute and chronic infections, which are often associated with severe liver diseases, including cirrhosis and hepatocellular carcinoma (HCC) (Ganem &Prince, 2004). It is estimated that approximately 350 million individuals worldwide suffer from chronic HBV infection, despite the availability of an effective vaccine for more than 25 years.

HBV is transmissible through several routes: (i) percutaneous - injecting drug use, exposure to contaminated blood or body fluid; (ii) sexual - heterosexual or male homosexual activity; (iii) vertical - from mother to infant; and (iv) horizontal - between children and household contacts through skin lesions or sharing of blood-contaminated toothbrushes and razors (CDC,2004; Shepard *et al.*,2006).

Among the estimated 40 million persons infected with HIV worldwide, approximately 2-4 million (up to 10%) are chronically infected with HBV. This prevalence varies with geographic region (McGovern *et al.*, 2007).

2.21.2 Virology of HBV

The hepatitis B virus (HBV) is an enveloped DNA virus of the hepatotropic DNA virus family (*hepadnaviridae*) and has the smallest (3.2 kb) genome among DNA viruses (Wieland *et al.,* 2004). The viral DNA is a closed, circular, partially double stranded molecule of 3.2 kilobases (relaxed circular DNA (rcDNA). This genome contains four partially overlapping open reading frames: the S gene, coding for the envelope proteins; the Core gene, coding for the core and "e" proteins (Figure 2.5).

The P gene, codes for a protein with multiple functions including reverse transcriptase and DNA polymerase activities.The X gene, codes for the "X" protein of yet not well defined functions, but with transcriptional transactivating properties and a likely important role in the viral replication (Ganem & Prince, 2004). Once it has penetrated into the hepatocyte, the viral core is transported to the nucleus and the rcDNA is converted into a circular, covalently closed, fully double stranded supercoiled DNA (cccDNA) that is the template for the production of the virus messenger RNAs including RNA pregenome. Similarly to the retroviruses, the RNA is reverse transcribed in the cytoplasm of the infected cells for the synthesis of the viral DNA (Ganem & Prince, 2004; Wieland *et al.,* 2004).

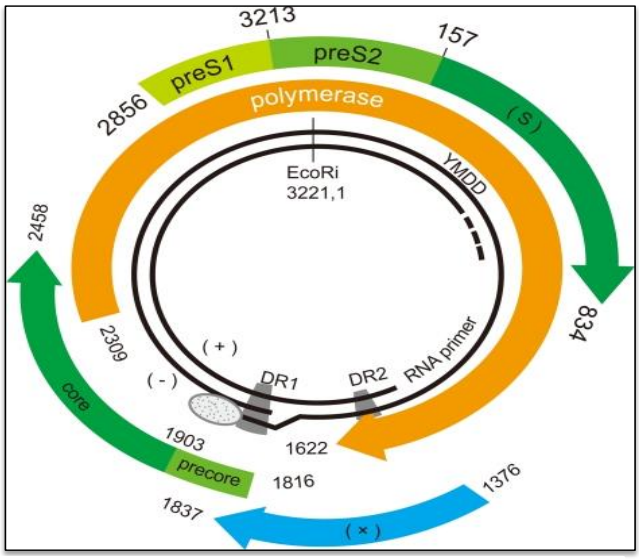

Figure 2.5: Hepatitis B virus genome

[source:https://www.wjgnet.com/1007.htm]

HBV cccDNA molecules bind histons and other proteins and are organized as a stable, long-term persisting chromatinized episomes that - together with the long half-life of hepatocytes - imply that HBV infection, once it has occurred, may continue indefinitely over time (Werle *et al.*, 2004; Levrero *et al.*, 2009)

2.21.3 Genetic Diversity of HBV

In 1988 it was first suggested by Okamoto *et al.*, 1998) that HBV could be divided into 4 genotypes based on a divergence of ≥ 8% in the complete genomic sequence and genotypes A,B,C and D were identified. Following the initial description of genotypes A-D, Norder *et al.*, (1992) also proposed genotypes E and F which differed by more than 4% in the *S* gene from the other genotype groups and this has become an alternative criterion for classification of distinct genotypes. Genotype G is the least common of the genotypes and was reported in 2000 from samples of French and American patients (Stuyver *et al.*,2000) but its geographic origin is still unknown (Lindh, 2005).

Currently, based on an intergroup divergence >7.5% across the complete genome, HBV has been classified phylogenetically into 9 genotypes, A-I (Panduro *et al.,* 2013), with a putative 10th genotype, 'J', isolated from a single individual. With between approximately 4 and 8% intergroup nucleotide difference across the complete genome and good bootstrap support, genotypes A-D, F, H, and I are classified further into at least 35 subgenotypes (Sakamoto *et al.*,2013).

Research on HBV genotypes during the last decade has significantly associated the HBV genotypes with the severity of liver disease, clinical outcomes and the response to antiviral therapies (Yin *et al.,* 2010; Mello *et al.,* 2012; Yang *et al.,* 2013). It has been documented that HBV genotypes or subgenotypes vary in geographical distribution. Genotype A prevails in Europe, Americas and Africa. Genotypes B and C are prevalent in Asia. Genotype D has global distribution, but is predominant in the Mediterranean area. Genotype E spreads commonly in West Africa (Kramvis *et al.,* 2005; Hübschen *et al.,* 2008; Garmiri *et al.,* 2009). Genotypes F and H are restricted to Central and South America. HBV genotype G is found mainly in the United States and Europe (Norder *et al.,* 2004).

2.21.4 Virology of HCV

HCV is a small, enveloped RNA virus that belongs to the Hepacivirus genus of the Flaviviridae family (Houghton *et al.,* 1991; Robertson *et al.,* 1998). Its genome consists of a single-strand of positive-sense RNA of approximately 9.6 kb which consists of 5' and 3' non coding regions and a single open reading frame that encodes a single viral polyprotein of 3010-3033 amino acids (Penin *et al.,* 2003; Thomson et *al.*, 2000; Choo *et al.,* 2012).

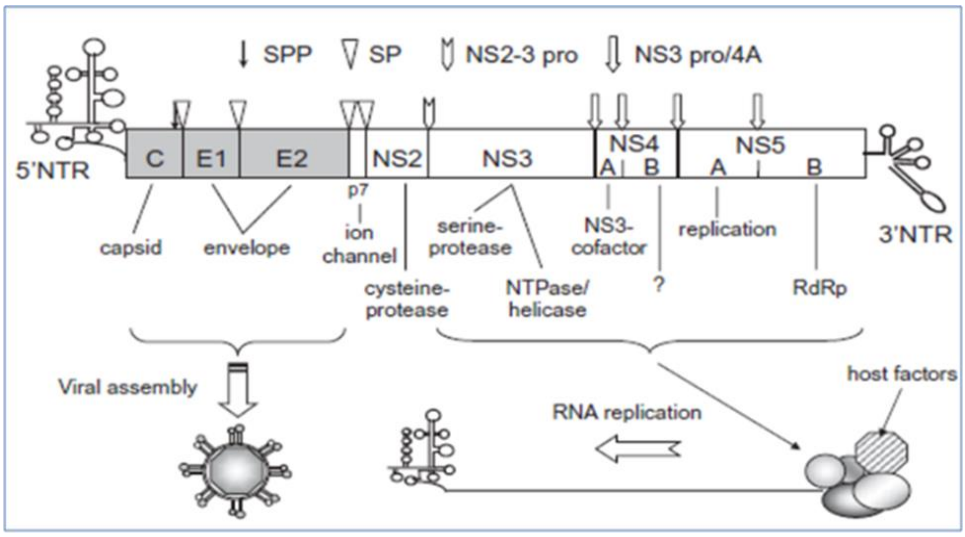

Figure 2.6: Hepatitis C virus genome

[Source www.nature.com/articles/s41598-018]

The precursor is cleaved into at least ten different proteins: the 3 structural proteins core, E1, E2 and the 7 nonstructural Proteins NS2, NS3, NS4A, NS4B, NS5A, NS5B and p7. Short untranslated regions at each end of the genome (5'-NCR and 3'-NCR) are required for its translation and replication (Natalia *et al*., 2015) (Fig. 2.6).

Because of its rapid replication and the high rate of error insertion of the RNA-dependent RNA polymerase, HCV spontaneously mutates within a given infected individual, resulting in related but distinct "quasispecies (Farci *et al.,* 2011; Natalia *et al.,* 2015).

The generation of these mutants appears to be one of the key mechanisms by which HCV escape the host's immunoresponse, maintaining persistent infection (Pawlotsky et al., 2003). Very importantly, the replication cycle of the HCV occurs totally in the cytoplasm and - once the replication is stopped - the virus can be cleared from the cells and thus the infection definitively cured (Orland *et al*., 2001).

2.21.4 Genetic Diversity of HCV

The high error rate of RNA-dependent RNA polymerase and the pressure exerted by the host immune system, has driven the evolution of HCV towards the development of a global diversity that revealed the existence of seven genetic lineages (genotypes 1 to 7) (Figure (Figure2)(Martell *et al.*,1992).

On average, the complete genome differs in 31%-33% of nucleotide sites (Saito *et al.*, 1990; Pawlotsky *et al.,* 2006). Genotypes 1 to 6 of HCV contain a series of more closely related sub-types (67 accepted subtypes and many more to be confirmed) that typically differ from each other by at least 15% in nucleotide positions within the coding region (Lauer *et al.,* 2001). Subtypes 1a, 1b and 3a are widely distributed and account for the vast majority of infections in Western countries (Kuo and Gish, 2012).

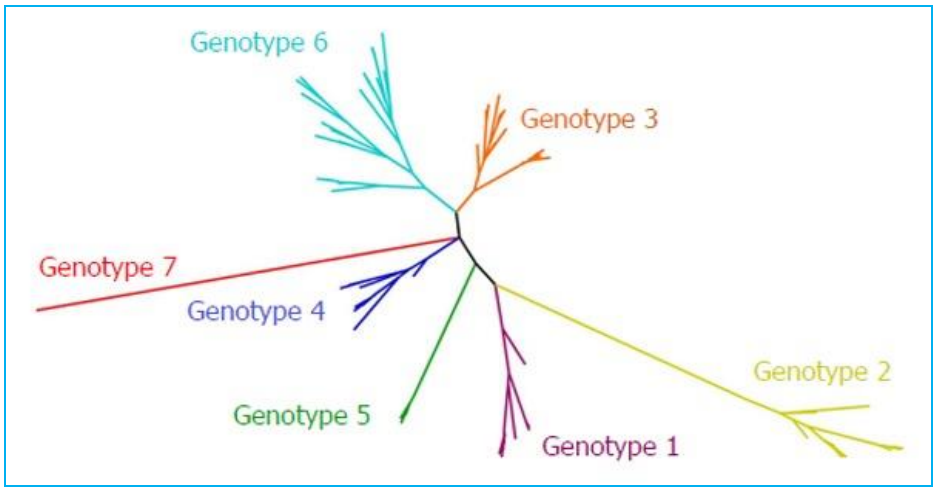

Figure 2.7: Hepatitis C virus genotypes

[Source www.nature.com/articles/s41598-018]

Seven major HCV genotypes and several subtypes have been identified **(**Figure 2.6 and 2.7) (Smith *et al.,* 2014). HCV exhibits a complex taxonomic structure (Smith *et al.,*

2014). Genetic diversity between HCV genotypes is about 30%, while subtypes differ by about 15% (Bukh *et al.,* 1995; Simmonds *et al.,* 1993). HCV genotypes show a characteristic distribution in different geographical regions (Lavanchy *et al.,* 2011). Genotypes 1, 2 and 3 exhibit a worldwide distribution. Genotypes 1 and 2 are endemic in West Africa while genotype 3 is endemic to the Indian subcontinent. Genotypes 4 and 5 are primarily found in Africa, and genotype 4 is particularly endemic in Egypt and Central Africa.

Genotype 6 is endemic of Asia whereas the distribution of genotype 7 has not been fully assessed (McOmish *et al.,* 1994; Kao *et al.,* 1995; Agha *et al.,* 2004; Lavanchy *et al.,* 2011; Simmonds *et al.,* 2013). Genotype 2 is the oldest lineage, followed by genotypes 3, 5 and 6, while genotypes 1 and 4 emerged more recently. Globally distributed genotypes are referred as epidemic, and their rapid dissemination over the last century is primarily attributed to modes of transmission including the use of intravenous drugs, nosocomial transmission and blood transfusions (Magiorkinis *et al.,* 2009). Endemic genotypes are usually highly divergent and limited to well defined geographic regions (Jackowiak *et al.,* 2004).

2.21.5 Epidemiology of Herpes Simplex

HSV-1 and HSV-2 belong to the genus *Simplex virus* within the subfamily Alphaherpesvirinae under the family Herpesviridae (Desai *et al.,* 2015).

Herpesviruses are frequently found in humans, although their prevalence significantly varies depending on ethnicity, sex, and geographical location of individuals, among others (CDC, 2005; Looker *et al.,* 2008; Yawn *et al.,* 2013; Dickson *et al.,* 2014; Suazo *et al.,* 2015). Currently, eight Herpesviridae family members are known to infect humans: Herpes Simplex Viruses (HSV) -1 and -2 (HSV-1, HHV-1 and HSV-2, HHV-2, resp.), Varicella Zoster Virus (VZV, HHV-3), Epstein Barr (EBV, HHV-4), Cytomegalovirus (CMV, HHV-5), Human Herpesvirus 6 (HHV-6), Human Herpesvirus

7 (HHV-7), and Kaposis Sarcoma-associated Virus (KSV or HHV-8). All Herpes Viruses harbor large genomes encoding >70 genes and share the capacity to establish lifelong persistent infections in the host (Grinde *et al.,* 2013).

Herpes Simplex Virus type 2 (HSV-2) is a linear double-stranded DNA virus of the *Herpesviridae* family and a major cause of genital ulcer diseases, is transmitted by sexual contact or via the maternal-neonatal relationship. Despite multiple measures adopted to control the diseases, this virus still infects at least 500 million people around the world (Looker *et al.,* 2008). HSV-2 reaches a latent state in the sensory nerve root ganglia and reactivates when the immune function of the body declines, causing recurrent episodes (Gupta *et al.,* 2007). The rapidly cleared episodes of HSV shedding are present in HIV co-infected persons (Mark *et al.,* 2010). However, the mechanisms of the latency are still unknown. Recent studies show that HSV-2 increases the risk of HIV-1 acquisition (Freeman *et al.,* 2006; Horbul *et al.,* 2011; Barnabas *et al.,* 2011).

The potential biological mechanisms by which HSV-2 increases risk of HIV-1 infection include disruption of the genital epithelium, recruiting activated target cells for HIV-1, decreasing innate mucosal immunity and inducing a mucosal inflammatory response (Thurman *et al.,*2012). HSV-2-infected monocyte-derived dendritic cells (moDCs) increase retinoic acid production and high α4β7 expression on $CD4^+$ T cell, which can be spotted by HIV-1 (Martinelli *et al.,* 2011). HSV-2 can also damage the protective function of mucosal Langerhans cells (LCs) through abrogating the function of langerin, which enhances the susceptibility of HIV-1 (De Jong *et al.,* 2010).

2.21.6 Epidemiology of syphilis

Syphilis is a multi-stage disease that is capable of being sexually transmitted, has the potential to invade the central nervous system, and, when untreated in woman, can lead to frequent transplacental congenital infections. Syphilis can lead to devastating diseases including cardiovascular syphilis, benign gummatous syphilis and neurosyphilis (Cole *et*

al., 2009). The vertical transmission of *Treponema pallidum* to the unborn child may result in congenital syphilis. In addition, syphilis infection increases the risk of transmission and acquisition of HIVas well as exacerbate the course of HIV infection (Lynn *et al.,* 2004; Kofoed *et al.,* 2006; Pialoux *et al.,* 2008; Cole *et al.,* 2009).

This chronic infectious disease is caused by the spirochaete *Treponema pallidum that* results in multiple patterns of skin and visceral disease. Human is the only known natural host of *Treponema pallidum.* Syphilis is usually transmitted through sexual contact or from mother to fetus or newborn infant. Studies have shown that 16 to 30% of individuals who have had sexual contact with a syphilis-infected person in the preceding 30 days become infected (Rebecca *et al.,* 2006); actual transmission rates may be much higher (Rebecca *et al.,* 2006). In a pregnancy, infection occurs when the organisms cross the placenta to the fetus.

The World Health Organization (WHO) estimated that 11 million new cases of syphilis occurred in adults in 2018, majority of them in developing countries (WHO, 2018). Although a worldwide battle against syphilis has evolved for several years, syphilis remains on the rise, particularly in Africa, Asia, and middle or southern America.(Gare *et al.,*2005; Pando *et al.,*2006; Hagan *et al.,*2007;).Syphilis imposes considerable health and economic burden on many countries, especially the developing countries.(Chen *et al.,*2012; Chico *et al.,*2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Sites

Blood samples for this study were collected from participants in ten health facilities conveniently selected from at least 7 provinces in Kenya (Table 3.1).Reference to provinces unlike counties is maintained in order to reflect the regional geo-location of sites as representative of that region (Figure 3.1). The facilities selected were those providing comprehensive care services (specialized clinics for HIV and AIDS). In addition, a blood transfusion center was also included to provide known positive samples for testing control purposes.

Table 3.1: Study sites

NO.	PROVINCE/REGION	FACILITY/SITE
1.	Western Kenya	Kakamega PGH
2.	Nyanza	Kisii hospital
		Kisumu, JOTH
3.	Rift valley	Kitale Hospital
		Nakuru PGH
4.	Nairobi:	Mbagathi Hospital
5.	Central Province	Nyahururu Hospital
		Karatina hosp
6.	Coast province	Malindi Hospital
7.	Eastern Kenya	Meru Hospital

Location of study sites

Study sites were distributed in 7 regions across Kenya (Figure 3.1).

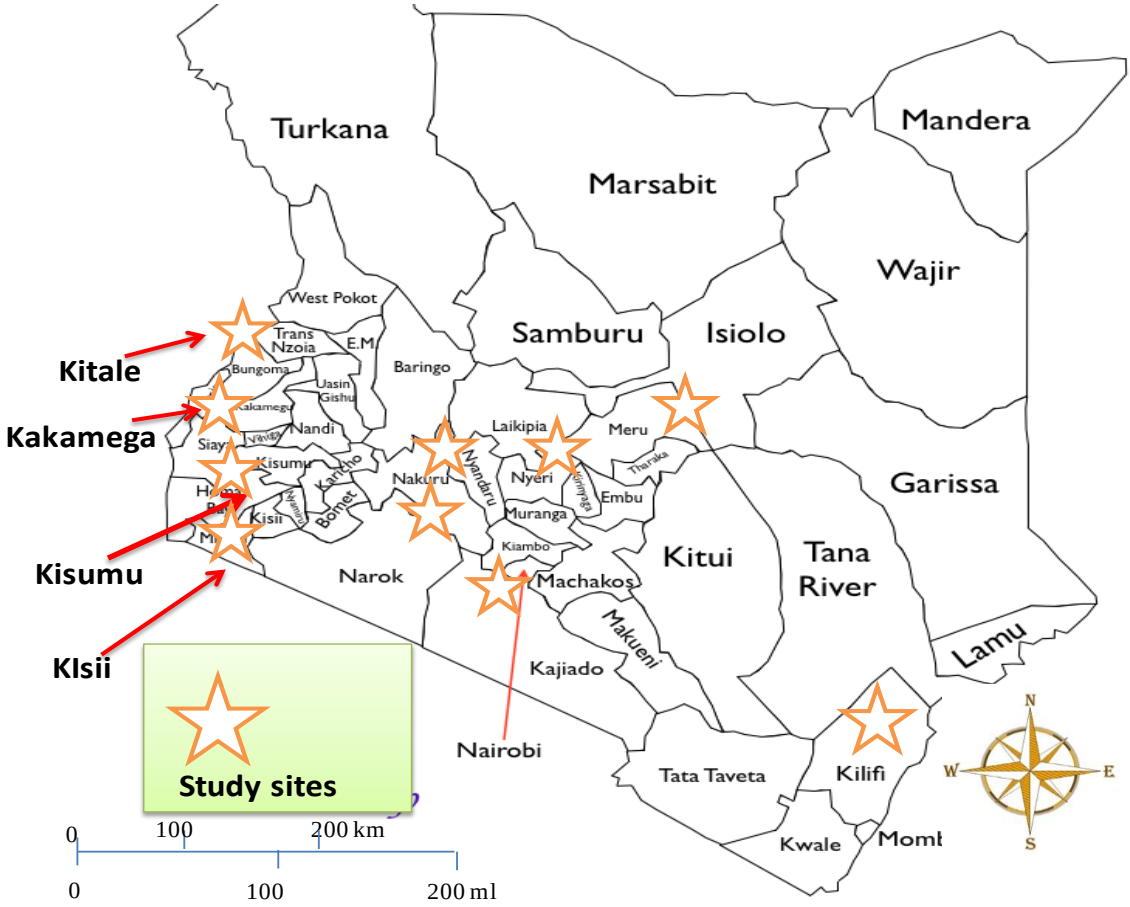

Figure 3.1: Map of Kenya indicating study sites. Sites are indicated by a star

Source, map of Kenya: https://opendata.go.ke/facet/counties

3.2 Study population

Study participants were HIV positive individuals attending the HIV comprehensive care clinics. These patients attend the clinics on a regular basis and are provided with care and management of AIDS. Blood samples were collected from these patients during their routine clinic visits. In addition blood samples to be used as test control samples

were obtained from the blood donors through donated blood at a blood transfusion center. Upon collection and routine laboratory testing at the clinical laboratory, samples were then de-identified and anonymised by unique codes assigned for the study.

3.3 Inclusion Criteria

- Individuals attending comprehensive care clinics
- Samples were collected from HIV positive individual over a 6 month period
- Samples reported as positive for HBV, HCV and syphilis at the blood transfusion centers for use as controls for the study

3.4 Exclusion Criteria

Individuals who were not on care for HIV at the collection site were excluded from the study.

3.5 Study Design

This was a cross sectional study involving 12 health facilities from 7 provinces in Kenya. Comprehensive care clinics, depending on their location serve populations beyond the provinces of their locality. Health facilities were conveniently selected based on availability of AIDS comprehensive care clinics (CCC) services. Samples were collected once from study sites during the study period.

