PREVALENCE OF HEPATITIS B SURFACE ANTIGEN AND SYPHILIS AMONG VCT AND PICT CLIENTS IN ST. PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE, ADDIS ABABA, ETHIOPIA

BY

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ABREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
DREC	Department of Research and Ethical Review Committee
FTA-ABS	Fluorescent Treponemal Antibody Absorption
GUD	Genital Ulcer Disease
HBV	Hepatitis B virus
HIV	Human Immuno-deficiency Virus
MHA-T	Micro- Hemaglutination Test
PCR	Polymerase Chain Reaction
PICT	Provider Initiated Counseling and Testing
SPSS	Statistical Package for the Social Science
STDs	Sexually Transmitted Disease
STIs	Sexually Transmitted Infections
TPPA	*T. pallidum* Particle Agglutination
VCT	Voluntary Counseling and Testing

ABSTRACT

Background: - Syphilis, HIV and HBV are the most common public health problems in Sub-Saharan countries. Ethiopia is among the countries where syphilis, HIV and HBV infections are highly prevalent. These infections are interrelated, since syphilis enhances HIV and, HBV infection is complicated by HIV co-infection. Although syphilis, HBV and HIV have the similar route of transmission, screening services for syphilis and HBV are not common in most of the voluntary counseling and testing centers (VCT). Therefore, more information is required on prevalence of HBV and syphilis in VCT centers, and the rate of co-infection of syphilis and HBV in HIV positive individuals.

Objectives: To assess sero-prevalence of hepatitis B surface antigen (HBsAg) and syphilis among provider initiated counseling and testing (PICT) and VCT clients. It is designed to determine the sero-prevalence of Hepatitis B Virus and syphilis among HIV positive and negative and co-infection of these infections with HIV. Hepatitis B virus infections and HIV are highly prevalent and they are among the major public health concern in developing countries including Ethiopia investigating this problem is of paramount benefit.

METHODS: A cross-sectional study was conducted from November 2011 to February 2012 in St. Paul's Hospital Millennium Medical College. 292 consecutive samples were collected using convenient sampling method from PICT and VCT clients. Data on socio-demographic characteristics and sexual behaviors were collected using a questionnaire. Blood specimen was tested for the presence Hepatitis B surface antigen using commercial test kits and syphilis serology was examined using rapid plasma reagin (RPR) and the data was analyzed using version16 SPSS (statistical package for the social science) software.

Results

The prevalence of HIV was 8/292 (2.7%), HBsAg was 43/292 (14.7%), and syphilis was 5/292(1.7%). HBs antigen prevalence among HIV positive clients was 2/8 (25%) while among HIV negative was 41/287 (14.3%). All HIV positive clients were negative for

syphilis. Sex statistically affects HBV infection (p=0.02), but there was no significant association with syphilis.

Conclusion

In this study syphilis prevalence was low. But a substantial percentage of the attendants seen in the PICT and VCT centers have HBV and HIV infections, which otherwise would remain undiagnosed without serological screening.

Recommendation

Therefore, strong HBV control strategies should be designed parallel with HIV and actions should be taken to avert the extent of the problem including provision of better health education, screening services in PICT and VCT centers and provision of vaccination for HBV.

CHAPTER I

INTRODUCTION

1.1. GENERAL INRODUCTION

Sexually transmitted diseases (STD) are diseases caused by microorganisms those that can transmit from one person to another through sexual intercourse. Sexually transmitted infections (STI) represent major causes of morbidity, public health burdens and socioeconomic cost in both developing and industrialized nations (Ramos *et al*., 2011). The high transmission of sexually transmitted diseases in developing countries is poverty driven prostitution (Memish and Osoba, 2006).

Sexually transmitted diseases are major health problems in Sub-Saharan countries, including Ethiopia (Moges *et al.*,2006). Many people suffer from STDs due to lack of access to appropriate health care (Moges *et al*., 2006). STDs are a major cause of acute illness, long-term disability and death, with severe medical and psychological consequences (Gutiérrez *et al*., 2004).

Most STD share the common property of inducing inflammation and resulting in what is believed to be a risk for HIV acquisition (Vermund *et al*., 2001). Previous data indicates that, during the past decades, STDs enhance transmission of HIV-1 infection (Heiner *et al*., 2000). Different age groups are affected by STD especially adolescents are at higher risk of acquiring sexually transmitted diseases, and adolescent girls in particular are disproportionately affected (Dawn and Yasamin., 1998).

The challenge of dealing with sexually transmitted diseases and their sequelae is an increasing concern for medical professionals and public health officials as they struggle to deal with the swelling pandemic (Genuis., 2004). Sexually transmitted diseases impose an enormous burden of morbidity and mortality in many developing countries, both directly through their impact on reproductive and child health, and indirectly through their role in facilitating the sexual transmission of HIV infection (Philippe *et al*., 1998). HIV, HBV, and

T. pallidum represent major public health problems throughout the world. These infections can be transmitted from mother to fetus and may cause severe morbidities in their offspring (Moges *et al*., 2006). The information generated from this study is expected to help in designing control strategies on HIV/AIDS and other STIs at VCT and PICT centers in the country as a whole.

1.2. BACKGROUND OF THE PROJECT

1.2.1 LITERATURE REVIEW

1.2.2. HEPATITIS B VIRUS (HBV)

Hepatitis B virus is one of the most common sexually transmitted diseases. HBV occurs worldwide and constitutes a serious public health problem. Globally, more than 2 billion people have been infected with HBV at some time in their lives (Moges *et al.*, 2006). Of these, about 350 million people remain infected chronically and become carriers of the virus, and 1.5 million deaths occur from HBV related liver diseases, including end stage cirrhosis and hepatocellular carcinoma each year (Awole and GebreSelassie., 2005; Firnhaber *et al*., 2009).

Africa is highly affected region next to Asia (Barth *et al.*, 2010). There are more than 50 million chronic carriers of hepatitis B Virus with 25% mortality risk. In Sub-Saharan Africa, even worse health problem and falls to high endemicity between 56% and 98% of the adult population show that previous exposure (Kiire., 1996).

HBV is highly transmissible and relatively easy to be transmitted from one infected individual to another. The three major routes of HBV spread are : perinatal, horizontal, and sexual transmission (Edmunds *et al*., 1996). In developing countries, the main routes of transmission are: neonatal with HBV carrier mother infecting her infant usually during birth or soon after birth following close contact, transfer of HBV via cuts, sexual transmission, transfusion of infected blood or blood products, contamination of eye, re-use of HBV contaminated needles and lancets (Moges *et al*., 2006).

Infection with HBV causes mortality, morbidity and financial burden. Individuals with chronic infection have a high risk of developing liver cirrhosis and hepatocellular carcinoma (Bhattacharya *et al*., 2007). Adults infected with HBV usually acquire acute hepatitis B virus and recover, but Infected children rarely develop acute disease (Juszozyk., 2000). Neonates who contract hepatitis will have an almost 90% risk of developing chronic HBsAg carriage and chronic liver disease (Sriprakash and Anil., 1997).

The global prevalence of chronic HBV infection varies widely, from >8%, in Africa, Asia and Western Pacific to <2%, in Western Europe, North America and Australia (Vermund *et al*., 2001). In Pune, India, in a STD clinic attendees show that from a total of 497 participants 3.6% were positive for HBsAg (Risbud *et al*., 2002). A similar study done in urban clinic in Johannesburg, South Africa, prevalence of HBV from 502 participants 4.8% were positive for HBsAg (Firnhaber *et al*., 2009).

Ethiopia is one of the Sub-Saharan countries and HBV infection is endemic. However; the distribution is highly variable because of the cultural and geographical diversity and it is more common in urban areas. The overall prevalence of hepatitis B virus markers among the general adult population of Ethiopia is about 80%, and the carrier state of hepatitis B surface antigen (HBsAg) is ll-12% (Tesga *et al.*, 1988). Acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma accounted 12% of the hospital admissions in Ethiopia. One study showed that from 238 study participants 27% were with chronic hepatitis and positive for HBsAg (Tsega *et al.,* 1995).

In an earlier study done to define the mode of transmission of Hepatitis B infection in Ethiopia, 5% of pregnant women were reported to be positive for HBsAg (Tesga *et al*., 1988). A similar study done to determine the prevalence and significance of sexually transmitted diseases among Ethiopian women attending antenatal care in Addis Ababa hospitals, the prevalence of HBsAg among pregnant women was similar (5%) to the above study (Duncan *et al*., 1995). A survey of Ethiopian blood donors showed that

occurrence of HBsAg was 8% (Kefene *et al*., 1988). A similar study in Northwest Ethiopia reported that HBsAg was detected in 14.4% of blood donors (Kebede., 1983).

Moreover, a community-based sero-epidemiological survey of Addis Ababa was conducted to inform on the transmission dynamics and control of HBV infection, and HBsAg prevalence was 7%. Overall HBV sero-prevalence , rose steadily with age to over 70% in 40-49 years old, indicating significant childhood and adult transmission (Abebe ., 2003). A study done in pregnant mothers in a rural hospital in Southern Ethiopia, to assess the risk of transmitting to the newborn and the sero-prevalence of HBsAg was 6% (Ramos *et al*., 2011). A study conducted at Debre-Tabor Hospital, Northwest Ethiopia to determine the sero-prevalence of HBsAg and associated risk factors among pregnant women attending antenatal care service indicates that the sero-prevalence of HBsAg was 5.3% and HBV is highly important in areas where HIV is epidemic (Walle *et al*., 2008).

