Chapter One

1. Introduction

1.1. Introduction

Syphilis is a bacterial disease caused by the bacteria *Treponema pallidum* which enter the body through mucous membranes or abraded skin. Once get inside the body, the bacteria enter the blood stream and attaches to cells damaging organs over time. Syphilis is still a public health problem in the world. The world health organization (WHO) estimated that approximately 12 million new cases are reported each year in the world with more than 90% from developing countries (WHO, 2001 and CDC, 2007).

The infection is transmitted from person to person through contact with syphilis ulcer during sexual contact. Infected mothers can infect their fetuses via the placenta, furthermore intravenous drugs addicts or other infected blood or blood products. (Workowski and Berman, 2006).

Moreover, syphilis has acquired a higher potential of morbidity and mortality with the increasing prevalence of HIV infection. If syphilis is rare in developed countries, it is much more common in developing countries where prevalence can reach 25% amongst blood donor. (Tagny *et al.*,2009 and Tagny *et al*.,2010). Syphilis is believed to have infect 12 millions people in 1999, with greater than 90% of cases in the developing world. It affects between 700,000 and 1.6 million pregnant women a year, resulting in spontaneous abortions, stillbirths and congenital syphilis. InSub-SaharanAfrica, syphilis contributes to approximately 20% of prenatal deaths. Rates are proportionally higher among intravenous drug users, those who are infected with HIV and men who have sex with men. In the United States, rates of syphilis in 2007 were six times greater in men than women, while they were nearly equal in 1997.

African Americans accounted for almost half of all cases in 2010.
(Tagny *et al*. ,2010).

Blood transfusion is a life-saving intervention and millions of lives are saved each year globally through this procedure. However, blood transfusions are associated with certain risks which can lead to adverse consequences. It may cause acute or delayed complication and carries the risk of the transmission of infections. Unsafe blood remains a major threat for the global spread of transfusion transmissible infections (TTIs). According to WHO, safe blood is a universal right, which means that blood that will not cause any harm to the recipient and that has been fully screened and is not contaminated by any blood-home disease such as HIV, hepatitis, malaria, or syphilis(WHO, 2008).

1.2. Rationale:

Syphilis is a serious bacterial infection caused by *Treponema pallidum*. The diseases' transmitted from person to person by direct contact with syphilis sore during vagina, anal or oral sexual contact. Pregnant women with the disease can pass it to their unborne children. It can also be transmitted through blood transfusion and this will make it increasingly difficult to get safe blood because of this blood born infection. Syphilis has adverse complication, it can cause rash, mild fever, fatigue, headache, sore throat, patchy hair loss and swollen glands through the body. If left untreated syphilis can spread to all areas of the body and most likely will affect the heart, eyes, brain, nervous system, bones and joints. It can cause mental illness, blindness, neurologic problem, heart disease and this greatly increases the risk of miscarriage, stillbirth or newborn’s death within a few days after birth, Due to that the syphilis infection is problem in Sudan and World wide. This study expected to highlight the problem in order to reduce the risks of infection.

1.3. Objectives:

1.3.1. General objective:

To determine the frequency of syphilis among Ethiopian residents in Khartoum.

1.3.2. Specific objectives

1.3.2.1. To detect syphilis antibodies among Ethiopian residents in Khartoum using Immune Chromatography Test (ICT) and Enzyme linked Immune Sorbent Assay (ELISA) Technique.

1.3.2.2. To relate the prevalence of syphilis according to age group and sex.

1.3.2.3. To assess the prevalence of syphilis among HIV infected individuals.

Chapter Two

2. Literature Review

2.1. History of syphilis:

The exact origin of syphilis is unknown. Of two primary hypotheses were proposed, one proposed syphilis was carried to Europe by the returning crewmen from Christopher Columbus voyage to the Americans, the other proposed syphilis existed in Europe previously, but went unrecognized. These are referred to as the "Columbian" and" pre-Columbian"hypotheses, respectively. The Columbian hypothesis is best supported by the available evidence. The first written records of an outbreak of syphilis in Europe occurred in 1494/1495 in Naples, Italy, during a French invasion. Due to it is being spread by returning French troops, it was initially known as the "French disease", as it is still traditionally referred. In 1530,the name "syphilis" was first used by the Italian physician and poet Girolamo Fracastoro as the title of his Latin poem in dactylic hexameter describing the ravages of the disease in Italy. It was also known historically as the "Great pox" (Romanelli,2008).

The causative organism *Treponema pallidum*, was first identified by Fritz Schaudinn and Hoffmann in 1905.The first effective treatment (Salvarsan) was developed in 1910 by Paul Ehrlich, which was followed by trials of penicillin and confirmation of its effectiveness in 1943(Knell, 2004). Before the advent of effective treatment, mercury and isolation were commonly used, with treatments often worse than the disease. Many famous historical figures, including Franz Schubert, Arthur Schopenhauer, Edouard Manet and Adolf Hitler are believed to have had the disease (Farhiand Dubin, 2010).

2.*2. Treponema pallidum*:

2.2.1. Structure and metabolism:

T. pallidum is a Gram-negative bacteria consisting of an inner membrane, a thin

peptidoglycan cell wall and an outer membrane. It is very small in size with a length that ranges from 5 to 15 μm and a diameter range of 0.1-0.5 μm.

T. pallidum is a member of the spirochetes family which are characterized by their distinct helical shape. Probably the most interesting property of *T. pallidums* structure is the endo flagella found in the periplasmic space between its two membranes. These organelles give *T. pallidum* its distinctive corkscrew motility (Rebecca and Sheila, 2006).

T. pallidum is a chemoheterotroph which encodes few proteins; therefore, it has very limited metabolic capacity. *T. pallidum* is also microaerophilic, meaning that it requires a very low concentration of oxygen (Norris, 1993). It doesn't have the ability to code for many enzymes and as a consequence, it is unable to synthesize fatty acids, nucleotides, enzyme cofactors and most amino acids. although contains the enzymes necessary for glycolosis, it lacks those which are required for the citric acid cycle as well as those needed for oxidative phosphorylation; therefore it obtains all of its energy through glycolysis.

T. pallidum imports molecules via 18ATP-binding cassettes which are each specific for certain carbohydrates, amino acids or cofactors DNA replication, transcription, translation and repair mechanisms for *T. pallidum* are intact. Another consequence of *T. pallidum's* small coding capacity is that it must rely heavily on its host for nutrients making it extremely hard to culture in the lab (Radolf *et al*., 1999).