3.6. Sample Size Determination

The sample size was determined using the formula of Fisher *et al. (*1998)

$$n=\frac{Z^2P\ (1-P)}{d^2}$$

Where:

n=Minimum sample size required.

Z=1.96 Standard error.

P= assumed prevalence of Hepatitis, 0.08(8%) Prevalence of HBV in Kenya (Kathleen *et al*, (2016**)**

q= (1-p)

d= degree of accuracy desired .0.05 the inverse of 95% confidence limit.

D=Design effect = 11 Therefore:

$$N=\frac{1.96^2(0.08)(1-0.8)}{0.05^2}$$

Calculated sample size was then multplied by D to account for design effect (11sites)

Total 113 samples (minimum sample size) for each site (x11) = 1243

3.7 Sampling method

Systematic random sampling (the n^{th} name selection technique) was used. After the required sample size had been determined, every n^{th} participant was selected for sample collection.This was followed until the required samples size was met.The first participant was identified through random selection where client files were assigned blinded numbers 1 to 5; the number that was picked first became client number 1. Subsequently every other 2^{nd} client visiting the clinic and consented for the study was recruited.Study pariticipants were then refered to the laboratory for sample collection.

3.8 Blood sample collection and shipment

Briefly; 5ml of blood was collected in CD4 stabilizer anti-coagulated tubes with appropriate labels (BD Vacutainer CD4 Stabilization Tubes (STs), Becton Dickinson, Franklin Lakes, New Jersey). At the site of sample collection, unique codes were used for de-identification and confidentiality. Samples were then packaged and transported by courier services to KEMRI; Nairobi.During transportation cold chain was maintained through use of cooler boxes with ice packs. CD4 testing was first carried out and the remnant sample separated by centrifugation..About 3 mls Plasma was collected in separate vials and frozen while the remaining sample (whole blood) in the main tube refrigerated immediately for peripheral blood mononuclear separation (PBMCs). Preparation of BMCs was carried out same day after separation or following day depending on workload in the lab.Biosafety precautions during sample collection, packaging and transportation were adhered to. These included use of gloves; protective clothing and transport packaging as described in, in-house laboratory protocols and WHO recommendations**.** Separated samples at KEMRI, Centre for Virus Research, HIV Laboratories were stored in a -80˚C and 4˚C freezer respectively till use.

3.9 Serological testing of HIV, HBV, HCV, syphilis and HSV infections

3.9.1 Enzyme linked Immuno sorbent assay identification of HIV, HBV and HCV

The ELISA methods, which are a benchmark for quantitation of antigens/antibodies, were used for testing of HIV, HBV, HCV and HSV according to manufacturers' instructions. HIV esting was carried out using VIRONOSTIKA HIV Uni-Form II Plus 0 (Biomerieux, The Netherlands); Murex anti HCV version 3.0 (DiaSorin, Kyalami, South Africa); Hepanostika® HBsAg Ultra sensitive test for Hepatitis B infection (bioMérieux, the Netherlands) and HSVtype 2 IgG ELISA ("Kalon"; Kalon Biological Ltd, Surrey, UK) for HIV, HBV, HCV and HSV2 respectively were used (Lingappa *et al*, 2010; Kosack *et al*, 2016). Briefly, typical ELISA testing procedure involved a three-step assay protocol where a sample (100 µl) was incubated for 60 min with antigen- and antibody-coated plates, followed by three buffer washes.

In the next step, conjugate was added to the well and incubated for 30 min. This step was then followed by three buffer washes. The conjugate containing recombinant antigens and peptides from HIV-1 conjugated with horseradish peroxidase was then added and incubated for 30 minutes. The amount of conjugate bound was determined by incubation with hydrogen peroxide–3,3′,5,5′-tetramethylbenzidine (TMB) substrate for 30 min.Termination of incubation was carried out with H_2SO_4, and absorbance measured at 450 nm. All incubations were carried out at 37°C and addition of each reagent was monitored by a color change. The color change was then determined by a spectrophotometer by obtaining optical density (OD) values.Cutoff values were then calculated to determine and results reported as positive or negative for each of the target infection (Hornbeck, 2015**)**.Confirmation of results was carried out using in-country and WHO recommended protocol where use of two tests, either ELISA or a rapid test as described in section 3.9.2.

3.9.2 Rapid testing for HIV, Syphilis and HCV infections

Rapid tests,in addition to ELISA were used for detection of HIV, HCV and Syphilis infections. Test procedure for rapid test involved adding a 50ul sample into the rapid test kit cassette or strip sample pad. Two drops of sample buffer/dilute was then added onto the sample pad.The sample –buffer mixture was then given time to migrates through the conjugate pad for 15 minutes hence reconstituting and mixing with the selenium colloid-antigen conjugate. As the mixture continued to migrate through the solid phase it then mixed with immobilized recombinant antigens and synthetic peptides at the patient window site. A positive result was indicated by formation of a red line at the patient window (based on respective manufacturers instructions insert).

For HIV, Determine rapid test (Abbott Japan Co Ltd, Tokyo, Japan), SD Bioline HIV 1/2 3.0(Standard Diagnostics Inc, Kyonggi-do, Korea) and Uni-Gold HIV-1/2 test kit ((Trinity Biotech PLC,Bray, Ireland)kit were used.This rapid kits were by then the approved kits for HIV testing for the national testing algorithm. Rapid tests for HCV

used were; Bioline HCV rapid (Standard Diagnostics Inc, Kyonggi-do, Korea) and HCV PHA 2nd Generation (Dinabot Co., Ltd., Tokyo, Japan).Rapid testing for HBV involved use of Determine HBsAg (Alere, USA) and ICHEK rapid test.Syphilis rapid testing was done using Rapid Plasma Reagin (RPR) Card Tests (Becton Dickinson BD USA) and reactive results compared with VDRL (Thermo Fisher Scientific) (WHO list of prequalified in vitro diagnostic products, 2020).

3.9.3 Algorithm testing

Testing algorithm involves determination of a testing strategy using different test methods.Procedure for testing algorithm involved selection of a number of kits for each infection to be tested and comparison of their testing performamnce. Comparative results against a panel of samples determines which kit becomes the screening or confimatory testing.Selection of such kits is based on kits that are available in-country and their performamnce characteristics validated.For this study additional criteria was whether such kits are in operation in health facilities.

A testing algorithm for serologic diagnosis of HIV-infection is the sequence in which assays are performed to detect HIV antibody in a body fluid. For this study this definition is also applied in testing of Hepatitis B and C since most testing methods are also serological. Polymerase chain reaction is also a common molecular method applied in these infections. Various combinations of tests can be used including combinations of EIAs; combinations of rapid tests; or an EIA in conjunction with rapid tests. The choice of strategy and of tests should be determined by the quality of the tests and by the practicality of their implementation and logistics.

3.10 CD4 cells count

Determination of CD4 count was carried out by flow cytometry.Results obtained were then analysed to obtained distribution of CD4 by demographic variables.

BD FACSCalibur Cell analyzer was used as described by the manufacturer's instructions (Becton Dickinson immunocytometry systems, San Jose, califonia). Briefly,samples collected from study sites in BD tubes (BD Vacutainer CD4 Stabilization Tubes (STs), Becton Dickinson, Franklin Lakes, New Jersey) were first brough to room temperature by standing them on the table for 30 minutes in the laboratory. In tubes labeled with the sample identification number, 20ul of triTEST CD3/CD8/CD45 reagent was pipetted into the bottom and 50ul of well-mixed, anticoagulated whole blood added. This was vortexed gentle to mix and incubated for 15 minutes in the dark at room temperature (20-25° ºC).Then, 450ul of 1x FACS lysing solution was added to the tube and incubated in the dark at room temperature. Processed samples were then analysed in a flow cytometer (Becton Dickinson immunocytometry systems, San Jose, califonia) and as described by Thakar *et al*, 2013.

3.11 Genotyping of HIV, HBV and HCV

3.11.1Separation of PBMCs

Peripheral blood mononuclear cells separation was carried out using ammonium chloride extraction/ Ficoll-Paque Plus method according to manufacturer's instructions. Briefly, three milliliters of Ficoll-Paque Plus (Amersham Pharmacia Biotech AB, Uppsala Sweden) was added to a 15ml centrifuge tube. Four milliliters of blood sample were then carefully layered onto Ficoll- Paque Plus to avoid mixing. The tube was then centrifuged at 2500g for 30 minutes at 20 °C without braking. After centrifugation, the upper layer of plasma was drawn off using a clean Pasteur pipette. The lymphocyte layer was then aspirated into a clean tube. This was followed by washing of PBMCs with Phosphate Buffered Saline (PBS) by centrifugation.Cells pellet obtained was then stored at 20 °C till when ready for proviral DNA extraction (Amersham Pharmacia Biotech AB, Uppsala Sweden) and as described by Grievink *et al*,2016.

3.11.2 DNA extraction from PBMCs

DNA extraction was carried out by lysis of PBMCs using DNAzol reagent (molecular research centre, Inc, Cincinnati, Ohio) and ethanol precipitation according to manufacturer's instructions. Briefly, 500microliters of DNAzol genomic DNA extraction reagent was added to the pellet and dissolved completely by pipetting the reagent-pellet mixture up and down with a pipette according to the manufacturers' instructions. One millilitre of absolute ethanol was then added to the dissolved pellet and mixed gently without vortexing. This mix was spun at 12,000xg in a microfuge at 4°C for 5 minutes. The obtained supernatant was discarded and the pellet washed in 1 ml of 70% ethanol at 12000xg for 10 minutes. The pellet was then dried in a safety cabinet at room temperature for 20 minutes. After drying, DNA pellet was dissolved in distilled DNAse and RNase free water. Quantification of proviral DNA was done using a spectrophotomer (Beckman, USA) and stored at -30 °C till use.

3.11.3 RNA/DNA Extraction for HIV, HBV and HCV

Viral genome extraction from plasma was carried out using an extraction kit (SMITEST R&D RNA/DNA, Genome science Co.Ltd, Tokyo, Japan) according to the manufacturers' instructions. The extracted nucleic acid was resuspended in 20 ul of RNAse/DNAse free water and stored at – 80 °C till use.

Briefly, enzyme solution, precipitation solution and sample diluent were put in a 1.5 ml tube. A 100 ul of plasma (virus) was then added into the same tube. This was vortexed to mix and incubated for 55 °C, 30 minutes by dry thermo unit. For nucleic acid purification, protein lyses buffer was added, mixed gently and incubated at 55 °C. Afterwards, 600ul Cold 2-Propanol was added and put in ice for 15 minutes. The clear pellet obtained was air dried at room temperature for 15 min, resuspended with RNAase/DNAase free water and kept at –80°C till use (Instruction manual,Genome

science Co.Ltd; *Smitest EX-R&D kit*, Genome *Science* Laboratories *Co*., *Ltd*., Fukushima, Japan).

3.12 Polymerase Chain Reaction (PCR) PROCEDURES

Polymerase Chain Reaction was performed on extracted DNA/RNA using a GeneAmp 9700 machine (Applied Biosystems, Foster City CA, USA) using specifically designed primers for the regions encoding the various viral genes (Table 3.2). Briefly a master mix for each target infection [HBV, HCV, and HIV] was prepared separately as shown in table 3.3.Extracted DNA/RNA was used as the template in round 1. The mix was then loaded into a thermocycler under conditions shown in table 3.4 PCR round 1.In the second round, similar master mix and was prepared with round I PCR product as the template. After PCR cycles were completed in the second PCR round, the product was then ran on gel elctorphoresis for separation based on molecular size. Visualization of amplified target [HBV, HCV.HIV] was done under UV light.

3.12.1 Primers for PCR

Primer sequences that are highly conserved (Table 3.2) for HIV and Hepatitis viruses were used for this work. For HIV primer pairs used are described by Lihana et al.,2009; for HCV by White *et al*;2000 and HBV by Stuyver *et al*; 2000 (Table 3.2).

Type specific master mix for the PCR was prepared for HIV, HBV and HCV viral genomes (Table 3.3). Amplification of the amplicons was done with one cycle of 94 ºC for 10 min and 35 cycles of 94 ºC for 30 s, 50 ºC for 30 s and 72 ºC for 30 s with a final extension of 72 ºC for 10 min in both rounds and as shown in Table 3.4.

Table 3.2: PCR primers used in this study

Primer	Primer nucleotide sequence	Fragmentsize(Bp)
	HIV Reverse transcriptase primers	2265–3180 (288)
RT 18 F	(5'-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3')	Sense
KS 104 R	(5'TGATTGCCCAATTTAGTTTTCCCACTAA-3')	Antisense
KS 101 F	(5'GTAGGACCTACACCTGTTCAACATAATTGGAAG-3')	Sense
KS 102 R	(5'CATCCAAAGAAATGGAGGAGGTTCTTTCTGATC-3')	Antisense
	HCV 5'UTR PRIMERS	9-252 (200)
KY80 F	5'-GCAGAAAGCGTCTAGCCATGGCGT-3',	Sense
KY78 R	5'-CTCGCAAGCACCCTATCAGGCAGT-3'	Antisense
hep21b F	5'-GAGTGTYGTRCAGCCTCCAGG-3'	Sense
hep22 R	5'-GCRACCCAACRCTACTCTCGGCT-3'	Antisense
	HBV preS1 PRIMERS	2850-3246 (400)
HBPr1 F	5'-GGGTCACCATATTCTTGGG-3'	sense
HBPr135 R	(5'-CA(A/G) AGACAAAAGAAAATTGG-3'	antisense
HBPr2 F	(5'-GAACAAGAGCTACAGCATGGG-3'	sense
HBPr3 R	(5'-CCACTGCATGGCCTGAGGATG-3'	antisense

Source of primers: Department of Viral Infection and International Health, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University

Table 3. 3: Typical master mix for HIV, HBV and HCV used in the study

Master Mix	Quantity									
	HIV Pol RT			**HBV preS1**			**HCV UTR ONE STEP RT**			
Ingredients	**1st PCR**	**2nd PCR**		**1st PCR**	**2nd PCR**			**RT/1ST PCR**		**Nested PCR**
H_2O	13.8ul		14.8ul	12.2ul	14.2ul			6ul		13.4ul
15Mmol $MgCl_2$	2.8ul		2.8ul	2.8ul	2.8ul		2x Rxn mix	12.5 ul		2.8ul
2 Mmol DNTPs	2.5ul		2.5ul	2.0ul	2.0ul					2.5ul
10X PCR Buffer	2.0ul		2.0ul	2.0ul	2.0ul					2.0ul
Primer F: Pr104	0.3u	**Pr101**	0.3u	**Pr135**	0.4ul	**Pr2**	0.4ul	**KY80**	0.5ul	Hep21b	0.5ul
Primer R: RT18	0.3ul	**Pr102**	0.3ul	**Pr1**	0.4ul	**Pr3**	0.4ul	**KY78**	0.5ul	Hep22	0.5ul
Taq Polyme.	0.3ul		0.3ul	0.2ul	0.2ul		RT Taq mix	0.5ul		0.3ul
Subtotal	**22ul**		**23ul**	**20ul**	**20ul**			**20ul**		**22ul**
Template (DNA)	3ul	1stPCR Ampli	2ul	DNA	5ul	Amp	3ul	RNA	5ul	1st PCR Ampl.	3ul
Reactn.Vol.	**25ul**		**25ul**	**25ul**	**25ul**			**25ul**		**25ul**

Master Mix for the PCR reactions varied with the primer used as well as volumes of specific ingredients as shown in this table (table 3.3).

Table 3.4: Typical cycling conditions during PCR testing

Cycling conditions for amplifying PCR products		
Step	**Time/cycles**	**Temperature**
Initial activation step	2 min	94°C
3-step cycling		
Denaturation	10 s	94°C
Annealing	1 min	55°C
Extension	1 min	72°C
Number of cycles	40 cycles	68°C
End of PCR cycling	Indefinite	4°C

Variations in cycle conditions varied with number of cycles; where 35 and 40 cycles were used for HIV/ HCV and HBV respectively.

3.13 Gel Electrophoresis of PCR Products

Once the PCR had been done electropheretic procedures were carried out as decribed by Pei Yun Lee and collegues 2012. Agarose gel was prepared on which the PCR products were loaded for visualization of the amplified DNA. Amplified PCR products electrophoretic analysis was carried out on 2% agarose gel followed by examination under UV light. In this procedure,an aliquot of the amplicons were mixed with the loading dye and loaded onto the gel together with the molecular weight marker (100bp). The gel was then put in an electrophoresis tank for electrophoresis. After running for 20 minutes the gel was then stained with 0.005% ethidium bromide in TBE for 20-30 minutes.This was followed by visualizion under ultraviolet light (Sambrook *et al*., 2001).

3.14 Sequencing and Phylogenetic Analysis

3.14.1 BigDye Terminator v3.1 Cycle Sequencing

Direct sequencing was carried out in an automated DNA sequencer (ABI3100 Applied Biosystems, Foster City, CA) with BigDye Terminator version 3.0 Cycle Sequencing Reaction Kits (Applied Biosystems, Foster City, CA).Briefly, a reaction mix for cycle sequencing was prepared with a terminator ready reaction mix of 4.0 μL mixed with Big Dye sequencing buffer (5x) 2 μL ,Template 2 μL, 2^{nd} round primer; 3 μL and De-ionized water;9μL to make a 20 μL reaction volume. This mixer was votexd and spun briefly, then loaded on a GeneAmp® PCR System 9700 thermocycling system under cycling conditions specified by the manufacturer (Applied Biosystems, Foster City, CA). After sequencing PCR, the products were purified before loading on sequencer, a 3100 Genetic Analyzer for sequence generation.

3.15 Sequence analysis

3.15.1 Sequences editing with Genetyx

Sequences obtained were edited using GENETYX Ver.9 Application (GENETYX, Tokyo, Japan). Forward and reverse PCR templates were sequenced for each sample in order to generate al larger sequence upon assembling. As a result two sequences were obtained for each sample.In the genetyx program sequences were uploaded and analyzed for sequence errors ('noise'). Where sequence errors were identified, correction was done to obtain clean sequences.The two corrected sequences were then re-assembled to generate a consensus sequence. The consensus sequence was copied and saved as a word or fasta format document. This process was repeated for all the sequence pairs from the samples until all the sequences were completed (Kotaki *et al*, 2015) .

3.16 Submission of sequences to genbank

After all sequences were corrected, A file for GenBank submission was prepared using Sequin application program version 13.70 (National Centre for Biotechnology information, National library of medicine, NIH (released Feb. 2015). Prepared file was then submitted to the genebank for accession numbers. The generated sequences in this study were deposited in the Genebank and given accession numbers for referencing. These accession numbers are shown in appendices 1 and 2.

3.17 Genotype determination

Sequences were used to construct the phylogenetic tree by neighbor – joining method. The reliability of the tree was estimated by 1000 bootstraps (Thomson *et al*, 1994). The resultant tree profiles were visualized with Tree View PPC version 1.6.5. Phylogenetic tree can be constructed using several program e.g. MEGA- a standalone program; ClustalW –a web based program by DDBJ (DNA Data Bank of Japan) available at

clustalw.ddbj.nig.ac.jp/. In this study both the programs were utilized in generation of Phylogenetic trees.

3.18 Methods for algorithm determination

Alogorithm determination method involved selection of approved serological test kits.A minimum of two kits each for HIV, HBV, HCV were identified based on their availability and use in Kenya.These kits were compared for their performamnce to identify each infection from the panel samples.Following this comparisons,determination of performance based on sensitivity and specificity,and Kappa[levels of agreement] determined.Kits whose sensitivity and specificity and Kappa results were high compared with the other kit was idenfied as the best/first kit for the algorithm. To calculate sentivity and specificity the formulae:

	Disease present	Disease absent	Total
Test positive	a	b	a+b
Test negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

Where **Sensitivity**= [a/ (a+c)] x100 and **Specificity**= [d/ (b+d)] x100

CHAPTER FOUR

RESULTS

4.1 Distribution of study participants by demographic characteristics

This study involved participants distributed across 10 study sites (Table 4.1) from across the regions in Kenya. In the study population 33% (604) were male while 67% (1225) were female (Table 4.2).There was almost an equal distribution of participants within age groups except for age group <14 and >51 which had 9% and 12 % respectively (Table 4.3).The mean age among female was 31.97, Standard deviation 12.5, at 95% CI while that of males was 32.35 standard deviation 15.1 at 95% CI (Figure 4.1).

Nyahururu and Mbagathi had the highest proportion of participants at 17% and 16 % respectively while Kitale had the lowest at 3 % (Table 4.1). Female comprised the highest number of participants at 67% (1232) while distribution of participants by age group indicates age groups >14yrs and >60 yrs had the lowest number of participants at 12 and 9% respectively (Table 4.1).

Table 4.1: Distribution of study participants by study sites

	Health facilities	N	Percentage %
1	Malindi	195	10.6
2	Kitale	51	3
3	Kisumu	211	11.5
4	Kisii	137	7.5
5	Kakamega	162	9
6	Nyeri	80	4.3
7	Nyahururu	310	16.9
8	Nakuru	212	11.5
9	Meru	171	9.3
10	Mbagathi	300	16.4
Total		1829	100
	Gender		
	Female	1225	67%
	Male	604	33%
Total		1829	100%
	Age groups		
	>14	220	12%
	15-24	259	14%
	25-44	1015	56%
	45-59	281	15%
	>60	54	3%
Total		1829	100%

4.2 Age of participants by gender

Mean age difference between men and women in the study indicated that women mean age of women (31.79) was lower than that of men (32.35) (Figure 4.1).This difference was however not significant **(**p=0.061**).**

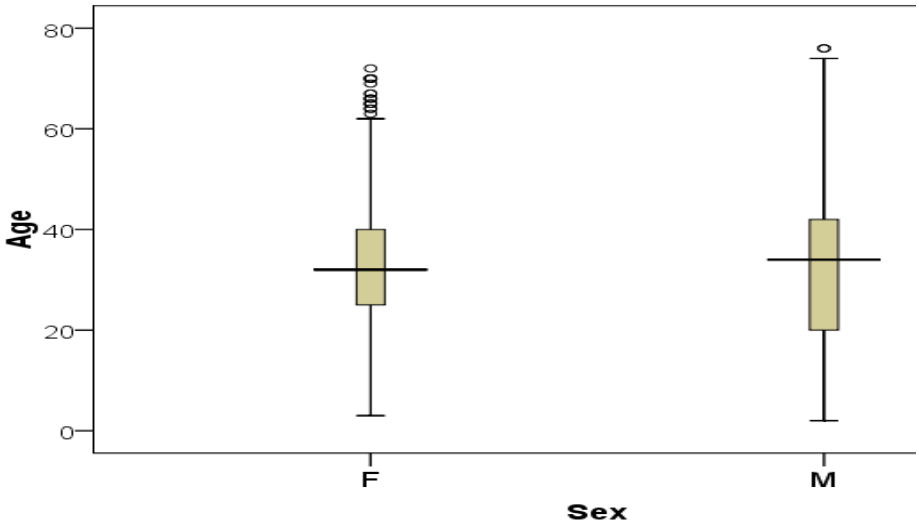

Figure 4.1: Mean age differences between gender

4.3. Prevalence of HBV, HCV, HSV and syphilis infections

The sero-prevalence of HSV2 was highest 860 (47%) followed by that of HBV 29% (530). Prevalence of HCV and syphilis was 256(14%) consequtively (Table 4.2). Majority of study participants had at least one co-infection or several 1299 (71% infected with HBV, HCV, HSV2 or syphilis), the rest of the study participants 530 (29%) had none of the co- infection.

