Comparison between Enzyme Linked Immunosorbent Assay (ELISA) and Immunochromatographic test (ICT) Methods for the Screening of Syphilis among Blood Donors

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B.Sc. (Hons.) in Medical Laboratory Sciences. Faculty of Medical Laboratory Sciences, University of Gezira, 2008

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Comparison between ELISA and ICT Methods for the Screening of Syphilis among Blood Donors

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Date of Examination: 16, March, 2016
Dedication

To My mother

My father

My Husband

My brothers

My Daughter

My son

And my colleagues

I dedicate this Study with my best wishes to all.
Acknowledgements

I would like to express my deep sincere gratitude and honest appreciation to my supervisor Dr. Mai Abdalla Ali, for her efforts on supervision and guidance, encouragement and support throughout this work in completing this study. Her ultimate help, and valuable comments and suggestions are highly appreciated.

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Comparison between Enzyme Linked Immunosorbent Assay (ELISA) and Immunochromatographic test (ICT) Methods for the Screening of Syphilis among Blood Donors

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Abstract

Blood transfusion is one of the most important routes for syphilis transmission, which is an infectious systemic disease caused by the *Treponema pallidum*, (Schaudinn and Hoffmann, 1905). The aim of this study was to compare between two methods used commonly in detecting syphilis among blood donors. The immune chromatographic test (ICT), a rapid chromatographic immunoassay for only the qualitative detection of antibodies or antigens in whole blood, serum and plasma versus the enzyme-linked immunosorbent assay (ELISA), which is both a qualitative and/or quantitative technique used for detection of antibodies or antigens in human serum or plasma. This comparative study was carried out in 200 samples collected from 200 blood donors in central blood bank, Wad Medani, Sudan during August 2015. The samples were subjected to syphilis test using both ICT and ELISA techniques. The study clearly showed that out of the 200 samples tested using the ICT test 191(95.5%) gave negative results with only 9(4.5%) samples reported positive. However, when the same 200 blood samples were tested using ELISA test 187(93.5 %) samples gave negative results and 13(6.5%) samples reacted positively. The 191 negative samples obtained by ICT gave 6 positive samples after tested by the ELISA, and the 9 positive samples obtained by ICT gave 7 positive samples and 2 negative samples after tested by ELISA. Thus, the outcome of this comparison clearly indicated that, ICT gave 2 false positive samples and 6 false negative samples compared to the ELISA test, a finding which reflects the possible hidden risk among the blood donors and can have a great impact in the case of blood transfusion. The study recommends the use of ICT in screening of syphilis but should be confirmed with other sensitive diagnostic tests. Further studies should be done in order to identify more sensitive, reliable techniques for syphilis detection in blood banks to avoid risks of false results.
مقارنة بين طريقتين المقايسة المناعية الإزمنية والاستشراب المناعي في فحص مرض الزهري وسط متبرع الدم

ميسان محمد البله يوسف

ملخص الدراسة

عُتبر نقل الدم من أهم طرق انتقال مرض الزهري وهو مرض جهازي مؤذي تسببه البكتيريا اللولبية الشاحبة (Treponema pallidum, Schaudinn and Hoffmann, 1905). تستخدمان بصورة عامة للكشف عن مرض الزهري (Syphilis) ووسط المتبرعين بالدم. اختبار الاستشراب المناعي (ICT) وهي تقنية سريعة نوعية فقط تستخدم للكشف عن الإمساك والاضداد والمضادات النوعي في الدم الكامل، المصل أو البلازما عكس طريقة المقايسة المناعية الإزمنية (ELISA) وهى تقنية نوعية وكمية للكشف عن الإمساك والاضداد والمضادات في المصل والبلزما. أجربت هذه الدراسة المقارنة في 200 عينة جمعت من 200 متبرع بالدم في بنك الدم المركزي ود مدني، السودان خلال شهر أغسطس للعام 2015. العينات تم اختبارها لمرض الزهري (Syphilis) بواسطة استخدام تقنيات الاستشراب المناعي (ICT) والمقاومة المناعية الإزمنية (ELISA). ووضعت من الدراسة ان الاستشراب المناعي (ELISA) اعطى 228(95.5%) عينة إيجابية و9(4.5%) عينة سلبية و9(4.5%) عينة إيجابية اما المقايسة المناعية الإزمنية (ICT) اعطت 191(95.5%) عينة سلبية و9(4.5)% عينة ايجابية. فحصت ال 191 عينة التي كانت سلبية بالاستشراب المناعي (ELISA) بواسطة المقايسة المناعية الإزمنية (ICT) واعتطت 6 عينات إيجابية. وسط فحص ال 9 عينات التي كانت إيجابية بالاستشراب المناعي (ELISA) اعطت 7 عينات إيجابية بواسطة المقايسة المناعية الإزمنية (ICT) و3 عينات سلبية. وبالتالي نجد ان الاستشراب المناعي (ICT) اعطى عينات إيجابية زائفة و6 عينات سلبية زائفة مقاير بالمقاومة المناعية الإزمنية (ELISA). تعكس النتيجة وجود خلل في وسط المتبرعين بالدم وينعكس ذلك على نقل الدم. اوصت الدراسة باستخدام الاستشراب المناعي (ICT) لعمل مسح لمراض الزهري (Syphilis) على ان يتم تأكيد باcence أكثر حساسية كما توصي بمزيد من الدراسات لتعريف المزيد من التقنيات الحساسة و التي يمكن الاعتماد عليها للكشف عن مرض الزهري (Syphilis) في بنوك الدم لتجنب خطر النتائج الزلقة.
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Chapter One
Introduction

Syphilis is a systemic disease caused by *Treponema pallidum*. The disease has been divided into stages based on clinical findings, helping to guide treatment and follow up. Persons who have syphilis might seek treatment for signs or symptoms of primary syphilis infection (i.e., ulcers or chancre at the infection site), secondary syphilis (i.e., skin rash, mucocutaneous lesions and lymphadenopathy), or tertiary syphilis (i.e., cardiac, gummatous lesions, tabes dorsalis and general paresis). Latent infections (i.e., those lacking clinical manifestations) are detected by serologic testing. Latent syphilis acquired within the preceding year is referred to as early latent syphilis; all other cases of latent syphilis are late latent syphilis or syphilis of unknown duration. *Treponema pallidum* can infect the central nervous system and result in neurosyphilis, which can occur at any stage of syphilis. Early neurologic clinical manifestations (i.e., cranial nerve dysfunction, meningitis, stroke, acute altered mental status and auditory or ophthalmic abnormalities) are usually present within the first few months or years of infection. Late neurologic manifestations (i.e., tabes dorsalis and general paresis) occur 10–30 years after infection (CDC, 2015).