T. pallidum is an obligate internal parasite, meaning that it requires a mammalian host for survival. In the absence of mammalian cells, *T. pallidum* will be killed by the absence of nutrients, exposure to oxygen and heat (Rebecca and Sheila, 2006).

T. pallidum causes the human disease syphilis. Since *T. pallidum* cannot be grown in culture, animal models are needed to study syphilis. Although mice and monkeys can be used, rabbits are animal model almost exclusively studied

in the laboratories. Rabbits are used because unlike monkeys they are inexpensive and unlike mice, rabbits develop the signs and symptoms of human primary and secondary syphilis (Sell *et al*., 1980).

T. pallidum initially infects the epithelial cells of the genitals during sexual intercourse. From this initial infection site, *T. pallidum* goes on, to infect almost any organ or tissue in the body (Rebecca and Sheila, 2006).

A study done using rabbits detected the presence of *T. pallidum* in the "lymph nodes, brain and aqueous humor and in the CSF" after only 18 hours post infection (Collart *et al*., 1971). Another study showed that *T. pallidum* was then able to travel from the CSF to the eye (Marra *et al*., 1991). *T. pallidum* has also been found in the blood and liver of infected rabbits (Collart *et al*., 1971).

2.2.2. Genome structure:

The complete genome for *T. pallidum* was sequenced in July of 1998.The genome was sequenced using "the whole genome random sequencing method". The genome consists of a single double stranded circular DNA chromosome 1,138,006 base pairs long. It contains approximately 1,090 genes which encode approximately 1,041 proteins. These open reading frames account for 92.9% of the genomic DNA. 55% of genes were assigned defined roles and 17% were categorized based on similarities to other organisms. The main organism used for comparison was *Borrelia burgdorferi* which is another pathogenic spirochete which causes lyme disease. 28% of genes were considered novel (unique to *T. pallidum*) and placed in a separate category. The average size of encoded proteins was estimated to range from 3235 to172,869 daltons.

(Fraser *et al*., 1998).

2.3.Transmission:

Human is natural (Reservoir) host of *T. pallidum* and the infection can be transmitted from human to human through different routes.

2.3.1.Sexual Contact:

Syphilis is one of sexually transmitted infections (STIs). Approximately 90% of all syphilis is sexually transmitted. Exposure mainly occurs during oral, anal,

or vaginal intercourse. Transmission occurs through direct contact with infectious exudates from moist skin lesions or mucus membranes of infected persons during sexual contact (Heymann, 2004). Many lesions are unrecognized, and persons who are unaware of their infection may transmit the organism to other. Primary, secondary and early latent stages are considered infectious, with an estimated risk of transmission per partner of 60%. Early latent syphilis is considered infectious because of 25% chance of relapse to secondary stage (Public Health Agency of Canada, 2006).

The spirochete is able to pass through intact mucous membranes or compromised skin. Its infectivity is exemplified by the fact that an individual inoculated with only 57 organisms has a50% chance of being infected. Ulcerative STIs like syphilis promote HIV transmission and/or acquisition by augmenting HIV infectiousness and susceptibility. Syphilis increases the rate of HIV acquisition between two and four-fold and risk of transmission of HIV between two and nine-fold (Eccleston *et al*., 2008)

2.3.2. Vertical Transmission:

Pregnant women can transmit the infection transplacentally to the fetus at all stages during the course of untreated disease or during passage through the birth canal. The rate of vertical transmission is approximately 70-100% in untreated early syphilis (Tsui, 1997).Transmission is more likely with primary and secondary infections and less likely during latent infections. Breast feeding does not result in syphilis transmission unless an infectious lesion is present on the breast (Schulz, 1990).

2.3.3. Infected blood and blood products:

Syphilis is also a transfusion-transmitted infection (TTI). The bacteria is recipient. The transmissibility of syphilis by blood transfusion has been frequently reported, chiefly based on animal experiments. Cases of syphilis transmitted by blood have been described in literature, with more than a hundred cases since the first description. The main cases reported were shown to occur when donors were in the primary or secondary stage of the disease

(Gardella *et al*., 2002). *T. pallidum* may be found in the blood stream, but levels are variable, and bacteremia is often short-lived even in recent contamination. Moreover, the treponemes are relatively fragile and sensitive to cold; storage below 20^0C for more than 72 hours destroys the organism and reduces dramatically the infectious risk. Although clearly potentially infectious, the risk of transmission through the transfusion of blood and blood components stored below 20^0C is very low. Platelet concentrates usually stored at a temperature above 20^0C or blood directly transfused few hours after collection comprises a higher risk of transmitting syphilis. This is the case in many developing countries with limited blood supply where blood is collected from family donors and frequently transfused in the following hours or days. Thus, the screening test is considered essential as most blood transfusion services are concerned storage of blood products stored either above 20^0C or not stored below 20^0C for more than 4 days (Orton, 2001;Wendel, 1994).

Syphilis cannot be spread through contact with toilet seats, door knobs, swimming pools, hot tubs, bathtubs, shared clothing or eating utensils (Centers for Disease Control (CDC), 2006)

2.3.4. Period of communicability:

Syphilis is infectious during primary, secondary and early latent stages and also in mucocutaneous recurrences. Congenital transmission is most likely during primary and secondary maternal syphilis, but can occur in the latent period (Public Health Agency of Canada, 2006).

2.4. Risk Factors:

-Men who have sex with men

-Sex with multiple sex partners

-Drugs addict

-Sex workers

2.5. Signs and Symptoms:

The disease was referred to as “the great imitator “by Sir William Osler due to its varied presentations often been called “the great imitator “ and many signs

and symptoms are indistinguishable from those of other diseases. Many people infected with syphilis do not have any symptoms for years, yet remain at risk for late complications if they are not treated. Although transmission occurs from persons with sores who are in the primary or secondary stage, many of these sores are unrecognized. Thus, transmission may occur from persons who are unaware of their infection (Centers for Disease Control and Prevention (CDC), 2007). Syphilis can present in one of four different stages: primary, secondary, latent, and tertiary and may also occur congenitally (White, 2000).

2.6. Pathology:

2.6.1. Primary Syphilis:

Primary syphilis is typically acquired by direct sexual contact with the infectious lesions of another person usually presents itself as a single chancre at the site of infection (Committee on Infectious Diseases, 2006).