Table 4.2: Prevalence of infections in the study population

Infection status	Negative (%) (n= 530)	Positive (%) (n=1299)	Totals
HBV	71 (1299)	29 (530)	1829
HCV	86 (1573)	14 (256)	1829
HSV	53 (969)	47 (860)	1829
Syphilis	86 (1573)	14 (256)	1829
Totals	29 (530)	71 (1299)	1829

(*Co-infections with HBV, HCV, HSV2 and syphilis among study participants)*

4.3.1 Distribution of Hepatitis, herpes and syphilis by age group, gender and sites

Distribution of HBV, HCV, HSV2 and syphilis infections was determined by age, gender and study sites, occurring as either single or multiple infections. Results indicated that that age group greater than 60 years had the highest co-infections (75%) (Table 4.3).

Segregation by gender indicated that among women (within women category) 70% had at least any one of the 4 infections, compared to 72% among male. Distribution of any of the 4 infections by site indicated Mbagathi had the highest co-infections at 80% (Table 4.3).

Table 4.3: Status of HBV, HCV, HSV2 and syphilis co-infection by demographic variables.

	Presence of co-infections with HBV, HCV, HSV and syphilis or none N (%)	
Age in years	**No (n=530)**	**Yes (n=1299)**
<14	83 (38)	137 (62)
15-24	73 (29)	186 (70)
25-44	283 (28)	732 (72)
45-59	78 (29)	203 (72)
≥60	13 (24)	41 (75)
GENDER		
Female	365 (30)	860 (70)
Male	165 (27)	439 (73)
STUDY SITE		
Kakamega	49 (30.2)	113 (69)
Kisii	37 (27.0)	100 (73)
Kisumu	55 (26.1)	156 (73)

Kitale	14	(25.9)	40	(74.1)	
Malindi	79	(40.5)	116	(59)	
Mbagathi	59	(19.7)	241	(80)	**
Meru	79	(46.2)	92	(53)	
Nakuru	50	(23.6)	162	(76)	
Nyahururu	78	(25.2)			
Nyeri	33	(41.3)	47	(58)	

tes presence of any of the 4 infections and No, none at all; for distribution of the particular infections see table 4.4 and 4.5)

4.3.2 Number of combinations of the co- infection among the HIV infected

29% (530) of the participants had no co-infection; 44% (804) had only one co-infection while only 0.8(15) had all the four (HBV, HCV, HSV2 and syphilis infections) (Figure 4.4).

Table 4.4: Number of HIV Co-infection combinations

Number of Co-Infections	n	%
0	530	29
1	804	44
2	366	20
3	110	6
4	18	1
Total	**1829**	**100**

4.3.3 Distribution of co-infections into single, double, or multiple infections by demographic variables

Co-infections in this study were compared between age, gender and study sites (Table 4.5) based on the four infections studied regardless of which infection to identify single, double, triple and multiple infections. In addition, HBV; HCV; HSV2 and syphilis infections were then segregated into their combinations (Table 4.6). Single co-infections

were more prevalent in age group above 60 years (49.1%), males had more single infections at 44.7% than female while Nakuru and Nyahururu had more single infections at 54.2% compared to other sites (Table 4.5).

Double infections were more prevalent in age group >60 (22.6%), female had more double infections (21%), while Mbagathi with 30.7% had highest (table 4.6)

Multiple co-infections were more prevalent in age group 25-44 (6.9%), by gender female had more multiple infections (6.9%), while by site, Kakamega and Kisumu had higher at 9.9% and 9.0% (Table 4.5) respectively.

Combination of the 4 co-infections was more prevalent in age group 25-44 years (2.1%) and by site Kisii had the highest (2.9%) (Table 4.5).

Table 4.5: Distribution of HIV co-infection into single, double or multiple infections

Characteristics	Number of co-infections				
Age in years	**0, (n=530)**	**1, (n=797)**	**2, (n=370)**	**3, (n=115)**	**4, (n=15)**
>14	83 (37.7)	84 (38)	39 (17.7)	14 (6)	0 (0.0)
15-24	73 (29.4)	106 (42.7)	52 (21.0)	14 (5.6)	3 (1.2)
25-44	283 (27.8)	450 (43.8)	208 (20.3)	71 (6.9)	**12 (1.2)**
45-59	78 (27.8)	130 (46.3)	59 (21.0)	14 (5.0)	0 (0.0)
≥60	13 (24.5)	**27(49.1)**	**12 (22.6)**	2 (3.8)	0 (0.0)
Gender					
Female	368 (29.9)	529 (42.9)	**259 (21.0)**	66 (5.4)	10 (0.8)
Male	165 (27.6)	**267 (44.7)**	111 (18.6)	**49 (8.2)**	5 (0.8)
Study Site					
Kakamega	49 (30.2)	64 (39.5)	33 (20.4)	**16 (9.9)**	0 (0.0)
Kisii	37 (27.0)	53 (38.7)	35 (25.5)	8 (5.8)	**4 (2.9)**
Kisumu	55 (26.1)	87 (41.2)	46 (21.8)	**19 (9.0)**	4 (1.9)
Kitale	14 (27.5)	22 (43.1)	11 (21.6)	4 (7.8)	0 (0.0)
Malindi	79 (40.5)	65 (33.3)	33 (16.9)	15 (7.7)	3 (1.5)
Mbagathi	59 (19.7)	130 (43.3)	**92 (30.7)**	17 (5.7)	2 (0.7)
Meru	79 (46.2)	61 (35.7)	17 (9.9)	13 (7.6)	1 (0.6)
Nakuru	50 (23.6)	115 (54.2)	35 (16.5)	11 (5.2)	1 (0.5)
Nyahururu	78 (25.2)	168 (54.2)	56 (18.1)	8 (2.6)	0 (0.0)
Nyeri	33(41.3)	31(38.8)	12 (15.0)	4 (5.0)	0 (0.0)

Key: 0=no any infection; 1= one type; 2= two types; 3= three types

(Bolded and underlines figures indicate age group,gender and sites with highest percentage of single,double,triple of quadruple infection categories)

4.3.4 Co-infections and their combinations among the study participants over all (100%)

The highest prevalence of multiple infections was that of HBV, HCV and HSV2 at 2.6% (47), while the highest for double infections was HBV and HSV2 at 2.9% (168) (Table 4.6).

Table 4.6: Combination of infections in the total population(n=1829)

Combination of HIV co-Infections	n (1829)	(%) 100
No Co-infections	530	29.1
Syphilis	64	3.5
HSV2	496	27.1
HSV2 & Syphilis	80	(4.4)
HCV	66	3.6
HCV & Syphilis	7	0.4
HCV & HSV2	51	(2.8)
HCV & HSV2 & Syphilis	9	0.5
HBV	170	9.3
HBV & Syphilis	20	1.1
HBV & HSV2	168	9.2
HBV & HSV2) & Syphilis	46	2.5
HBV & HCV	44	2.4
HBV & HCV & Syphilis	13	0.7
HBV & HCV & HSV2	47	2.6
HBV & HCV & HSV2 & Syphilis	15	0.8

4.3.5 Dual and multiple combinations of co- infections with HBV, HCV, HSV2 and syphilis among those infected only (71%)

The highest prevalence of multiple combinations among the co-infected was HBV, HSV and syphilis combination at 9% while among the double infections HBVand HSV was the highest at 34% (Table 4.7).

Table 4.7: Dual and multiple combinations of HBV, HCV, HSV2 and syphilis

Distribution of the 1299 infected cases	%	n
HIV and HSV2	34%	442
HBV,HSV2 and Syphilis	9%	117
HBV and HCV	9%	117
HBV,HCV and syphilis	3%	39
HBV,HCV and HSV2	9%	116
HBV,HCV,HSV and syphilis	3%	39
HSV2 and syphilis	16%	208
HCV and syphilis	1%	13
HCV and HSV2	10%	130
HCV,HSV2 and syphilis	2%	26
HBV and syphilis	4%	52
TOTAL	**100**	**1,299**

4.4 Association of HIV co-infections and demographic characteristics

Association of HIV co-infection with age, gender and study sites indicated significant relationship for the sites (P=<0.001) but not for gender **(**p=0.325**)** and age (p=0.063) (Table 4.8).

Table 4.8: Associations of HIV co-infections and participants demographic variables

Presence of a viral co-infection Cases, n %)				
Characteristic	**Groups**	**No (n=530)**	**Yes (n=1299)**	**X^2 Test**
Age in years	0-4	1 (0.2)	5 (0.4)	**No X^2 value, used Fisher's exact due to low cell numbers p=0.063**
	5-14	82 (15.4)	132 (10.2)	
	15-24	73 (13.7)	175 (13.5)	
	25-44	283 (53.7)	744 (57.2)	
	45-59	78 (14.6)	203 (15.7)	
	≥60	13 (2.4)	40 (3.1)	
Gender	Female	365 (69.0)	860 (66.7)	**X^2 =0.97, 1 d.f p=0.325**
	Male	165 (31.0)	439 (33.3)	
Study Site	Kakamega	49 (9.2)	113 (8.7)	**X^2 = 62.01, 9 d.f p=<0.001**
	Kisii	37 (6.9)	100 (7.7)	
	Kisumu	55 (10.3)	156 (12.0)	
	Kitale	14 (2.6)	40 (2.9)	
	Malindi	79 (14.8)	116 (9.0)	
	Mbagathi	59 (11.1)	241 (18.6)	
	Meru	79 (14.8)	92 (7.1)	
	Nakuru	50 (9.4)	162 (12.5)	
	Nyahururu	78 (14.6)	232 (17.9)	
	Nyeri	33 (6.2)	47 (3.6)	

4.5 Logistic regression analysis on HIV co-infections and demographic variables

Odds ratio were determined for infection status and demographic variables (Table 4.9).Using Kakamega as a reference site, Mbagathi and Meru had statistically significant associations with HIV co-infection (P=0.000 and P=0.002 respectively) in binary logistic regression model (Table 4.8).

A participant at Mbagathi had 2.26 odds that of a patient in Kakamega of getting a viral co-infection and this relationship was statistically significant, (p=0.000, Odds Ratio= 2.26, at 95% C.I). Similarly, a patient at Meru had 0.49 odds that of a patient in Kakamega of getting a viral co-infection and this relationship was statistically significant, (p=0.002, Odds Ratio= 0.49). Thus, a patient at Meru had a 51% (0.51) less chance/odds of getting a viral co-infection as compared to patient at Kakamega (Table 4.9).

Table 4.9: Association of HIV co-infections and patient demographic characteristics

Characteristic	Odds Ratio	P-Value	(95% Confidence Interval) for the Odds Ratio
Age in years			
0-4	**Reference**		
5-14	0.14	0.076	(0.015 – 1.230)
15-24	0.20	0.156	(0.023 – 1.834)
25-44	0.29	0.271	(0.034 – 2.596)
45-59	0.30	0.279	(0.034 – 2.662)
≥60	0.30	0.300	(0.031 – 2.914)
Gender			
Female	**Reference**		
Male	1.15	0.221	(0.918 – 1.446)
Study Site			
Kakamega	**Reference**		
Kisii	1.33	0.275	(0.795 – 2.238)
Kisumu	1.25	0.336	(0.791 – 1.984)
Kitale	1.18	0.652	(0.579 – 2.394)
Malindi	0.65	0.058	(0.413 – 1.016)
Mbagathi	2.26	**0.000**	(1.434 – 3.570)
Meru	0.49	**0.002**	(0.307 – 0.722)
Nakuru	1.44	0.125	(0.904 – 2.288)
Nyahururu	1.35	0.169	(0.879 – 2.078)
Nyeri	0.61	0.086	(0.345 – 1.073)

4.6 Cross tabulation of age and infection status for HBV, HCV, HSV and syphilis

In the current study, there was no significant relationship between age and infection status for HBV, HCV, HSV and syphilis (Table 4.10).

Table 4.10: Relationship between age and infection status

HBV			**HCV**		**HSV**		**Syphilis**	
Sero-status	n = 1829	Age Mean/ Std. Dev.	n= 1829	AgeMean/ Std. Dev.	n = 1829	Age Mean/ Std.Dev.	n = 1829	AgeMean/ Std. Dev.
Neg	71 % (1299)	32.4±13.6	86.2% (1573)	32.5±13.5	(53.1%)969	31.9±13.6	86.1% (1573)	32.3±13.5
Pos	29% (530)	32.3±12.8	13.8% (256)	31.9±12.6	(46.9%)860	32.9±13.2	13.9% 256	33.1±12.7
Total	100% (1829)	32.4±13.4	100% (1829)	32.4±13.4	100% 1829	32.4±13.4	100% 1829	32.4±13.4
P-val		**0.850**		**0.575**		**0.1231**		**0.3319**

Analysis of association between gender and infection status for HBV, HCV, HSV2 and syphilis was also carried out. Results indicated there was no significant relationship between gender and infection status P>0.005 (Table 4.11)Table 4. 11: Gender and infection status with HBV, HCV, HSVand syphilis

Table 4.11: Gender and Infection Status with HBV, HCV, HSV2 and Syphilis

HBV				HCV			HSV			Syphilis		
Gender	Neg	Pos	n= 1829	Neg	Pos	n= 1829	Neg	Pos	n= 1829	Neg	Pos	n =1829
Female	72.8% (899)	27.2% (333)	100% (1,232)	86.9% (1,071)	13.1% (161)	1,232	49.6% (611)	50.4% 621	100% 1,232	86.8% (1,069)	13.2% (163)	100% (1,232)
Male	69.3% (414)	30.7% (183)	100% (597)	84.8% (506)	15.2% (91)	597	51.3% (306)	48.7% (291)	100% 597	84.8% (506)	15.2% (91)	100% (597)
Total	71.4% (1,306)	28.6% (523)	100% 1,829	86.2% (1,577)	13.8% (252)	1,829	50.1% (917)	49.9% (912)	1,829	86.1% (1,575)	3.9% (254)	100% (1,829
P-val	**0.175**			**0.206**			**0.505**			**0.243**		

4.7 Mapping of HIV Co- infections

4. 7.1 Maping of HBV by the regions in Kenya

Sero-prevalence of HBV was high across all sites ranging from 19 to 39% comparatively highest prevalence was recorded in Western and Nyanza provinces (above 31%) (Figure 4.2)

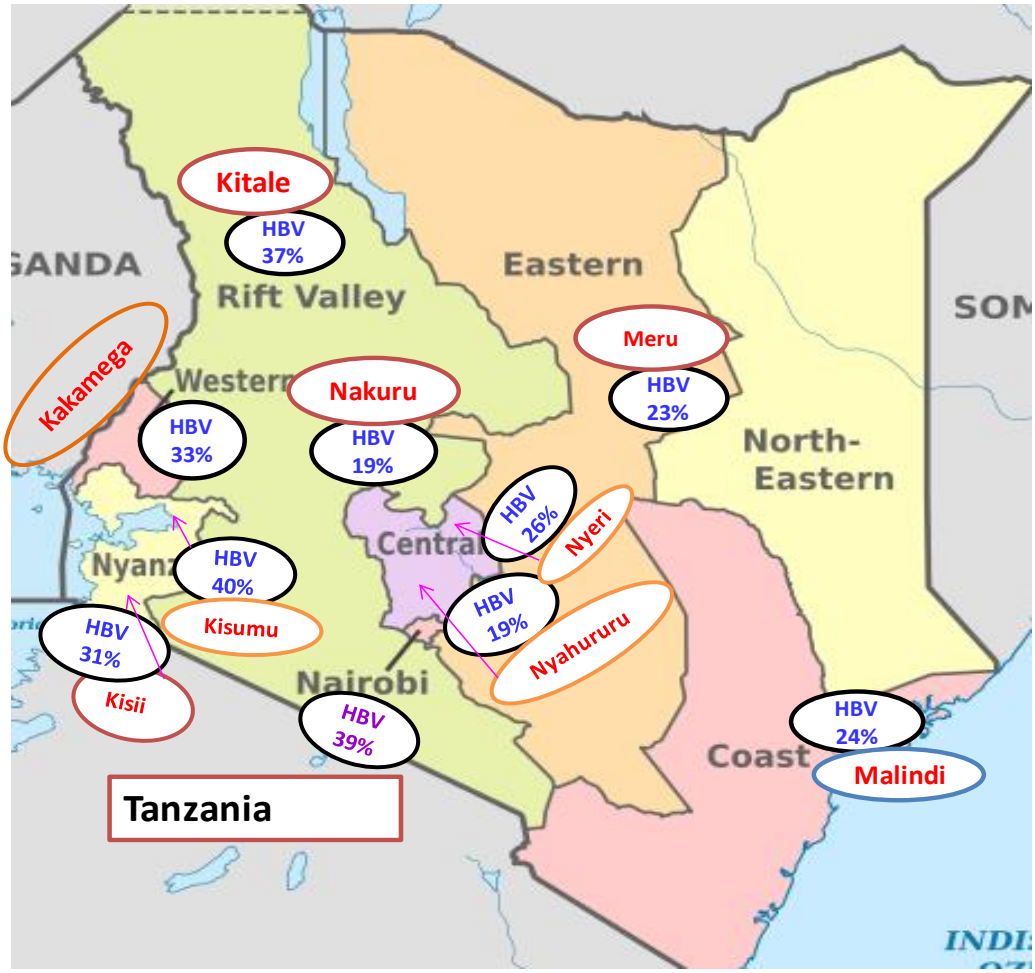

Figure 4.2: HBV Co-infection in Kenya

(Kenya administrative divisions - Image ID: EH1GHX)

4.7.3 Mapping of HCV by the regions in Kenya

Sero-prevalence of HCV showed a more homogenous pattern across the regions with Kisumu 19 %(40) (Nyeri19 %(15) (Kitale 18 %(33), Meru16 %(27) (Malindi 15% (29) and Kakamega 15%(24) (having the highest proportions.On average, the prevalence across the regions also indicate similar homogenonicity (Figure 4.3).

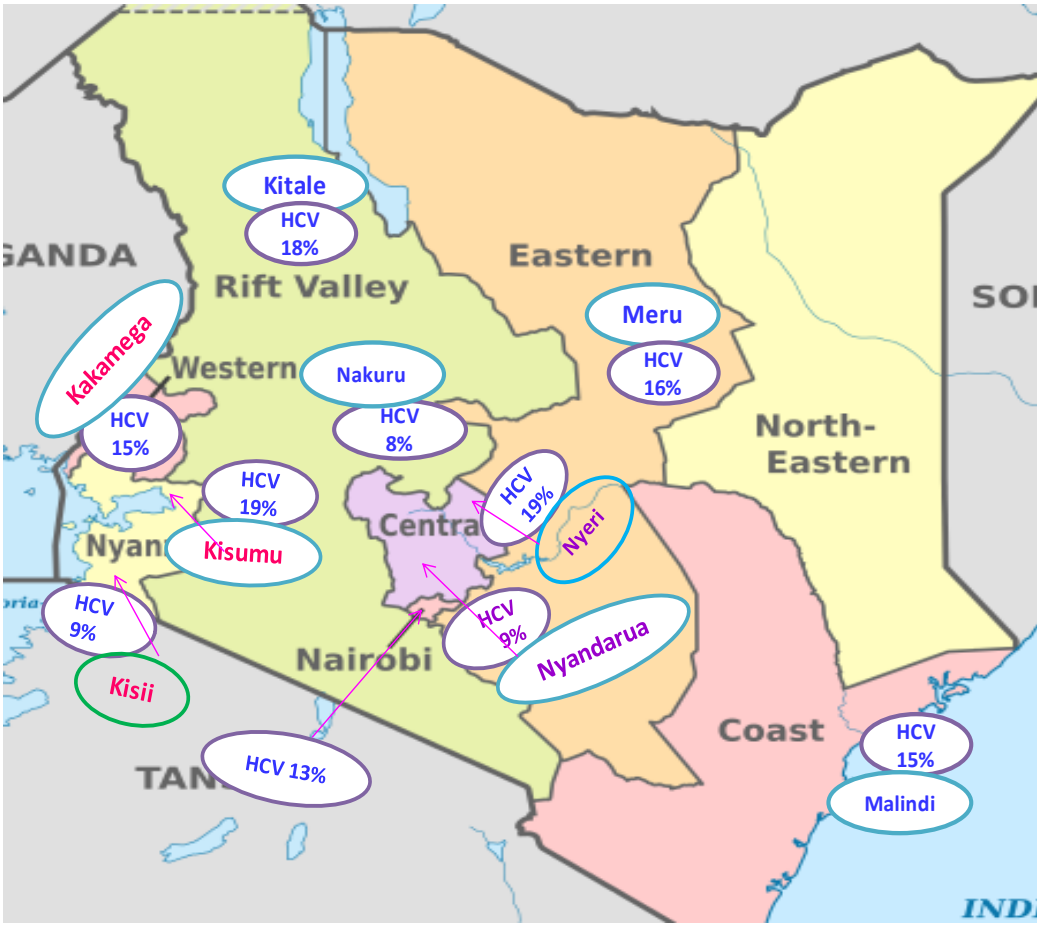

Figure 4.3: HCV co-infection in Kenya

4.7.4 Mapping of syphilis infections

Syphilis prevalence was highest in Western and Nyanza region (18% to 26%) compared to other regions (4% to15%), the lowest prevalence was recorded in Nyahururu and Nyeri, both in Central region. On average Nyanza region had 22% (76) followed by Western at 19 %(31) and Rift Valley at 17 %(45).The lowest regional average was that of Central 6.2 % (24) ((Figure 4.4).