Transmission occurs by sexual contact, Trans placental infection of a fetus may occur during the pregnancy of an infected woman. Fetal infection occurs with high frequency in untreated early infections of pregnant women and with lower frequency later in the disease or in late latency. Syphilis is also transmitted by transfusion of blood from infected individuals (Schmid, 2004).

An ELISA is a test use to determine if a particular protein is present in a sample and if so, how much. There are two main variations on this method: it can determine how much antibody is in a sample, or how much protein is bound by an antibody. The distinction is whether you are trying to quantify an antibody or the protein (Gao *et al*., 2009).

The immunochromatographic assays, also known as lateral flow immunochromatographic assays, are simple devices intended to detect the presence (or absence) of a target analyte in sample without the need for specialized and costly equipment, though many lab based applications exist that are supported by reading equipment the first of which was developed in 1956 by singer and plot strip tests ideal for applications such as home testing, rapid point of care testing, and testing in the field for various environmental and agricultural analyses. In
addition, they provide reliable testing that might not otherwise be available to third world countries (Yetisen, 2013).

**Rationales:**

- Syphilis is a serious infectious disease; late syphilis is a slowly progressive inflammatory stage in which granulomatous lesions (gummas) develop in skin, bones, liver, stomach and other organs, and degenerative changes occur in the central nervous system causing meningovascular syphilis, and general paralysis with cerebral atrophy, psychosis, Visual problems, deafness and dementia. Cardiovascular syphilis may lead to aortic aneurysm and aortic valve insufficiency.
- No vaccine is available now, so prevention is the corner stone in the management strategy. Since blood transfusion is the route of transmission all healthy donors must be screened accurately for syphilis.

**General objective:**

To compare between ICT technique and ELISA technique in the detection of Syphilis.

**Specific objectives:**

- To compare the sensitivity of both ICT and ELISA.
- To find out the appropriate diagnostic tool to diagnose Syphilis.
Chapter Two
Literature Review

2.1. Syphilis:
2.1.1. History:

The exact origin of syphilis is disputed. Syphilis was indisputably present in the Americas before European contact. The dispute is over whether or not syphilis was also present elsewhere in the world at that time. One of the two primary hypotheses proposes that syphilis was carried from the Americas to Europe by the returning crewmen from Christopher Columbus's voyage to the Americas. The other hypothesis says that syphilis existed in Europe previously, but went unrecognized until shortly after Columbus' return. These are referred to as the Columbian and pre-Columbian hypotheses, respectively (Farhi and Dupin, 2010).

The Columbian hypothesis is best supported by the available evidence. The first written records of an outbreak of syphilis in Europe occurred in 1494 or 1495 in Naples, Italy, during a French invasion (Italian war of 1494–98). As it was claimed to have been spread by French troops, it was initially known as the "French disease" by the people of Naples (Winters and Adam, 2006). In 1530, the pastoral name "syphilis" (the name of a character) 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" (Dayan and Ooi, 2005).

The causative organism, *Treponema pallidum*, was first identified by Fritz Schaudinn and Erich 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. Before the discovery and use of antibiotics in the mid-twentieth century, mercury and isolation were commonly used, with treatments often worse than the disease (Dayan and Ooi, 2005).
2.1.2. Epidemiology:

Syphilis is believed to have infected 12 million additional people in 1999, with greater than 90% of cases in the developing world. It affects between 700,000 and 1.6 million pregnancies a year, resulting in spontaneous abortions, stillbirths, and congenital syphilis. During 2010 it caused about 113,000 deaths down from 202,000 in 1990 (Lozano, 2012).

In sub-Saharan Africa, syphilis contributes to approximately 20% of perinatal deaths. Rates are proportionally higher among intravenous drug users, those who are infected with HIV. In the United States, rates of syphilis as of 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 (Clement et al., 2014).

Syphilis was very common in Europe during the 18th and 19th centuries. In the developed world during the early 20th century, infections declined rapidly with the widespread use of antibiotics, until the 1980s and 1990s (Franzen, 2008).

Since the year 2000, rates of syphilis have been increasing in the USA, Canada, the UK, Australia and Europe, primarily among men who have sex with men. Rates of syphilis among American women have, however, remained stable during this time, and rates among UK women have increased, but at a rate less than that of men (Ficarra and Carlos, 2009).

Untreated, it has a mortality of 8% to 58%, with a greater death rate in males. The symptoms of syphilis have become less severe over the 19th and 20th centuries, in part due to widespread availability of effective treatment and partly due to decreasing virulence of the spirochetes. With early treatment, few complications result. Syphilis increases the risk of HIV transmission by two to five times, and co infection is common (30–60% in a number of urban centers). In 2015, Cuba became the first country in the world to eradicate syphilis (WHO, 2015).

2.1.3. Treponema pallidum characters:

Treponema pallidum subspecies pallidum is the causative agent of syphilis is a spiral-shaped, gram-negative, highly mobile bacterium; Humans are the only known natural reservoir for subspecies pallidum (Eccleston et al., 2008). Three other human diseases are caused by related Treponema pallidum, including yaws (subspecies per tenue), pinta
(subspecies *carateum*) and bejel (subspecies *endemicum*). Unlike subtype *pallidum*, they do not cause neurological disease. The treponemes have a cytoplasmic and an outer membrane. Using light microscopy, treponemes are only visible using dark field illumination, but some regard them too thin to be gram stained. This bacterium can be detected with special stains, such as the Dieterle stain. *Treponema pallidum* is also detected by serology (Kent and Romanelli, 2008). *Treponema pallidum* has one of the smallest bacterial genomes at 1.14 million base pairs, and has limited metabolic capabilities, reflecting its adaptation through genome reduction to the rich environment of mammalian tissue (Tomson et al., 2007).