1.2.3. HEPATITIS B VIRUS (HBV) AND HIV CO-INFECTION

Since HIV and hepatitis B virus share similar routes of transmission hence, co-infection is a frequent problem. In areas of low endemicity, such as North America, Australia and Europe, HBV and HIV infection are usually acquired in adulthood through sexual or percutaneous transmission. In areas of low endemicity, the prevalence of chronic co - infection is around 5-7% among HIV-infected individuals (Alter., 2006). In countries with intermediate and high HBV endemicity, the main routes of transmission of HBV are perinatal or in early childhood; in these countries HBV co- infection rates are 10-20% (Lee *et al*., 2008). Exposure to these viruses is followed by an immune response, which differs markedly in its ability to clear the infection. Clearance is maximal for adults exposed to HBV and negligible (or non-existent) for HIV (Lebovics *et al*., 1988).

The rate of progression and complications from viral hepatitis are accelerated in patients with HIV co-infection (Puoti *et al*., 2006). After acquiring HBV infection, HIV infected individuals are 6 times more likely to develop chronic hepatitis B than HIV negative individuals (Bodsworth *et al*., 1991). This was more likely to occur in HIV infected men with lower CD4 cells (Bodsworth *et al*., 1991).

Decreased rates of clearance of HBeAg and increased HBV replication are also seen, with higher HBV DNA viral load (Colin *et al*., 1999). In addition, HIV infected individuals are more likely to lose previously developed protective anti-HBs antibody and develop acute hepatitis B infection; this risk is also associated with lower CD4 counts (Konopnicki *et al*., 2005, Biggar *et al*., 1987).

In the setting of HIV co-infection, the mortality rate from chronic Hepatitis B Virus is higher than that of either infection alone. Specially there is high risk of developing hepatotoxicity following the initiation of antiretroviral therapy in subjects with underlying chronic hepatitis than in HIV-mono-infected individuals and is important to consider HBV therapy as a priority in HIV-co infected patients (Hoffmann and Thio., 2007).

Globally, 10% of the HIV-infected population suffers from chronic hepatitis B Virus. In many parts of Africa, HIV/HBV co-infection is common. In one study over 90% of patients with HIV had HBV markers of current or past infection (Lebovics *et al*., 1988).

Most of the researches done on HIV and HBV co-infection in low HBV endemic regions showed significantly higher prevalence of HBV infection in HIV positive individuals. Studies done in Sub-Saharan Africa have conflicting result; most of which don't support an increase prevalence of HBV in HIV positive individuals (Burnett *et al*., 2005). However there are few studies about HBV-HIV co-infection in Ethiopia (Rahlenbeck *et al*., 1997). This is supported by Rahlenbeck *et al*., 1997), where no association was found between HIV and HBV infection among blood donors. Another study done in Addis Ababa, prevalence of HBsAg and anti-HBc in VCT clients were 5.7% and 44.8%, respectively. Among HIV-infected persons, 3.9% were sero-positive for HBsAg; and there was no significant difference in HBsAg or anti-HBc sero-positivity between HIV-positive and HIV-negative subjects (Shimelis *et al*., 2008).

Accurate assessment of HBV infection in HIV co-infected individuals is necessary for therapeutic decisions (Thio., 2009). WHO advocates HBsAg testing especially in areas of high HBV prevalence (WHO., 2006). But additional testing for HBV markers such as HBeAg and HBV DNA and to assess stage of liver disease may not be widely available in many resource limited countries (Thio ., 2009). For HIV infected individuals with chronic HBV, additional screening of other markers; for hepatocellular carcinoma screening with alpha fetoprotein and imaging of liver every 6 months is being suggested by some but the cost-benefit of one or both tests as well as the frequency of monitoring in various health economies remain to be assessed especially in resource limited areas (Thio., 2009).

1.2.4. SYPHILIS

Syphilis is a systemic disease caused by *T. pallidum* which can be spread by sexual contact, blood transfusion and via vertical transmission (Moges *et al*., 2006). Syphilis, a sexually transmitted disease (STD) which seemed to have disappeared or had been controlled over the years, has re-emerged as a major public health problem in many rural, urban and sub-urban communities (William *et al*., 2001). The increasing incidence of the disease is caused by sexual promiscuity, drug abuse, poverty, increased population migration, and unemployment, which lead children and adolescents into prostitution. Decreasing government support for public health in developing countries also aggravates this situation (Antunes *et al*., 2006).

Syphilis is a global problem, with a 12 million people infected every year. Syphilis is endemic in areas of Africa, Asia and Latin America where it is estimated that 11 million of the 12 million new adult cases of syphilis occur annually (Klisch *et al*., 2007). During the early part of the 20th century, tertiary syphilis was the cause of illness in about a fifth of patients in institutions for the mentally ill in the USA (Walker and Walker., 2002).

Congenital syphilis causes adverse outcomes in up to 80% of cases and is estimated to affect over 1 million pregnancies annually (Fenton *et al*., 2008). Africa in the 1980s appeared to

be facing problems associated with syphilis during pregnancy similar in severity and magnitude to those faced by the industrialized world in the early 1900s. Reported prevalence of syphilis in pregnant women in Africa frequently ranges from 10 - 15%, with 50 - 80% of those new positive women experiencing an adverse outcome (WHO, 1996). In Ethiopia previous study showed a prevalence among pregnant women was 2.2% in different parts of the county (Moges *et al*., 2006).

Syphilis clinical manifestations are classified in multiple stages as primary, secondary, latent (early and late), and tertiary syphilis (Antunes *et al*., 2006). Primary syphilis facilitates both the transmission and the acquisition of HIV infection and it can contribute to the spread of HIV infection. However, to date, there is no clear evidence of increased transmission of HIV infection (Nicola and Jeffrey., 2007). The annual incidence of HIV infection at San Francisco, California, municipal STD clinic decreased between 1999 and 2001 from 5.4 cases per 100 person/years to 2.5 cases per 100 person years, despite there being dramatic increases in the rates of primary and secondary syphilis during the same period (Nicola and Jeffrey., 2007).

Although there are minor differences, syphilis presents similarly in HIV-infected and HIV uninfected patients. In primary syphilis, HIV-infected patients may present with two or more chancre and with larger and deeper lesions. Approximately one-fourth of HIV-infected patients present with concomitant lesions of both primary and secondary stages of syphilis at the time of diagnosis (Nicola and Jeffrey., 2007).

In Ethiopia a survey done in 1952 showed an average of 48% sero-positivity in cardiolipin test, and using venereal disease research laboratory (VDRL) and fluorescent treponemal antibody (FTA) test showed that 31% of sera from unselected population gave positive results (Friedmann and Wright., 1997). According to Friedmann, a survey done in 1976 in unselected group of women admitted to one of the largest obstetric units in Addis Ababa (St. Paul's Hospital) showed sero-positivity of 12.7% for VDRL and 10.9% for fluorescent treponemal antibody absorption (FTA - AB). He concluded that syphilis was still

prevalent in Addis Ababa although it was decreasing probably because of the wide spread use of penicillin (Friedmann and Wright., 1997).

Another study done in 1975/76 in Addis Ababa among pregnant women attending antenatal clinic showed the sero-prevalence rate of 27% to TPHA and 28% to VDRL (Duncan *et al*., 1995). Another study done in rural hospital, in northwest Ethiopia, in September 1994, showed VDRL sero-positivity of 13.7% in ante-natal attendees (Azeze *et al*., 1995).

In Ethiopia twenty one percent of children of sero-positive mothers develop signs of syphilis, while stillbirth and abortion rate of infected women was almost double than among the general population (Larsen *et al*., 1995).. A contemporary study to this found that syphilis was the fourth most common cause of perinatal death and accounted for 10% of the 70 perinatal deaths per 1000 births and almost five percent of all postnatal deaths (Naeye *et al*., 1977). Rates for congenital syphilis have been reported to be 3,200/100,000 live births in Addis Ababa (Ratnam., 1982).

1.2.5. SYPHILIS AND HIV CO-INFECTION

Syphilis and HIV are both transmitted sexually and so it is no surprise that a substantial number of people are infected with both agents. HIV has several effects on the presentation, diagnosis, disease progression, and therapy of syphilis. Syphilis may increase the risk of HIV transmission and acquisition by causing genital ulcers (Lynn and Lightman., 2004). So, Syphilis is an important infection in contemporary medicine because of the morbidity it causes and its ability to enhance the transmission of HIV (James., 2005). Patients co-infected with HIV and *T. pallidum*, cutaneous lesions may be more severe, symptomatic neuro-syphilis may be more likely to develop, the latency period before the development of meningo-vascular syphilis may be shorter, and the efficacy of standard therapy for early syphilis may be reduced (David., 2000).

The rate of HIV and syphilis co-infection will vary depending on the prevalence of both infections in the community or the patient group being studied, along with individual risk

factors. Blocker *et al*., (2000) reviewed 30 studies that looked at HIV rates in people with Syphilis in the USA. They reported an overall median sero-prevalence for HIV of 15·7% (27·5% in men and 12·4% in women).

Furthermore, much higher rates of HIV co-infection were detected in relation to specific risk factors, for example intravenous drug use (22·5–70·6%) and gay sex (68–90%) (Blocker *et al*., 2000). The relative risks for HIV infection associated with syphilis and other genital ulcer disease (GUD) range between 2 and 11, with the strongest evidence to date reported from studies conducted in Africa (Jeffrey *et al*., 2005). For example, one mathematical model has suggested that approximately 1000 additional cases of heterosexual HIV transmission occur annually in the USA as a result of syphilis (Lynn and Lightman., 2004). In several developed countries, the prevalence of HIV among patients with syphilis has ranged from 15.7% to 43% and as high as 64–90% (Mark *et al*., 2010, Nicola and Jeffrey., 2007).

A study done in New Jersey Medical School, USA in pregnant mothers 1.7% of the 735 study subjects were tested positive for syphilis. Seven (0.95%) patients tested HIV positive. One patient was HIV co-infected with syphilis (Lisa *et al*., 1994). A similar study in HIV infected women at the University of Rocheiter, USA who contracted syphilis infection during pregnancy were noted to deliver HIV infected newborns at a high rate (Lee *et al*., 1998). A study done among neuro-syphilis and ocular syphilis cases, 36% of the patients were HIV co-infected (David., 2000).