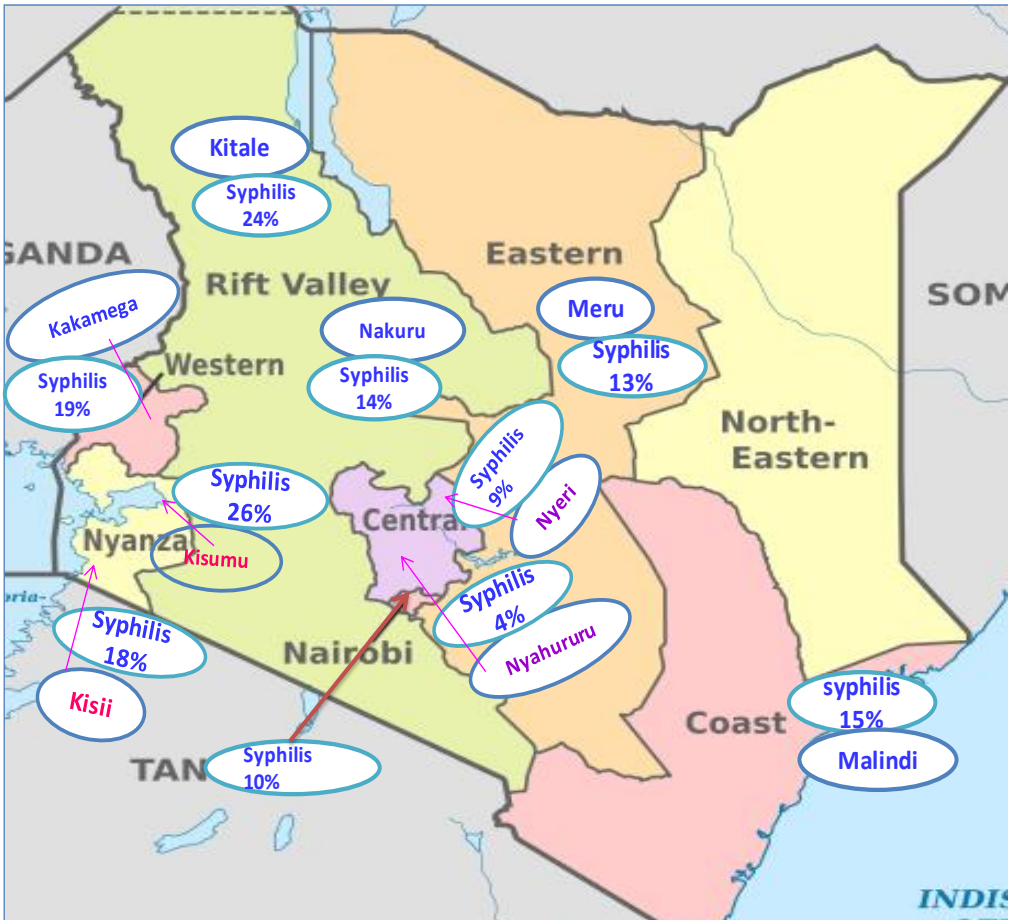

Figure 4.4: Syphilis co-infection in Kenya

4.7.5 Mapping of HSV infections

HSV2 prevalence ranged from 29% to 67% in all regions. The lowest was in Meru at 29% (50) and the highest Nyahururu at 67% (208). Regional averages indicate Nairobi was leading at 62% (186), Central at 48.5% and Rift Valley at 47.5%. Lowets prevalence of HSV2 was at eastern region (29%) (Figure 4.5).

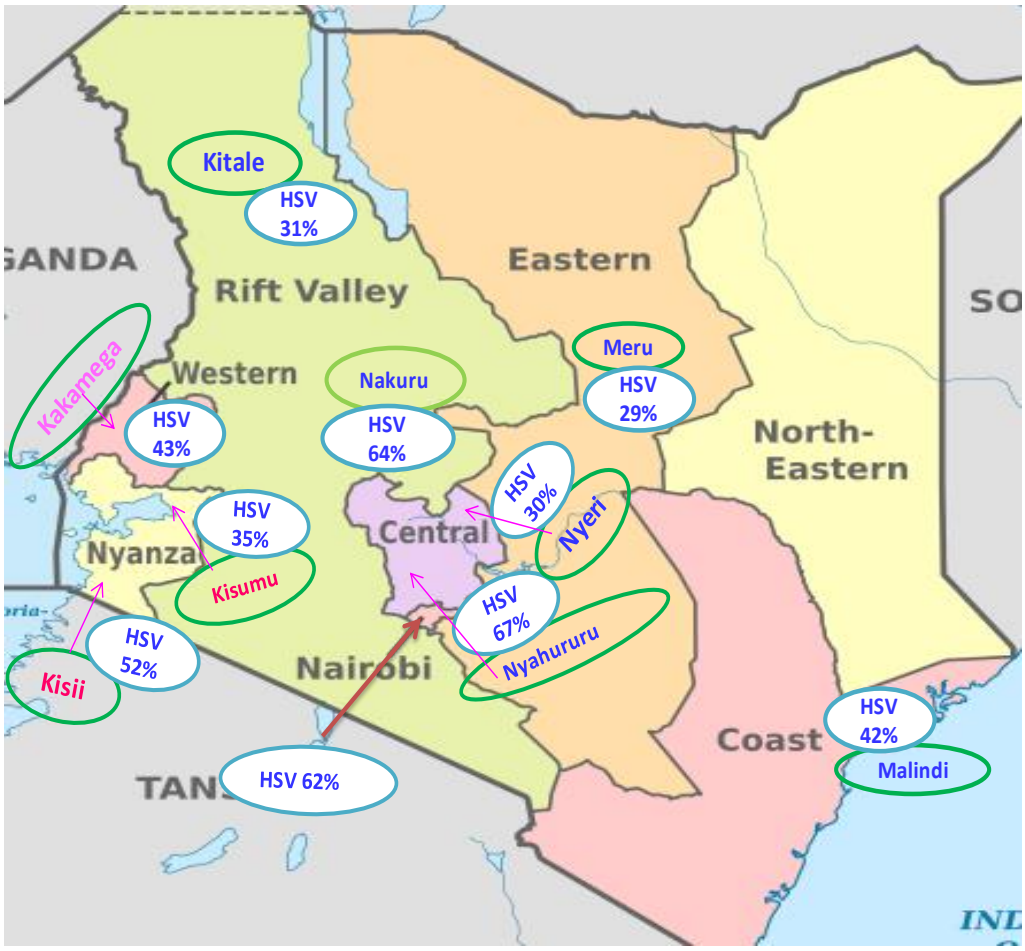

Figure 4.5: HSV2 co-infectionin Kenya

4.8 Corelation between HBV, HCV, HSV2 and syphilis infections and study sites

Corelation on infection status and study site was carried out for each of the 4 infections.There was significant association between HBV, HSV and syphilis infection and study sites (p-value 0.000) except for HCV (p-value-0.008), (Table 4.12 A, B, C and D).Within sites, Kisumu, Mbagathi and Kitale had highest sero-prevalence (40,39 and 37 respectively) of HBV while Nyahururu had the lowest at 19%.Sero-prevalence of HCV was highest in Kisumu and Nyeri(19%) and lowest in Nakuru(8%).

Table 4.12: Corelation between HBV, HCV, HSV and syphilis co-infections and study sites

Table A

HBV			
Site	Neg	Pos	Total
Kakamega	67% (108)	‡33%(54)	162
Kisii	69% (93)	‡31%(44)	137
Kisumu	60% (126)	‡‡40%(86)	212
Kitale	63% (32)	‡37%(19)	51
Malindi	76% (145)	24%(50)	195
Mbagathi	61%(183)	‡‡39%(117)	300
Meru	77%(131)	23%(40)	171
Nakuru	81%(171)	19%(41)	212
Nyahururu	81%(251)	19%(59)	310
Nyeri	74%(59)	26%(21)	80
Total	71%(1,299)	29%(530)	1,829

Chi-square test; p-val=0.000

Table B

HCV			
Site	Neg	Pos	Total
Kakamega	85% (137)	‡15% (25)	162
Kisii	91% (112)	9% (25)	137
Kisumu	81% (171)	‡‡19% (40)	211
Kitale	82% (42)	‡‡18% (9)	51
Malindi	85% (166)	‡15% (29)	195
Mbagathi	87% (262)	13% (38)	300
Meru	84% (145)	‡16 (26)	171
Nakuru	92% (191)	8% (21)	212
Nyahururu	91% (282)	9% (28)	310
Nyeri	81% (65)	‡‡19% (15)	80
Total	86%(1,573)	14% (252)	1,829

Chi-square test; p-val=0.008

TABLE C

Syphilis			
Site	Neg	Pos	Total
Kakamega	81% (131)	‡19% (31)	162
Kisii	82% (113)	‡18% (24)	137
Kisumu	74% (156)	‡‡26%(5)	211
Kitale	76%(39)	‡‡24%(1)	51
Malindi	84% (164)	15% (31)	195
Mbagathi	90% (270)	10%(30)	300
Meru	87%(149)	13% (22)	171
Nakuru	86%(182)	14% (30)	212
Nyahurur	96 %(296)	4% (14)	310
Nyeri	91%(73)	9% (7)	80
Total	86%(1,573)	14%(256	1,829

Chi-square test; p-val=0.000

TABLE D

HSV2			
Site	Neg	Pos	Total
Kakamega	57% (93)	43% (69)	162
Kisii	48% (66)	‡52% (71)	137
Kisumu	65%(138)	35% (73)	211
Kitale	69% (35)	31% (16)	51
Malindi	58%(114)	42% (81)	195
Mbagathi	38% (13)	‡‡62% (187)	300
Meru	71%(121)	29% (50)	171
Nakuru	36% (77)	‡‡64% (135)	212
Nyahururu	33%(104)	‡‡67% (206)	310
Nyeri	70%(56)	30% (24)	80
Total	53%(969)	47% (860)	1,829

Chi-square test; p-val=0.000

4.9 CD4 count and co-infections with HBV, HCV, HSV and syphilis

4. 9.1 Distribution of CD4 counts by mean age and sites

Mean age of study participants by site was determined (Table 4.15). Study participants in Kakamega and Malindi had the highest mean age at 35.8 years (SD 11.9) and 35.6 yrs (SD 13.9) respectively, while Mbagathi had the lowest at 24.4 years (SD 14.2).The over all Mean CD4 count was 382. Study participants in Malindi and Mbagathi had the highest mean CD4 count at 468 and 464 respectively, while Kitale had the lowest at 234 (Table 4.13).

Table 4.13: CD4 count by demographic variables

Item No.	Sites	N= Samples	Age Mean, (SD)	Mean CD4, (SD)
1	Kakamega	162	35.8 (11.9)	386.8 (263)
2	Kisii	137	29.7 (12.1)	372.5 (282)
3	Kisumu	211	33.3 (11.4)	300.8 (259)
4	Kitale	51	33.8 (12.4)	233.6 (182)
5	Malindi	195	35.6 (13.9)	468.8 (307)
6	Mbagathi	300	24.4 (14.2)	464 (377)
7	Meru	171	31.1 (13.47	367.7 (262)
8	Nakuru	212	32.9 (12.6)	317.7 (293)
9	Nyahururu	310	34.4 (13.5)	381.9 (330)
10	Nyeri	80	34.6 (10.5)	377.7 (253)

Kakamega and Malindi sites had higher partipants mean ages compared to other sites while Mbagathi had the lowest.Mean CD4 count was highets in Malindi, Mbagathi and Kakamega and lowest at Kisii.

4.9.2 CD4 distribution by WHO treatment guidlines among participants

CD4 counts were grouped into <250,251-350,351-500 and >501 where the total number of individuals in each category were ; 34%(624),18%(348),19%(329) and 29%(530) respectively (Figure 4.6).

CD4 count results were also compared to WHO recommendations for ART initiation.WHO guidelines recommendation for CD4 counts threshhold for ART initiation has changed over time rising from 200cells/mm^3 in 2002 to 350 cell/ mm^3 in 2013 and again to 500 mm^3 in 2015.By 2017 the guidelines recommended initiation of treatment once one is HIV positive regardless of CD4 count.These guidelines also recomended use of CD4 count as a measure of advanced HIV disease (WHO, 2017). Based on these changes, CD4 count among the study participants indicated there was a steady increase in demand for treatment with each change in eligibility criteria based on WHO cut-off for treatment initiation (Figure 4. 6).Thus 34 % of the participants met the criteria of CD4 count monitoring regardless of viral load measurements).

At less than 250 CD4 count treatment eligibility criteria, 34 %(624) of the study population was eligible for treatment.This however increased by 18% (624 to 955, 19 %(955 to 1297) and 29 %(1297 to 1829) at 350,500 and above 500 CD4 count (treat all infected) WHO criteria respectively (Figure 4.6).

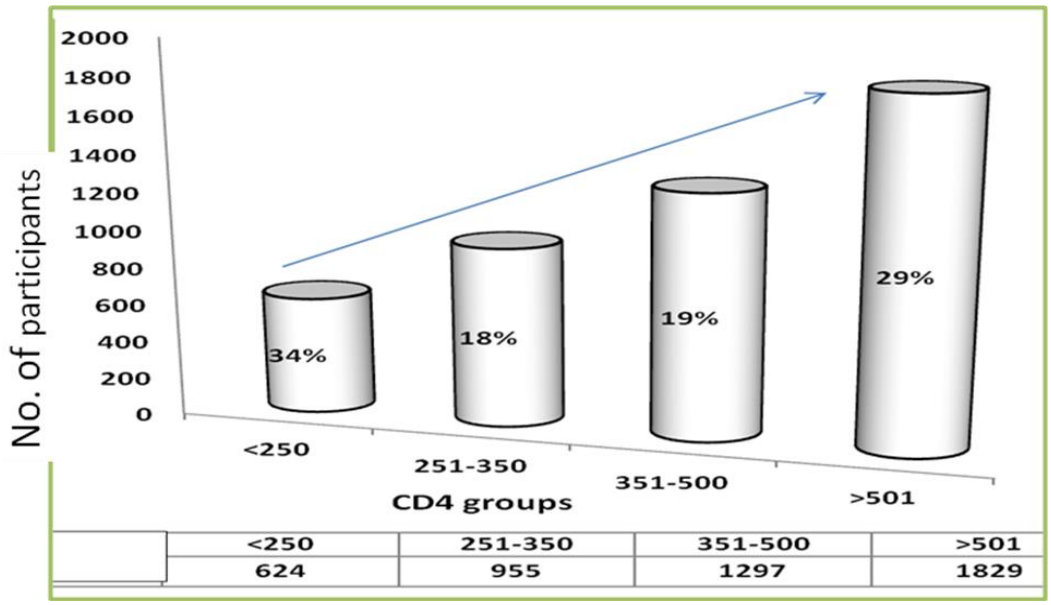

Figure 4.6: CD4 counts among **participants.**

(624 participants had CD4 count below 250 cell/ml indicating an advanced HIV disease).

4.9.3 Variation of mean CD4 by gender within study sites

Gender disparity was detected at combined mean CD4 count of 382 (participants) where in most sites (8) males had lower mean against 3 sites for females (Table 4.14)

Table 4.14: Variation of mean CD4 by gender within study sites

Study site	Mean CD4 count Female	Male
Kakamega	420	380
Kisii	425	300
Kisumu	320	370
Kitale	250	200
Malindi	500	500
Mbagathi	510	490
Meru	400	380
Nakuru	370	370
Nyahururu	440	381
Nyeri	410	369

4.9.3 Relationship between CD4 count and the HBV, HCV, HSV and Syphilis status

Relationship between the four infections indicate HSV2 affects CD4 count (p-value=0.0021; mean Pos 365±306/ul vs. mean Neg 413±309/ul. HBV, HCV and syphilis did not affect CD4 count (p>0.005) (Table 4.15).There was therefore no statistical significant relationship between participants with HBV, HCV and syphilis and CD4 count.Infection with HSV2 significantly affected CD4 count.

Table 4.15: CD4 count and HBV, HCV, HSV and syphilis co-infection status

A) CD4 and HBV co-infection					**B) CD4 and HCV co-infection**			
HBV	Mean	Std. Dev.	Freq.		**HCV**	Mean	Std. Dev.	
Neg	412.4	314.0	1306		Neg	404.8	309.6	1577
Pos	392.5	296.8	523		Pos	418.6	307.5	252
Total	406.7	309.2	1829		Total	406.7	309.2	1829
T-test; p-val=0.2136					**T-test; p-val=0.5116**			
c) CD4 and syphilis co-infection					**d) CD4 and HSV2 co-infection**			
Syphil	Mean	Std. Dev.	Freq.		**HSV2**	Mean	Std. Dev.	
Neg	420.1	322.3	917		Neg	413.4	309.3	1575
Pos	393.3	295.1	912		Pos	365.2	306.4	254
Total	406.7	309.2	1829		Total	406.7	309.2	1829
T-test; p-val=0.0643					**T-test; p-val=0.0021**			

4.10 Testing Algorithm for HIV, HBV, HCV, syphilis and HIV incidence

Objective 3 of this study involved determining suitability of testing algorithm for HIV, HBV, HCV and syphilis.

4.10.1 HIV testing

For HIV, four test methods (test kits) were selected based on the national testing guidelines that were operational at the time of the study .Vironostika HIV ELISA used as the reference test method; Determine HIV; Bioline HIV and Unigold HIV rapid tests.

All samples received for the study were retested in house with ELISA kit (Vironostika HIV).This was to confirm HIV status. A total of 243 of 1829 samples tested negative. The negative samples (243) were subjected to additional testing for HIV using an in-house algorithm for discrepant test results.This involved repeat testing with vironostika and and Murex HIV Ag/Ab Combination assay (Murex Biotech)where 181 were conclusively resolved as being positive.To determine performance characteristic of the different test methods against this 243 samples,four different test Kits;Vironostika,determine rapid HIV kit,Unigold HIV test kit and Bioiline HIV kit were selected since they are the most commonly used in Kenya (Table 4.16). Results indicated that 62 out of 243 samples remained discrepant. All the 62 samples were then re-tested by PCR9as described earlier in 3.11.3 to 3.13(PCR procedure) where, 26 were confirmed positive. The rest, 36 of 62 samples were consistently HIV negative (Table 4.16).

Table 4.16: comparative HIV testing using four different test methods

	Vironostika		
		Frequency	Percent
	POS	217	89.3
Valid	NEG	26	10.7
	Total	243	100.0

	Determine HIV		
		Frequency	Percent
Valid	POS	185	76.1
	NEG	58	23.9
	Total	243	100.0

11 % samples were Neg

24% samples were Neg

Unigold HIV Test Kit			
		Frequency	Percent
Valid	POS	183	75.3
	NEG	60	24.7
	Total	243	100.0

		Bioline HIV kit	
		Frequency	Percent
Valid	POS	181	74.5
	NEG	62	25.5
	Total	243	100.0

25% of samples were Neg

26% of the samples were Negative

4.10.2 HIV ELISA and Rapid test assays characteristics

Performance characteristics of the HIV rapid tests used in the study (Table 4.17) were evaluated. Low sensitivity and specificity with the reference test (ELISA) was detected for determine, bioline and unigold rapid test kits (4.18a-4.18d). Levels of agreement (kappa) between the tests were analyzed, where bioline rapid test had the least level of agreement (kappa 0.251) with ELISA as well as with the other rapid tests (Table 4.17). Bioline rapid test also had the highest number of false positives (46) (Table 4.17) compared to ELISA, unigold and determine HIV rapid tests.

Table 4.17: Rapid Kit's performance characteristics based on ELISA reference test.

Determine HIV 1&2 * Vironostika HIV 1&2 Kit Cross tabulation				
		Vironostika		
		Pos	Neg	Total
Determine HIV 1&2	Pos	175	10	185
	Neg	42	16	58
	Total	**217**	**26**	**243**
Sensitivity 80.6%	**specificity** 61.5%		**Kappa**0.274	

UniGold HIV Test Kit * Vironostika HIV 1&2 Kit comparison				
		Vironostika		
		Pos	Neg	Total
UniGold test	Pos	173	10	183
	Neg	44	16	60
	Total	**217**	**26**	**243**
Sensitivity 79.7%	**specificity** 61.5%		**Kappa** 0.262	
Kappa	UniGold Vs Determine		0.799	

Bioline * Vironostika HIV 1&2 Kit comparison				
		Vironostika		
		Pos	Neg	Total
Bioline test	Pos	171	10	181
	Neg	46	16	62
	Total	**217**	**26**	**243**
Sensitivity	78.8% **Specificity** 61.5% **Kappa** 0.251			
Kappa	Bioline vs. determine		0.491	
Kappa	Bioline vs. Unigold		0.562	

Kappa key
Poor agreement = Less than 0.20 ; Fair agreement = 0.20 to 0.40
Moderate agreement = 0.40 to 0.60 ; Good agreement = 0.60 to 0.80
Very good agreement = 0.80 to 1.00

4.11 Hepatitis testing algorithm

4.11.1 Hepatitis B testing algorithm results

Of the 530 (29) samples positively for HBV in this study 320 samples were randomly selected for HBV testing strategy investigation.These samples were then comparatively tested with PCR, Determine HBVand Ichek HBV test kits for algorithm determination.