![Figure 2.1: Electron micrograph of Treponema pallidum, source (Wikipedia.org)](image)

2.1.4. Signs and symptoms:

The signs and symptoms of syphilis vary depending in which of the four stages it presents (primary, secondary, latent, and late stage) (Coffin et al., 2010).

2.1.4.1. Primary syphilis:

Within about 3 weeks of infection an ulcer, known as a chancre forms at the site of infection usually on the genital area (about 10% of ulcers are extra genital). The ulcer is shallow, well-defined with indurated edges and smooth red surface that exudes serum. It is usually painless. Treponemes can be found in the chancre fluid. There is enlargement of nearby lymph nodes. The chancre heals after 3-6 weeks, and healing may be delayed in those co-infected with Human Immunodeficiency Virus HIV (Cheesbrough, 2006).
2.1.4.2. Secondary syphilis:

This occurs 4-8 weeks after the primary infection, a wide spread non-itchy maculopapular rash appears, corresponding to the spread of organisms in the body. The secondary skin lesions contain treponemes and are highly infectious. Treponemes are also present in the blood. Mucus membranes are also infected and mouth ulcers are common. There is generalized enlargement of lymph nodes, head ache, joints pain, fever, malaise and other symptoms. In those co-infected with HIV, the syphilitic rash may be sever with ulceration (Cheesbrough, 2006).

2.1.4.3. Latent syphilis:

During the latent stage, there are no clinical manifestations of syphilis but there is serological evidence of infection. Latent syphilis is described as early when the infection is under 2 years or late latent syphilis when the infection is more than 2 years (Cheesbrough, 2006).

2.1.4.4. Late stage syphilis:

Late stage syphilis is a slowly progressive inflammatory stage in which granulomatous lesions (gummas) develop in skin, bones, liver, stomach and other organs, and degenerative changes occur in the central nervous system causing meningovascular syphilis, and general paralysis with cerebral atrophy, psychosis and dementia. Cardiovascular syphilis may lead to aortic aneurysm and aortic valve insufficiency. Treponemes are not present in late stage syphilitic lesions. In those co-infected with HIV, late stage syphilis may progress more rapidly with neurosyphilis developing early (Cheesbrough, 2006).

2.1.5. Congenital syphilis:

Transmitted during pregnancy or during birth. Two-thirds of syphilitic infants are born without symptoms. Common symptoms that develop over the first couple of years of life include enlargement of the liver and spleen (70%), rash (70%), fever (40%), neurosyphilis (20%), and lung inflammation (20%). If untreated, late congenital syphilis may occur in 40%, including saddle nose deformation (Woods, 2009).
2.1.6. Neurosyphilis:

Can occur during any stage of syphilis including primary and secondary syphilis. Ocular syphilis is a clinical manifestation of neurosyphilis, can involve almost any eye structure, but posterior uveitis and panuveitis are the most common. Additional manifestations may include anterior uveitis, optic neuropathy, retinal vasculitis and interstitial keratitis. Ocular syphilis may lead to decreased visual acuity including permanent blindness. While previous research supports evidence of neuropathogenic strains of syphilis, it remains unknown if some *Treponema pallidum* strains have a greater likelihood of causing ocular infections (CDC, 2012).

2.1.7. Complications:

Without treatment, syphilis can lead to damage throughout the body. Syphilis also increases the risk of HIV infection and for women, can cause problems during pregnancy. Treatment can help preventing future damage but can't repair or reverse damage that's already occurred.

2.1.7.1. Small bumps or tumors:

Called gummas, these bumps can develop on skin, bones, liver or any other organ in the late stage of syphilis. Gummas usually disappear after treatment with antibiotics (Winter and Adam, 2006).

2.1.7.2. Neurological problems:

Syphilis can cause a number of problems with nervous system, including stroke, meningitis, deafness, visual problems and dementia (Winter and Adam, 2006).

2.1.7.3. Cardiovascular problems:

These may include bulging (aneurysm) and inflammation of the aorta (body’s major artery) and of other blood vessels. Syphilis may also damage heart valves (Electson, 2011).
2.1.7.4. Human Immunodeficiency Virus (HIV) infection:

Adults with sexually transmitted syphilis or other genital ulcers have an estimated two to fivefold increased risk of contracting HIV. A syphilis sore can bleed easily, providing an easy way for HIV to enter the bloodstream during sexual activity (Karp et al., 2009).

2.1.7.5. Pregnancy and childbirth complications:

In pregnant, may pass syphilis to the unborn baby. Congenital syphilis greatly increases the risk of miscarriage, stillbirth or newborn's death within a few days after birth (Electson, 2011).

2.1.8. Transmission:

Syphilis is transmitted primarily by sexual contact or during pregnancy from a mother to her fetus; the spirochetes is able to pass through intact mucous membranes or compromised skin. It is thus transmissible by kissing near a lesion, as well as oral, vaginal, and anal sex (Radolf and Lukehart, 2006).

Approximately 30 to 60% of those exposed to primary or secondary syphilis will get the disease. Syphilis also is transmitted by transfusion of blood from infected individuals. The risk of transmission from sharing needles appears limited (Kent and Romanelli, 2008).

It is not generally possible to contract syphilis through toilet seats, daily activities, hot tubs, or sharing eating utensils or clothing. This is mainly because the bacteria die very quickly outside of the body, making transmission via objects extremely difficult (Larry, 2006).