2.6.2. Secondary Syphilis:

Secondary syphilis occurs approximately four to ten weeks after primary infection. This stage characterized by the appearance of a red, maculopapular rash on almost any part of the body, including the palm of the hands and the soles of the feet. Also present as pale, moist flat seen primarily in the genital region, armpits and mouth**.** Bacteria are highly infectious. Other symptoms may include fever, sore throat, malaise, weight loss, hair loss and headache. Rare manifestations include hepatitis, kidney disease, arthritis, optic neuritis and interstitial keratitis. The acute symptoms usually resolve after three to six weeks, however, about 25% of people may present with a recurrence of secondary symptoms. Many people who present with secondary syphilis (40-85% of women, 20-65% of men) do not report previously having had the classic chancre of primary syphilis (Bhatti, 2007).

2.6.3. Latent Syphilis:

In this stage the lesions of secondary syphilis gradually resolve and period latent infection is entered; in which no clinical manifestations are evident, but serological evidence of infection persists. Relapse of the lesions of secondary

syphilis is common and latent syphilis is classified as early (high likelihood of relapse) or late (recurrence unlikely). In the United States latent syphilis is described as early (less than 1 year after secondary syphilis) or late (more than1 year after secondary syphilis). The United Kingdom uses a cut-off of two years for early and late latent syphilis. Individuals with late latent syphilis are not generally considered infectious, but may still transmit infection to the fetus during pregnancy and their blood may remain infectious (Edward *et al*., 2003).

2.6.4. Tertiary (late) Syphilis:

Tertiary syphilis is slowly progressive. Destructive inflammatory disease that may occur approximately three to 15 years after the initial infection and may be divided into three different forms: gummatous syphilis (15%), late neurosyphilis (6.5%) and cardiovascular syphilis (10%). Without treatment, a third of infection people develop tertiary disease. People with tertiary syphilis are not infectious (Romanelli, 2008).

Gummatous syphilis or late benign syphilis usually occurs 1 to 46 years after the initial infection, with an average of 15 years .This stages is characterized by the formation of chronic gummas, which are soft, tumor-like balls of inflammation which may vary considerably in size. They typically affect the skin, bone, and liver, but can occur anywhere. Neurosyphlilis refers to an infection involving the central nervous system. It may occur early, being either asymptomatic or in the form of syphilitic meningitis, or late as meningovascular syphilis, general paresis, or tabesdorsalis, which is associated with poor balance and lighting pains in the lower extremities. Late neurosyphilis typically occurs 4 to 25 years after the initial infection.

Meningiovascular syphilis typically presents with apathy and seizure, and general paresis with dementia and tabesdorsalis. Also, there may be Argy11 Robertson pupils, which are bilateral small pupils that constrict when the person focuses on near objects, but do not constrict when exposed to bright light (Romanelli, 2008).

Cardiovascular syphilis usually occurs 10-30years after the initial infection.

The most common complication is syphilitic aortitis, which may results from destruction of the elastic tissue of the aorta which leads to aneurysms that, rarely, rupture. The ascending aorta is most affected, with the potential complication of valve insufficiency and coronary artery stenosis. Approximately 11%of untreated patients progress to cardiovascular syphilis (Bhatti, 2007).

2.6.5. Syphilis in Pregnancy and Congenital Syphilis:

Syphilis can be transmitted transplacentally to the fetus at all stages during course of untreated maternal disease from incubating syphilis to primary, secondary, tertiary and latent disease. Syphilis can also be transmitted during passage through the birth canal when the newborn infant contacts a genital lesion (Tsui,1997*).* Breastfeeding does not result in transmission of syphilis unless an infection lesion is present on the breast. Pregnancy has no known effect on the clinical curse of syphilis. The rate of vertical transmission in untreated women is 70-100 % in primary syphilis, 40% for early latent syphilis and 10% for latent disease.

The longer the interval between infection and pregnancy, the more benign is outcome in the infant. All pregnant women should be screened for syphilis (with nontreponemal test) and other STIs(including HIV) on their first prenatal visit. High seroconversion rates for both syphilis and HIV in high risk populations during pregnancy has led some experts to suggest repeat screening of women during late pregnancy and delivery (Qolohle, 1995).

Syphilis in pregnancy can cause widespread complications for both the infected mother and fetus. At least two-thirds of babies born to untreated women with syphilis are infected. If evidence of syphilis is present, treatment should be initiated immediately according to the stage of the disease. Efficiency of syphilis treatment in pregnancy considers resolution of maternal infection and prevention of congenital syphilis. (Zenker and Rolfs,1989).

Clinical manifestations of congenital syphilis are divided into early (appear within the first two years of life) and late (after first two years of life) stages.

Late congenital syphilis usually manifests near puberty. Most clinical signs of early congenital syphilis develop within the first three months of life. Snuffles or persistent rhinitis is one of the earliest clinical manifestations occurring in 4-22% of infants. The nasal discharge may be profuse, purulent, or blood tinged and is highly infectious. Hepatomegaly with or without splenomegaly occurs in 33-100% of patients. Asymptomatic central nervous system involvement manifesting in CSF abnormalities of lymphocytosis, elevated protein levels, and positive serologic tests occur in up to 80% of infected infants. Symptomatic neurosyphilis develops rarely. Bone lesions develop within eight months of birth in early congenital syphilis. Late manifestations of congenital syphilis include Hutchinson's triad of interstitial keratitis, peg shaped upper incisors, and eighth cranial nerve deafness. The hearing loss can be sudden and usually occurs at 8 to10 years of age. The optimal treatment of an infant born with congenital syphilis is not known (Tsui, 1997).

2.6.6. HIV and Syphilis:

Coinfection is common as both syphilis and HIV are sexually transmitted infections. The two diseases can affect each other in several ways. As with other ulcer-causing infections, syphilis can enhance the-acquisition of HIV. Syphilis in the HIV- infected individual can be highly aggressive. Patients can progress from primary to tertiary syphilis over several years. As opposed to several decades in individuals not infected with HIV. Despite this progression,
The conventional staging of syphilis is similar with HIV coinfection. Although patients with syphilis and HIV conifection have shown no distinctive or unique features from those without concurrent HIV infection, they are at increased risk to manifest amore protracted and malignant course. This includes greater constitutional symptoms, greater organ involvement, atypical and florid skin rashes, multiple genital ulcers, develop symptomatic neurosyphilis, especially uveitis. Coinfected patients should be managed in consultation with an infectious disease specialist or physician knowledgeable in HIV/AID
(Tramont, 2005).

2.7. Diagnosis of Syphilis:

The inability to grow most pathogenic treponemes in vitro, coupled with transitory nature of many of the lesions, makes diagnosis of treponemal infection impossible by routine bacteriological methods. Although spirochetes are detectable by microscopy in primary and secondary lesions, diagnosis is based primarily on clinical observations and confirmed by serological tests (Egglestone and Turner, 2000).