4.11.2 Comparative performance between Determine HBV,Ichek HBV and PCR tests against ELISA positive samples

Determine test kit captured the highest number of positives samples (265/320) from the ELISA positives compared to PCR and I-check tests. I-check test captured the least with 154/320 samples positive by ELISA being negative (Figure 4.7)**.**

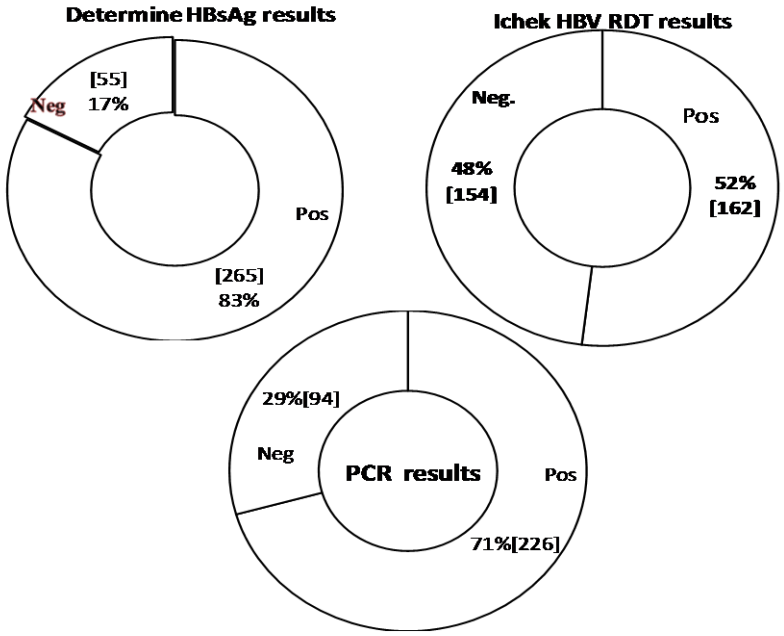

Figure 4.7: Performance results **of 3 different test kits for HBV**

4.11.3 Sensitivity and specificity Ichek and PCR tests on hepatitis B

Using HBV determine test kit as the gold standard, Ichek had the lowest performance characteristics (Sensitivity 61%, and specificity at 89%). Level of agreement between the tests was higher between determine and PCR at 39.2 % compared to Ichek (28.9%) (Table 4.18)

Table 4.17: Sensitivity and specificity of Determine and Ichek test for HBV testing

Ichek HBV RDT * Determine HBsAg Cross tabulation				
		DETERMINE HBsAg		
		Neg	Pos	Total
Ichek HBV RDT	Pos	6	160	166
	Neg	49	105	154
Total		**55**	**265**	**320**
Sensitivity 61% , Specificity 89% , Kappa .289				

PCR * Determine HBsAg Cross tabulation				
		DETERMINE HBsAg		
		Neg	Pos	Total
PCR	Pos	16	210	226
	Neg	39	55	94
Total		**55**	**265**	**320**
Sensitivity 79% , Specificity 71% , Kappa .392				

4.12 Hepatitis C testing algorithm results

The most suitable test method for HCV was SD Bioline HCV rapid and Anti HCV Particle hemagglutination tests ((PHA), where positivity rate was at 15% for both the tests respectively. Murex anti HCV had more positives at 22% (false positives) than the other two kits (15%) (Figure 4.8).

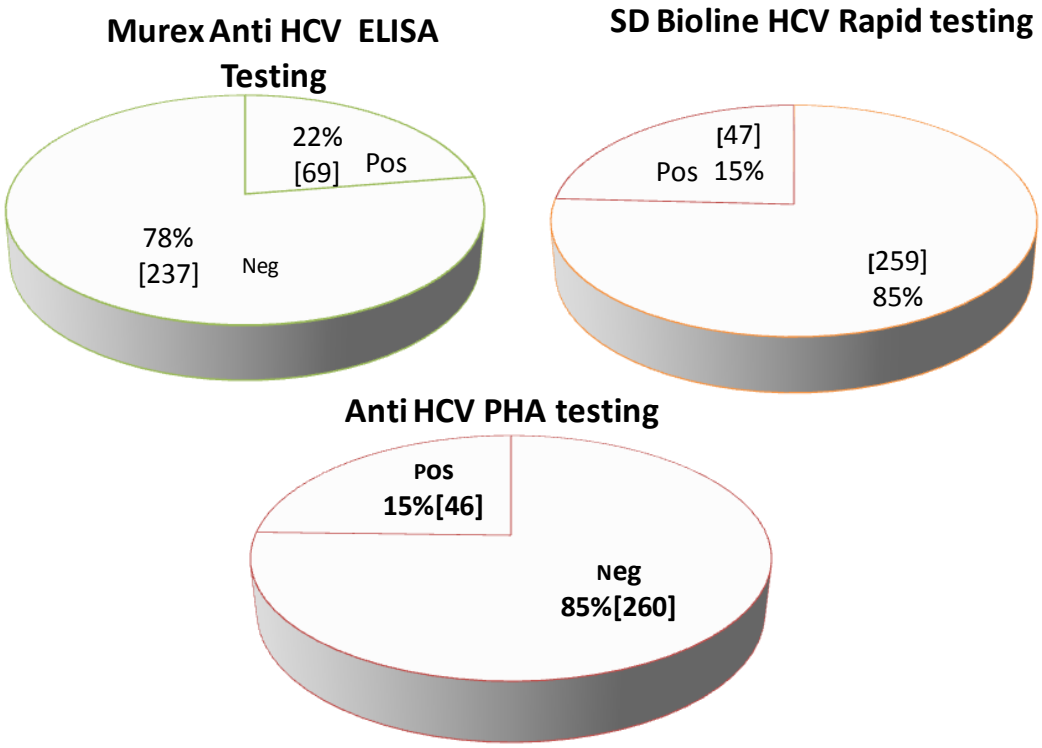

Figure 4.8: Hepatitis C testing using Murex ELISA,SD Bioline PHA tests

4.13 Comparative performance of HCV PHA,HCV ELISA and HCV Bioline test kits

Comparing the three kits used for HCV testing, Bioline HCV, with sensitivity of 94% and specificity of 99% had better performance.The highest level of agreement was between bioline and HCV PHA kit (91.1%) (Table 4.19).

Table 4.18: Sensitivity and specificity of HCV test Kits

Bioline HCV * HCV PHA Cross tabulation				
		HCV PHA		
		Neg	Pos	Total
Bioline HCV	Pos	4	43	47
	Neg	256	3	259
Total		**260**	**46**	**306**

Sensitivity 94%, Specificity 99%,Kappa .911

HCV ELISA * HCV PHA Cross tabulation				
		HCV PHA		
		Neg	Pos	Total
HCV ELISA	Pos	26	43	69
	Neg	234	3	237
Total		**260**	**46**	**306**

Sensitivity 94%,Specificity 90%,Kappa .692

Bioline HCV * HCV ELISA Cross tabulation				
		HCV ELISA		
		Neg	Pos	Total
Bioline HCV	Pos	3	44	47
	Neg	234	25	259
Total		**237**	**69**	**306**

Sensitivity 64%, Specificity 90%, Kappa .705

HCV PHA * HCV ELISA Cross tabulation				
		HCV ELISA		
		Neg	Pos	Total
HCV PHA	Pos	3	43	46
	Neg	234	26	260
Total		**237**	**69**	**306**

Sensitivity 62% , Specificity 99% , Kappa .692

HCV PHA * HCV Bioline Cross tabulation				
		Bioline HCV		
		Neg	Pos	Total
HCV PHA	Pos	43	3	46
	Neg	4	256	260
Total		**47**	**259**	**306**
Sensitivity 92% , Specificity 99% , Kappa .911				

4.14 HIV subtypes among study participants

A 150 HIV positive samples were subjected to PCR Pol-RT testing and sequencing.Out of this, 120 samples were prified for sequencing.Analysis of the sequences indicated

HIV Genotypes A(70%),D(18%),G(7%) and C(5%) were present in this study(Figure 4.10).The other 30 samples did not give clear PCR products even upon repeat PCR testing and were therefore not used for sequencing.Of the sequenced samples,19 did not yield clean sequences(had molecular noise-poor quality) and were therefore not conclusive in genotype determination. Figure 4.10 therefore shows 101 samples that were succesfuly analysed.

HIV genotypes A1 clustered with references from Tanzania and Rwanda.Genotype D clustered with references genotypes from Democratic republic of Congo and Uganda while the rest group of isolates; genotypes A2,G and C clustered with references from Cameroon, Uganda, Kenya, South Africa and Tanzania (Figure 4.9).

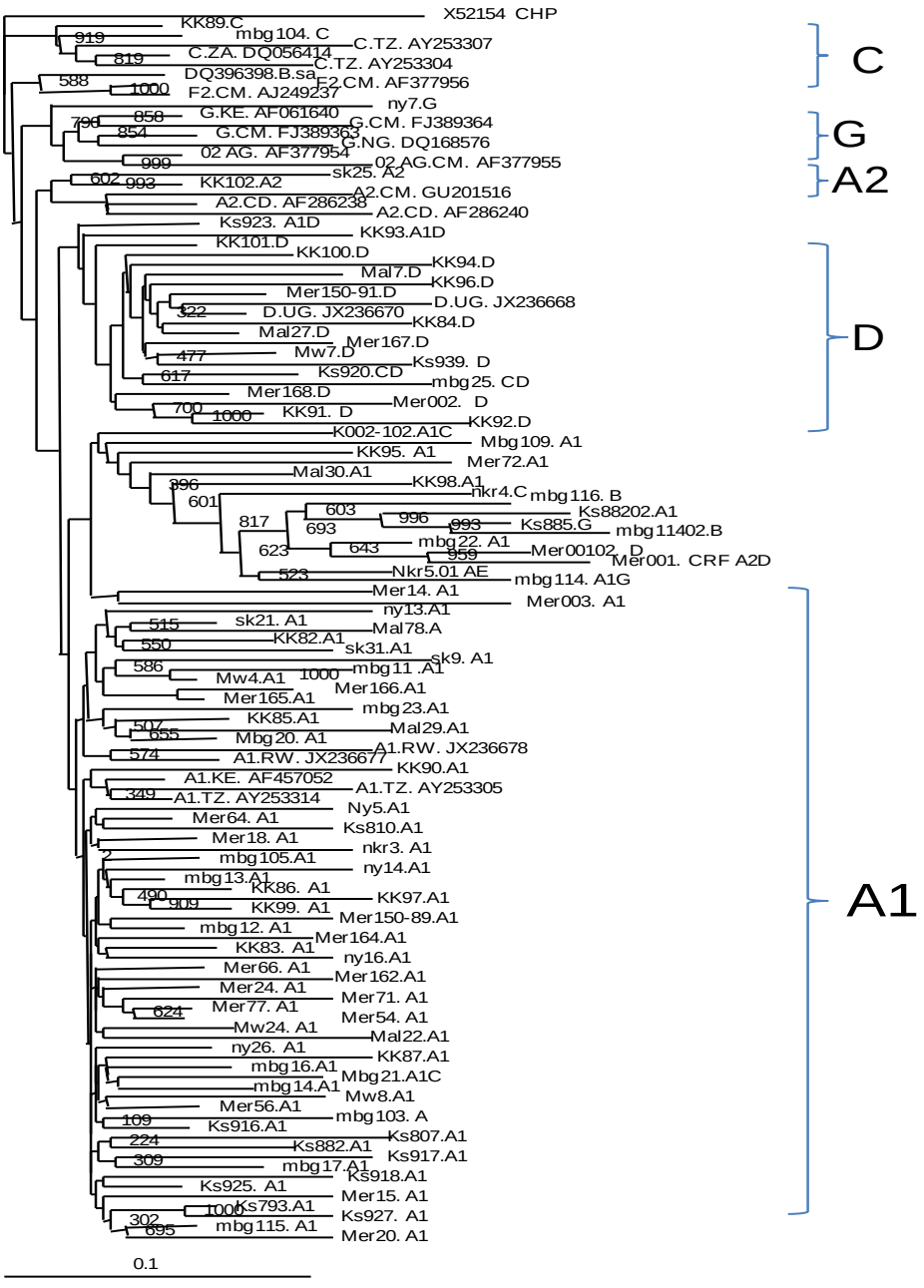

Figure 4.9: Phylogenetic tree 1. HIV subtypes.

(Kenya samples are indicated by prefixes: Mer; Mbg; Ks; SK; NY; Mal; Nkr and KK)

4.14.1 Distribution of HIV genotypes by sites

HIV genotype A was the main genotype identified in all the study sites, followed by genotype D and C in that order (Table 4.20)

Table 4.19: Distribution of HIV genotypes by study sites

	HIV	**Subtypes**								
	Sites	A	B	C	D	E	G	AD	AE	CD
1	Kakamega -KK	√		√	√			√		√
2	Mbagathi -Mbg	√	√	√						√
3	Nyeri-Nyr	√					√			
4	Kisii -Sk	√						√		
5	Kisumu Ks-	√		√			√			√
6	Malindi	√			√					
7	Meru	√			√			√		
8	Mw=Nyahu	√			√					
9	Nakuru	√							√	
10	Kitale	√								
11	BTC, Nairobi	√			√	√				

4.14.1 Subtypes of HBV among study participants

Hepatitis B seropositive samples were tested by PCR and those positive sequenced to determine genotypes based on PreS1 region. A total of 133 samples were succesfully sequenced and analysed for HBV genotypes.Subtypes are presented in 3 phylogentic trees (Figure 4.10, 4.11 and 4.12). This was to avoid tree overcrowding. Genotypes of HBV detected were A (82%), C (9%); E (6%) and D (3%) (Figure 4.10; 4.11 and 4.12). Identified Genotypes A1 clustered closely with refernces from Kenya, Uganda, Somalia, Malawi and South Africa (Fig 4.10; 4.11; and 4.12)

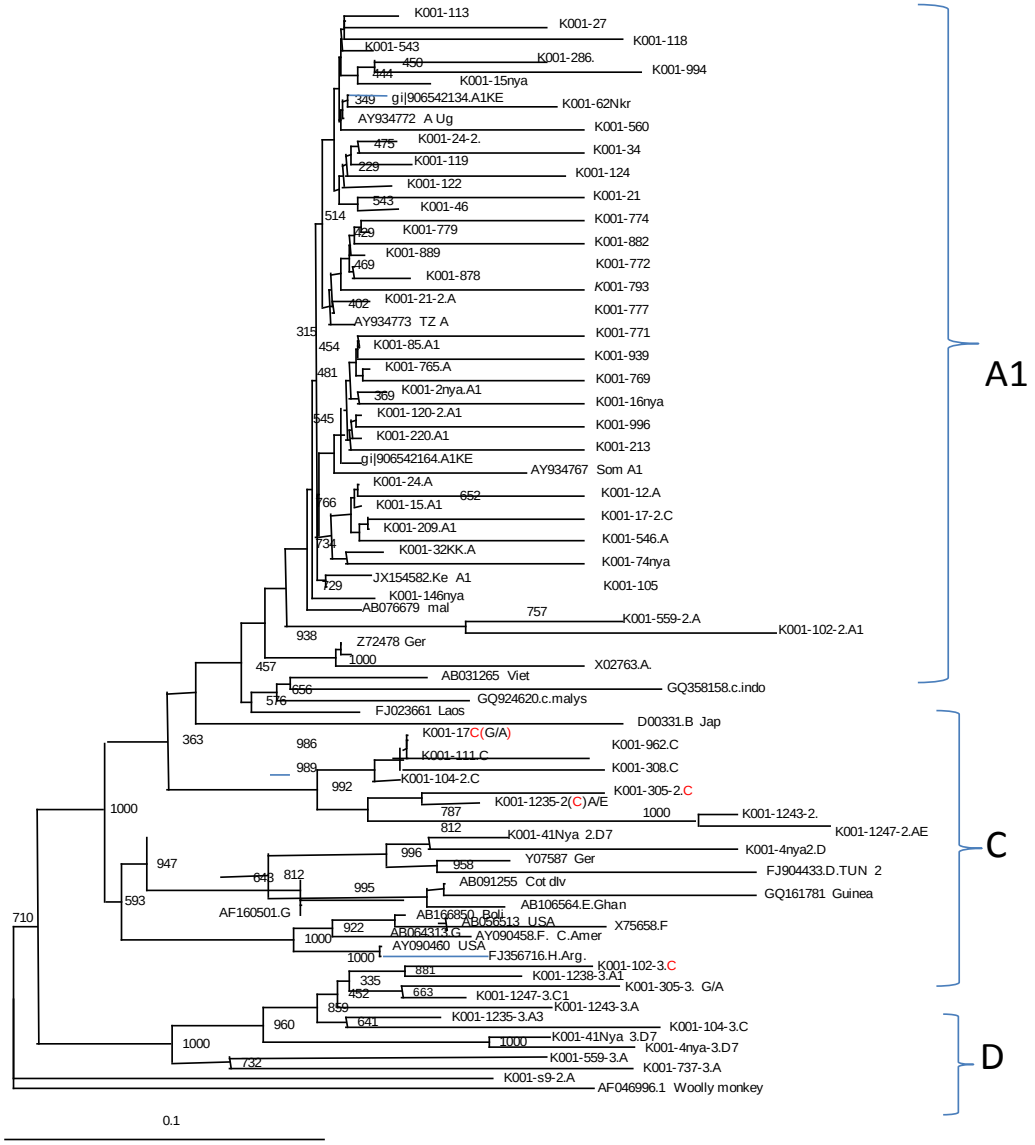

Figure 4.10: Phylogenetic tree of HBV genotypes (1)

(Kenya samples are represented by code K001---Genotypes D,C and were identified).

Phylogenic tree of HBV genotypes based on PreS1 region (Kenyan samples are underlined) indicate genotype A1 and A2 were detected (Figure 4.11).

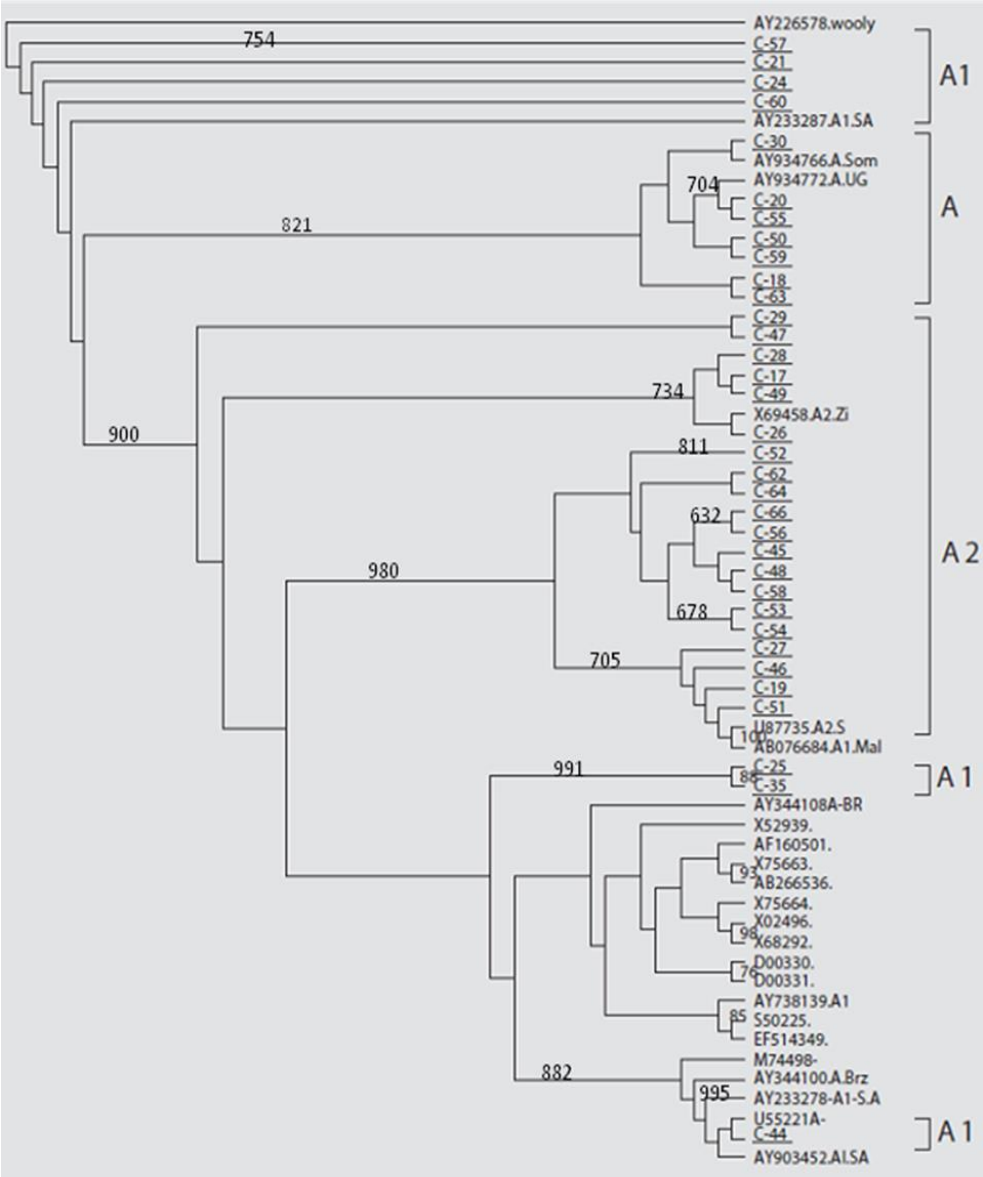

Figure 4.11: Phylogenetic tree of HBV genotypes (2).

(Phylogenic tree of HBV genotypes based on PreS1 region (Samples are underlined) indicate genotype A1 and A2 were detected).

Phylogenic tree of HBV genotypes based on PreS1 region.Results indicate genotype A1 and A2 were detected (Figure 4.12).