2.1.9. Diagnosis:

Syphilis is difficult to diagnose clinically early in its presentation. Confirmation is either via blood tests or direct visual inspection using microscopy. Blood tests are more commonly used, as they are easier to perform. Diagnostic tests are, however, unable to distinguish between the stages of the disease (Farhi and Dupin 2010).
2.1.9.1. Blood tests:

Blood tests are divided into non treponemal and treponemal tests. Non treponemal tests are used initially, and include venereal disease research laboratory (VDRL) and rapid plasma reagin tests. However, as these tests are occasionally false positives, confirmation is required with a treponemal test, such as *T. pallidum* particle agglutination (TPHA) or fluorescent treponemal antibody absorption test (FTA-Abs). False positives on the non treponemal tests can occur with some viral infections such as varicella and measles, as well as with lymphoma, tuberculosis, malaria, endocarditis, connective tissue disease and pregnancy. Treponemal antibody tests usually become positive two to five weeks after the initial infection. Neurosyphilis is diagnosed by finding high numbers of leukocytes (predominately lymphocytes) and high protein levels in the cerebrospinal fluid in the setting of a known syphilis infection (Kent and Romanelli, 2008).

2.1.9.2. Direct testing:

Dark ground microscopy of serous fluid from a chancre may be used to make an immediate diagnosis. However, hospitals do not always have equipment or experienced staff members, whereas testing must be done within 10 minutes of acquiring the sample. Sensitivity has been reported to be nearly 80%, thus can only be used to confirm a diagnosis but not rule one out. Two other tests can be carried out on a sample from the chancre: direct fluorescent antibody testing and nucleic acid amplification tests. Direct fluorescent testing uses antibodies tagged with fluorescein, which attach to specific syphilis proteins, while nucleic acid amplification uses techniques, such as the polymerase chain reaction, to detect the presence of specific syphilis genes. These tests are not as time-sensitive, as they do not require living bacteria to make the diagnosis (Eccleston *et al.*, 2008).

2.1.10. Prevention:

There is no vaccine effective for prevention. Abstinence from intimate physical contact with an infected person is effective at reducing the transmission of syphilis, as is the proper use of a latex condom. Condom use, however, does not completely eliminate the risk (Koss *et al.*, 2009).
Congenital syphilis in the newborn can be prevented by screening mothers during early pregnancy and treating those who are infected. The United States Preventive Services Task Force (USPSTF) strongly recommends universal screening of all pregnant women, while the World Health Organization recommends all women be tested at their first antenatal visit and again in the third trimester. If they are positive, they recommend their partners also be treated. Congenital syphilis is, however, still common in the developing world, as many women do not receive antenatal care at all (Hawkes et al., 2011).

2.1.11. Treatment:

2.1.11.1. Early infections:

The first choice treatment for uncomplicated syphilis remains a single dose of intramuscular benzathine penicillin G. Doxycycline and tetracycline are alternative choices for those allergic to penicillin; however, due to the risk of birth defects these are not recommended for pregnant women (CDC, 2015). Resistance to macrolides, rifampin, and clindamycin is often present. Ceftriaxone, a third-generation cephalosporin antibiotic, may be as effective as penicillin-based treatment (CDC, 2010).

2.1.11.2. Late infections:

For neurosyphilis, due to the poor penetration of penicillin G into the central nervous system, those affected are recommended to be given large doses of intravenous penicillin for a minimum of 10 days (Stamm, 2010).

If a person is allergic, ceftriaxone may be used or penicillin desensitization attempted. Other late presentations may be treated with once weekly intramuscular penicillin G for three weeks. If allergic, as in the case of early disease, doxycycline or tetracycline may be used, albeit for a longer duration. Treatment at this stage limits further progression, but has only slight effect on damage which has already occurred (Kent and Romanelli, 2008).

2.1.11.3. Jarisch-Herxheimer reaction:

One of the potential side effects of treatment is the Jarisch-Herxheimer reaction. It frequently starts within one hour and lasts for 24 hours, with symptoms of fever, muscle pains, headache, and a fast heart rate. It is caused by cytokines released by the immune
system in response to lipoproteins released from rupturing syphilis bacteria (Radolf and Lukehart, 2006).

2.1.12. Society and culture:

The earliest known depiction of an individual with syphilis is Albrecht Dürer's Syphilitic Man, a woodcut believed to represent a Landsknecht, a Northern European mercenary. The myth of the femme fatale or "poison women" of the 19th century is believed to be partly derived from the devastation of syphilis, with classic examples in literature including John Keats' La Belle Damesans Merci (Hughes, 2007).

The artist Jan van der Street painted a scene of a wealthy man receiving treatment for syphilis with the tropical wood guaiacum sometime around 1580. The title of the work is "Preparation and Use of Guayaco for Treating Syphilis". That the artist chose to include this image in a series of works celebrating the New World indicates how important a treatment, however ineffective, for syphilis was to the European elite at that time. The richly colored and detailed work depicts four servants preparing the concoction while a physician looks on, hiding something behind his back while the hapless patient drinks (Reid and Basil, 2009).

2.2. Detection methods:

2.2.1 ELISA test:

As a "wet lab" analytic biochemistry assay, ELISA involves detection of an "analyte" (i.e. the specific substance whose presence is being quantitatively or qualitatively analyzed) in a liquid sample by a method that continues to use liquid reagents during the "analysis" that stays liquid and remains inside a reaction chamber or well needed to keep the reactants contained; It is opposed to "dry lab" that can use dry strips and even if the sample is liquid (e.g. a measured small drop), the final detection step in "dry" analysis involves reading of a dried strip by methods such as reflectometry and does not need a reaction containment chamber to prevent spillover or mixing between samples (Leng et al., 2008).

ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result (yes or no) for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation (error inherent in a test) is often used to distinguish positive from negative samples. In quantitative ELISA, the optical density (OD) of the sample is compared to a standard
curve, which is typically a serial dilution of a known-concentration solution of the target molecule (Plus, 2007).