2.7.1. Dark Field Microscopy and Direct or Indirect Fluorescent Antibody Test (DFA/IFA):

Dark field Microscopy and DFA/IFA testing of lesion exudates or tissues are the definitive methods for diagnosing early syphilis when an active chancre, mucouspatch, or condylomalatum is present. It is also useful for testing nasal discharge in a neonate with snuffles. The treponemes are recognized by their characteristic morphology and motility. Dark field microscope is often not practical (it is not available in most laboratories) as it requires a skilled technician on-site. In addition, specimens must be appropriately collected and quickly examined within 5-20 minutes of collection. Positive tests on these materials for immunofluorescent (DFA) testing are diagnostic (Fiumara,1995).

2.7.2. Histochemistry and Immunohistochemistry:

Treponemes can be observed in formalin-fixed and paraffin embedded biopsy material using silver impregnation methods such as the whar-tin-starry9 or the Steiner method. Immuno-logical recognition of *T. pallidum* in paraffin embedded tissues has been described (Ito *et al*., 1991).

2.7.3. Demonstration of Antibodies:

Serologic tests for syphilis are essential for diagnosis of individuals, for following the efficacy of therapy, and for screening purposes. They detect antibodies formed during the course of syphilitic infection. A presumptive diagnosis is possible with the use of two types of serologic tests for syphilis ;

nonspecific nontreponemal antibody tests (VDRL and RPR) and specific treponemal antibody tests (FTA-ABS and TP-PA).To establish a diagnosis of syphilis, both types of serologic tests are usually necessary. It should be emphasized that a serologic test results for syphilis on rare occasions may be negative in active cases, especially in older patients, or very early in primary infections. Routine screening of umbilical blood is not recommended for serological testing where a diagnosis of congenital syphilis is considered .Testing of maternal serum is preferred to testing infant serum because infant serum can be nonreactive if maternal serology is low titer or the infection was late in pregnancy. Cord blood that is contaminated with maternal blood may lead to a false positive test result (Larsen *et al*., 1998).

2.7.3.1. Nontreponemal Tests (VDRL and RPR):

Syphilitic infection leads to the production antibodies (1gM and 1Gg) directed against a lipoid antigen resulting from the interaction of host tissues with *T. pallidum* or from *T. pallidum* itself. This antibody –antigen reaction is the basis of nontreponemal tests such as the Venereal Disease Research laboratory slide test (VDRL) and the Rapid Plasma Regain test (RPR).The RPR test is more sensitive than the VDRL (Larsen *et al*., 1998).

After adequate treguate treatment of syphilis, notreponemal test (NTT) eventually become nonreactive. However, even with sufficient treatment. Patients sometimes have a persistent low-level positive nontreponemal test referred to as aserofast conversion. Nontreponemal test titers of persons who have been treated for latent or late stages of syphilis or who have become reinfected do not decrease as rapidly as a do those of persons in the early stages of their first infection. In fact these persons may remain serofast for life (Larsen *et al* ., 1998) .

VDRL and RPR become positive one to four weeks after the appearance of the primary chancre or six weeks after exposure. Biologic false positive reactions occur at a rate of 1-2% in the general population and have different causes (Hepatitis, Measles, Malaria, Immunization,…etc). Acute false positive tests

lasting less than six months can occur following a febrile illness or immunization. As a rule, 90% of false positive titers are less then 1:8, but low titers are also seen in latent infection. False positive rates in pregnancy are similar to the general population. More than 10% of Intravenus Drug User (IDU) may have false positive result. HIV infection has not been associated with increased false positive NTT in individuals at low risk of IDU, serial nontreponemal tests are useful to determine the stage of the diseases, a four-fold rise in titer may indicate recent infection, reinfection in an adequately treated person, or relapse in an inadequately treated person. Adequate treatment of infectious syphilis is indicated by a four –fold or greater or greater decline in titer within one year. Titers should generally become non-reactive or weakly reactive within one year following primary syphilis and within two years after treatment for secondary syphilis. Treatment of late latent or late syphilis usually has little or no effect on the titer and should not be used togauge the adequacy of the treatment. Titers tend to become lower with time, but serum frequently remains reactive, usually in low titer. As with all quantitative serologic tests, only a four-fold or greater change in titer is meaningful (Larsen *et al*., 1998).

2.7.3.2. Specific Treponemal Tests:

These tests measure antibodies against specific *T. pallidum* antigens and are used primarily to confirm the diagnosis of syphilis in patient with a reactive nontreponemal test. The principal specific treponemal antibody tests most laboratories are the *T. pallidum* particle agglutination tests (TP-PA) and fluorescent treponemal antibody-absorption test (FTA-ABS). Most patients who have reactive treponemal tests will have reactive tests for the remainder of their lives. Regardless of treatment or disease activity, however, reversion to a nonreactive status may occur in up to 10% of patients, especially in those who are treated early. Trepnemal test antibody titers correlate poorly with disease activity and should not be used to assess treatment response. False positive result can occur especially when the FTA-ABS test is used in patients with lyme disease, HIV, pregnancy, drug addiction, toxoplasmosis, *H. pylori*,

autoimmune disorders like lupus and rheumatoid arthritis, and persons with other treponemal diseases such as yaws, pinta or bejel (Tramont, 2005).

2.7.4. Rabbit Infectivity Test (RIT):

RIT is probably the most sensitive methods for detecting infectious treponeme. Any source of specimen can be used for RIT as long as the materials is less than 1 hour old or was flash-frozen immediately after collection and maintained in liquid nitrogen or at temperature of -78C or bellow. The RIT remains a research tool of academic interest for detection of virulent organisms in clinical specimens because of the need for animal, the very long incubation time after infection (several weeks to month), and variation in rabbits susceptibility to infection (Grimprel *et al*., 1991).

This technique was also used as a gold standard for measuring the sensitivity of method such as PCR. The RIT, using susceptible rabbit, has a sensitivity of 10 to 50 organisms, similar to that of DNA PCR (Grimprel *et al*., 1991).

2.7.5. Test For Congenital Syphilis:

Venous samples should be obtained from both mother and baby for serology (treponemal and nontreponemal tests). Cord blood is not suitable for testing. The interpretation of reactive antibodies in neonate must take into consideration the maternal history, including stage of syphilis, history of treatment and syphilis serology results. Placenta, neonatal nasal discharge, or skin lesions may be examined by darkfield microscopy or DFA\IFA for *T. pallidum*. CSF examination should be performed on all infants with suspected congenital syphilis. Long bone x-rays should also be performed. (Egglestone and Turner, 2000).