Syphilis causes a transient increase in HIV viral load and decreases in CD4 cell count that can resolve after the infection is treated (Malone *et al*., 1995). Ethiopia, like many African countries, is experiencing a severe HIV/AIDS epidemic and the prevalence of syphilis sero-reactivity in Ethiopia is 20–30% in men and 2–27% in women measured in different regions of the country. So, co-infection of HIV and syphilis may be high which needs further study (Marrazo., 2004).

1.2.6. HIV

Human Immunodeficiency Virus (HIV) was first recognized in 1981 cases from United States(Kalemli-Ozcan and Turan., 2011). Since the epidemiology and microbial characteristic of HIV was not known, HIV was spreading at an alarming rate worldwide (Cohen., 2004). In 1985 HIV was found in every regions of the world, infecting 1.5 million people globally. By 2007 HIV has infected 33.2 million people and greater than 20 million people had died of acquired immune deficiency syndrome (AIDS). AIDS is now the leading cause of death among people 15–59 years old and the world's most urgent public health challenge (Kebede., 1983).

Political and economic problems of Africa has led to the wide spread of HIV infection (Genuis., 2004). HIV causes an economic burden to Africa, since, HIV affects the productive age which will lead to higher capital labor ratios in the affected countries of Africa. This affects growth in the region (Sebnem and Belgi., 2010). Majority of people infected with HIV/AIDS are found in Sub-Saharan Africa. During 2002, 29.4 million people were infected with HIV and 2.4 million people died accounting 20% of the deaths and disabilities in Africa (Lawn, 2004). In 2008 22.4 million people were infected with HIV in Sub-Saharan Africa (Friend and Doncel., 2010). In Sub-Saharan Africa risk for HIV infection is associated with history of medical injections, blood transfusions and antenatal care (Deuchert and Brody., 2007).

Ethiopia is classified along Nigeria, India, China and Russia, where large populations are at risk for HIV infection. By the end of 2003 HIV has infected 1-2.3 million and 1.7 million people had died from AIDS (Kebede *et al*., 2000). During that time, it was estimated that, there were 700, 0000 children under the age of 17 who have lost either one or both parents to AIDS (Kebede *et al*., 2000). In Addis Ababa, 68% of all deaths in the age group 20-54 were due to AIDS in 2001 (Araya *et al*., 2004). HIV prevalence is between 14% and 20% among urban pregnant women, 12% in patients treated for STDs, and 74% in commercial sex workers (Kebede *et al*., 2000).

In 2006, HIV adult prevalence was 4.4%. High prevalence in urban (12.6%) and low in rural areas (2.6%) (Moges *et al*., 2006). Similarly data in 2009, national prevalence of HIV/AIDS was 2.1% (Reda *et al*., 2009). A similar Study in pregnant mothers from rural hospitals in Southern Ethiopia in 2011, of 165 study individuals 1.8% was positive for HIV (Ramos *et al*., 2011).

1.3. LABORATORY DIAGNOSIS

A. SYPHILIS LABORATORY DIAGNOSIS

Different laboratory methods are used to detect *T. pallidum;*

1. Direct microscopy

Microscopy of fluid from the primary and secondary lesions using dark-field microscope and fluorescent antibody testing can diagnose treponemal disease with better accuracy. But as there are other treponemes that may be confused with *T.pallidum*, care must be taken in evaluating with microscopy to correlate symptoms with the correct disease. Because, its accuracy is limited by the experience of the operator performing the test, the number of live treponemes in the lesion, and the presence of non-pathologic treponemes in oral or anal lesion (David *et al*., 2003).

2. Serologic tests

The Present-day syphilis screening tests, such as Rapid Plasma Reagin (RPR) and Venereal Disease Research Laboratory (VDRL) tests are cheap and fast but not completely specific, because many other conditions can cause a positive result (Table 1.1). These tests are routinely used to screen blood specimens. There are two types of serological tests: treponemal and non-treponemal tests (David *et al*., 2003).

a. NON- TREPONEMAL TESTS

The VDRL test is micro-flocculation test and is read under a microscope. A disadvantage of the VDRL test is; it requires daily preparation of antigen suspension. VDRL test is the only non-treponemal test that can be used to test CSF due to the limited sensitivity and specificity of the other non-treponemal tests.

The RPR test is macroscopic flocculation test and requires no microscope. The RPR test uses a stabilized suspension of VDRL antigen to which charcoal particles are added to aid in the visualization of the test reaction. The RPR test is one of the most commonly used non-treponemal tests, and is a simplified version of the VDRL test.

Each of the above tests can be used as a quantitative test. Quantitative tests allow for the establishment of a baseline titer, which allows evaluation of recent infection and response to treatment. This also allows for the detection of reinfection or relapse in persons with a persistently reactive titer. However, the numerical values obtained may vary between tests; thus, when a patient is being followed with serial titers, the same test and preferably the same laboratory should be used.

b. TREPONEMAL TEST

Treponemal test detects the antibody to the bacterium that causes Syphilis (*T. pallidum*) in blood, body fluid, or tissue. The tests are used to confirm Syphilis infection (Table 1.1).

Table.1.1. Sensitivity and specificity of the different types of serological tests in different stages of syphilis.

	Test	Sensitivity % (range)				Specificity %(range)	Reference
		Primary syphilis	Secondary	Latent syphilis	Late syphilis		
Non-treponemal	VDRL	78(74-87)	100	96(88-100)	71(37-94)	98(96-99)	(Larsen*etal.*, 1995).
	RPR	86(77-99)	100	98(95-100)	73	98(93-99)	(Larsen *et al.*, 1995).
Treponemal Test	MHA-TP	76(69-90)	100	97(97-100)	94	99(98-100)	(Larsen *et al.*, 1995)
	TPPA	88(86-100)	100	100	NA	96(95-100)	Ratnam., 2005)
	TPHA	86	100	100	99	96	(Lesinski *et al.*, 1974)
	FTHA-ABS	84(70-100)	100	100	96	97(94-100)	(Larsen *et al.*, 1995).

Note VDRL – Venereal disease research laboratory, RPR- Rapid plasma reagin, MHA-TP-Microhaemaglutination *T.pallidum*, TPPA- *T.pallidum* particle agglutination, TPHA-*T.pallidum* hemaglutination, FTHA-ABS-Flourescent treponemal hemaglutination absorbtion,

Nucleic acid amplification methods

A number of PCR-based methods have been developed for the detection of *T. pallidum* in clinical specimens. Although these methods are not standardized, they have been found to be highly sensitive, able to detect as low as one to 10 organisms per specimen with high specificity (Ratnam., 2005). These methods are also the most practical in certain settings.

PCR undoubtedly holds promise as a test of choice for congenital syphilis, neuro-syphilis and early primary syphilis when traditional tests have limited sensitivity. This method could be also used to monitor treatment effects (Ratnam., 2005).

B. HBV LABORATORY DIAGNOSIS

Different Laboratory methods are used for the diagnosis of acute and chronic Hepatitis B Virus infection.

SEROLOGY

There are different serological markers used for HBV diagnosis (Selby., 2009). The interpretation and types of tests are listed in table (Table 1.2) (Selby., 2009). These serological tests have different sensitivity and specificity (Table 1.3).

Table 1.2. The different Serological diagnosis methods of HBV and their interpretation.

HBV markers	Results	Interpretation	Vaccinated?
HBsAg anti-HBc anti-HBs	Negative Negative Negative	Susceptible	Vaccinate if indicated
HBsAg anti-HBc anti-HBs	Negative Negative Positive	Immune due to Vaccination	No Vaccination Necessary
HBsAg anti-HBc anti-HBs	Negative Positive Positive	Immune due to Natural infection	No vaccination necessary
HBsAg anti-HBc IgM anti-HBc anti-HBs	Positive Positive Positive Negative	acutely infected	No Vaccination Necessary
HBsAg anti-HBc IgM anti-HBc anti-HBs	Positive Positive Negative Negative	chronically infected	No vaccination necessary (may need treatment)
HBsAg	Negative	Four interpretations	

anti-HBc anti-HBs	Positive Negative	possible	Use clinical judgment

Table 1.3. Sensitivity and specificity of EIA and rapid tests for HBV laboratory diagnosis

	EIA test		**Rapid test**		
	HBsAg	**anti-HBc**	**Anti-HBs**	**HBsAg**	**anti-HBc**
Sensitivity (%)	100(99.1-100)	99.53(98.3-99.9)	74.2%	>99.0(97.6-100)	96.3(94.1-97.8)
Specificity (%)	99.94(99.9-100)	99.9(99.8-100)	86.9%	>99.0(97.6-100)	96.8(91.9-99.1)
Reference	(Bjoerkvoll *et al*., 2010)	(Bjoerkvoll *et al*., 2010)	(Eng Lee *et al*., 2011)	(Bjoerkvoll *et al*., 2010)	(Bjoerkvoll *et al*., 2010)

Note EIA- Enzyme-linked immune-assay

HBV-DNA detection- indicates active replication of virus, more accurate than HBeAg especially in cases of escape mutants. Used mainly for monitoring response to therapy.

C. HIV LABORATORY DIAGNOSIS

Table 1.4. Different Laboratory methods are used for the diagnosis of HIV.

1. HIV RAPID TESTS

HIV rapid tests are used for detecting antibody to HIV is a screening test that produces very quick results, usually in 5 to 30 minutes. The sensitivity and specificity of these rapid tests are listed in (Table1.4).