2.2.2. Types of ELISA:

2.2.2.1. Direct ELISA:

The steps of direct ELISA follow the mechanism below:

- A buffered solution of the antigen to be tested for is added to each well of a micro titer plate, where it is given time to adhere to the plastic through charge interactions.
- A solution of non-reacting protein, such as bovine serum albumin or casein, is added to well (96-well plates) any plastic surface in the well that remains uncoated by the antigen.
- The primary antibody is added, which binds specifically to the test antigen coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.
- A secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody. In other cases, the primary antibody itself is conjugated to the enzyme.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows the secondary antibody has bound to primary antibody, which strongly implies the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in research.
- The higher the concentration of the primary antibody presents in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.
A major disadvantage of the indirect ELISA is the method of antigen immobilization is not specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the micro titer plate well, and so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich or direct ELISA provides a solution to this problem, by using a "capture" antibody specific for the test antigen to pull it out of the serum's molecular mixture (Lequin, 2005).

### 2.2.2.2. Sandwich ELISA:

A "sandwich" ELISA is used to detect sample antigen. The steps are:

1. A surface is prepared to which a known quantity of capture antibody is bound.
2. Any nonspecific binding sites on the surface are blocked.
3. The antigen-containing sample is applied to the plate.
4. The plate is washed to remove unbound antigen.
5. A specific antibody is added, and binds to antigen (hence the 'sandwich': the Ag is stuck between two antibodies)

6. Enzyme-linked secondary antibodies are applied as detection antibodies that also bind specifically to the antibody's Fc region (nonspecific).

7. The plate is washed to remove the unbound antibody-enzyme conjugates.

8. A chemical is added to be converted by the enzyme into a color or fluorescent or electrochemical signal.

9. The absorbency or fluorescence or electrochemical signal (e.g., current) of the plate wells is measured to determine the presence and quantity of antigen (Plus, 2007).

---

**Figure (2.3): Sandwich ELISA, source (Wikipedia.org)**

### 2.2.2.3. Competitive ELISA:

A third use of ELISA is through competitive binding. The steps for this ELISA are somewhat different from the first two examples:

1. Unlabeled antibody is incubated in the presence of its antigen (sample).

2. These bound antibody/antigen complexes are then added to an antigen-coated well.

3. The plate is washed, so unbound antibody is removed. (The more antigens in the sample, the fewer antibodies will be able to bind to the antigen in the well, hence "competition").
4. The secondary antibody, specific to the primary antibody, is added. This second antibody is coupled to the enzyme.

5. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.

6. The reaction is stopped to prevent eventual saturation of the signal.

Some competitive ELISA kits include enzyme-linked antigen rather than enzyme-linked antibody. The labeled antigen competes for primary antibody binding sites with the sample antigen (unlabeled). The fewer antigens in the sample, the more labeled antigen is retained in the well and the stronger the signal commonly, the antigen is not first positioned in the well (Plus, 2007).

2.2.2.4. Multiple and portable ELISA:

A new technique uses a solid phase made up of an immunosorbent polystyrene rod with eight to 12 protruding ogives. The entire device is immersed in a test tube containing the collected sample and the following steps (washing, incubation in conjugate and incubation in chromogens) are carried out by dipping the ogives in micro wells of standard micro plates filled with reagents.

The advantages of this technique are:

1. The ogives can each be sensitized to a different reagent, allowing the simultaneous detection of different antibodies and/or different antigens for multiple-target assays.
2. The sample volume can be increased to improve the test sensitivity in clinical (blood, saliva, urine), food (bulk milk, pooled eggs) and environmental (water) samples.
3. One ogive is left un sensitized to measure the nonspecific reactions of the sample.
4. The use of laboratory supplies for dispensing sample aliquots, washing solution and reagents in micro wells is not required, facilitating the development of ready-to-use lab kits and on-site testing (Plus, 2007).

2.2.2.5. Applications:

Because the ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations (such as with the HIV test or West Nile virus) (Plus, 2007).

It has also applications in the food industry in detecting potential food allergens, such as milk, peanuts, walnuts, almonds, and eggs. ELISA can also be used in toxicology as a rapid presumptive screen for certain classes of drugs. The ELISA was the first screening test widely used for HIV because of its high sensitivity (Plus, 2007).
Dr. Bidwell and Voller created the ELISA test to detect various kinds of diseases, such as malaria, Chagas disease, and Johne's disease (Frank et al., 2005). ELISA tests also are used as in vitro diagnostics in medical laboratories. The other uses of ELISA include:

- Detection of Mycobacterium antibodies in tuberculosis.
- Detection of rotavirus in feces.
- Detection of hepatitis B markers in serum.
- Detection of entero toxin of E. coli in feces.
- Detection of HIV antibodies in blood samples (Lequin, 2005).

2.3. Immunochromatographic test (Lateral flow test):

Also known as Lateral Flow Immuno-chromatographic Assays and they are simple devices intended to detect the presence (or absence) of a target analyte in sample (matrix) without the need for specialized and costly equipment, though many lab based applications exist that are supported by reading equipment (Link, 1999).

Typically, these tests are used for medical diagnostics either for home testing, point of care testing, or laboratory use. A widely spread and well known application is the home pregnancy test. The technology is based on a series of capillary beds, such as pieces of porous paper or sintered polymer. Each of these elements has the capacity to transport fluid (e.g., urine) spontaneously. The first element (the sample pad) acts as a sponge and holds an excess
of sample fluid. Once soaked, the fluid migrates to the second element (conjugate pad) in which the manufacturer has stored the so-called conjugate, a dried format of bio-active particles in a salt-sugar matrix that contains everything to guarantee an optimized chemical reaction between the target molecule (e.g., antigen) and its chemical partner (e.g., antibody) that has been immobilized on the particle's surface. While the sample fluid dissolves the salt-sugar matrix, it also dissolves the particles and in one combined transport action the sample and conjugate mix while flowing through the porous structure. In this way, the analyte binds to the particles while migrating further through the third capillary bed. This material has one or more areas (often called stripes) where a third molecule has been immobilized by the manufacturer. By the time the sample-conjugate mix reaches these strips, analyte has been bound on the particle and the third 'capture' molecule binds the complex. After a while, when more and more fluid has passed the stripes, particles accumulate and the stripe-area changes color. Typically there are at least two stripes: one (the control) that captures any particle and thereby shows that reaction conditions and technology worked fine, the second contains a specific capture molecule and only captures those particles onto which an analyte molecule has been immobilized. After passing these reaction zones the fluid enters the final porous material, the wick that simply acts as a waste container. Lateral Flow Tests can operate as either competitive or sandwich assays (Yetisen, 2013).