2.7.6. Application of Enzyme Linked Immunosorbent Assays:

The enzyme linked immunosorbent assay (ELISA) are widely used in the field of infectious diseases owing to the possibility of automation and the broad potential to detect antibodies, antigens and haptens. After the first use of the indirect ELISA in the serodiagnosis of syphilis, several related tests in which detergent extracted or sonicated *T. pallidum* (Nichols) were used as antigen

have been reported. Indirect ELISA in which components from non pathogenic treponemes or modified VDRL antigen where used as an antigen have been developed. Most ELISA designed to detect antibodies against treponemal antigens perform well when used for screening. Theoretically, ELISA that detect both IgG and IgM should be more sensitive in early disease than those that detect only IgG. There appears to be a benefit of having multiple recombinant antigens rather than antigens obtained from whole cell lyses. Currently available ELISAs are based on the use of recombinant antigens to avoid cross reactivity with antigens from other treponemal species.
(Sambri *et al*., 2001).

2.7.7. Polymerase Chain Reaction (PCR):

A number of PCR based methods have been developed for the detection of *T. pallidum* DNA or RNA in clinical specimens. These assays are based on the detection of various target genes like tmpA (a 45KDa membrane protein Hay, 1990a); bmp, 39-KDa basic membrane protein (Noordhoek *et al*., 1991); tpp47, a 47KDa membrane immunogen (Burstain *et al*.,1991).

The first application of PCR in clinical samples from patient with syphilis was reported by (Hay *et al*., 1990).They used primers derived from the gene sequence of TmpA and the 4D antigen (oligomeric protein with multiple forms). The detection limit was 65 organisms. When PCR was used to detect *T. pallidum* DNA in cerebrospinal fluid (CSF) of patients with and without syphilis, sensitivity was 47% and specificity 93% (Hay *et al*., 1990).

PCR was applied to detect *T. pallidum* DNA in CSF form neurosyphilis patients before and after antibiotic treatment. Prior to treatment, PCR was positive in 71% (5/7) patient with acute symptomatic and 12% (2/16) asymptomatic neurosyphilis. However, they found treponemal DNA in CSF up to 3 years after treatment (Noordhoek *et al*., 1991).

The ttp47gene PCR to detect *T. pallidum* DNA in clinical samples was developed by (Burstain *et al*., 1991). A 658-bp (648-1305) portion of tpp47 gene was amplified and the PCR products were probed by DNA-DNA

hybridization with a 496-bp (713-1208) fragment internal to the amplified DNA. The assay detected approximately 0.01pg of purified *T. pallidum* DNA, and it was able to detect as low as one to 10 organisms per specimen with high sensitivity.

T. palldium DNA was detected in serum, CSF and amniotic fluids from syphilis patients but not in non syphilitic controls (Buristain *et al*., 1991).

The same technique was applied in several clinical materials in order to investigate the potential of PCR in diagnosis of congenital syphilis. The PCR was 100% specific for *T. pallidum* compared with the sensitive rabbit infectivity test (RIT) for all clinical materials tested. Sensitivities for amniotic fluids, CSF and serum were 100%, 60% and 67% respectively.

(Grimprel *et al*., 1991).

Later several methods of PCR were developed with different target of genes and had different sensitivities when they were applied in several clinical materials in order to investigate the potential of PCR in diagnosis of various stages of syphilis.

2.8. Control :

Management of cases:

Close collaboration between Public Health and Primary Care in addition to timely completion of STD forms are crucial to ensure there is sufficient in formation to identify and locate sexual contacts in a timely manner. Seroreactive persons should be expeditiously evaluated, this evaluation should include a history and physical examination, laboratory testing, risk assessment and promotion of safer sex practices.

All persons with syphilis should be counseled concerning the risks of the infection and other STIs and testing for this infection should be offered.

Cases with infectious syphilis (primary, secondary, and early latent) should interviewed for sexual contacts.

The following principles of case management should be applied:

Treat all cases of infectious syphilis immediately.

Interview cases with in one working day whenever possible.

Evaluate cases with in one week after treatment to document clinical.

(Garnett *et al*., 1997).

2.9.Treatment:

Penicillin G, administed parenterally is the preferred drug for treatment all stages of syphilis.

Injectable benzathine penicillin G (Biclliin L-A) is available for the treat incubating, primary, secondary, latent, and tertiary syphilis.

(Katsambas *et al*.,1987).

Chapter Three

3. Materials and Methods

3.1. Study design:

3.1.1. Study type:

The presented work was cross- sectional study.

3.1.2. Study area:

This study was carried out in Khartoum State. blood sample was collected from Ethiopian patients attended Khartoum Teaching Hospital, Bahrey Teaching Hospital and Omdurman Teaching Hospital.

3.1.3. Study duration:

The study was conducted from April to November 2014.

3.1.4. Study population:

3.1.4.1. Inclusion criteria:

Individuals who were included in the study were Ethiopians, male and female aged between 15 and 45 years and some of them infected with HIV infection.

3.1.4.2. Exclusion criteria:

Patients with current history of medication and those with a history of operational serious illness, jaundice, blood transfusion radiotherapy or any form of cancer therapy were excluded.

3.2. Data Collection:

After explaining the purpose of the study, data were collected from each subject by interviewing questionnaire (appendix). The data included the demographic information (age and residence), history of previous infection with HIV and genital ulcer.

3.3. Sample size:

One hundred blood samples were collected.

3.4. Specimen Collection and Preparation:

Using sterile disposable syringes, about 5 ml of blood were drawn from the

anticubital vein under aseptic condition. The blood samples were collected in sterile containers without any additives, and left to clot at room temperature for 15 minutes. Each blood sample was, then, centrifuged at 1500 rpm for 5 minutes, and each serum was separated in another sterile plain container. Samples were labeled by giving laboratory numbers. Serum samples were kept frozen at -20^0C without addition of preservatives, until the time of analysis.

3.5. Laboratory Examination:

All the specimens were tested for syphilis using two screening serological tests, Rapid Syphilis Test (RST), using ICT and enzyme linked immunosorbent assay (ELISA).

3.5.1 .Immuno Chromatography Test (ICT):

The sample was considered positive if it showed two red lines in the test strip, and negative if it showed one red line, similar to control.

3.5.1.1. Determination of ICT (Strip method):

The SD BOILING syphilis 3.0 test is a solid phase immune chromatographic assay for qualitative detection of antibodies of tall isotypes (IgG, IgM, and IgA) against *Treponema pallidum* (TP).