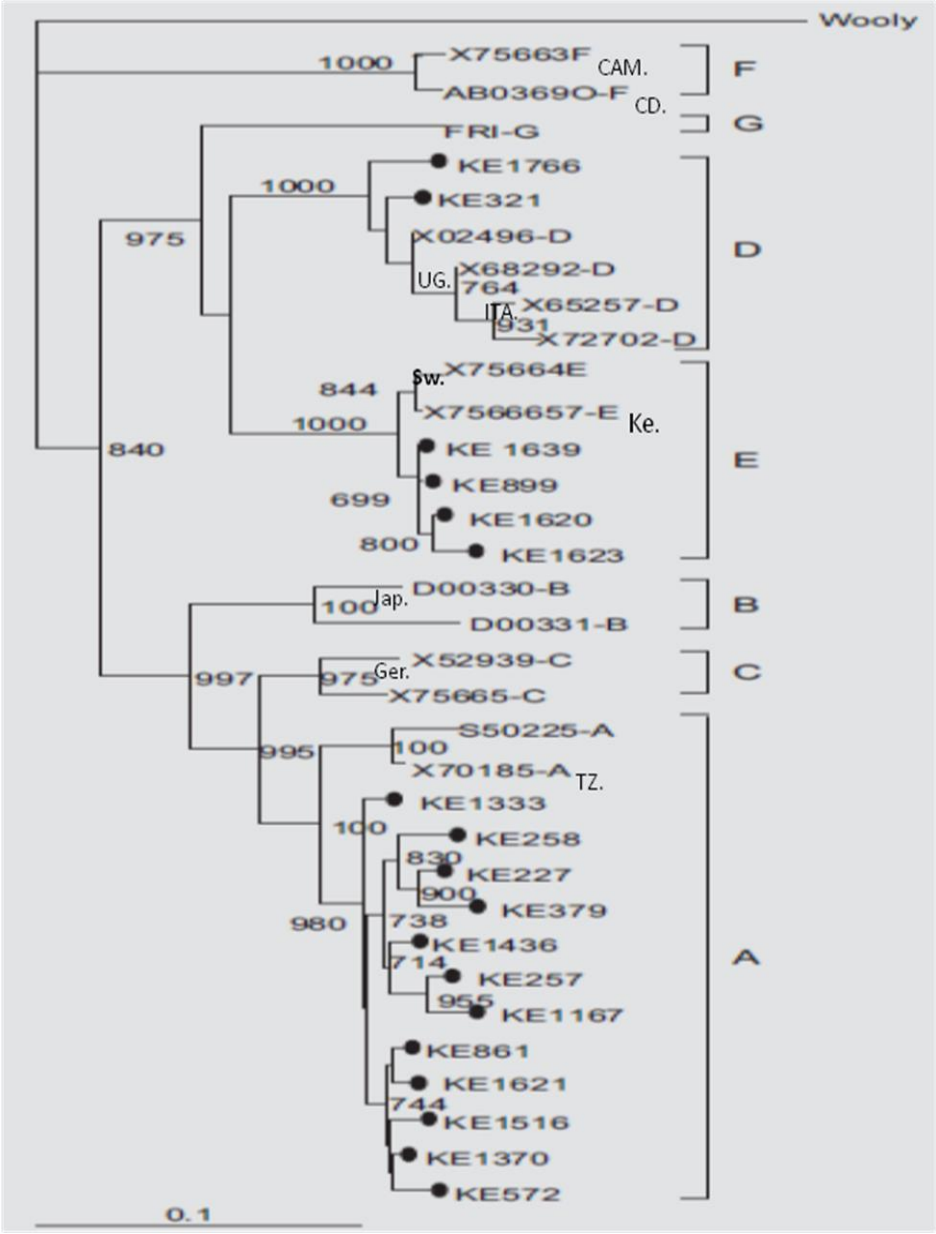

Figure 4.12: Phylogenetic tree (3) of HBV genotypes

(PreS1 region (Samples are bulleted) indicate genotypes D, E, A were detected)

4.14.2 Subtypes of HCV based on UTR gene among study participants

Hepatitis C positive were tested by Polymerase chain reaction and 14 of these samples sequenced to determine genotypes (Figure 4.13).On phylogentic analysis HCV Genotypes 1a(14%) and 2b(86%) were identified.The Kenyan samples clustered closely with those from USA,Canada and Japan.

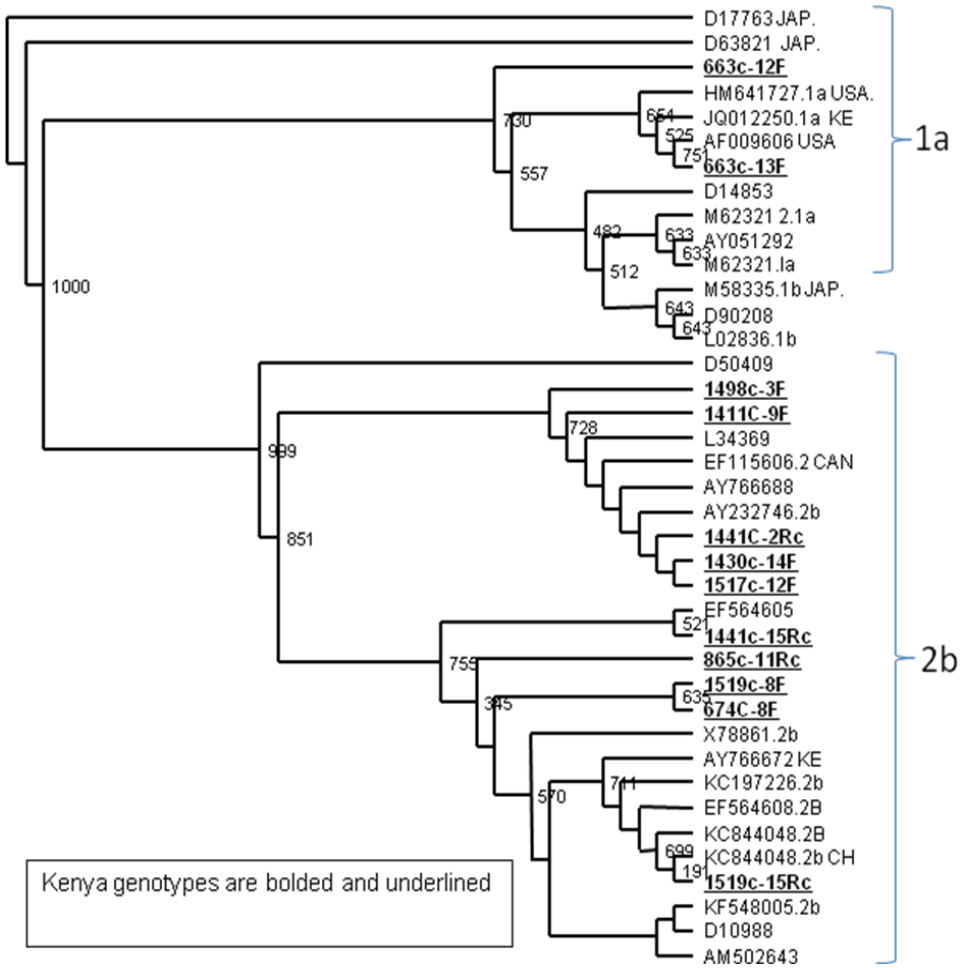

Figure 4.93: Phylogenetic tree HCV.

(HCV Genotypes 1 and 2b were detected in this study)

CHAPTER FIVE

DISCUSSION

5.1 Introduction

The findings of this study demonstrates variations in seroprevalence of hepatitis B virus (26%), hepatitis C (14%), Herpes simples virus 2(47%) and syphilis (14%) among HIV infected population in Kenya.Regional difference in seroprevalence was also shown in this study.To the best of my knowledge, this is the first study that provided information on HBV genotypes circulating in Kenya for the first time, through a paper published in 2008 as well as the first study to report on presence of HCV genotype 2b in Kenya. This is important information to inform tailor made interventions especially in areas of high prevalence for HBV, HCV, HSV2 and syphilis in Kenya, considering now that health services is a devolved function.Four papers were published in peer reviewed journals from this work.

This study thus, carried out investigation on co-infections which are an important concern in health care management and disease control. A population of patients attending comprehensive care clinics was targeted to determine HIV co-infections including Hepatitis B, Hepatitis C, Herpes simplex and syphilis-These infections were selected for the study since they share common modes of transmission and also affect HIV and AIDS disease progression and management outcomes . In addition an analysis of CD4 counts, an important immunological parameter in HIV and AIDS was examined. Testing algorithm was also determined in a sample of the study population in order to collate data that could improve utility of testing as well as patient management.

The study was unique in two aspects; it was a multicentre study, with study sites drawn from across regions (formerly provinces) in the country. In addition the study also employed a relatively large sample size. It thus provides results that represent a HIV

population as well as regional diversity within Kenya. This study also provides information on 5 infections unlike other studies in Kenya that include HIV, HBV and or HCV. The current study had HSV2 and syphilis in addition. These results therefore are likely to form the basis for inferences on HIV co-infections in Kenya.

The study population selected from at least 10 sites within the high population belt in Kenya had more female participants than males (67% vs. 33%).This is despite the random selection within the target population and similar demographic profile. Since gender is a social determinant of health, perhaps, this is an indication of disparity in health seeking behavior between female and males in the population. Notably, the population in Kenya consist of almost equal proportions of females and males; 24,014,716 million female vs. 23,548,056 million males (KBS, 2019). Since respondents were participants seeking HIV comprehensive care services, this could also be a reflection of high infection rates among females. The latest HIV surveillance data, the Kenya AIDS Indicator survey 2012, demonstrated higher HIV prevalence among female (6.9% vs 4.4%; overall 5.6 % prevalence) (KAIS, 2012). Risk factors and vulnerability, HIV prevalence rates and the impact of HIV/AIDS are important factors that place gender issues at the core of the HIV/AIDS. Of the global HIV infected population, 24.5 million people are in Africa where 12.9 million (53%) are women. This represents 82% of the world's women with HIV/AIDS. Africa is the only continent where HIV prevalence is higher for women than for men.

Characterization of the study participants by age and age groups indicated a homogenous population with comparable mean age between females and males at 31.97 and 32.35 respectively. Most of the study participants were of middle age as indicated by age group categories. This is important when considering the distribution of co-infections across the population demographics. Importantly, age is a determinant factor in HIV transmission. For example, HIV prevalence increased with advancing age, peaking at 35–39 years and 45–49 years among women and men respectively in Kenya

according to the latest HIV population based survey (KENPHIA, 2018).The youth (15-24 years) contributed 51% of adult new HIV infections in 2015.

5.2 Co-infections: Viral co-infections and syphilis among participants

In this study, HSV2 was the most prevalent co-infection (47%) among the HIV infected followed by HBV (29%), HCV (14%) and syphilis (14%). Disparities were prominent in the distribution of these infections by gender, age and locality. Analysis of multiple co-infections indicated, majority of individuals (71%) had one infection or another. This finding indicates the magnitude and complexity of co-infections in HIV. A spectrum of co-infections which included; single (43.5%, dual (20.2%), multiple (6.3%) and quadruple (0.8%) infection among the HIV infected were identified in this population. Single and double co-infections were common among the older participants (>60 years) with 49% and 23% respectively while multiple and quadruple infections were highest among 25-44 year old (6.9% and 2.2% respectively). This could be due to higher risk factors such as multiple sexual partners and low condom use associated with the latter age group.Highest mono co-infections were by HSV 2 followed by HBV, HCV and syphilis in that order.

Such multiple co-infections have been reported previously among HIV/AIDS patients co-infected with five viruses including; HBV,HCV, Epstein-Bar virus (EBV), and HSV 1 and 2 in china by He and team.In that study; 1.0% of participants had five different infections; 15.9% had four viruses, 55.0% had three , 25.9% had two and 2.1% with single infections (He *et al*.,2011).Shared mode of transmission and the impact co-infections have on disease progression are important public health considerations informed by the results of the current study.

Data on co-infection in this study is timely to inform intervention in care and management of HIV and AIDS. Co-infections have been shown to have a bidirectional pathological relationship with HIV since they accelerate disease progression in HIV/

AIDS and vice versa (White *et al.,* 2006). Syphilis, for example, is related to both increased concentrations of HIV RNA in blood plasma (Buchacz *et al.,* 2004). Infections with HSV-2 is linked to increased concentrations of HIV in blood plasma and genital fluids (Duffus *et al.,* 2005). Local inflammation of the genital tract caused by viral and non-viral STI promotes HIV shedding, therefore increasing HIV infectiousness (Cohen *et al.,* 1997). The increase in mortalities and morbidities from liver diseases amongst HIV patients is in part due to co-infection with hepatitis B and C viruses (HBV and HCV) as these viruses promote liver fibrosis by increasing intra-hepatic apoptosis (Iser *et al.,* 2011; Chiekulie *et al.,* 2013).

Hepatitis viruses have been associated with worse HIV outcomes (Attia *et al.*, 2012). Since some antiretrovirals are utilized in treatment of both HIV and Hepatitis,similarly, antiviral therapy can also be complex in the context of co-infection. This is because HIV-infected individuals typically do not respond well to treatment for HBV or HCV. The co-infections reported in this study therefore indicate the need to factor co-infections in management strategies for HIV patients in Kenya.

5.3 Hepatitis B co-infection

In this study, hepatitis B co-infections with HIV were the second most prevalent (29%). Previous studies in Kenya have reported varied HBV prevalence including; 22.9 % among blood donors (Nyairo *et al*., 2016), 50% among patients with symptoms of liver disease (Ochwoto *et al*, 2016); 13.3 % in informal settlements (Kerubo *et al*, 2015); 7% among women on ART (Day *et al*., 2013) and 3.8% among ANC women in Kenya. Results obtained in the current study indicate a higher prevalence for HBV than previously reported in Kenya. These previous studies were of segregated population groups while the current study represented, a 'homogenous' population (HIV infected). The study by Ochwoto and group was a targeted study, where higher prevalence (50% reported among liver disease patients) is expected. The current study findings are crucial since data on co-infections among the HIV infected is limited in Kenya. Globally,

approximately 10% of HIV-infected patients are co-infected with HBV. The prevalence is higher in countries endemic for chronic hepatitis B infection (CHB). This is the case in Asia and Africa, where the prevalence of co-infection has been as high as 25% (98 Chang *et al*., 2013).

Across the study sites, there was significant association between study sites and seroprevalence. Sero prevalence of hepatitis ranged from 19% to 40% among the HIV infected.This variation is important to note since there is scarcity of information on distribution of hepatitis across counties in Kenya.This pattern of variation is also noted in HIV prevalence in Kenya across the counties and is attributed to multiple sexual patners,disparities in purchasing power,condom use, traditional practices including circumscision, wife inheritance among other factors.For hepatitis this risk factors may not be responsible for variation in distribution.This study was not able to determine risk or exposure factors to account for variation in HBV prevalence across the counties. However factors such as shared equipments, unprotected sex and vaccination coverage could account for these variations.

Hepatitis B and HIV co-infection complicates management of patients since HIV alters the course of HBV disease progression (Singhatiraj *et al*., 2012). Chronic HBV infections are 3.5 times more likely to progress to liver disease than those with no HBV infection (Osbon *et al*., 2007). HIV infected persons are less likely to clear acute HBV infection spontaneously and face a higher risk of liver-related death than those who are infected with only HIV (Singhatiraj *et al*., 2012). The presence of chronic HBV can also lead to an increased risk of hepatotoxicity related to the administration of HAART. Individuals co-infected with HIV and HBV, especially those with low cluster of differentiation 4 (CD4) T lymphocyte counts, are at increased risk for liver-related mortality (Osbon *et al*., 2007). Management of HBV in co-infected patients is complicated not only by the differences in natural history, but also, by the activity of many of the drugs active against both viruses and by the development of drug-resistant

HIV and HBV variants. This therefore has a great implication on co-infected patients in comprehensive care clinics in Kenya.

5.4 Hepatitis C Virus

This study represents one of the most comprehensive efforts to provide data on HCV infection in Kenya. A HCV sero-prevalence of 14% among the HIV infected was realized. Across study sites HCV seroprevalence ranged from 9 in nyahururu to 19% in Kisumu.There was however no significant association between study sites and infection status. Previous studies from selected population groups have shown that HCV prevalence ranges from 0.7 % to 0.9 % among blood donors; 3.9% among patients with symptoms of liver disease (Ochwoto *et al*., 2016); 1.0% (Harania *et al*., 2008); 22% and 16, 4% among IDUs (Muasya *et al*., 2008; Mwatela *et al*, 2015;); 0.76 among informal settlements (Kerubo *et al*., 2015).The finding of the current study is therefore consistent with previous studies by Mwatela and Muasya. In Kenya, the assumptions have been that HCV prevalence is mostly higher among most at risk populations only. This study however indicates the case could be different. Since risk factors for HCV were not evaluated in this study, the high seroprevalence could be attributed to same risk factors for HIV transmission in the study population. It is most probable that exposure to HCV occurred alongside HIV acquisition especially if the HCV positive individuals were also HIV new infections.This was however not determined, a limitation of this study.

Hepatitis C virus (HCV) infection is one of the main global health burdens (Razavi *et al*., 2013). A country-specific policy of prevention, diagnosis and treatment could reduce this disease burden, but unfortunately, in Kenya, there is a lack of robust epidemiological data upon which to base these strategies.

Currently in Kenya, there is an ongoing focus on Hepatitis infections among IDUs especially, HCV. A program on special population is exploring treatment options and so far has initiated treatment within a few sites. Availability of data on HCV, as shown in this study is therefore crucial in order to plan for treatment as well as manage the infections.

5.5 Characterization of co-infection status by age, gender and study sites

In multivariate analysis, the HBV, HCV, HSV2 and syphilis infection status categorized by gender and age showed no significant relationship (P >0.005).This findings suggests gender and mean age may not be an important determinant of risk of infection. However, for HIV, gender and age have been shown to be important risk factors.

This study identified that majority of participants had one or more additional infections (71%) to HIV compared to 29 % who had none (HIV positive with none of the other infections).The rates of co-infections in this study ranged from 43% for single infection to 20, 6.3 and 0.8% for double multiple and quadruplet infections respectively .This is an indication of high rate of co-infections in this population. Regardless, co-infections are not tested for among patients on HIV care and treatment. This suggests that many underlying infections remain undetected till late when the infections may have progressed to chronicity. This failure is occasioned by lack of testing policy on co-infections, under estimation due to lack of diagnostic strategies and specific surveillance system for many infections. Policy guidelines available are mostly for blood transfusion, not general population. As a result, Hepatitis B and C, while recognized as silent killers are not yet recognized as major public health challenges in many developing countries, Kenya included. The only infection given this priority is tuberculosis.

The distribution of these co-infections by number (single, double, multiple and quadruple) varied with demographic characteristics. Single and double co-infections were higher among those in age group ≥ 60 years, while multiple and quadruple

infections were more common among age group 24 to 44 years(8 vs. 3.8%). The rate of sexual activity and probably exposure from multiple partners could explain these findings with respect to age group 24-44 years. Between gender; single and doudle infections were common among men, while multiple infections were common among women. These variations were however not significant (Fisher's exact test p=0.063 for age groups; Chisquare = 0.97, 1 d.f, p=0.325). Logistic regression analysis of the variables with respect to sero-status, age and gender did not show any significant correlations

Comparison between study sites and regions indicated positive relationship, with significant differences (X^2 = 62.01, 9 d.f, p=<0.001).This indicates that study sites have a statistically significant relationship (p<0.001), with the presence or absence of viral co-infections for a patient. For example a patient at Mbagathi had an odds 2.26 times that of a patient in Kakamega of getting a viral co-infection (p=0.000, Odds Ratio= 2.26**;** 95% C. I (1.434 – 3.570), while a patient at Meru had an odds 0.49 of getting a viral co-infection (p=0.002, Odds Ratio= 0.49; 95% C.I (0.307 – 0.722). It is therefore possible that in Kenya the risks of co-infections are associated with geographical region. This is important and validates the mapping of infections as carried out in this study. The variations detected with various demographic characteristics in this study, however, could not be attributed to any particular factors since this was beyond the scope of the study. Likely factors include inadequate vaccine coverage (for HBV), variation in education, poverty, inadequate prevention and screening strategies, frequent iatrogenic transmission, insufficient or uneven access to treatment (Lemoine *et al*., 2013).Other factors may include the presence of other population groups of interest e.g. injecting drug users, men who have sex with men, sex workers among the study regions in Kenya. The spread of such groups is not well establish and remains a subject of research. Regional variations in hepatitis infections have been documented across countries (Barth *et al*., 2010; Lemoine *et al*., 2013; Noubiap *et al*., 2013; Bagheri *et al*., 2016; Al-

Mughales *et al*., 2016;).However the current study documents a comprehensive variation across regions within Kenya.

5.6 Mapping of HBV, HCV, HSV2 and syphilis Infections in Kenya

Mapping distribution of HIV co-infections in Kenya is important and a quick guide to visualize estimates of the particular infections. This can also be used to predict outcomes and target clusters of exposed subpopulations or geographical localities as hotspots of transmission. Results of HBV, HCV, HSV2 and syphilis demonstrated in this study can be utilized to to initiate health interventions on HIV co-infections. Across the 10 study sites, Hepatitis B prevalence ranged from 4-26%. Study sites in the western region of the country had higher prevalence of HBV infections than other regions (range from 31-40%).This is way above the overall prevalence at 29%. The only other site with such high prevalence was Nairobi at 39%.Comparably, sites within the central region and neighborhood had lower prevalence (range 19-26%).Syphilis prevalence was also comparably higher in western region.

The pattern of HCV infections indicates a similar distribution within the regions with only 3/10 sites having below the overall prevalence (14%). On the other hand; the pattern of HSV infections seems different from HBV, HCV and syphilis. In contrast, sites within the central region and neighborhood had the highest HSV prevalence range 62-67%, above the overall prevalence of 50%). There were significant associations between infections and study sites (P-value at 0.000). The only exception to this association was with HCV infection.

This pattern of HBV, HCV, syphilis and HSV distribution in Kenya across regions is important since it provide data through which comparisons and deductions can be made with regard to HIV and viral co-infections. One such possible inference is that distribution of HIV infections could be a determinant factor in the distribution and pattern of other viral infections across regions in Kenya. HIV prevalence by regions

demonstrates variations with some regions reporting a low of less than 2.0% while others have high of 19.6 % (KENPHIA, 2018). A more closely related pattern is observed in the current study for HBV, HCV, HSV and syphilis. Pockets of HBV and HCV transmission have been identified in focal populations groups including people who inject drugs at 16.4% (Mwatela *et al*., 2015); 0.5% (Lizcano *et al*., 2017 and 22% (Muasya *et al*., 2008). Available data among patients includes_1.6% HCV and 5.7% HBV (Wambani *et al*., 2015). Prevalence of syphilis reported was 6.4 and 4.5% among patients (Otieno *et al*., 2011) and HSV2 at 53% and 35% in general population (Njuguna *et al*., 2012, Mugo *et al*., 2011).