2.3.1. Coloured particles:

In principle, any coloured particle can be used; however latex (blue colour) or nanometer sized particles of gold (red colour) are most commonly used. Fluorescent or magnetic labeled particles can also be used; however these require the use of an electronic reader to assess the test result (Point of Care Technologies, 2007).

2.3.2. Sandwich assays:

The sample first encounters coloured particles which are labeled with antibodies raised to the target analyte. The test line will also contain antibodies to the same target, although it may bind to a different epitope on the analyte. The test line will show as a coloured band in positive samples (Yetisen, 2013).

2.3.3. Competitive assays:

The sample first encounters coloured particles which are labeled with the target analyte or an analogue. The test line contains antibodies to the target/its analogue. Unlabeled analyte in the sample will block the binding sites on the antibodies preventing uptake of the coloured particles. The test line will show as a coloured band in negative samples (Yetisen, 2013).
2.3.4. Quantitative tests:

Most tests are intended to operate on a purely qualitative basis. However it is possible to measure the intensity of the test line to determine the quantity of analyte in the sample. Handheld diagnostic devices known as lateral flow readers are used by several companies to provide a fully quantitative assay result. By utilizing unique wavelengths of light for illumination in conjunction with either CMOS or CCD detection technology, a signal rich image can be produced of the actual test lines. Using image processing algorithms specifically designed for a particular test type and medium, line intensities can then be correlated with analyte concentrations. One such handheld lateral flow device platform is made by Detekt Biomedical L.L.C. Alternative non-optical techniques are also able to report quantitative assays results. One such example is a Magnetic immunoassay (MIA) in the lateral flow test form also allows for getting a quantified result (Link, 1999).

2.3.5. Control line:

While not strictly necessary, most tests will incorporate a second line which contains an antibody that picks up free latex/gold in order to confirm the test has operated correctly (Point of Care Technologies, 2007).

2.3.6. Speed and simplicity:

Time to obtain the test result is a key driver for these products. Tests can take as little as a few minutes to develop. Generally there is a tradeoff between time and sensitivity - so more sensitive tests may take longer to develop. The other key advantage of this format of test compared to other immunoassays is the simplicity of the test typically requiring little or no sample or reagent preparation (Point of Care Technologies, 2007).
Chapter Three
Materials and Methods

3.1. Study design:
Is in vitro comparative study, conducted in August 2015 at Central blood bank, Gezira state, Sudan. 200 samples were collected from 18-40 blood donors in central blood bank.

3.2. Materials:
Test tubes, syringes, cotton, gloves, Micro pipette, centrifuge.

3.3. Methodology:

3.3.1. Enzyme Linked Immunosorbent Assay (ELISA):
ELISA was used for screening of blood donors, and diagnosis and management of clinical conditions of syphilis.

3.3.1.1. Principle of ELISA:
The detection of anti-TP antibodies will be achieved by antigen sandwich enzyme linked immune sorbent assay, where the micro wells were coated with recombinant Treponema pallidum antigens expressed in Escherichia coli. The samples were incubated in the micro wells together with recombinant TP antigens conjugated to HRP conjugate antigens, but are expressed in different hosts. In case of presence of anti-TP in the sample, during incubation the pre-coated and conjugated antigens were bound to the two variable domains of the antibody and the specific antigen-antibody immune complex is captured on the solid phase.

After washing to remove sample and unbound conjugates, chromogen solution containing TMB and urea peroxidase were added into the wells. In presence of the antigen-antibody sandwich complex, the colour less chromogen was hydrolyzed by the bound HRP conjugate to a blue coloured product, which turns yellow upon addition of the stop solution. This color is then red photo metrically and is directly proportional to the amount of the antibody in the sample. Wells containing samples negative for anti-TP remain colorless.
3.3.1.2. Kits contents:

<table>
<thead>
<tr>
<th>Kit contents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell plate 96 test</td>
<td>1 plate (12x8/8x12 well strips per plate)</td>
</tr>
<tr>
<td>Negative control</td>
<td>1x0.5 ml</td>
</tr>
<tr>
<td>Positive control</td>
<td>1x0.5 ml</td>
</tr>
<tr>
<td>HRP- Conjugate reagent</td>
<td>1X13 ml</td>
</tr>
<tr>
<td>Stock wash buffer</td>
<td>1x50 ml (Dilute 1 to 20 with distilled water before use. Once diluted stable for two weeks at 2-8°C)</td>
</tr>
<tr>
<td>Chromogen solution A</td>
<td>1x7 ml (Ready to use and once open stable for one month at 2-8°C)</td>
</tr>
<tr>
<td>Chromogen solution B</td>
<td>1x7 ml (Ready to use and once open stable for one month at 2-8°C)</td>
</tr>
<tr>
<td>Stop solution</td>
<td>1x7 ml</td>
</tr>
<tr>
<td>Plastic sealable bag</td>
<td>1 unit</td>
</tr>
<tr>
<td>Plate cover</td>
<td>1sheet</td>
</tr>
<tr>
<td>Package insert</td>
<td>1 copy</td>
</tr>
</tbody>
</table>

3.3.1.3. Samples collection:

Fresh serum samples were used for this assay. Blood collected by venipuncture was allowed to clot naturally and completely, the serum was separated from the clot as early as possible to avoid hemolysis on the RBC. Care was taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample was removed by centrifugation at 3000 rpm for at least 20 minutes at room temperature, but highly lipaemic, icteric, or hemolized samples were not be used as they could give erroneous results.

3.3.1.4. Stability of reagent:

The components of the kit were stored between 2-8°C and were not freeze. To assure maximum performance of this anti-TP ELISA kit, during storage was protected the reagents from contamination with microorganism or chemicals.
3.3.1.5. Assay procedure:

**Step 1 Reagents preparation:**

The reagents and samples were allowed to reach room temperature (18-30°C) for at least 15-30 minutes.