3.5.1.2. Principle :

The SD BOILING syphilis 3.0 contain a memberance strip, which is precoated with recombinant *Treponema pallidum* antigen (17,15KDa) on test band region. The recombinant *Treponema pallidum* antigens colloid gold conjugate (17,15KDa), patient and sample diluents move along the memberane chromatographically to the test region (T) and from the visible line as antigen - antibody - antigen cold particle complex forms.

The formation of visible line in the test region indicates a positive result for detection of specific treponemal antibodies (IgG, IgM, IgA). When the *Treponema pallidum* specific antibodies (IgG, IgM, IgA) are absent in the samples, no visible color band in the test band region will be formed.

3.5.1.3. procedure:

1. The test device was removed from foil pouch and was placed on a flat, dry surface .

2. Then20µl of blood specimen was drawn with 20µl capillary peppite place into the sample well (s).

3. Four drops (about 120µl) of assay diluents were added into the sample well (s).

4. As the test begins to work, purple color was moved across the result window in the center of the test device.

5. Test result was interpreted in 5-20 minutes

3.5.2. Enzyme linked Immunosorbent Assay ELISA:

3.5.2.1. Determination of ELISA:

The reagents were supplied by Biorex, United Kingdom, ELISA gen, fourth generation ELISA for in vitro determination of specific antibodies to syphilis in human plasma and serum.

3.5.2.2. Principle of the test:

Syphilis EIAII96 test kits use three recombinant antigens in a sandiwish test to produce a test. The antigen will detect *T. pallidum*-specific IgG, IgM, and IgA; enabling the test to detect antibodies during all stages of infection. All reagents except the wash solution are supplied ready to use and colour-coded, and the procedure uses undiluted samples and standard volumes for ease of both manual and automated use. The assay can be used with both serum and plasma. The plastic wells are coated with a mixture of the 15Kd, 17Kd, and 47Kd recombinant antigens of *T. pallidum*. Specific antibodies in the serum or plasma specimens combine with these antigens and with the same antigens conjugated to horsredish peroxidase, when conjugate is added to well in which the specimen has been incubated. After unreacted materials have been removed by washing, the presence of bound enzyme indicating the presence in the specimens of specific antibodies is revealed by a colour change in the substrate/chromogen mixture. The intensity of the colour is compared to that in

control wells to determine the presence or absence of specific antibody.

3.5.2.3.Procedure:

3.5.2.3.1. Control :

Three negative controls and two positive controls were included with each batch of samples to be tested. The controls were treated as patient samples.

3.5.2.3.2. Conjugate and samples incubation:

Fifty microliter of undiluted sample, negative control (three) and positive control (two) were added to each well. Then 50 µl of conjugate was added to each well. The conjugate was supplied at working strength. Then mixed on a plate shaker for 30 seconds, and the plate was covered and then incubated at 37^0C for 30 minutes.

3.5.2.3.3. Wash:

The plate was washed5 times with working strength wash buffer, it was soaked for about 30 seconds between each wash cycle, and excess liquid was tapped out.

3.5.2.3.4. Substrate incubation:

Fifty microliter of the substrate/chromogen was added to each well and incubated away from light at room temperature (RT) for 30min. In the presence of positive sample, the colour turns blue.

3.5.2.3.5. Stop colour development:

Fifty microliter of stop solution of (H_2SO_4-0.5M) was added to each well, blue colour will change to yellow.

3.5.2.3.6. Reading:

Carefully, the plate bottom was wiped and the OD was read at 450/(620-690nm) using a plate reader within 30minutes of stopping the reaction.

3.5.2.4 . Calculation and interpretation of results:

3.5.2.4.1. Assay validation:

Each negative control OD should be lower or equal to 0.080,

and each positive control OD should be greater than or equal to 1.000 according to the manufacture.

3.5.2.4.2 . Cut-off-value:

Calculated as the mean of the negative control values plus 0.100

i.e. NC + 0.100

3.5.2.4.3 . Interpretation:

Samples with OD less than the cut-off value were considered negative by syphilis EIA. Results just below the cut-off value (C.O-10% <OD<C.O) were interpretated with a caution.

Samples with OD greater than or equal to the cut-off value are considered positive by syphilis EIAII.

Chapter Four

4. Results

4.1. Results:

The individuals in this study were males and females with an age ranging from 15-45 years most of them between 15-25 years (Table 1).

Table1: Distribution of population according to the age group (n=100)

Age	Frequency	Percent
15-25	**48**	**48%**
26-35	**45**	**45%**
36-45	**7**	**7%**
Total	**100**	**100%**

Eight (8%) of them were syphilis seropositive by the two methods (Table2).**Table 2: Total syphilis positive cases tested by ICT and ELISA (n=100)**

Results	ICT	ELISA	Percent
Positive	**8**	**8**	**8%**
Negative	**92**	**92**	**92%**
Total	**100**	**100**	**100**

The seroprevelence differed with age and there was higher prevalence observe among age group of 26-35 years (Table3).

Table 3: Syphilis positive cases according to age group (n=100)

Age	Positive Frequency	Percent
15-25	**3**	**37.5%**
26-35	**4**	**50%**
36-45	**1**	**12.5%**
Total	**8**	**100**

Five (62.5%) of them were males and three (37.5%) were females (Table4).

Table 4: Syphilis positive cases according to sex (n=100)

Sex	Positive Frequency	Percent
Female	**3**	**37.5%**
Male	**5**	**62.5%**
Total	**8**	**100**

The cases with HIV were six (75%) and without HIV were two (25%) (Table5).

Table 5: Related risk factor (HIV infection) among syphilis positive cases (n=100)

Subject	Positive	Percent
With HIV	**6**	**75%**
Without HIV	**2**	**25%**
Total	**8**	**100%**

Chapter Five

5. Discussion

5.1. Discussion:

The seroprevalence of syphilis obtained in this study among Ethiopian residents in Khartoum was (8%) this result is considered slightly different in comparison to the result reported for seroprevalence of HIV, hepatitis B infections and syphilis among street dwellers in Gondar city, northwest Ethiopia (10.9%) (Moges *et al.,* 2004). However, it is lower when compared with a (28.9%) prevalence among factory workers in Addis Ababa in Ethiopia (Sahlu *et al*., 1999).

The proportion of males who were found to be seropositive for syphilis (62.5%) is higher than females (37.5%) this is in line with other report indicating a higher prevalence of syphilis in males than females. Reported seroprevalence of syphilis among HIV infected individuals in Addis Ababa in Ethiopia, (11%) in males and (8 %) in females (Begna *et al* ., 2013).