Table 1.4. Sensitivity and specificity of HIV rapid test kits.

tests	Sensitivity % (range)	Specificity % (range)	Reference
KHB	100(97.7-100)	100(98.8-100)	(WHO, 2004)
STAT-PACK	99.7(98.9-100)	99.9 (98.6 –100)	(Branson., 2007)
UNIGOLD	100 (99.5 –100)	99.7 (99.0 -100)	(Branson., 2007)

Viral antigen and Viral RNA/DNA. These diagnosis methods are used for infants. There have been a few reports using RNA qualitative detection assay, DNA PCR and HIV-1 p24 antigen assay for the early diagnosis of infants born to HIV-1-infected mothers. HIV-1 p24 antigen detection was less sensitive than HIV-1 RNA and DNA detection. These diagnostic methods have a sensitivity and specificity of 100% which is similar in three of the diagnosis but there is sensitivity variation under infants of less than 2 years of old (Sutthent *et al*., 2003).

1.4. SIGNIFICANCE OF THE STUDY

It is estimated that 340 million episodes of curable STDs occur annually. Sexually transmitted diseases are huge burdens in terms of health, social and economic cost (Memish and Osoba, 2006). In most developing countries, the incidence and prevalence of sexually transmitted infections is 20 times higher than those in developed countries (Anvikar *et al*., 2009). In Sub-Saharan Africa STD accounts 17% of the total burden of diseases of women of reproductive age (Philippe *et al*., 1998).

The importance of the present study is to determine the prevalence of HBV and Syphilis in HIV-infected and HIV-uninfected individuals which is very important during therapeutic management. Furthermore, this study will clarify whether prevalence and associated risk factors of these infections differ between HIV-infected and HIV-uninfected individuals. Consequently, in the present study, we sought to provide some initial estimates regarding the rate of co-infection between HIV, Syphilis and HBV, and to contribute additional information to the scientific community on syphilis and HBV serology among HIV-positive and HIV-negative individuals in Ethiopia.

1.5. OBJECTIVES OF THE STUDY

GENERAL OBJECTIVE

- To determine the prevalence of Syphilis and Hepatitis B surface antigen among VCT and PICT attendants.

SPECIFIC OBJECTIVES OF THE STUDY

- To determine the prevalence of syphilis in HIV positive and negative individuals among VCT and PICT clients.
- To determine the prevalence of HBsAg in HIV positive and negative individuals among VCT and PICT clients.
- To determine the rate of co-infection of Syphilis and HIV.
- To determine the rate of co-infection of HBV and HIV.
- To determine the associated risk factors with Syphilis and Hepatitis B Virus.

CHAPTER II

METHODS AND MATERIALS

2.1. STUDY DESIGN AND AREA

A cross-sectional study was conducted from November 2011 to February 2012 involving VCT and PICT centers in St. Paul's Hospital Millennium Medical College Addis Ababa, Ethiopia. This institution provides health care service for approximately 100,000 patients per year to the Addis Ababa and the surrounding population and those referred from different parts of the country. The study site also provides voluntary counseling and testing services.

2.2. STUDY SUBJECTS

Using convenient sampling method, 292 study subjects were selected from PICT and VCT centers. All consecutive volunteers for HIV, HBV and syphilis testing were recruited. The study participants were between the ages of 18 and 75. Sample size was determined by using single population formula considering the following assumptions: Proportion (prevalence) of syphilis 10.9% (Moges *et al*., 2006), Prevalence of HBsAg 5.7% (Shimelis *et al*., 2008), Level of significance = 0.04, Degree of accuracy desired (d) =4%, Non-response rate =10%. Note: n= sample size, Z (a/2) = Z-score at 96% confidence interval =2.05

$$n = \frac{(z_{\frac{\alpha}{2}})^2 p(1-p)}{d^2} = (2.05)^2 * 0.109 * 0.891 / (0.04)^2 = 255 + 10\% = 280$$

2.3. Specimen Collection, Handling and Transport

After pre-test counseling, 4ml of venous blood was aseptically collected from each of the study participants. These blood samples collected for HIV test were used to test for syphilis and HBsAg. First, one of the arms of the clients were tied with tourniquet and then the arm was cleaned with gauze soaked with 70% alcohol then after cleaning with dry cotton blood samples were collected with five ml syringe. The collected blood samples have been transferred to the test tube and then span in a centrifuge to separate serum from blood cells.

2.4. HIV testing

The hospital routinely uses commercial HIV test kits according to national algorithm. These rapid test kits are KHB, STAT-PAK and UNIGOLD (see appendix IV).

2.5. HBs antigen determination

HBsAg test kit (ACON Laboratories, Inc., USA) is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B surface Antigen in serum or plasma (see appendix V).

2.6. Screening for Syphilis

Serum samples were tested for the presence of non-treponemal antibodies using rapid plasma reagin test (RPR) following the manufacturer's instructions (RPR, Wampole Laboratories, and Princeton, N.J., USA) (see appendix VI).

2.7. VARIABLES

Table 2.1. The dependent and independent Variables

A. INDEPENDENT VARIABLES	**B. DEPENDENT VARIABLES**
Age	Syphilis status
Sex	HBsAg status
Educational status	
Needle stick injury	
Multiple sex partners	
History of blood transfusion	
History of medical procedure	
Living area	
Marital status	
Abstinence	
Condom use	

2.8. STATISTICAL ANALYSIS

The data collected were entered in computer to compile, organize and analyze using computer-based statistical analysis software, SPSS version 16. The prevalence of syphilis and HBV in HIV positive and negative individuals was determined using bi-, and multi-variate analysis, considering 4% level of significance. The associated risk factors and demographic data collected by a questionnaire were analyzed by logistic regression and by descriptive statistics respectively. As the designed & proposed cross-sectional study is a prevalence study, the prevalence of HBV and syphilis among HIV positive and negative individuals were compared. Descriptive statistics for laboratory results and questionnaire variables were calculated.

Frequency tables were used to evaluate demographic data. Too few individuals were infected with HIV and Syphilis to conduct a statistical assessment of HIV risk factors. First, uni-variate analysis was done to determine the frequency of positive serological test, then bivariate analysis was done to determine predictors for infection and finally multivariate analysis was done to determine independent predictors of infection using logistic regression model. P- Value < 0.04 was taken to be statistically significant.

2.9. ETHICAL CONSIDERATION

The M.Sc. research project proposal was ethically cleared by Department of Research and Ethical Review Committee (DREC) and approved by Department of Microbiology, Immunology and Parasitology, Written informed consent was obtained from study participants and letter of permission from St. Paul's Hospital Millennium Medical College.

CHAPTER III

RESULTS

3.1. Study Subjects

The socio-demographic characteristics of the study subjects (n=292) were described in (Table 3.1).

Table 3.1. Socio-demographic characteristics of study participants (n=292) in St. Paul's Hospital Millennium Medical College (November 2011 to February 2012).

VARIABLES		**NUMBER (%)**
Sex	Male	92(31.5)
	Female	200(68.5)
Age (in years)	18-25	87(29.8)
	26-35	109(37.4)
	36-45	55(18.8)
	>45	41(14.0)
Living Area	Addis Ababa	162(55.6)
	Outside Addis Ababa	130(44.4)
Educational status	Literate< 4^{th} grade	186(63.7)
	Illiterate $\geq$ 4^{th} grade	106(36.3)
Marital status	Single	60(20.3)
	Married	210 (72.0)
	Widow	8(2.7)
	Divorced	14 (4.7)

The socio-demographic characteristics of the study subjects showed that 92/292(31.5%) of the study participants were males and 200/292 (68.5%) of them were females. The mean age of the study participants was 33 years and the highest age category was between 26 to 35 years 109/292(37.7%) followed by <26 years of age 87/292 (30.0%). 162/292 (55.14%) of the study participants were from Addis Ababa and 130/292 (44.86%) of them were from Outside Addis Ababa. Majority of study participants were married 210/292 (72.3%), 60/292 (20.4%) single, 14/292(4.7) were divorced and few of them were widow 8/292(2.7%). In

educational background 186/292(63.7%) of the study participants were literate and 106/292(36.4%) were illiterate.

3.2. HIV status

Prevalence of HIV infection among males and females, and among the different age groups is listed in table 3.2.

Table.3.2. Prevalence of HIV among the study subjects attending PICT and VCT center at St. Paul's Hospital Millennium Medical College, Addis Ababa Ethiopia (November 2011 to February 2012).

Variables		HIV status		p-value	Odds ratio	Confidence interval (CI 96%)
		Positive No. (%)	**Negative No. (%)**			
Sex	Male	3(3.3)	89/92(96.74)	0.71	1.31	0.29-6.02
	Female	5(2.5)	195/200(97.5)	0.00	1	
	Total	8(2.7)	284/292(97.3)			
Age (in years)	18-25	1(1.15)	86(98.85)	0.59	0.46	0.03-8.62
	26-35	5(4.59)	104/109(95.41)	0.57	1.89	0.19_18.48
	36-45	1(1.82)	54/55(98.18)	0.83	0.74	0.04-13.95
	>45	1(2.44)	40/41(97.56)	000	1	

Prevalence of HIV infection in this study population was 2.7%. 3/92 (3.3%) of the 92 males and 5/200 (2.5%) of the 200 females were positive for HIV infection (p=0.71) and analysis of HIV infection by age group revealed slight increase with age and highest 26-35 age groups 5/109(4.59%). But there was no significant difference among these age groups (p=0.57).

3.3. HBs antigen

The prevalence of HBsAg among males and females and in different age groups is listed in (Table 3.3).

Table 3.3. Prevalence of HBs antigen among the study subjects attending PICT and VCT center at St. Paul's Hospital Millennium Medical College, Addis Ababa Ethiopia (November 2011 to February 2012).

Variables		**HBsAg status**		**p-value**	**Odds ratio**	**Confidence interval (96%)**
		Positive No (%)	**Negative No (%)**			
Sex	Male	20(21.74)	72(78.26)	0.02	2.13	1.07- 4.25
	Female	23(11.5)	177(88.5%)	0.00	1	
	Total	43(14.7)	249(85.3)			
Age (in years)	18-25	12(13.79)	75(86.21)	0.88	0.92	0.30-2.79
	26-35	15(13.76)	94(86.24)	0.86	0.91	0.31-2.66
	36-45	10(18.18)	45(81.82)	0.65	1.30	0.41-2.12
	>45	6(14.63)	35(85.37)	000	1	

Among the 292 PICT and VCT clients, a total of 43/292(14.7%) were HBsAg positive. The highest prevalence of HBV infection was observed among males 20/92 (21.74%) (p= 0.02, OR=2.13) compared to females which accounts for 23/200 (11.5%). HBsAg is most prevalent among the 26-35 age groups 15/109(13.76%) (p=0.57).