**Step 2 Wells numbering:**

The strips needed were Seted in strip-holder, and numbered sufficient number of wells including three negative controls (e.g. B1, C1 D1), tow positive control (e.g., E1, F1) and one blank (e.g. A1, neither samples nor HRP-Conjugate) was added into the blank well.

**Step 3 Added HRP conjugate:**

100 µl HRP conjugate was added into each well except the blank well.

**Step 4 Added sample:**

20 µl of Positive Control, negative Control and Specimen was added into their respective wells. Upon addition of the sample the HRP Conjugate – sample was mixed well will appear blue. A separate disposable tip was used for each specimen, Negative control and positive control to avoid cross – contamination.

**Step 5 Incubating:**

The plate was mixed by tapping gently; the plate was covered with the plate cover and incubated for 60 minutes at 37°C.

**Step 6 Washing:**

At the end of the incubation, the plate cover was removed and discards; each well was washed 6 times with diluted wash buffer.

**Step 7 Colouring:**

50 µl of chromogen A and 50 µl chromogen B were dispensed into each well including the Blank, and mixed by tapping the gently, the plate was Incubated at 37°C for 15 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HPR Conjugate produced blue color in positive Control and anti – TP positive sample wells.
Step 8 Stopping reactions:

50 µl stop solution was added into each well and mixed gently. Intense yellow color develops in positive control and anti TP positive sample wells.

Step 9 Measuring the absorbance:

The plate reader was calibrated with the blank well and the absorbance was read at 450 nm. The Cut-off value was calculated and evaluated the results (Note: The absorbance was read within 5 minutes after stopping the reaction).

3.3.1.6. Interpretation of results:

Each micro plate was considered separately when calculated and interpreted result of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O) of the plate. (S = the individual absorbance (OD) of each specimen)

Negative results (S/C.O.<1): Samples giving an absorbance less than the cut-off value were considered negative, which indicates that anti-TP antibodies was detected with this anti HP ELISA kit. And there are no serological indications for past infection with TP.

Positive results (S/C.O. ≥ 1): Samples giving an absorbance great than or equal to the cut-off value were considered initially reactive, which indicates that anti-TP antibodies was detected. Repeatedly reactive samples were considered positive for antibodies to anti-TP; there for three are serological indication for current or past infection with TP. any blood unit containing antibodies to T. pallidum was immediately discarded.

Borderline (S/C.O = 0.9-1.1 ) : Samples with absorbance to cut-off ratio between 0.9 and 1.10 are considered borderline samples and retesting is recommended, repeatedly positive samples can be considered positive for anti- TP antibodies.

3.3.1.7. Calculation of cut-off value:

\[(\text{C.O.}) = *\text{NC} + 0.18: \]

*Nc= the mean absorbance value of three negative controls.
3.3.1.8. Quality control range:

- The OD value of the blank well, which contains only chromogens and stop solution, is less than 0.080 at 450 nm.
- The OD value of the positive control was equal to or greater than 0.800 at 450/630 nm or at 450 nm after blanking.
- The OD value of the negative control was less than 0.100 at 450 \( \square \) 630 nm or at 450 nm after blanking.

3.3.2. ICT:

3.3.2.1. Intended use:

The Syphilis Ultra Rapid Test Strip (whole blood/ serum/ plasma) is a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgM and IgG) to *Treponema Pallidum* (TP) in whole blood, serum or plasma to aid in the diagnosis of Syphilis.

3.3.2.2. Principle:

The Syphilis Ultra Rapid Test Strip (whole blood / serum / plasma) is a qualitative membrane strip based immunoassay for the detection of TP antibodies (IgG and IgM) in whole blood, serum or plasma, in this test procedure, recombinant syphilis antigen is immobilized in the test line region of the strip. After a specimen is added to the specimen pad it reacts with syphilis antigen coated particles that have been applied to the specimen pad, this mixture migrates chromatographically along the length of the test strip and interacts with the immobilized syphilis antigen, the double antigen test format can detect both IgG and IgM in specimens, if the specimen contains TP antibodies, a red line will appear in the test line region, indicating a positive result, if the specimen does not contain TP antibodies, a red line will not appear in this region, indicating a negative result. To serve as a procedural control, a pink line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

3.3.2.3. Reagents:

The test strip contains syphilis antigen coated particles and syphilis antigen coated on the membrane.
3.3.2.4. Storage and stability:

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C), the test strip is stable through the expiration date printed on the sealed pouch. The test strip must remain in the sealed pouch until use.

3.3.2.5. Specimen collection and preparation:

- Fresh serum samples were used for this assay. Blood collected by vein puncture was allowed to clot naturally and completely; the serum was separated from the clot as early as possible to avoid hemolytic on the RBC. Testing was performed immediately after specimens have been collected.
- Bring Specimens to room temperature prior to testing.

3.3.2.6. Materials:

Materials were used:

- Test strips.
- Disposable Specimen droppers.
- Buffer.
- Test cards.
- Packages insert.
- Specimen collection container.
- Centrifuge.
- Timer.

3.3.2.7. Directions for use:

- The test strip, specimen, and buffer were allowed to equilibrate to room temperature (15-30°C) prior to testing.
- The test strips were removed from the sealed foil and used as soon as possible.
- The tape from the test card was peeled off, and stuck the test strip in the middle of the test card with arrows pointing downwards as illustrated.
- For Serum or Plasma specimens: the dropper was held vertically and 2 drops of serum or Plasma (approximately 50 µl) was transferred onto the specimens pad of the
test strip, then 1 drop of buffer (approximately 40 µl) was added and the timer was started.

- Wait for the red line(s) to appear. The result was read at 10 minutes.

3.3.2.8. Interpretation of results:

**POSTITIVE:** Two distinct red lines were appeared. One line in the control line region (C) and another line in the test line region (T).

*Note:* The intensity of the red color in the test line region (T) will vary depending on the concentration of TP antibodies present in the specimen. Therefore, any shade of red in the test line region (T) was considered positive.

**NEGATIVE:** One red line was appeared in the control line Region (C). No apparent red or pink line appears in the test line region (T).