In this study the prevalence of syphilis among HIV positive was (75%),this finding appear to be higher than syphilis- HIV coinfection among street dwellers (7.6%) (Moges *et al*., 2004) and the HIV infected individual in Addis Ababa (9.8%) (Begna *et al* ., 2013).

The seroprevalence of syphilis obtained in this study lower when to compared to study conducted by Atif and Magzoub (2010) among Sudanese blood donors in South Western Sudan (23.5%) and with other study reported by Elfaki (2008) in Elobied in West Sudan (15%).

5.2. Conclusion:

The results of this study concluded that:

. The seroprevalence of syphilis obtained in this study among Ethiopian residents in Khartoum was 8%.

. The high prevalence of syphilis among people with HIV infection highlighted the need to target this population to prevent the transmission of both infections.

. There is direct relationship between the age of patient and susceptibility to the disease; the younger of the patients have high susceptibility to the disease.

. Both genders are susceptible to infection with almost the same rate.

5.3. Recommendations:

. Efforts should be made to reduce illegal immigration in order to reduce the risks of syphilis infection.

. Screening all HIV infected people for syphilis and managing those infected would have clinical and epidemiological importance.

. Provision of many centers for voluntary screening for sexual transmitted diseases to detect sexually transmitted diseases before marriage for both male and female.

. All finding cases should be treated .

REFERENCES

1. **Atif A. Elagib and Magzoub A. Abdelmagid (2010).** Seroprevalence of Syphilis among Blood Donors in South Western Sudan. Tropical Medicine Research Institute.
2. **Begna T., Zufan S., Addisu A. and Techalew S. (2013).** Seroprevalence of syphilis among HIV- infected individuals in Addis Ababa, BMJ open 2013, 3:eoo2293. dio:10.1136/ bm jopen-2013- 002566.
3. **Bhatti M. T. (2007)** Optic neuropathy from viruses and spirochetes. *Int Ophthalmol Clin* **47**(4):37-66.
4. **Burstain JM., Grimprel E., Lukehart SA, Norgard MV and Radolf JD (1991).** Sensitive detection of *Treponema pallidum* by using the polymerase chain reaction. *J Clin Microdiol* **29**: 62-9.
5. **Center for Disease Control (CDC) (2004).**STD facts syphilis. Centers for Disease Control. Retrieved on 2007-5-30.
6. **Centers for Disease Control (CDC) (2006).** Sexually transmitted Diseases Treatment Guidelines: ***MMWR*** **55**(RR-11): 24-32.
7. **Centers for Disease Control and prevention (2007).** Syphilis Fact Sheet.
8. **Collart P., Franceschini P. and Durel P. (1971).** Experimental rabbit syphilis *Br Jvener Dis*. **47**(6): 389-400.
9. **Committee on Infectious Disease (2006).** Larry K. Pickering. ed. Red book 2006 Report of the Committee on Infectious Disease (27^{th} ed.) PP. 631-44.
10. **Eccleston K., Collin L. and Higgins S. (2008).** Primary Syphilis. *International Journal of STD and ADIS* **19**(3): 145-57.
11. **Edwards, AM., Dymock, D. and Jenkinson HF (2003).** from tooth to hoof: treponemes in tissue-destructive diseases. *J. of Appli. Microb*. **94**:767-780.
12. **Egglestone SI and Turner AJ (2000).** Serological Diagnosis of syphilis: PHLS Syphilis Serology Working Group. *Communicable Disease and Publi Health* **3**: 158-162.

13. **Elfaki A.M, Eldour A.A and Elsheikh N.M (2008).** Seroprevalence of Immunodeficiency Virus, hepatitis B and C and Syphilis among blood donors at Elobied Teaching Hospital, West Sudan. *Sudan Journal of Medical Sciences* **3** (4): 333-338.
14. **Farhi, D. and Dupin, N (2010).** Origin of syphilis and management in the immuncompetent patient: Facts and controversies: *Clinics in Dermatology* **28** (5): 533-8.
15. **Fiumara, NJ., (1995).** The Diagnosis and Treatment of Infection Syphilis .*Comprehensive Therapy* **21**(11) :639-644.
16. **- Fraser CM, Norris SJ, Wein Stock GM and white O. (1998).** Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* **281**: 375-388.
17. **Gardella, C., Marfin, A.A., Kahn, RH, Swint, E. and Markowitz, LE. (2002).** Persons with early syphilis identified through blood or plasma donation screening in the United States. *j. Infect. Dis*.**185:** 545-549.
18. **Garnett GP., Aral SO., Hoyle DV., Cates W. Jr, Anderson R.M (1997).** The Natural History of Syphilis. Implication for Transmission Dynamics and Control of Infection. *Sexually Transmitted Disease*; **24**: 185-200.
19. **Grimprel E, Sanchez J and Wendel GD (1991).** Use of polymerase chain reaction and rabbit infectivity testing to detect *Treponema pallidum* in amniotic fluid, fetal and neonatal sera and cerebrospinal fluid. *J. Clin Micr biol* **29**: 1711-8.
20. **Hay PE, Clarke JR, Taylor – Robinson D and Goldmeier D. (1990).** Detection of treponemal DNA in the CSF of patients with syphilis and HIV infection using polymerase chain reaction. *Genitourin Med*. **66**: 428-32.
21. **Heymann DL. (2004).** Syphilis in Control of Communicable Diseases Manual 18th ed. American Public Health Association, Washington, 518-524.