3.4. Syphilis

Prevalence of Syphilis among males and females, and among the different age groups is listed in table 3.4**.**

Table 3.4. Prevalence of syphilis among the study subjects attending PICT and VCT center at St. Paul's Hospital Millennium Medical College, Addis Ababa Ethiopia (November 2011 to February 2012).

Variables		**Syphilis status**		**p-value**	**Odds ratio**	**(96% CI)**
		Reactive No. (%)	**Non-reactive No. (%)**			
Sex	Male	2(2.2)	90(97.8)	0.66	1.46	0.2-9.7
	Female	3(1.5)	197(98.5)	0.00	1	
	Total	5(1.7)	287(98.3)			
Age (in years)	18-25	0(0.0)	87(100)	1.00	1.0	0.00
	26-35	3(2.8)	106(97.2)	0.998	4.5	0.00
	36-45	2(3.6)	53(86.4)	0.998	6.1	0.00
	>45	0(0.0)	41(100)	0.00	1	

The prevalence of Syphilis among 292 study subject was 5/292(1.7%) and the prevalence of Syphilis among males and females were 2/92 (2.2%) and 3/200(1.5%) respectively. The males seem to be more affected than females, although it was not statistically significant (P=0.99). Syphilis is most prevalent among 36-45 age groups 2/55(3.6). But the prevalence of Syphilis among the different age groups was not significantly different (p=1.00).

3.5. HBV and HIV co-infections

The prevalence of HBV among HIV positive and HIV negative clients is listed in table 3.5.

Table 3.5. Prevalence of HBsAg among HIV Positive and Negative individuals

		HIV status				
		Positive No (%)	**Negative No (%)**	**P-value**	**Odds ratio**	**(96% CI)**
HBsAg	Positive	2(25)	41(14.44)	0.41	2.00	0.36-11.08
	Negative	6(75)	243(85.56)	0.000	1	

Prevalence of HBV among HIV positive was 2/8(25%) and among HIV negative was 41/284(14.4%).The prevalence rate of HBV was higher in those having HIV infection as compared to those without the infection.

3.6. Syphilis and HIV co-infection

Table 3.6. Indicates that prevalence of syphilis among HIV positive and negative individuals.

Table 3.6. Prevalence of Syphilis among HIV Positive and Negative individuals.

		HIV Status				
		Positive No (%)	**Negative No (%)**	**P-value**	**Odds ratio**	**(96% CI)**
Syphilis status	Reactive	0(0.00)	5/284(1.76)	0.99	0.000	0.000
	Non-reactive	8/8(100)	279/284(98.24)	0.000	1	

All the HIV positive clients were negative for syphilis and among the HIV negative 5/284(1.76%) were positive for syphilis.

3.7. HBV, Syphilis and HIV co-infection

Prevalence of syphilis and HBV infection and their co-infection with HIV is listed in table 3.7.

Table 3.7. HBV and Syphilis Prevalence among HIV Positive and Negative individuals.

		HIV status				
		Positive No (%)	**Negative No (%)**	**P-value**	**Odds ratio**	**(96% CI)**
HBsAg	Positive	2/8(25)	41/284(14.4)	0.38	2.08	0.37-11.58
	Negative	6/8(75)	243/284(85.6)	0.000		
Syphilis	Reactive	0(0.00)	5(1.8)	0.99	000	000
	Non-reactive	8/8(100)	279/284(98.2)	0.000		

The prevalence of HBV and Syphilis among HIV infected VCT and PICT clients was 2/8 (25%) and 0/8(0.000%), respectively, compared with the prevalence rate of 41/284 (14.4%), and 5/284(1.8%) among HIV-sero-negative clients. Furthermore, no statistically significant association was observed between syphilis and HIV infection (P =0.0.99), and HBV and HIV infection (P = 0.41).

3.8. Risk factors for HBV and Syphilis infections.

Some risk factors that can influence prevalence of Syphilis and HBV are involved in this study. The risk factors and their relationship with prevalence of Syphilis and HBV are listed in table 3.8.

Table 3.8. Significance of risk factors in HBV and Syphilis prevalence

Variable		**HBsAg (HBV)**				**RPR (Syphilis)**			
		Positive No (%)	**Negative No (%)**	**P-value**	**OR(96% CI)**	**Reactive No (%)**	**Non-reactive No (%)**	**P-value**	**OR(96% CI)**
Needle stick injury	No	39(14.44)	231(85.56)	0.00	1	5(18.52)	265(98.2)	0.00	1
	Yes	4(18.18)	18(81.82)	0.62	1.33(0.4-4.2)	0(0.00)	22(100)	1.00	0.0(0.0)
Multiple sex partner	No	32(12.08)	233(87.92)	0.00	1	3(1.13)	262(98.9)	0.00	1
	Yes	11(40.74)	16(59.26)	0.56	1.98(.18-21.7)	2(7.41)	25(92.59)	1.00	0.0(0.0)
History of medical Procedure	No	40(14.60)	234(85.40)	0.00	1	5(1.82)	269(98.2)	0.00	1
	Yes	3(16.67)	15(83.33)	0.8	1.19(0.31-4.56)	0(0.00)	18(100)	1.00	0.0(0.0)
Use of Sharp Materials	No	39(14.55)	229(85.45)	0.00	1	5(1.87)	263(91.6)	0.00	1
	Yes	4(16.7)	20(83.3)	0.76	1.19(0.4-3.9)	0(0.000)	24(100)	0.99	0.0(0.0)

P= <0.05 statistically significant

Prevalence HBV among those with needle stick injury was 18.2% compared to 14.4% those with no history of needle stick injury. Similarly prevalence of Syphilis in those with no history of needle stick injury was 18.5% but no clients were positive for syphilis with history of needle stick injury. Prevalence of HBV in those with multiple sex partners was 40.7% while the prevalence of Syphilis was 7.4% in these groups.

CHAPTER IV

DISCUSSION

Sexually transmitted infections (STI) represent major public health problems globally. STIs are a major global cause of acute illness, infertility, long term disability and death, with severe medical and psychological consequences for millions of men, women and infants. HIV, HBV and Syphilis are the most common types of STIs throughout the world. These infections are endemic and constitute a huge health and economic burden in Sub-Saharan countries including Ethiopia (WHO., 2003).

Previous epidemiologic studies conducted among VCT clients, assessed individual infections of HIV, Syphilis or HBV, or clients from PICT were not included in their study. This study has tried to ascertain association of HIV infection with HBV and Syphilis among VCT and PICT clients, but as the study population is selected group; PICT and VCT clients, conclusion might not be drawn to general population. The results of this study showed the risk of acquiring major sexually transmitted infections (HIV, HBV and Syphilis) from PICT and VCT centers in St. Paul's Hospital. In addition this cross-sectional study provides an important opportunity to assess the status of the HIV infection among clients from Addis Ababa and outside Addis Ababa, since this study area is a referral hospital.

In this study, the HIV sero-prevalence among VCT and PICT clients was found to be 8/292(2.7%) which is by far greater than that of a study conducted in New Delhi, India (0.56%) and similar to the study done in Nigeria (2.7%) (Abdus-Salam AA *et al*., 2008). This HIV prevalence was also lower than the national adult prevalence for the year 2003 which was estimated as 4.4% (UNAIDS/WHO., 2002). Similar findings was found in antenatal care surveillance in rural areas from Ethiopia that found 2.6% sero-prevalence (Moges *et al*., 2006). From data in 2009, national prevalence of HIV/AIDS was 2.1% which is relatively in line with the present study (Reda *et al.*, 2009).

These data contrast with a study conducted among urban areas of Ethiopia that showed a high sero-prevalence (12.6%) while in this study the prevalence of HIV in Addis Ababa was very low (1.8%) even though this study did not include other cities of the country (Moges *et al*., 2006).

The prevalence of HIV in this study in Addis Ababa was 1.8%. But it was very low compare to the urban prevalence 12.6% (UNAIDS/WHO., 2002). In this study HIV-1 sero-prevalence from clients outside Addis Ababa was (2.31%). This is in agreement with recent antenatal care surveillance in rural areas from Ethiopia that found 2.2% sero-prevalence (Montana *et al.*, 2008). In contrast data from rural areas of Ethiopia a relatively low sero-prevalence of HIV-1 infection was found (1.8%). These data contrast with a recent study conducted among pregnant women from rural sites in the northwest of Ethiopia that showed a high sero-prevalence (9.4%) (Tiruneh., 2008).

There was no association between sex and HIV infection in the present study. The proportion of females who were found to be seropositive for HIV infection 5/200(2.5%) was lower than males 3/92 (3.3%). This was in line with study done among blood donors (Yami *et al*., 2011). In contrast previous study done in Gondar showed prevalence among adult women double that of men (Moges *et al*., 2006).. Another study indicated that, HIV prevalence was higher among female blood donors (4.19%) compared to males (3.43% (Damtie *et al*., 2006). This difference could be due to the different study population used.

In this study age specific HIV prevalence was particularly high in the age group between 26-35 years. This was in line with reports from rural Ethiopia which showed a higher prevalence of HIV in this age group (Moges *et al.*, 2006). This result was different from national reports which showed the peak age for HIV infection started from as early as 15-19 age range in females and 20-24 in males (MOH., 2004).