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

3.3.2.9. Quality control:

A procedural control was included in the test. A red line appearing in the control line Region (C) is considered an internal procedural control. It confirms sufficient specimen volume and correct procedural technique.

3.3.2.10. Expected values:

The Syphilis Ultra Rapid Test Strip (whole blood / serum / plasma) was compared with a leading commercial TPHA Syphilis test, demonstrating an overall accuracy greater than or equal to 99.7%.
Chapter Four
Results and Discussion

4.1. Results:

4.1.1. Positive and negative cases for ELISA and ICT tests:

The number of cases that was used for running ELISA test was 200 blood samples, as same as that for running by ICT test. The negative cases were 187 for ELISA test, while they were 191 cases for ICT test. 6 cases from these positive by ELISA test (False negative). The positive cases were 13 for ELISA test, while they were 9 cases for ICT test (2 cases from these negative by ELISA test (False positive)).

Table (4-1) Positive and negative cases of syphilis using ELISA and ICT test

<table>
<thead>
<tr>
<th>Test type</th>
<th>Cases Tested</th>
<th>Cases Negative</th>
<th>Cases Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>N 200, Percent 100.0%</td>
<td>N 187, Percent 93.5%</td>
<td>N 13, Percent 6.5%</td>
</tr>
<tr>
<td>ICT</td>
<td>N 200, Percent 100.0%</td>
<td>N 191, Percent 95.5%</td>
<td>N 9, Percent 4.5%</td>
</tr>
</tbody>
</table>

Table (4-2) Case processing Summary

<table>
<thead>
<tr>
<th>Cases</th>
<th>Valid</th>
<th>Missing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Percent</td>
<td>N</td>
<td>Percent</td>
</tr>
<tr>
<td>ELISA* ICT</td>
<td>200, Percent 100.0%</td>
<td>0, Percent 0%</td>
<td>200, Percent 100%</td>
</tr>
</tbody>
</table>
Table (4-3) ELISA* ICT tests cross tabulation

<table>
<thead>
<tr>
<th>ICT</th>
<th>ELISA Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>9</td>
<td>191</td>
</tr>
</tbody>
</table>

Table (4-4) Symmetric Measures

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Approx. significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal by nominal Contingency</td>
<td>0.532</td>
<td>0.000</td>
</tr>
<tr>
<td>N of valid cases</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

Prob. Value ≤ 0.05 (Significant) *
Prob. Value ≤ 0.01 (Highly Significant) **
Prob. Value > 0.05 (No Significant)

The ICT test detected 6 cases false negative and 2 cases false positive when compared with the confirmatory ELISA test. There was highly significant difference between the ELISA and ICT test.

4.2. Discussions:

The study clearly showed that out of the 200 samples tested using the ICT test 191 (95.5%) gave negative results with only 9 (4.5%) samples reported positive. However, when the same 200 blood samples were tested using ELISA test 187 (93.5 %) samples gave negative results and 13 (6.5%) samples reacted positively. The 191 negative samples obtained by ICT gave 6 positive samples after tested by the ELISA, and the 9 positive samples obtained by ICT gave 7 positive samples and 2 negative samples after tested by ELISA. Thus, the outcome of this comparison clearly indicated can that, ICT gave 2 false positive
samples and 6 false negative samples compared to the ELISA test, a finding which reflects the possible hidden risk among the blood donors and can have a great impact in the case of blood transfusion. These findings, agreed with Hasab Elrasoul and Nafi (2014) enrolled 90 blood donors in their study they found 6 out of the 90 samples were positive by ICT and 4 positive by ELISA.

Elagib and Abdelmaged (2009), studied 451 blood donors, the ELISA test showed that 23.5 % from blood donors were positive for syphilis; while the ICT test showed that only 16.4 % from the blood donors were positive for syphilis. ELISA test showed that 106 individuals were syphilis positive, whereas ICT test showed that 74 individuals were syphilis positive. The ICT test detected 32 individuals false negative when compared with the confirmatory ELISA test. There was significant difference between the ELISA and ICT test.

Kilany et al., (2015), found that; depending on donor selection criteria, voluntary non-remunerated 7267 blood donors (26 females (0.36%, median age of 28) and 7241 males (99.64%, median age of 30) when selected to donate their blood, the serological screening of the samples resulted in positivity of many different markers. Two (0.028%) positive cases to anti-\textit{Treponema pallidum} antibodies, one is 33 years old and the second is 36 years old both with positive markers for HBcAb.

PCR can also be used to detect \textit{T. pallidum} genetic material, most often the PolA gene. To date, it is most commonly used to diagnose congenital syphilis. However, it has also been shown to be effective at diagnosing primary syphilis with sensitivities between 73% and 95% and specificities 95%. For the diagnosis of congenital syphilis, frozen tissue and formalin-fixed, paraffin-embedded tissue and, for the diagnosis of primary syphilis, swabs of the ulcer can be submitted to the CDC for processing with prior approval from the local health department. Other specimen types are acceptable but have less diagnostic value. Sensitivities of blood samples in primary syphilis are as low as 18% (Chaurasia \textit{et al.}, 2014).
Chapter Five
Conclusion and Recommendations

5.1. Conclusion:
A positive result for syphilis antibodies gotten with an immune-chromatographic rapid strip test does not warrants that treatment should begin. False positive and negative results are common. Therefore the presence of the disease should be investigated further using a more sensitive and specific assay prior to treatment. Although PCR assays are very expensive to be incorporated, an ELISA which is less expensive and more affordable can be implemented to give more valid results. A negative result too dose not exclude the presence of the infection. If symptoms persist, then the infection should be investigated further with a PCR assay. It is important that diagnosis should be done together with the patient medical history since the major risk factors for infection syphilis.

5.2. Recommendations:
- ICT is not perfect to diagnose syphilis because the false results are probable.
- To confirm the diagnosis of syphilis ELISA must be used.
- ELISA test should be used in blood bank to avoid any probability of false blood transfusion and spreading the disease.
- Further studies should be done in order to identify more sensitive and reliable techniques for syphilis detection in blood banks.
References