22. **Ito F, Hunter E, George RW, Swisher BI and Larsen SA. (1991).** Specific Immunofluorescence Staining of *Treponema pallidum* in smears and tissue. *J. Clin Microbiol*.**29**: 444-8.
23. **Kastambas A., Adonious C., Katsarou A., Kerkidou A. and Stratigos J. (1987).** Comparative Study of Ceftriaxone and Benzathine Penicillin G in Treatment of Primary and Secondary Syphilis. *Chemiotherapia* **6**:549-50.
24. **Knell, RJ (2004).** Syphilis in renaissance Europe: rapid evolution of an introduced sexually transmitted disease (http:// rsb. Royal Society Publishing. Org). Biological Sciences /The Royal Society **271 supp 14.**
25. **Larsen SA., Hunter EF and Creighton ET., (1990).** Syphilis In: *Sexually Transmitted Diseases*, 2nded. New York: Mc Graw, Hill .924-34.
26. **Larsen SA., Pope V, Johnson RE and Kennedy EJ (1998).** A Manual of Tests for syphilis 9th edition American public Health Association Washington.
27. **Marra C., Baker S.A, Hook EW. and Lukehart SA (1991).** An experimental model of early central nervous system syphilis. *J. Infect. Dis*.**163**:825-829.
28. **Moges F., Kebede Y., Kassu A., Mulu A., Tiruneh M. and Degu G. (2004).** Seroprevalence of HIV, hepatitis B infections and syphilis among street dwellers in Gondar city, Northwest Ethiopia. *J. Health Dev*. 20(**3**):160-165.
29. **Noordhoek GT, Wieles B, Sluis JJ and Embden JD (1991).** Detection of Treponemal DNA in the CSF of patient with syphilis and HIV infection using the polymerase chain reaction and synthetic DNA probes. *Infect. Immu* ;**58**:2011-3.
30. **Norris (1993).** Polypeptides of *Treponema pallidum*: Progress toward understanding their structural, functional and immunologic roles. *Treponema Pallidum* Polypeptide Research Group. *Microbiol Rev.***57**: 750-779.

31. **Orton, S. (2001).** Syphilis and blood donors: What we know, what we do not know, and what we need to know. *Trans fus Med Rev.* **15**: 282-291.
32. **Public Health Agency of Canada's Sexual Health and Sexually Transmitted Infection (2006).** Edition available at: WWW. Phac-aspc,gc,ca/std-mts/sti-2006/pdf/sti.pdf.
33. **Qolohle DC. (1995).** Serological Screening for Sexually Transmitted Infections in Pregnancy: Is There Any Value in Rescreening for HIV and syphilis at the Tim of Delivery? *Genitourinary Medicine* **71**:65-67.
34. **Radolf, J., Steiner, B. and Shevchenko, D. (1999).** *Treponema pallidum* : Doing a remarkable job with what it's got. *Trends in Microbiology,***7**: 135-40.
35. **Rebecca E. Lafond and Sheila A. Lukehart (2006).** Biological Basis for Syphilis. Departments of Pathobiology, Medicine, University Basis Washington, Seattle. *American society for Microbiology* **19**:232-245.
36. **Romanelli F (2008).** Reexamining Syphilis: an update on epidemiology Clinical Manifestation and Management. *Ann Pharmacother* **42:** 124-128.
37. **Sahlu T., Rinkede Wit, Tsegaye A., Mekonnen Y., Beyene A., Hailu B.,Coutinho A. and Fontanet A. (1999).** Syphilis among factory workers in Ethiopia, *Sex Transm Infect* ,**78**:123-126.
38. **Sambri, V., Marangoni, A., Simone, M.A., D'Antuono, A., Negoanti, M.,& Cevenin, R., (2001).** Evaluation of recom Well Treponema, a novel Recombinant antigen-based enzyme-linked immunosorbent assay for the diagnosis of syphilis. *Clin Miicrobiol Infect,* 7:200-205.
39. **Schulz KF. (1990).**Congenital syphilis in: Sexually Transmitted Diseases: 2nd ed. Mc Graw-HILLInc New York, 821-42.
40. **Sell S, Gamboa D, Baker-zander SA, Lukehart SA and Miller JN (1980).** Host response to *Treponema pallidum* in intradermally- Infected rabbits. *J. Invest Dermatol*.**75**:470-5.

41. **Tagny, CT., Diarra, A., Yahaya, R., Hakizimana, M., Ngussanm A., Mbensa, G., *et al.*,(2009).** Characteristics of blood donors and donated blood in Sub- Saharan Francophone Africa. *Transfusion*, **49 :**1592-9.
42. **Tagny, CT., Owusu-Ofori, S., Mbanya, D. and Deneys, V. (2010).** The blood donors in Sub –Saharan Africa: a review. *Transf . Med*. **20**:1-10.
43. **Tramont EC. (2005).** *Treponema pallidum* (syphilis). In: principles and practice of Infectious Diseases. 6th ed. Elsevier Churchill Livingston, Philadelphia 276-2785.
44. **Tsui, AO. (1997).** Reproductive Health in Developing Countries. National Academy Press , Washington.
45. **Wendel, S. (1994).** Current concepts on transmission of bacteria and parasites by blood components, *Vox Sang.* **67**: 161-174.
46. **White, RM (2000).** Unravelling the Tuskegee Study of untreated syphilis. *Archives of Internal Medicine***160**(5): 585-98.
47. **WHO Guidelines of Blood Transfusion safety (2008).** Appia, CH-1211 Geneva **27**, Switzerland.
48. **Workowski, K.A and Berman, S.M. (2006).** Sexually transmitted diseases treatment guidelines. *MMWR Recommed .Rep.***55**:1-94.
49. **World Health Organization (2001).** Global prevalence and incidence of selected curable sexually transmitted infections: Overview and estimates. World Health Organization Geneva, Switzerland.
50. **Zenker PN and Rolfs RT (1989).** Treatment of Syphilis. *Review of Infectious Diseases***12**: s590-s605.

Appendix (1)

1.1.Equipments:

Centrifuge	hettich- Germany
Automatic micropipette	axiom- German
Alarm clock	quartz- Japan
Microwell reader (450nm)	stat-fax-USA
Microwell washes system	stat-fax- USA
Automatic pipette (multichannel)	axiom-Germany
Dry incubator 37^0C	torrpicenardi-Italy

Appendix (2)

Reagents:

Deionized or distilled water

Alcohol

ELISA kit atlas medical- Cambridge-(UK)

Concentrated washing solution (20x)

Negative control (human) yellow

Positive control (human) red

Conjugate ready-to-use blue rAg conjugated to Horseradish peroxidase

Substrate pink

Stop solution H_2SO_4-0.5M

Appendix (3)

Other materials:

VDRL ICT	SD- Germany
Cotton	medical- sanitary
Disposable pipette tips	China
Vacuum tubes	China
Syringes	tuttlingen- Germany
Alcohol swab	Saudi Saches-Services-KSA-Saudi
Plan containers	China
Disposable gloves	India
Absorbent tissue	Sudan

Microplate: 12stripsof 8 wells coated with *T. pallidum* rAg

Erlenmeyer flasks: 100 ml, 400ml, 1 L and 2L

Automatic microtiter plate washer or squirt bottle

Bag for storing unused wells

Test tubes and racks

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Name: **Serial No:**

Age:

Sex:

Male….. **Female….**

Residence:

Occupation:

Previous Infection (s) or Disease (s):

Treatment(s):

Yes…. **No….**

Type of Treatment (s):