The findings of the current study indicate that the prevalence of HBV, HCV, HSV2 and syphilis infections is higher among HIV infected close to what has been reported among special population groups including injecting drug users. Considering the regional HIV prevalence, population social dynamics, and social networks, it is likely transmission of any or several of the infections could occur concurrently. Since available data on population based studies on HIV co-infections including hepatitis, syphilis and HSV2 is limited, it is likely that the rates of these infections could also be high in the general population. The only two population based studies in Kenya on HBV reported prevalence of 31.5% (KAIS, 2007) and 13.3% (Kerubo *et al*., 2015). Except for HIV and syphilis, testing for these infections is not routinely done.Among the infectious diseases, some of the program priority diseases in Kenya over the years have been HIV, tuberculosis and malaria.These diseases have routine testing and treatment programs. For HBV, HCV and HSV, this has not been the case mostly due to lack of sufficient data to support prioritising programs for these infections.This study provides additional data to support inclusion of screening,prevention and treatment program for HBV,HCV,HSV and syphilis in the general population in Kenya.

Based on the findings of this study, it is possible that the high seroprevalence of the four infections is indicative of high rates of exposure in the population.High prevalence could also indicate failure of uptake of prevention measures or even lack of non in the

population. Since hepatitis, HSV2 and syphilis ar not a program priority disease in Kenya, targeted interventions are not readily available. This may account for the overall high prevalence of these infections. In the past, syphilis was a key component of STI programs in many countries. However demand for expansion of HIV prevention, care and treatment and focused funding in this direction resulted in reduced priority for syphilis. Syphilis transmission could have re-emerged and continued unnoticed due to limited screening and monitoring interventions. Could the findings of this study be an indicator of resurgence of other STIs besides HIV? It is probably true and a new focus on all STIS may be required. For HBV infections, there is also the question of effectiveness of vaccination program. Currently vaccination for hepatitis B is available, though routinely for infants only. Vaccine access to high risk population for HBV is facilitated in order to avoid the impending health risk associated with HBV transmission in the population.

5.7 Performance of testing algorithms for HIV, HBV and HCV

This study determined the utility of the test kits for hepatitis, syphilis and HIV by comparingtheir relative performance in order to inform a testing strategy for co-infections. Testing for HBV, HCV and HSV2 is not provided as a routine test in HIV care clinics as well as in hospital laboratories in the study sites. Where available, these tests, especially for HBV and HCV are, only requested in special circumstances like incase of suspected liver disease. The only testing routinely available at the time of this study was that of HIV and syphilis. Testing for HSV2 was largely not available, except in private health facilities. Lack of routine testing for these infections is a disparity in health care considering the impact co-infections have in HIV and AIDS.

With regard to testing algorithm for HIV, HBV and HCV, two approaches were tested in this study. Relative performance of testing kits for HBV, HIV and HCV and best fit combination for testing that is likely to provide screening and confirmatory platform. The primary goal of this study therefore, was not to determine performance of test kits

used for HIV, HBV, HCV and syphilis. However variations in their performance characteristics including sensitivity, specificity and levels of agreement when compared are reported. In addition, levels of agreement between the test methods indicated a suitable combination for use of the test as a screening method for HIV.HBV, HCV and syphilis

5.8 HIV testing algorithm

In this study, discrepancies between HIV ELISA (A reference method), rapid tests and combinations of rapid tests was demonstrated. A considerable high number of samples were discrepant (n=243) among the study participants (n=1896).These discrepancies (13%} in test results could be accounted for by difference in relative performance of the tests kits used. Probably, of great concern were samples that were conclusively confirmed as negative (n=36).This is likely to be a significant misdiagnosis considering that this is about 2% of the study population. Since the study participants were patients on HIV comprehensive care, it was unexpected that any should be HIV negative. It may be that some patients are on care and treatment while their HIV status is negative. Such patients may find themselves in care and treatment due to a number of reasons; either in cases of false diagnosis, system failure in referral and linkages, incomplete testing especially confirmatory as may happen when recommended test algorithm is not adhered to. Kalou *et al*., (2012) and Shanks *et al*., (2013) cite that misdiagnosis in HIV can result from limitations of the assay itself, operator-related factors, facility-related factors such as inappropriate storage of reagents and selection of inappropriate testing strategies, and suboptimal national testing algorithms. A few cases of this nature have been reported in Kenya (unpublished data), including the findings of this study which have been used to advocate for a repeat testing before initiation of therapy.

5.9 HBV testing algorithm

To identify suitable strategy for hepatitis B diagnosis, all samples in this study were initially screened and then re-tested with three additional test methods; determine rapid kit, Ichek rapid kit and Polymerase chain reaction. In Kenya there is no standard recommended testing strategy for Hepatitis B in clinical setting. The only standardized testing is for blood transfusion centers where ELISA based testing is routinely carried out. All other test sites use methods of their choice.

Sensitivity and specificity of the test were wide-ranging; with determine rapid kit emerging as the most suitable test method. While ELISA is a method of choice in many testing strategies, for hepatitis testing, this method falls short in performance. It's demonstrated in this study that ELISA testing had many false positives, where 17% and 29% of them were negative with determine test and PCR test respectively.

Determine test kit, which had a higher sensitivity and specificity was the method of choice with which the other test methods could be compared to. Ichek HBV rapid tests and PCR had lower sensitivity and specific. Ichek kit is used in many setting in Kenya,yet its performance is greatly reduced. This indicates that almost half the number of people tested with this kit could be getting unreliable results. Patients may therefore end up missing the opportunity for referral, further testing, linkage to care or even end up developing chronic infections. The standard diagnostic enzyme immune assays, where available, have limitations (Cruz *et al*., 2015), that may compromise their routine use in low- and middle-income countries: they require trained personnel as well as the availability of all necessary infrastructures.

As alternatives, rapid tests (RTs) may have several advantages over standard procedures because they are easy to perform and can provide conclusive results within a few minutes (Chevalier *et al*., 2014). Additionally, these tests may be performed on a case-by-case basis and do not require laboratory infrastructure. Variations in performance

characteristics of kits and levels of results agreements (kappa statistics) have been reported elsewhere. Khuroo *et al*., (2014) and Franzeck *et al*., (2013), indicated variations in sensitivity ranging from 43% to 98%.This is in agreement with the current study where low sensitivity was detected for Hepatitis B testing, especially with Icheck (61%).With this findings it is apparent that testing for hepatitis B will require scrutiny in order to identify best strategy for the benefit of hepatitis B programming.

In absence of other test methods, the findings of this study indicate the correct testing algorithm for hepatitis B would be that which utilizes determine as the screening test and PCR as a confirmatory test. This is justifiable since unlike the combination of Ichek and determine, the sensitivity of PCR is high than specificity. This implies a reduction in number of false positive and false negatives. Ichek has a lower sensitivity and a higher specificity. The level of agreement between the determine-PCR combination is also higher (39.2%) unlike that of Ichek determine combination (28.9%).World health organization recommends use of determine for HBV screening in high prevalence areas. However no options are recommended for confirmatory testing. In absence of such recommendation, and in ensuring that patients get the most benefit from testing, an algorithm where PCR becomes a confirmatory test is currently recommendable in clinical settings as shown in this study.

5.10 HCV testing algorithm

The testing algorithm for HCV recommendable in this study would involve a combination of Bioline HCV test kit and particle hemagglutination test (PHA). Murex Anti HCV ELISA seems to give high number of false positives unlike the other two tests. It is also not a rapid test and thus not suitable for out of-laboratory testing where majority of testing is likely to occur. The entire test menu used in this algorithm had a higher specificity than sensitivity. As a result the number of false negatives is greatly reduced except for Murex anti HCV ELISA. This is a key performance indicator in diagnosis. The high number of false positives in Murex could be attributed to samples

with low optical density and near cutoff that may have been labeled as positives. This scenario is not applicable in rapid testing where results are read visually. The 26 samples identified as positive by ELISA were mostly negative by both Bioline and PHA Kit and were therefore considered to be false positives. These samples were also negative by PCR, further indicating the reliability of the results.

In search of a suitable algorithm for HCV therefore, results of the current study indicate bioline would be the method of choice as first line test (Sensitivity 94%, Specificity 99%). Confirmatory testing would involve use of Ant HCV PHA with sensitivity of 92% and specificity of 99%.The Kappa test for the two test is .911 indicating a level of agreement between Bioline and PHA at 91.1%.This is the highest level of agreement among the combination of HCV test kits used in this study.

Testing for HIV, HBV and HCV is an important prevention intervention in developing countries and especially in Africa, where the prevalence of these infections is considered high. Testing of these infections is also the entry to care and treatment. With this dual benefit, testing of the three infections requires well determined test algorithms that will ensure better health outcomes in the population. The current study has demonstrated the importance and utility of a suitable algorithm towards achievement of diagnostic goals. In line with WHO recommendations, (WHO; 2016[a]; 2016[b]; 2015) testing for hepatitis in selected population groups is recommended. However the choice of test is limited based on WHO approved test kits. Other studies have demonstrated such limitation in testing for HCV due to lack of suitable testing algorithm and complexity of available test kits.Most available test kits have wide variability in clinical performamnce ,low sensitivity, specificity and predictive value(Shivkumar *et al*.,2012; Khuroo *et al*., 2015; Chevaliez *et al*.,2016)

This implies that for countries rolling out testing for hepatitis, limitation in choice of test is a drawback they will need to deal with. However this is not the case for HIV, where algorithm for testing has been operational for years (WHO, 2015). Such algorithm,

however continue to have shortcoming and hence the need for post market surveillance in order to maintain and improve testing services.

5.11 CD4 count distribution among the HIV infected

CD4 count was measured to determine immunological status of the HIV infected in presence or absence of co-infection with HBV, HCV, HSV2 and syphilis.CD4 cell count is an important parameter in HIV management monitoring, where increase in count from baseline indicates positive response to HIV treatment and care. Findings of this study indicate co-infections with HSV2 seemed to result in reduction of CD4 count. There was a significant difference between the CD4 count for those positive for HSV2 and those negative (p-0.0021). The mean CD4 count difference between the Positive and negative participants were not significant in case of HCV, HBV and syphilis. This finding is important since almost half of the study population was infected with HSV2. Herpes simplex virus type 2 (HSV-2) has been postulated to increase risk of HIV acquisition and disease progression. The immunologic impact of viral coinfections has the amplitude to increase viral replication leading to expedited atrophy in immune function (Munawwar and Singh, 2016; Darrell *et al*, 2013).

Based on the finding of the current study, it is probable that while a CD4 increase upon intake of ART is expected, for individuals co-infected with HSV-2 this may not be the case. It implies therefore, that for those being enrolled for ART, testing for HSV may be crucial in order to factor monitoring for response to treatment and improved management. Although co-infection with HBV and HCV may not show a significant difference in CD4, these infections are known to increase mortality among the HIV infected (Than *et al*, 2012).

Unlike in this study, other studies have shown that syphilis co-infections is associated with a significant increase in plasma HIV viral load and a significant decrease in CD4 cell counts (Jarzebowski et al 2012; Spagnuolo et al, 2014; Kotsafti et al, 2015; and

Sadala et al, 2017). CD4 cells are important in the immunological defense mechanisms and loss of significant numbers of these cells therefore affects immune responses. Even in absence of the further effect of the co-infections on CD4 count. Progressive CD4+ cell depletion is the main landmark of untreated HIV infection. The immunological goal of antiretroviral therapy is to restore the levels of those lymphocytes subpopulation to normal range.While currently viral load is the main parameter for ART initiation and treatment monitoring, by the time of this study, viral load was less available and CD4 was the main parameter used.This study demonstrated that majority of HIV patients required antiretroviarl treatment based on the outcome of CD4 count. Then, At CD4 count >250, WHO ART treatement eligibility criteria, 624 particpants were eligible. However this doubled to 1297 at CD4 count 350- 500 cell/mm3 eligibility criteria.Thus by the time of this study, 71% of study participants were eligible for ART, indicating the increasing demand for the life saving treatment in the country. Based on WHO guidline in operation to date (updated July 2017), besides viral load monitoring, CD4 count measeurement is relevant in assessing advanced HIV disease. Patients entering or re-entering care are required to receive a CD4 test at treatment baseline and as clinically indicated for patients who are unstable or with advanced HIV disease. It is strongly recommended that patients with advanced HIV disease (CD4 count below 200 cells/mm3) receive a package of care as defined in the 2017 WHO Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy. Of the approximately 2 million people starting or restarting ART every year, as many as 20%–35% of them have a CD4 cell count less than 200 cells/μl and meet the definition of advanced HIV disease [Carmona et al, 2018; Chammartin et al, 2018].Therfore CD4 count remains relevant in such cases.In the current study about 34% of the study participants had CD4 count lower than 250 cells/ul, a sign of advanced disease.

5.12 Genotypes of HIV, HBV and HCVamong study participants

The last objective in this study focused on investigation of genotypes of HIV, HBV and HCV. Variations of genotypes in co-infections are an important cause of death in human

immunodeficiency virus (HIV) infected patients on antiretroviral therapy. The distribution of genotypes varies with geographical regions and time, and limited studies have focused on the genotype in HIV co-infections.The findings of this study provide information on genotypes of HIV, HBV and HCV in Kenya.

5.12.1 HIV subtypes among participants

In this study 7 different HIV genotypes were identified.This includes genotypes; A, C, D and G as single genotypes and CD, AD and AE as posible recombinants. Since study population was diverse and drawn from across the country, this could be an indication of how diverse HIV genotypes are in Kenya.

The findings of the current study indicate that HIV genotype A is widely distributed followed by genotype D and C. Globally HIV subtype A accounts for 50% of HIV infections and in some countries up to 80% of all infection are due to it (Buonaguro *et al*., 2007).

Differences in viral diversity between geographical regions point to differences in the drivers of HIV-1 transmission and movement of people across the regions. In addition, genotype diversity has been shown to have significant impact on epidemiological and clinical management of HIV, efficiency in transmission, early onset of disease progression and development of drug resistance are also factors associated with different HIV genotypes and their recombinants (Edsel *et al*., 2017). For example genotype C,identified in the current study has been shown to be more effectively transmitted and is faster in disease progression (José ,2011). Its existence in the country could probably account for increased transmission of HIV and remains one of the commonest genotype identified in many studies in Kenya.

Subtype D was also identified in this study.Although transmissibility of this subtype is considered to be low however disease progression may be faster (José, 2011).Subtype D

has been associated with neurocognitive disorders leading to dementia (Sacktor *et al*., 2009).This could explain the neurological complication experienced by some HIV patients attending comprehensive care clinics in Kenya.

Genotypes are also an important consideration in vaccine design. Difficulties in development of effective HIV vaccine are partly due to limitations in eliciting immune responses against wide-ranging viral subtypes (Seaman *et al*., 2010).Increased data on genotype prevalence therefore would help inform vaccine research and development and targeted vaccination efforts tailored towards the HIV epidemic.

Previous studies in Kenya have reported genotypes A, C, D, E and their recombinants. Predominance of Genotype A has been shown in previous studies in Kenya (Kageha *et al*., 2012; Lihana *et al*., 2012; Nyagaka *et al*, 2011; Kebira *et al*., 2009 and Khamadi *et al*., 2009).The findings of the current study therefore collates these previous reports.

Globally; HIV-1 subtype's distribution is heterogeneous and varies even within regions of the same continent. HIV-1 subtype A1 is prevalent in Central Africa, Eastern Europe and Central Asia. Subtypes A2 and A3 are found primarily in Africa (Maartens *et al*, 2014). From the current study, subtype A was predominant, indicating a similar pattern.

5.12.2 Genotypes of HCV among participants

Based on the finding of this study presence of genotypes 1a and 2b is reported in Kenya. The predominant genotype (90 %) in circulation is 2b.To my knowledge this is also the first study to report on presence of genotype 2b in Kenya. Prior to this suty,the only other study on HCV genotypes in Kenya reported presence of genotypes 1a and 4, with genotype 1a being more prevalent at 73% (Muasya *et al*,2008). Globally, six major genotypes and more than 83 subtypes of HCV have been identified with genotypes 1, 2 and 3 being the most prevalent (Chao *et al*., 2011; Bunchorntavakul *et al*., 2013). In Africa various genotypes have been reported including; 4a-Egypt, 4c and 5a Central

Africa (Demetriou *et al*., 2009), 3a, 1, 2,4c and subtype 4g (Prabdial-sing *et al*., 2008) in South Africa. Genotype 4 subtypes' 4e, 4c, 4p and 4r have been reported in Gabon (Kamal *et al*., 2008). Closer to Kenya, subtype 4a has been reported in Tanzania (Rapicetta *et al*., 1998) and genotype 4 in Uganda (Biggar *et al*., 2006).Based on distributions of various genotypes in africa,international travels and cross border movements, it is most likely that diverse genotypes of HCV could be circulating in Kenya.

Presence of genotype 2b in Kenya is of particular interest. HCV genotypes 1a, 1b, and 3a are highly prevalent "epidemic" strains that are found globally (Verbeeck *et al*., 2006.).There is glowing evidence to support origin of genotype 2 being West Africa (Markov *et al*., 2009), (Djomou *et al*., 2003.). In the last few years bilateral relations between different countries and Kenya has been growing significantly. There are direct flights to many countries including those in West Africa. A population of West Africans and Congolese from Zaire constitute a significant business community in Kenya, especially in the small scale market sector. A number Countries in West Africa and those from central Africa have reported diverse genotypes in their populations. This could explain the emergence of related genotypes in Kenya.

Muasya *et al*, (2008) reported presence of HCV genotype 1a and 4 from a population of injecting drug users in Kenya. In that previous study, the genotypes identified; formed two clusters of related isolates. In the current study, isolates clustered into 3 different groups. Since the two study populations are different; the current one being blood donors, it is most likely that HCV genotypes among blood donors could be more diverse than those in the previous group. Sharing of needles and close association among individuals injecting drugs could contribute to similar genotypes among the group, whereas this may not be the case in a diverse population among whom blood donations are normally obtained.

Although the two studies done in Kenya may not provide HCV genotypes topography, due their limitations, they are however a pointer to an unfolding situation where diverse genotypes could be existing in the country. Notably, Kenya is host to over 600,000 refugees from different countries including; Somalia, Ethiopia, Sudan, Democratic Republic of the Congo among other countries who have experienced internal civil war and unrest. Moreover, bilateral relations with Far East countries have increased in the recent past with citizens from countries like china, now playing a major role in the business and construction sector. These populations could constitute a hot spot for divergent genotypes of HCV, perhaps pointing to a scenario where additional genotypes could be identified in Kenya.

Genotypes 1a and 3a have been linked to intravenous drug use and the main risk factor for infection by genotypes 1b and 2 as parenteral contact (Tamalet *et al*., 2003). In this study, the samples analysed were from blood donors suggesting the validity of the findings by Tamalet *et al*. Currently, Injection drug use (IDU) has been a problem in some settings in Kenya. The only study targeting this population reported presence of genotype 1a (73%) and genotype 4 (27%) (Muasya *et al*., 2008). In the current study there was only one sample with genotype 1a while the rest were 2b.This probably further confirms the subtype difference with risk factors as earlier indicated. It would be interesting to see the genotypes and subtypes detectable among the drug users and correlation with other population groups where infection rate with HCV could be higher.

5.12.3 Genotypes of HBV among study participants

This study identified four HBV genotypes in all, namely genotypes A, C, D and recombinant C/D among the patients on care and treatment. For blood donor samples, genotypes; A, E and D were identified. This suggests that diversity of HBV genotypes could be wider in Kenya. Important to note that there is a possibility that genotypes A and D could be the most prevalent as indicated by both blood donor and patients isolates. In a population of high HIV prevalence including Kenya, diversity of HBV

genotypes together with HIV can complicate the management of patient. While some antiretroviral drugs (including Lamivudine) used for HIV management also manage hepatitis, prolonged exposure to the drugs for management of either infections could result in drug resistance. Genotypes could also cross populations like for example, those exposed to treatment to drug naïve and this could have implications on future management of such patients.

While HBV is differentiated into different genotypes including A-H, according to genome sequence,,the current study did not identify the other genotypes of HBV (besides A, C, D, and E). The rest of genotypes may still be circulating in the population as well.

In a previous study,Nyairo *et al* identified only genotype A and its variants in a in a population of blood donors (Nyairo *et al*, 2016). Genotypes identified in the current study have also been reported in other countries in Africa includingGenotype E in central and west Africa (liu *et al*, 2013) and genotype D that predominates in up to 80% of patients in Egypt, Algeria and Libya) (Moura *et al*, 2013).

At a global and regional perspective, the identitification of various genotypes in the current study seems to confirm geomapping of genotypes in the African region. Genotype A which appears to be a resident in Africa was detected in the current study while genotype D, a more global cosmolitant genotypes, also detected seems a resident here too. Genotype E also identified in the study is mostly confined to western Africa and distributed to other regions of the world following emigration from Africa. Identification of the various genotypes could therefore be linked to migration of populations.This is likey to happen due to globalization of economies leading to movement of people from regions to another or due to refuges as happens due political instabilities in some. Kenya experiences both of these situations as a regional business hub as well as having enjoyed political stability in a region of political instability.It is

therefore higly likely that even those genotypes ,not identified in this study may be circulating in the country and are likely to be detected as studies continue to evolve.

In the public health perspectives, genotypes are important in control and prevention as well as in management of acute and chronic infections.Dynamics of transmission and circulation of gnetoypes in different population groups like the drug users, perinatal mothers, infants and health workers is an important consideration in this regard.Development of vaccines, vaccination and immunization programs is another important public health concern. Mutations and risk of drug resistance including transmission of resistant strains is also a major health concern. Development of mutations can be influenced by the HBV genotype or subgenotype.

Genotypes have been shown to affect recombinations, which indicates why certain recombinants are more frequent in some regions and not others (Shi *et al*, 2012),genotypes have effect on HBeAg(-a classical marker for HBV)expression,frequency of transmission (Pournik *et al*, 2013; Madejon *et al*,2016),frequency of transmission(Anna,2016) and modes of transmission, mainly because of seroconversion(early or delayed),viral load variations and HBeAg positivity(Candotti *et al*,2007). The findings of the current study thus provide important information attributatble to topography of hepatitis in global settings as well as in Kenya.