HBsAg sero-prevalence obtained in the present study was remarkable 43/292(14.7%). In contrast a study from the general population and from pregnant HIV infected women in the

USA indicated a much lower prevalence of HBV, 0.4% and 1.5%, respectively, as compared to the present study (Lisa *et al*., 1994). This could be due to the fact that in most developed parts of the world (Western Europe, USA, Australia), the endemicity of HBV infection is low (Vermund *et al*., 2001). The sero-prevalence of HBV (14.7%) is higher than the previous reports, 10.4% in Nigeria (Mustapha and Jibrin., 2004) and similar to 15.0% in Ghana (Ampofo *et al*., 2002).

Although VCT and PICT clients are not usually representative of the general population, these numbers confirm the high endemicity of HBsAg in Ethiopia. According to (Abebe , 2003) prevalence of HBV which is greater than or equal to 8% is considered to be endemic. This study result was Similar to the finding in Northwest Ethiopia with 14.4% HBsAg prevalence (Kebede, 1983). In contrast this study result was higher than a study conducted on HBV prevalence in Ethiopia 12% (Tesga *et al*., 1988).

In contrast, a study done to determine the prevalence and significance of sexually transmitted diseases in Addis Ababa showed very low (5%) (Duncan *et al.*, 1995). Another Study in Northwest Ethiopia to determine the sero-prevalence of HBsAg and associated risk factors indicated that the sero-prevalence of HBsAg was (5.3%) which was quite lower than this study (Walle *et al.*, 2008).

In Shashemene hospital a cross-sectional study done among VCT clients a 5.7% prevalence of HBsAg was determined which was relatively low compared to the current data. Similarly, a study conducted in Addis Ababa revealed a 5.7% HBsAg prevalence among VCT clients. These data indicate that a low prevalence of HBsAg as compared to the present study (Shimelis *et al*., 2008).

Similarly, a study conducted in Addis Ababa revealed a 5.7% HBsAg prevalence among VCT clients (Shimelis *et al*., 2008).Another community based sero-epidemiological survey that addresses the transmission dynamics and control of hepatitis B virus in Addis Ababa,

reported HBsAg prevalence of 7.0% from the general population (Abebe *et al*., 2003). These data showed that the prevalence of HBV is low compared to the present study.

Significant association has been found between HBsAg positivity and sex like other studies done in the country from VCT centers and general population, where males were two times at high risk than females OR=2.13 (96% CI, 1.07-4.26, p=0.02). This was in line with previous studies done among VCT clients where men were at high risk than that of women (Shimelis *et al*., 2008, Yami *et al.*, 2011). This was also supported by another study done at Gondar University teaching Hospital showed that HBV sero-prevalence rate was higher among males than females (Damtie *et al*., 2006).

An increased sero-prevalence of HBV was observed in the age groups of 26 - 35 and 36 - 45 years compared to the age group of greater than 45 years. This was in accordance with previous reports by (Ejele *et al*., 2005, Baba *et al*., 2000) in which higher prevalence was observed among 18_27 years. This observation is worrisome since the most productive and economically viable age group of the populations is worst hit. There is the need for renewed intensification of preventive programs aimed at high risk behavioral change.

The prevalence of syphilis in this study was 5/292(1.7%) which was lower than to the prevalence of syphilis in Gondar (10.9%) (Moges *et al*., 2006), reports on blood donors (4.8%) (Yami *et al*., 2011) in Gondar and 28.8 % prevalence among factory workers in Ethiopia (Sahlu *et al*., 2002).

This relatively few cases of syphilis (1.7%) found in this study as compared to previous studies may be due to the marked declining trend observed in the prevalence of *T. pallidum* in the last decade among VCT as well as in the general population. This fall was probably due to use of antibiotics for minor complaints and the more easy availability of drugs among population (Friedmann and Wright., 1997).

There was no significant association between Syphilis and sex like HBV. According to this result, men were more often affected than women (2.2% vs. 1.5%). In contrast, studies from drug addicts, the risk was especially increased in women as compared to men. A study among IDUs in Spain showed a 4 times higher prevalence of syphilis and other STDs among women than men (Scherbaum *et al*., 2005).

The overall prevalence rate of syphilis in this study was 1.7% with the highest infection in the age group 36 – 45 years, but there was no significant difference between the age group and infection. In contrast studies from pregnant mothers highest prevalence of syphilis was observed in the age group 21-30 (Ojo and Oyetunji., 2007)

The prevalence of HIV/HBV co-infection in the VCT and PICT population was relatively high (25%), as compared to that of individual HIV or HBV infections. This was comparable with a study done by Burnett *et al*., (2005*)* which documented an increased occurrence of HBV among HIV positive individuals. This could be due to the shared transmission route of both HBV and HIV infection.

In contrast studies from ART and VCT centers, HIV-positive and HIV-negative people had similar exposure to HBV infections (Shimelis *et al*., 2008) while in this study HIV positive individuals were at high risk compared to HIV negative individuals but there was no significant association. Likewise, several studies from areas of high HBV endemicity reported the absence of any association between HIV and HBV infection (Rouet *et al*., 2004).

Furthermore, some studies from this region found an association, but it was not as large as results in countries of low HBV endemic region. In the USA, for instance, a more than fourfold increase in HBV infection was reported in patients with HIV (Rogers *et al*., 2000). This may be because in people living in areas of high HBV endemicity are infected early in childhood. When they reach sexual maturity at which stage they have a higher risk of acquiring HIV, early acquired immunity may protect them from HBV infection. In contrast,

most adolescents and adults in low-endemic areas are not protected by antibodies to natural HBV infection; thus, the shared transmission routes result in specific risk groups to acquiring both HBV and HIV at almost the same time. Even though the rate of co-infection of HIV-HBV is not as high as that of low endemic countries, there is relatively high co-infection among HIV positive as compared to HIV negative individuals.

One of the major concerns associated with HIV-syphilis co-infection is that syphilis facilitates HIV acquisition and transmission. But in this study no cases of HIV- syphilis co-infection was found. This supports the marked declining trend observed in the prevalence of *T. pallidum* in the last decade among VCT and PICT clients as well as in general population. This fall is probably due to an over-the-counter use of antibiotics for minor complaints and the more easy availability of drugs among population. In contrast to this study an estimated 16% of all patients and 28% of men infected with syphilis have co-infection with HIV in the United States (Zetola *et al*., 2007).

The sero-prevalence rate for syphilis was highly associated with HIV infection in previous studies. This may be because syphilis agents may increase the susceptibility of subjects for HIV infection, probably through the increased incidence of genital ulcer (Lynn and Lightman., 2004). In contrast, a study from HIV clinic significant increase in syphilis/HIV co-infection (15.2%) was observed, which is quite different from this study where there were no cases found with HIV/Syphilis co-infection (Levin *et al.*, 2010).

In this study, high prevalence rate of HIV-HBV (25%) and no cases of HIV-Syphilis co-infections was revealed among VCT and PICT clients. One of the major concerns associated with HIV-syphilis co-infection in previous studies was that syphilis facilitates HIV acquisition and transmission. While in this study, there were no cases of HIV and Syphilis co-infection due to the decrease in the prevalence of both infections. This may be due to extensive prevention programs to HIV and the wide use of penicillin and its derivatives which decreased prevalence of Syphilis (Zetola *et al*., 2007). But studies from Gondar

University teaching hospital statistically significant association was observed between syphilis and HIV infection (Tessema *et al.,* 2010).

On the other hand, in this study 25% co-infection of HIV-HBV was found. This may be due to increased prevalence of hepatitis B virus. One may conclude that the transmission of HIV and syphilis in this population, therefore, may take place mainly through sexual contact with an infected person. While, HBV transmission takes place through many different routes, like perinatal transmission in developing countries where it is the most common route of transmission (Lee *et al*., 2008).

Unlike sex which had a significant association with HBV, no association was observed with other socio-demographic variables like, illiteracy and age. However, a study done in Jimma among pregnant women has shown high HBsAg positivity rate among the illiterate and those with low incomes (Awole and GebreSelassie., 2005, Firnhaber *et al*., 2009).

In this study no significant association was found between HBV, HIV and Syphilis and number of sexual partner. In contrast data from previous study prevalence of HIV infection and syphilis correlated with the number of sexual partners where all HIV positive cases except one and 84.1% of all subjects that were seropositive for syphilis had a history of sexual exposure which was contrary to the present study. In-line with this study, the reported use of condom was not significantly associated with reduced prevalence of HIV infection and syphilis. Similarly, result from other study prevalence of HBV infection did not show correlation with the number of sexual partners and condom use (Moges *et al*., 2006).

Similar result was found from study done in VCT clients who had multiple sexual partners were infected with HBV at higher rate, although the difference was not significant (Sisay *et al*., 2011). In-contrast, another study showed significantly high prevalence of HBsAg among individuals with history of invasive procedures, like history of medical procedure , blood transfusion history and needle stick injury compared to this study (Martin *et al*., 2001).

LIMITATIONS

Because this study was based on a convenient sample rather than on a random sampling frame, and the 2 populations were not matched, selection bias cannot be ruled out. In addition, because our sample was limited to a population of PICT and VCT clients, the generalizability of these results is somewhat limited. The limitations of the majority of serological tests were also considerable to the test kit used by this study. This particularly holds true for *T.pallidum*-infection because the RPR anti-body determination may not completely detect clients with *T.pallidum* –infection and the HBsAg for HBV due to low specificity and sensitivity compared to the molecular techniques. Another major limitation of this study was the inability to determine the actual stage of HBV infection among the VCT and PICT with further test (anti-Hbc, anti-HBe, and anti-HBs antibodies) and the inability to measure the HBV viral load. Thus, a single determination of HBsAg may not be the ideal way of defining the carrier state. Nevertheless, a prevalence of 14.7% for HBsAg, 2.7% for HIV and 1.7% for Syphilis among healthy clients is still important and significant.

CONCLUSION AND RECOMMENDATION

CONCLUSION: Results from this study indicated that prevalence of HIV, HBs antigen and Syphilis were 2.7%, 14.7% and 1.7% respectively. 25% of the HIV positive were co-infected with HBV. None of the study participants were co-infected with Syphilis and HIV. Males were two times at high risk for HBV infection than females (p=0.02). The low sero-prevalence of HIV-1/2 and syphilis in VCT and PICT clients observed in the present study may be associated with the expansion of health programs that Ethiopia has implemented in the last decade. In contrast, HBV continues to be a challenge and requires intensified prevention measures to prevent the transmission of this infection.

RECOMMENDATION: The high prevalence of HBV as well as HIV/HBV co-infection is concerning. Formal guidelines and interventions for hepatitis prevention and management of both HBV and HIV/HBV co-infection are needed. Guidelines and interventions should be comprehensive and include: hepatitis education and prevention, HIV–hepatitis counseling,

screening for HBV parallel with HIV testing and overall integration of hepatitis prevention into HIV prevention programs. The usefulness of HBV–HIV co-infection during clinical management and treatment programs should be explored.

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APPENDIX I. QUESTIONNAIRE

Questionnaire for the determination of syphilis and Hepatitis B surface Antigen among HIV positive and Negative individuals.

I. Demographic data

A. ID NO___ Age___ Sex___

B. Study site PICT ___ VCT_____

C. AREA

1. Addis Ababa 2. Outside Addis Ababa

D. Marital Status

1. Single 2. Married 3.Widow 4. Divorced

B. Educational Status

1. Illiterate 2.Literate

II. Risk Factor

1. Needle stick injury 2. Multiple sex partners 3. History of medical procedure 4. Use of Sharp Materials

III. LABORATORY DATA

A. HIV Status 1. Negative 2. Positive

B. Syphilis (RPR) 1. Non- Reactive 2. Reactive 3.weak reactive

C. HBsAg 1.Negative 2.Positive

APPENDIX II. INFORMATION SHEET

(To be translated into Amharic)

This study includes 280 study participants and you are kindly invited to participate in this study. The aim of this study is to determine the prevalence of syphilis and Hepatitis B surface Antigen in HIV positive and negative individuals in St. Paul's Hospital Millennium Medical College at VCT center. Infection of Syphilis enhances HIV transmission and HIV complicates Hepatitis B virus infection. So, this study will help in the control and prevention of HIV and therapeutic management of HIV and Hepatitis B Virus co-infection. Results from this study will provide current information to be used as base line for policy makers and agencies involved in the control and prevention of Sexually transmitted diseases.

A. PURPOSE: This study will determine the prevalence of Syphilis and Hepatitis B surface Antigen in HIV positive and negative individuals.

B.DURATION: This study may take four months.

C. PROCEDURE TO BE CARRIED ON: In this study no additional blood samples will be collected. Blood samples collected for HIV test will be used for this study.

D. RISKS AND DISCOMFORTS: Since blood samples collected for HIV test will be used for this study. So, there is no harm or discomfort.

E. EXPECTED BENEFITS: The study participants will not benefit directly from this research. But, results of this study will be used by policy makers and agencies involved in control and prevention of sexually transmitted diseases.

F. CONFIDENTIALITY: All personal information collected for this study will be kept confidentially.

G. COMPENSATION: No compensation will be provided for participating in this study.

H. TERMINATION OF THE STUDY: At any time study participants have the right to refuse from the study and refusal will not follow penalty or loss of benefit.

Study participants have the following rights:

- Keep hold information
- Decline to cooperate in the study
- To refuse provision of specimens

I would also like to inform you that this study was approved by the Department of Research and Ethical Review Committee (DERC) and ethically cleared by Institutional Review Board (IRB). If you have any question about the study the address is:

Faculty of Medicine Addis Ababa University

Office of Associate Dean, Postgraduate Programs and Research

P.O. Box 9086. Addis Ababa, Ethiopia

Tel. 251-011-551-28-765

If you have question about the study the address of the principal investigator is:

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Tel: 0913064132

<u>የጥናቱ ተሳታፊዎች የመረጃ ቅጽ</u>

በዝህ ጥናት ሁለት መቶ ሰማንያ ተሳታፊዎች ይኖራሉ፡፡ የጥናቱ ዓላማም በቅዱስ ጳውሎስ ሆስፒታል ሚሊንየም መዲካል ኮለጅ ነፃ የኤች ኣይቪ ምርመራና የምክር ኣገልግሎት ተጠቃሚዎች ላይ የለው የጨብጥና ሄፓታቴስ ቢ ቫይረስ መጠንና ስርጭት ለማወቅ የምደረግ ጥናት ነው፡፡ በጨብጥ በሽታ የተያዘ ሰው በኤች ኣይቪ የመያዝ ዕድል ስለምጨምር እንድሁም ኤች ኣይቪ የሄፓተቴስ ቢ ቫይረስ በሽታ ስለምያባብስ ስለሆነም ይህ ጥናት ለኤች ኣይቪ መከላከልና ቁጥጥር እንድሁም የኤች ኣይቪና የሄፓታቴስ ቢ ቫይረስ ኮእንፈክሽን ህክምና ኣሰጣጥ ላይ ያግዛል፡፡ ከዝህ ጥናት ውጤት ለፖሊሲ ኣውጪዎችና በኤች ኣይቪ መከላከልና ቁጥጥር የምሰሩ ድርጂቶች መሰረታዊ ጥናት ሁኖ ልያገለግል ይችላል፡፡

ሀ. <u>የጥናቱ ዓላማ፡-</u> የዝህ ጥናት ዓላማ ነፃ የኤች ኣይቪ ቫይረስ ምርመራና የምክር ኣገልግሎት ተጠቃሚዎች ላይ ያለው የጨብጥ እና የሄፓታቴስ ብ ቫይረስ ስርጭት እና መጠን ለማወቅ የሚደረግ ጥናት ነው፡፡

ለ. <u>የሚፈጀው ጊዜ</u>፡- ይህ ጥናት እስከ ኣራት ወር ልደርስ ይችላል፡:

ሐ. <u>ኣጠቃቀም</u>፡-በዚህ ጥናት ተሳታፊ ከሆኑ ሰዎች ለኤች ኣይቪ ከተስጠው ደም ለዝሁ ጥናት ይውላል፡፡

መ. <u>ሊደርስ የሚችል ኣደጋ</u>፡- በዚህ የጥናት ጊዜ ኣደጋ የሚያደርስ ድርጊት የለም፡፡

ሠ. <u>የሚገኝበት ጥቅም</u> ፡- ከዚህ ጥናት ውጤት በተለይ ኤች ኣይቪ ከጨብጥ እና ከሄፓታቴስ ብ ቫይረስ የምፈጠረው የበሽታ መባባስ ኣስቀድሞ ለመከላከል ያስችላል፡፡

ረ. <u>ሚስጥራዊነት</u>፡- ማንኛውም የጥናቱ ተሳታፊ መረጃ በሚስጥራዊነት ይያዛል፡፡

የእያንዳንዱን ግለሰብ መረጃ ከዋናው ተመራማሪ

በስተቀር ማንም ሊያገኘው ኣይችልም፡፡

ሰ. **<u>ፈቃደኝነትን ስለማቋረጥ</u>** ፡- የጥናቱ ተሳታፊዎች፤መረጃ ያለመስጠት፣በጥናቱ

ለመሳተፍ ፈቃደኝነት ያለማሳየት እንዲሁም ናሙና

ያለመስጠት መብታቸው የተጠበቀ ነው፡፡

አድራሻ ማወቅ ካስፈለግዎ፡-

ህክምና ፋክሊቲ፤አዲስ አበባ ዩኒቨርሲቲ

የድህር ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ.251-011-551-28-765

የዋናው ተመራማሪ አድራሻ፤

ሓብታሙ ወልደሰንበት

ህክምና ፋክሊቲ፤አዲስ አበባ ዩኒቨርሲቲ

የመ.ሳ.ቁ. 9086 አዲስ አበባ

የማይክሮ ባዮሎጂ፤እምኖሎጂ እና ፓረሳይቶሎጂ ትምህርት ክፍል

ህክምና ፋክሊቲ፤አዲስ አበባ ዩኒቨርሲቲ

የመ.ሳ.ቁ. 9086 አዲስ አበባ

ስልክ 0913064132

APPENDIX III CONSENT FORM

(To be translated into Amharic)

ID NO……..age……Sex……

I have been informed that the objective of this study is to assess the prevalence of syphilis and HBsAg in HIV positive and negative individuals in St. Paul's Hospital Millennium Medical College from VCT clients. The sample will be 3_4 ml blood samples. The result of this study will be used by Policy makers and organizations involved in the control and prevention of Sexually transmitted diseases. I have been informed that about the confidentiality. I have been also informed that my right to keep hold of information and to drop out in any inconvenient conditions. Therefore, with full understanding of the importance of the study, I agree voluntarily to provide the requested samples.

I_____ hereby give my consent for giving of the requested information and blood specimen as the researcher find best to do the research.

Participants signature _____Date_____

<u>የፈቃደኝነት መጠየቂያ ቅጽ</u>

ተራ ቁጥር.....ዕድሜ.....ፆታ.....

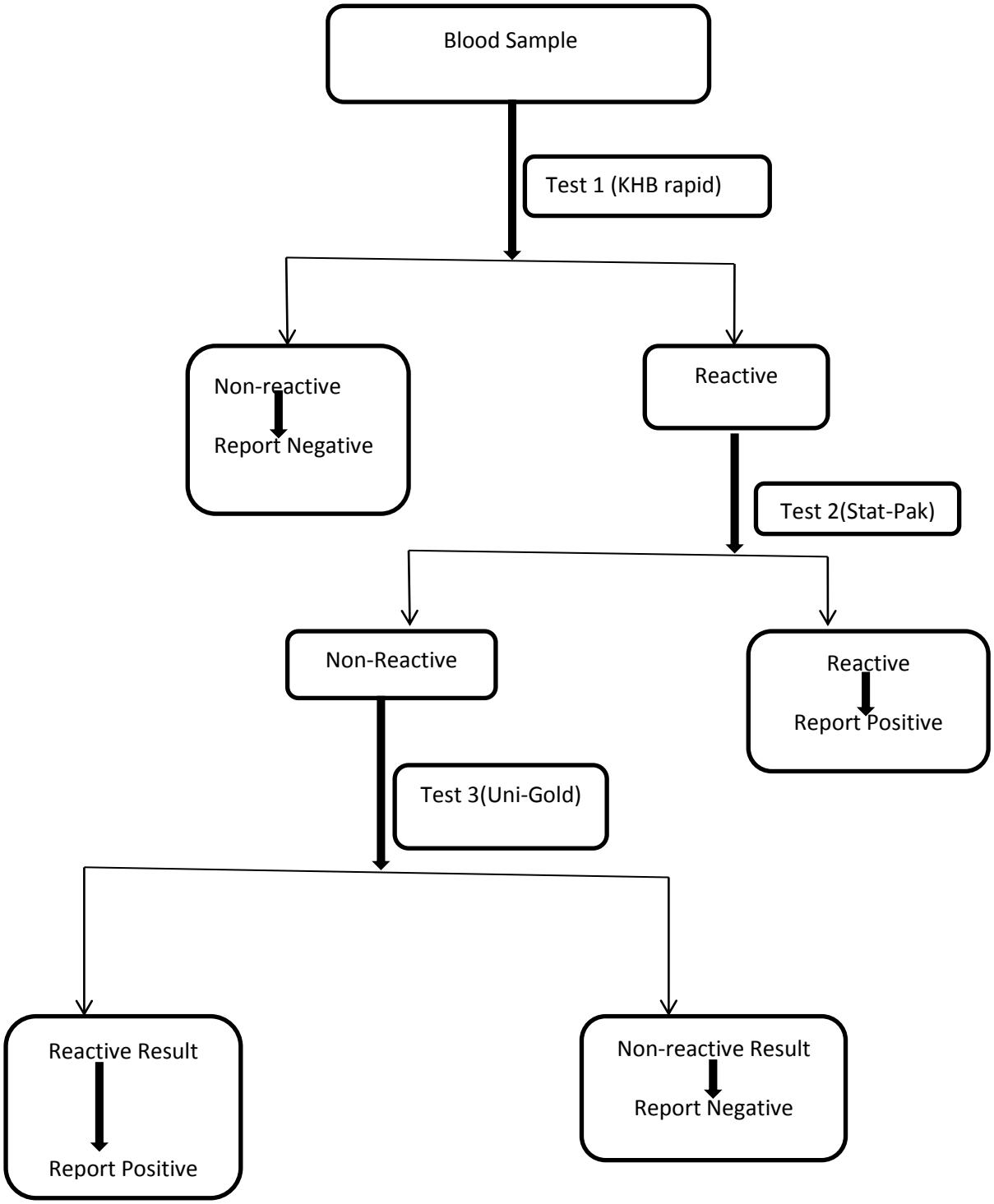
የዝህ ጥናት አላማ ነፃ የኤች ኣይቪ ቫይረስ ምርመራና የምክር ኣገልግሎት ተጠቃሚዎች ላይ ያለው የጨብጥ እና የሄፓታቴስ ብ ቫይረስ eርጭት እና መጠን እንድሁም መንስኤዎች ለማውቅ የሚደረግ ጥናት ነው፡፡ ከላይ የተጠቀሱት ተሃዋስያን ከኤች ኣይቪ የመተላለፊያ መንገድ ተመሳሳይ በመሆናቸው ኤች ኣይቪ ያለው ሰው ከላይ በተጠቀሱ ተሃዋስያን ልያዝ ስለመቸል እንድሁም በነዚህ በሽታዎች የተያዘ ሰው በኤች ኣይቪ የመያዝ እድል ልያባብሱ ስለምችሉ ቅድመ ህክምና ለማድረግ ነው፡፡ በዚህ ጥናት ውስጥ ምንም ኣይነት ጎጂ ድርጊት የለም፡፡ እንዲሁም ማንኛውም መረጃ በሚስጥር ይያዛል፡፡ ስለዚህ በዚህ ጥናት በመሳተፍ ኣስፈላጊውን ናሙና / 3-4ሚሊ የደም ናሙና/ የሚወሰድ በተጨመርም ለቃለ መጠይቅ እንድትተባበሩኝ እጠይቃለሁ፡፡

እኔ_____ከላይ የተጠቀሰውን ኣድምጩ ጥናቱ ጠቃሚ መሆኑን ስለተረዳሁ በጥናቱ በመሳተፍ የሚፈለግብኝን ለማድረግ ተስማምቻለሁ፡፡

ፍርማ .....

ቀን.....

APPENDIX IV NATIONAL HIV TESTING ALGORITHM



APPENDIX.V HIV Testing procedure

A. KHB

KHB (KHB, Shanghai Kehua Bio-engineering Co., Ltd. China) is used as screening purpose in Ethiopia as national HIV testing procedure.

Test Procedure

1. Collect the test items and other necessary laboratory supplies
2. Remove the KHB packaging and label it using a code number or a client identification number.
3. Collect the specimen using test tube.
4. Add a drop of blood enough to cover the sample port using capillary tube.
5. Add a drop of buffer to the sample port
6. Wait for 30 minutes the test to develop.

Result Interpretation:

After 30 minutes if both the control line and test line are seen, the result is considered to be reactive. If only the control line appears and no test line is seen, the result is considered to be non-reactive. If the control line is not seen, the test has not worked correctly and the result is considered to be invalid. This result will be repeated using a new KHB device. Those clients with KHB non- reactive result will be considered to be Negative and those with KHB reactive results are confirmed with STAT-PAK testing device (WHO, 2004).

B. STAT-PAK

Reactive samples with KHB test device were re-tested with STAT-PACK (Chembio HIV 1/2 STAT-PAK™ Assay, CHEMBIO DIAGNOSTIC SYSTEMS, INC., MEDFORD, NY, USA). This test kit is used as confirmatory test nationally. The procedure for this test is very similar to that used for KHB.

Test procedure

1. Remove the STAT-PAK packaging and label it using a code number or a client identification number.
2. Place some of the blood to microscopic slide.
3. Using the special applicator collect the blood from the microscopic slide and then transfer it to the sample port.

4. Now add three drops of the running buffer.
5. Wait for ten minutes for the test to develop.

Result interpretation

Result interpretation is the same as used for KHB device. If two lines of any intensity appear in both the control and test areas, it is reactive. If one line appears in the control area and no line in the test area, it is non-reactive. If no line appears in the control area, it is invalid test result and this test will be repeated with a new test device even if a line appears in the test area (WHO, 2005).

C. UNIGOLD

Samples giving discordant results in the two tests were re-examined using tie-breaker, (Uni-Gold HIV, Trinity Biotech PLC, Co. Wicklow, Ireland). The procedure for this test is very similar to that used for the KHB and STAT-PAK tests.

Procedure

1. Collect the test items and other necessary laboratory supplies
2. Remove the Unigold packaging and label it using a code number or a client identification number.
3. Take 60ul of blood from the test tube and then transfer to the sample port
4. Add two drops of running buffer to the sample port.
5. Wait for 10 minutes not longer than 20 minute to develop.

Interpretation of results:

This is very similar to the interpretation used for KHB and STAT-PAK. But, those samples reactive with Unigold are considered to be Positive and those samples with Non-reactive with Unigold results are considered to be negative.

APPENDIX VI HBs antigen testing procedure

Test procedure

The test procedure was followed according the manufacturer's protocol: -

1. With arrows pointing towards the serum specimen, each test strip was immersed vertically to each serum sample for 15 seconds. Care was taken not to pass the maximum line (MAX) on the test strip while immersing the strip
2. A time of 15 minutes was allowed until the red line (s) appears on the test strip
3. The results were interpreted within 15 minutes.

Interpretation of results

POSITIVE: - Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T). NOTE: The intensity of the red color in the test line region (T) will vary depending on the concentration of HBsAg present in the specimen. Therefore, any shade of red in the test region (T) should be considered positive. **NEGATIVE**: One red line appears on the control region (C). No apparent red or pink line appears in the test region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

This one step strip test kit has a relative sensitivity of > 99.0%, relative specificity 99.7% and an accuracy of 99.8%. However, the test limitation includes, the following: Neither the quantitative value nor the rate of HBsAg concentration can be determined by this qualitative test. A negative result at any time does not preclude the possibility of hepatitis B infection. ACON one- step test strip has a precision (replica of results) of 99% (ACON Lab. INC. IHBSG-u 301, USA, 2003).

APPENDIX VII Syphilis (RPR) testing procedure

Test procedure

1. Place 50 µl of serum on to an 18-mm circle of the RPR test card
2. Spread the serum to fill the entire circle.
3. Gently shake the antigen dispensing bottle to resuspend the particles.
4. Add exactly 1 free-falling drop (17 µl) of antigen suspension to each circle containing serum.
5. Place the card on the mechanical rotator under a humidifying cover. Rotate the card for 8 minutes at 200 rpm.
6. Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (three or four to-and-fro motions) to aid in differentiating nonreactive from minimally reactive results.

Result interpretation

Characteristic clumping ranging from marked and intense reactive to slight but definite (minimally to moderately) reactive or no clumping Non-reactive (Larsen *et al*., 1995).

APPENDIX. VIII DECLARATION

I, under signed, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Haftamu Weldesenbet Hadgu (B.Sc.)

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia

1. Supervisor: Daniel Asrat (MD, M.Sc., PhD)

   Signature: _____

   Date and place _____ _____

   Addis Ababa, Ethiopia

2. Supervisor: YimtubezinashW/Amanuel (MD, M.Sc., PhD)

   Signature: _____

   Date and place _____

   Addis Ababa, Ethiopia