5.13 Limitations of the study

This study had some limitations.While it would have been desirable to do viral load along side the CD4 count,this was not included due to cost constrains.At the time of the study,CD4 count was the main indicator of HIV treatment initiation and monitoring.This however, was replaced by viral load testing in 2017. Notwithstanding, introduction of viral load testing was gradual in most resource constrained settings and CD4 count remained in operation till these setting could secure support to replace with viral load for

purposes of treatment initiation.To date CD4 count is still relevant since its an immunological marker, unlike viral load, a virological one.Based on WHO recommendation CD4 is important to determine advanced HIV disease and an important determinant in opportunistic prophylaxis management.This study did not determine if the HSV2 infections were current or past infections.However its detection was important since presence of HSV2 has implications on HIV transmission,disease progression and a marker of sexual activity among young people.Although syphilis,caused by *treponema pallidum* is a bacterial infection,its inclusion in this study was important since it's a major HIV co-infection which share similar mode of transmission and facilitates HIV acquisition.Other co-infections could have, as well been included in the study,this however could not be done due to cost.It was important to narrow the focus of the study at this level.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- The prevalence of HBV(26%), HCV(14%), HSV(47%) and syphilis(14%) in the population of HIV infected individuals
- Distribution of the 4 infections varied across the regions with sites in western region of Kenya having greater than 31% HBV and 18-26% of syphilis.
- 47% of the study population was HSV positive with regional variations ranging from 29%-67%.
- Determine HBV rapid, SD Bioline HCV rapid and Determine HIV rapid test were identified as the suitable first line test kits for HBV, HCV and HIV testing algorithm respectively
- In this study, 34 % of particpants met the criteria for advanced HIV disease based on WHO recomendations indicating the need for CD4 count among clients on HIV care .
- HIV genotypes identified were A, C, D, and G, HBV genotypes; A, C, E and D while for HCV genotypes 1 and 2b.
- 36 participants whose results were HIV negative were identified yet they were attending comprehensive care clinic.This indicate a number of uninfected individuals on care for HIV could have a misdiagnosis.

6.2 Recommendations

- In view of the findings of this study, it is recommended that:
- Since this study did not cover all counties,a country wide study on prevalence and distribution of HBV, HCV, HSV and syphilis is required in order to provide more detailed data on these infections
- A national testing algorithm for HBV and HCV is required
- It is recommended that HBV and HCV be considered as priority infections and a Hepatitis program be established to deal with the emergence of this infections
- Available testing starategy for HBV, HCV and HIV requires continous monitoring and improvement
- Theres is a need for CD4 count in HIV population to estimate the threshold criteria for advance HIV disease.
- Repeat testing for HIV is recomedable for patient's referd to CCC in order to rule out any misreferal or false positive cases
- Monitoring of genotypes is necessary since the country could be developing a diversity of molecular types.This is likely to complicate management and control efforts
- The suitable algorithm for HCV testing is one, where bioline anti HCV rapid test becomes the first line test (screening) and the second line testing carried out by Anti HCV Particle hemagglutinaton test (HCV PHA) as a confirmatory test.

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APPENDICES

Appendix I: Accession numbers for HIV Sequences

HIV SEQUENCES K002-102 KR8 72425

HIV SEQUENCES KK82

HIV SEQUENCES KK83 KR872427

HIV SEQUENCES KK84 KR872428

HIV SEQUENCES KK85 KR872429

HIV SEQUENCES KK86 KR872430

HIV SEQUENCES KK87 KR872431

HIV SEQUENCES KK89 KR872432

HIV SEQUENCES KK90 KR872433

HIV SEQUENCES KK91 KR872434

HIV SEQUENCES KK9102 KR872435

HIV SEQUENCES KK92 KR872436

HIV SEQUENCES KK93

HIV SEQUENCES KK94 KR872438

HIV SEQUENCES KK9402 KR872439

HIV SEQUENCES KK95 KR872440

HIV SEQUENCES KK9502

HIV SEQUENCES KK96 KR872442

HIV SEQUENCES KK97 KR872443

HIV SEQUENCES KK98 KR872444

HIV SEQUENCES KK99 KR872445

HIV SEQUENCES KK9902 KR872446

HIV SEQUENCES KK100 KR872447

HIV SEQUENCES KK101 KR872448

HIV SEQUENCES KK102 KR872449

HIV SEQUENCES Ks793 KR872450

HIV SEQUENCES Ks807 KR872451

HIV SEQUENCES Ks810 KR872452

HIV SEQUENCES Ks882 KR872453

HIV SEQUENCES Ks88202 KR872454

HIV SEQUENCES Ks885 KR872455

HIV SEQUENCES Ks917 KR872456

HIV SEQUENCES Ks918 KR872457

HIV SEQUENCES Ks91802 KR872458

HIV SEQUENCES Ks920 KR872459

HIV SEQUENCES Ks923 KR872460

HIV SEQUENCES Ks925 KR872461

HIV SEQUENCES Ks927 KR872462

HIV SEQUENCES Ks939 KR872463

HIV SEQUENCES mbg11 KR872464

HIV SEQUENCES mbg1102 KR872465

HIV SEQUENCES mbg12

HIV SEQUENCES mbg13

HIV SEQUENCES mbg14 KR872468

HIV SEQUENCES mbg1402 KR872469

HIV SEQUENCES mbg16 KR872470

HIV SEQUENCES mbg1602 KR872471

HIV SEQUENCES mbg17 KR872472

HIV SEQUENCES Mbg20 KR872473

HIV SEQUENCES Mbg2002 KR872474

HIV SEQUENCES Mbg21 KR872475

HIV SEQUENCES Mbg2102 KR872476

HIV SEQUENCES mbg22 KR872477

HIV SEQUENCES mbg23 KR872478

HIV SEQUENCES mbg25 KR872479

HIV SEQUENCES mbg103 KR872480

HIV SEQUENCES mbg104 KR872481

HIV SEQUENCES mbg105 KR872482

HIV SEQUENCES Mbg109 KR872483

HIV SEQUENCES Mbg10902 KR872484

HIV SEQUENCES mbg114 KR872485

HIV SEQUENCES mbg11402 KR872486

HIV SEQUENCES mbg115 KR872487

HIV SEQUENCES mbg116 KR872488

HIV SEQUENCES Mer001 KR872489

HIV SEQUENCES Mer00102 KR872490

HIV SEQUENCES Mer002 KR872491

HIV SEQUENCES Mer003 KR872492

HIV SEQUENCES Mer00302 KR872493

HIV SEQUENCES Mer14 KR872494

HIV SEQUENCES Mer1402 KR872495

HIV SEQUENCES Mer15 KR872496

HIV SEQUENCES Mer18 KR872497

HIV SEQUENCES Mer20 KR872498

HIV SEQUENCES Mer24 KR872499

HIV SEQUENCES Mer54 KR872500

HIV SEQUENCES Mer56 KR872501

HIV SEQUENCES Mer64 KR872502

HIV SEQUENCES Mer66 KR872503

HIV SEQUENCES Mer71 KR872504

HIV SEQUENCES Mer7102 KR872505

HIV SEQUENCES Mer72 KR872506

HIV SEQUENCES Mer7202 KR872507

HIV SEQUENCES Mer77 KR872508

HIV SEQUENCES Mer150-89 KR872509

HIV SEQUENCES Mer150-91 KR872510

HIV SEQUENCES Mer162 KR872511

HIV SEQUENCES Mer16202 KR872512

HIV SEQUENCES Mer164 KR872513

HIV SEQUENCES Mer165 KR872514

HIV SEQUENCES Mer166 KR872515

HIV SEQUENCES Mer167 KR872516

HIV SEQUENCES Mer168 KR872517

HIV SEQUENCES Mal7 KR872518

HIV SEQUENCES Mal702 KR872519

HIV SEQUENCES Mal22 KR872520

HIV SEQUENCES Mal27 KR872521

HIV SEQUENCES Mal29 KR872522

HIV SEQUENCES Mal30 KR872523

HIV SEQUENCES Mal3002 KR872524

HIV SEQUENCES Mal78 KR872525

HIV SEQUENCES Mw4 KR872526

HIV SEQUENCES Mw7 KR872527

HIV SEQUENCES Mw8 KR872528

HIV SEQUENCES Mw802 KR872529

HIV SEQUENCES Mw24 KR872530

HIV SEQUENCES nkr3 KR872531

HIV SEQUENCES nkr4 KR872532

HIV SEQUENCES nkr402 KR872533

HIV SEQUENCES Nkr5 KR872534

HIV SEQUENCES Ny5 KR872535

HIV SEQUENCES ny7 KR872536

HIV SEQUENCES ny13 KR872537

HIV SEQUENCES ny14 KR872538

HIV SEQUENCES ny16 KR872539

HIV SEQUENCES ny26 KR872540

HIV SEQUENCES sk9	KR872541
HIV SEQUENCES sk21	KR872542
HIV SEQUENCES sk25	KR872543
HIV SEQUENCES sk31	KR872544

Appendix II: Accession numbers for HBV sequences

HBV FINAL SEQ K001-8-2 KR815915

HBV FINAL SEQ K001-4nya2 KR815916

HBV FINAL SEQ K001-2nya KR815917

HBV FINAL SEQ K001-12 KR815918

HBV FINAL SEQ K001-15nya KR815919

HBV FINAL SEQ K001-15 KR815920

HBV FINAL SEQ K001-16nya KR815921

HBV FINAL SEQ K001-17 KR815922

HBV FINAL SEQ K001-17-2 KR815923

HBV FINAL SEQ K001-21 KR815924

HBV FINAL SEQ K001-21-2 KR815925

HBV FINAL SEQ K001-24 KR815926

HBV FINAL SEQ K001-24-2 KR815927

HBV FINAL SEQ K001-27 KR815928

HBV FINAL SEQ K001-32KK KR815929

HBV FINAL SEQ K001-34 KR815930

HBV FINAL SEQ K001-34-2 KR815931

HBV FINAL SEQ K001-41Nya_2 KR815932

HBV FINAL SEQ K001-41Nya_3 KR815933

HBV FINAL SEQ K001-46 KR815934

HBV FINAL SEQ K001-62Nkr KR815935

HBV FINAL SEQ K001-74nya KR815936

HBV FINAL SEQ K001-85 KR815937

HBV FINAL SEQ K001-102-2 KR815938

HBV FINAL SEQ K001-102-3 KR815939

HBV FINAL SEQ K001-104-2 KR815940

HBV FINAL SEQ K001-104-3 KR815941

HBV FINAL SEQ K001-105 KR815942

HBV FINAL SEQ K001-111 KR815943

HBV FINAL SEQ K001-113 KR815944

HBV FINAL SEQ K001-118 KR815945

HBV FINAL SEQ K001-119 KR815946

HBV FINAL SEQ K001-120-2 KR815947

HBV FINAL SEQ K001-122 KR815948

HBV FINAL SEQ K001-124 KR815949

HBV FINAL SEQ K001-146nya KR815950

HBV FINAL SEQ K001-209 KR815951

HBV FINAL SEQ K001-213 KR815952

HBV FINAL SEQ K001-220 KR815953

HBV FINAL SEQ K001-286 KR815954

HBV FINAL SEQ K001-305-2 KR815955

HBV FINAL SEQ K001-305-3 KR815956

HBV FINAL SEQ K001-308 KR815957

HBV FINAL SEQ K001-543 KR815958

HBV FINAL SEQ K001-546 KR815959	HBV FINAL SEQ K001-771 KR815967	HBV FINAL SEQ K001-889 KR815975
HBV FINAL SEQ K001-559-2 KR815960	HBV FINAL SEQ K001-772 KR815968	HBV FINAL SEQ K001-939 KR815976
HBV FINAL SEQ K001-559-3 KR815961	HBV FINAL SEQ K001-774 KR815969	HBV FINAL SEQ K001-962 KR815977
HBV FINAL SEQ K001-560 KR815962	HBV FINAL SEQ K001-777 KR815970	HBV FINAL SEQ K001-994 KR815978
HBV FINAL SEQ K001-737-2 KR815963	HBV FINAL SEQ K001-779 KR815971	HBV FINAL SEQ K001-996 KR815979
HBV FINAL SEQ K001-737-3 KR815964	HBV FINAL SEQ K001-793 KR815972	HBV FINAL SEQ K001-1235-2 KR815980
HBV FINAL SEQ K001-765 KR815965	HBV FINAL SEQ K001-878 KR815973	HBV FINAL SEQ K001-1235-3 KR815981
HBV FINAL SEQ K001-769 KR815966	HBV FINAL SEQ K001-882 KR815974	HBV FINAL SEQ K001-1238-3 KR815982

HBV FINAL SEQ K001-1243-2 KR815983	HBV FINAL SEQ K001-1247-2 KR815985	HBV FINAL SEQ K001-s9-2 KR815987
HBV FINAL SEQ K001-1243-3 KR815984	HBV FINAL SEQ K001-1247-3 KR815986	HBV FINAL SEQ K001-4nya-3 KR8159

Appendix III: Accession numbers for HCV genotypes

DQ460666, FJ710115,

FJ710110. FJ710116,

FJ710111, FJ710118,

FJ710112, FJ710119

FJ710114,

Appendix IV: Publications

Publication 1

Journal of Biology, Agriculture and Healthcare
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Sero-Mapping of HBV, HCV, HSV2 and Syphilis Across Regions in Kenya

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Abstract

Hepatitis B and C,herpes Simplex Virus 2 and syphilis are important infections that share common modes of transmission. The global burden of these infections and their occurrence as co-infection of HIV in high risk population groups calls for more attention in control and management. While HIV has been a program priority disease in many low resource settings, hepatis B and C have not had as much attention. Yet there is substantial indication that transmission of hepatitis, herpes virus 2 and syphilis is on the increase. This study aimed at determining the prevalence of hepatitis B, hepatitis C, Herpes simples virus 2 and syphilis and their distribution across different regions in Kenya. Samples were collected from HIV positive study participants in 11 counties (out of total 47) distributed in 7 out of the 8 regions (provinces).A total of 1829 samples were tested for the 4 infections and their distribution analysised. Mapping of the infection was presented by study sites and regions . Overall prevalence of HSV2 was the highest (at 50%) followed by that of HBV (29%) and HCV and Syphilis (14% repectively). Majority of study participants (71%) had co-infections while the rest of the study participants (29%) had none of the four infections. Western and Nyanza regions of the country had higher prevalence of HBV (above 31%). Sero-prevalence of HCV across the regions ranged between 8 to 19 percent. Syphilis sero-prevalence was highest in western and Nyanza region (Range 18% to 26%) compared to other regions (4% to15%), the lowest prevalence was in Central region. Distribution of HSV2 indicated Nairobi was leading at 62%, Central at 48.5% and Rift Valley at 47.5%. Lowest prevalence of HSV2 was at eastern region with an average of 29%. There was significant relation between infections and study sites. Since routine testing for HBV, HCV and HSV is not readily available, there is need to develop a strategy for prevention and control of these infections. Integration of services would be an important consideration in this regard.

Keywords: HBV, HCV, HSV, Syphilis, prevalence, co-infection, distribution
DOI: 10.7176/JBAH/9-12-06
Publication date:June 30th 2019

Publication 2

Molecular Genetic Diversity of Hepatitis B Virus in Kenya

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Key Words
Genotypes · Hepatitis B virus · Kenya · Nuclear acid testing

Abstract
Eight genotypes of hepatitis B virus (A-H) and subgenotypes have been recognized worldwide. However, there is limited information on prevalent genotypes in many countries in Africa. This study was undertaken to determine the hepatitis B virus (HBV) genotypes in Kenya. Seropositive HBV blood samples from a blood donor setting were used in the study. HBV genotypes were determined in 52 nucleic acid-positive samples using specific primer in a nested PCR and sequencing employed in the HBV genotyping. This study shows presence of HBV variants with genotypes A (88%), E (8%) and D (4%). In conclusion, we found that HBV genotype A is the most predominant genotype in Kenya with both subgenotype A1 and A2 present. Genotype D and E are also present in our population. This demonstrates that there could be a high genetic diversity of HBV in Kenya.

Hepatitis B virus (HBV), a well-known agent of acute and chronic hepatitis, is endemic in many parts of the world, especially Asia and Africa. More than 2 billion people have had contact with the virus and more than 350 million are chronic carriers globally [1, 2], making the infection a public health problem.

HBV is the prototype member of the genus *Orthohepadnaviridae* of the family *Hepadnaviridae* and the viral genome is about 3.2 kb long. It circulates in the serum as a Dane particle which is a round structure consisting of an envelope and an inner core of nucleocapsid protein, enclosing both a polymerase and the partly double-stranded circular viral DNA [3].

The highly compact genome contains the four major open reading frames (ORFs) encoding the envelope (preS1, preS2 and surface antigen HBsAg), core (preCore precursor protein, HBeAg and HBcAg), polymerase (HBPol) and X (HBX) proteins, respectively [4]. Earlier, before the genotype definition, HBV strains were distinguished into 9 hepatitis B surface antigen (HBsAg) subtypes designated *ayw1*, *ayw2*, *ayw3*, *ayw4*, *ayr*, *adw2*, *adw4q–*, *adrq+*, and *adrq–* by serological analysis [3].

The new classification based on a comparison of the complete genomic sequence classifies HBV into 8 geno-

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Publication 3

PanAfrican Medical Journal

Open Access

Research

Switch from 200 to 350 CD4 baseline count: what it means to HIV care and treatment programs in Kenya

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Key words: CD4, New criteria, HIV, AIDS, care and treatment, ARV initiation

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Abstract

Introduction: With the increasing population of infected individuals in Africa and constrained resources for care and treatment, antiretroviral management continues to be an important public health challenge. Since the announcement of World Health Organization recommendation and guidelines for initiation of antiretroviral Treatment at CD4 count below 350, many developing countries are adopting this strategy in their country specific guidelines to care and treatment of HIV and AIDS. Despite the benefits to these recommendations, what does this switch from 200 to 350 CD4 count mean in antiretroviral treatment demand? **Methods:** A Multi-centre study involving 1376 patients in health care settings in Kenya. CD4 count was carried out by flow cytometry among the HIV infected individuals in Kenya and results analyzed in view of the In-country and the new CD4 recommendation for initiation of antiretroviral treatment. **Results:** Across sites, 32% of the individual required antiretroviral at <200 CD4 Baseline, 40% at <250 baseline count and 58% based on the new criteria of <350 CD4 Count. There were more female (68%) than Male (32%).Different from <200 and <250 CD4 baseline criteria, over 50% of all age groups required antiretroviral at 350 CD4 baseline. Age groups between 41-62 led in demand for ART. **Conclusion:** With the new guidelines, demand for ARVs has more than doubled with variations noted within regions and age groups. As A result, HIV Care and Treatment Programs should prepare for this expansion for the benefits to be realized.

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This article is available online at: http://www.panafrican-med-journal.com/content/article/12/80/full/

Publication 4

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ORIGINAL ARTICLE

Hepatitis C virus genotypes in Kenya

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Abstract Hepatitis C virus is a great public-health concern worldwide. Phylogenetic analysis of the HCV genome has identified six different genotypes that have generally been divided into several subtypes. There is very little information on HCV seroprevalence and genotypes in Kenya. To determine the genotypes of HCV circulating in Kenya, blood donor samples were serologically tested and confirmed by polymerase chain reaction (PCR). Positive samples were cloned and sequenced, and phylogenetic analysis conducted to determine the HCV genotypes. One hundred Murex-seropositive samples were re-tested using a passive hemagglutination test, and 16 of these were identified as seropositive. Further testing of all of the samples by PCR identified only 10 of the 16 samples as positive. Thus, only 10 % (10/100) of the samples were viremic. Six were from females (60 %), and four were from males (40 %). The mean age of the positive donors was considerably low, at 25 +/- 9 years. Genotypic testing indicated the presence of genotype 1a (10 %) and genotype 2b (90 %). This study reports on HCV genotypes in a blood donor population in Kenya where little had been done to provide information on HCV genotypes.

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The results of this study have not been submitted to any other journal for publication.

This research work has received approval from national research and ethical review committees in Kenya.

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Introduction

Hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV) are viruses that cause great public-health concern worldwide. These three viruses cause over 562 million infections globally, affecting 9.4 % of the world population. Approximately 3 % of the world's population (170 million people) is infected with HCV, which is a serious cause of chronic liver disease that may progress to liver cirrhosis and hepatocarcinoma. Thus, HCV continues to be a major disease burden on the world.

The prevalence of HCV varies in different regions of the world. In Europe, the general prevalence of HCV is about 1 % but varies among the different countries [1], ranging from 0.87 % in Belgium [2], 3.2 % and 8.4 %-22.4 % in Italy [3] and 1.3 % in France [4]. In Central and South America, the estimated prevalence of HCV is 6.3 % [5]. In Japan, it is 0.49 %-0.98 % [6, 7], and in Thailand it is 3.2-5.6 % [8, 9].

In Africa, there have been few studies on genotypes of HCV when compared to other parts of the world, but

Appendix V: Study approval letter by IRB

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **3 SEPTEMBER 2008**

FROM: **SECRETARY, KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE**

THROUGH: **DR. F. OKOTH,**
CENTRE DIRECTOR, CVR,
NAIROBI

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P. O. Box 54628
NAIROBI

TO: **MR. JOSEPH MWANGI (PRINCIPAL INVESTIGATOR)**

RE: **SSC PROTOCOL No. 1396 (*REVISED*): MOLECULAR EPIDEMIOLOGY AND INTERREGIONAL GENETIC DIVERSITY OF HIV, HBV AND HCV IN KENYA: HOST/VIRAL CHARACTERIZATION, DIAGNOSIS AND DRUG RESISTANCE**

Dear Sir,

Reference is made to your letter dated 1 September 2008. We acknowledge receipt of the revised proposal and a copy of the letter from Mr. David L. Ruto, the Chief Medical Laboratory Technologist, Ministry of Health endorsing the collection of remnant blood samples for purposes stipulated in the proposal.

The Committee notes the following:
a) The institution of appropriate biosafety measures in the level 3 laboratory;
b) A narrative justification of the modular budget is provided;
c) All investigators on the study are affiliated with KEMRI;
d) The description of the responsibilities of all investigators on the study on page 22 of the document.

The Committee is satisfied that the issues raised at the 157th meeting of 26 August 2008, have been adequately addressed. The study is hereby granted approval for implementation effective today, the 3rd day of September 2008 to 2 September 2009.

Please note that you are responsible for reporting any changes to the research study to the Scientific Steering Committee and to the Ethical Review Committee prior to implementation. This includes changes to research design, personnel and funding and

CENTRE FOR VIRUS RESEARCH
03 SEP 2008
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In Search of Better Health

procedures that could introduce new or more than minimum risk to research participants. You may embark on the study.

Sincerely,

C. WASUNNA,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE