EVALUATION OF IMMUNOCHROMATOGRAPHIC STRIP TEST FOR RAPID DIAGNOSIS OF ANTENATAL WOMEN SYPHILIS IN ELDORET, KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN IMMUNOLOGY IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

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DECLARATION

I, Lydia Bonareri Nyamwamu, do declare that this thesis is my original work and has not been presented for a degree in any other University or for any other award.

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DEDICATION

I dedicate this thesis to my Mum Joyce, husband Albert, our sons Ian and Jerry and all Kenyan women.

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GLOSSARY OF ABBREVIATIONS AND ACRONYMS

ANC	Antenatal Clinic
BFP	Biological False Positive
CD	Cluster of Differentiation
CDC	Centers for Disease Control and Prevention
CSF	Cerebrospinal Fluid
FTA-ABS	Fluorescent Treponemal Antibody Absorption
DFA-TP	Direct Fluorescent Antibody Test for *Treponema pallidum*
DNA	Deoxyribonucleic Acid
HIV	Human Immunodeficiency Virus
ICS	Immunochromatographic Strip
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
INF-γ	Interferon Gamma
MHA-TP	Microhaemagglutination Assay for Antibody to *Treponema pallidum*
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
RPM	Revolutions Per Minute
RPR	Rapid Plasma Reagin
SC	Sample Collection
SLE	Systemic Lupus Erythematosus
STD	Sexually Transmitted Disease

Th	T helper
TLR	Toll Like Receptor
TPHA	*Treponema pallidum* Haemagglutination Assay
TPI	*Treponema pallidum* Immobilization Test
TPPA	*Treponema pallidum* Particle Agglutination Assay
Tpr	*Treponema pallidum* Repeated Genes
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organization

ABSTRACT

Antenatal syphilis is a major cause of perinatal morbidity and mortality. Programmes to control syphilis in developing countries are hampered by lack of laboratory services, delayed diagnosis and doubts about the accuracy of the current screening methods. In Kenya, the Venereal Disease Research Laboratory (VDRL) test is currently used as the primary screening test for syphilis in health facilities. This method is labour intensive in the screening of large numbers of serum specimens and is carried out in a laboratory setting resulting in delays in identification and treatment of infected pregnant mothers. In addition, the reagents for the VDRL test require cold storage and electricity to operate a centrifuge, most of which are unavailable at the periphery of the health care systems. The limitations associated with VDRL testing at clinics have led to the development of technologically simple immunochromatographic strip (ICS) tests. This study sought to evaluate the performance of a rapid simple point-of-care ICS test and the VDRL test as a gold standard with *Treponema pallidum* haemagglutination assay (TPHA) as a confirmatory test to screen antenatal clinic attendants for syphilis. One hundred and fifty women were drawn from a population of pregnant women aged 18 years to 42 years old making their first antenatal visit or follow-up visits but without a previous syphilis test during that pregnancy. Using a gold standard of the VDRL test, the prevalence rate of syphilis was shown to be 3%. In addition, the results of this study demonstrate that there was no significant difference between the ICS and the VDRL tests ($P>0.05$). The sensitivity and specificity of the ICS test was 80% and 98.62% respectively while the negative predictive value (NPV) and positive predictive value (PPV) were both 100%. In conclusion, the diagnostic accuracy of the ICS compared favourably with the gold standard. The use of the ICS test in Kenya may improve the diagnosis of syphilis in health facilities with or without laboratories and allow community health care workers to make rapid diagnosis of the disease and consequently make immediate therapeutic decisions.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Syphilis remains a significant cause of preventable perinatal death in developing countries with many women remaining untested and thus untreated (Myer *et al.*, 2003). Transmission of *Treponema pallidum* occurs during sexual intercourse and other intimate contact with infected lesions (Gerbase *et al*., 1998). Congenital syphilis occurs following vertical transmission of *T. pallidum* from an infected mother to the fetus in utero, but neonates may also be infected during passage through an infected birth canal at delivery (Rawstron *et al*., 1993). Syphilis has been acquired rarely by transfusion of blood (Cockayne, 2002). Syphilis causes a variety of symptoms corresponding to stages of infection (primary, secondary, tertiary) and no symptoms during latent stages (Kinghorn, 2004). Undiagnosed and untreated syphilis increases the risk of the Human Immunodeficiency Virus (HIV) transmission (Walker and Walker, 2002; Buchacz *et al*., 2004). The World Health organization (WHO) estimates that 1.6 million pregnant women remain undiagnosed in sub-Saharan Africa including more than one million attending antenatal care (Gloyd *et al*., 2001; Montoya *et al*., 2006).

Screening for syphilis in antenatal clinic attendants remains a very important priority for preventing adverse outcomes on pregnancy due to syphilis such as spontaneous abortion, perinatal or infant death with congenital syphilis (Temmerman *et al*., 2000). Definitive diagnosis of syphilis has been difficult because *T. pallidum* subspecies cannot be cultured by standard bacteriological techniques (Sato *et al*., 2003).

Serological diagnosis can be carried out by two methods. First, sera are initially screened with relatively inexpensive quantitative non-treponemal assays, which include the venereal

disease research laboratory (VDRL) and the rapid plasma reagin (RPR) tests (Zarakolu *et al.*, 2002). Confirmatory tests for sera that are reactive in non-treponemal assay are often carried out with a second treponemal test such as the *Treponema pallidum* haemagglutination assay (TPHA), the fluorescent treponemal antibody-absorption test (FTA-ABS), the microhaemagglutination assay for antibody to *Treponema pallidum* (MHA-TP) and various enzyme–linked immunosorbent assays (ELISA; Ebbel *et al*., 2000). *Treponema pallidum* western blot is available in some research laboratories and has similar sensitivity and specificity to those of fluorescent treponemal antibody-absorption test (FTA-ABS; Kinghorn, 2004). All these tests require laboratory settings to perform and results take considerable time to obtain (Sato *et al*., 2003).

In developing countries and areas with limited resources, laboratory facilities are often unavailable for standard syphilis tests. Blood samples may need to be transported to centralized laboratories or patients may be referred to these laboratories that are far, thus delaying diagnosis (Deperthes *et al*., 2004). Consequently, infected individuals may go home untreated since some may not proceed to these centralized laboratories or may not return for their results (Diaz *et al*., 2004). The immunochromatographic strip (ICS) test is a new treponemal test, which detects antibodies to *T. pallidum* specific antigen (Nesteroff, 2004). It is a rapid test that requires no specialized equipment and can be performed in non-laboratory settings by non-specialized staff at room temperature (Montoya *et al*., 2006), and whole blood can be used as the test sample (Siedner *et al*., 2004). The ICS test can be of great use in the diagnosis of maternal syphilis by antenatal clinic nurses to prevent debilitating effects of syphilis on pregnancy. The ICS test is a 1-step test for syphilis that detects specific antibodies to *T. pallidum* through their binding to antigen-selenium colloid that is subsequently captured by immobilized antigen forming a red line on the test strip (Morse, 2003).

1.2 Global incidence and burden of syphilis

The World Health Organization (WHO) estimates that the annual global incidence of adult syphilis is approximately 12.2 million cases (Fig.1.1), most of which occur in developing countries (WHO, 2001). The incidence of maternal syphilis varies from one country to another (Schmid, 2004). It is now a rare disease in affluent countries but remains a cause of severe pregnancy outcome in many less developed countries (Walker and Walker, 2002). Despite the fact that congenital syphilis can be prevented by detection and treatment of infected pregnant mothers, it still occurs with distressing frequency in many parts of the world (Conway, 2007).

In North America and Western Europe, few babies are born with congenital syphilis (Peeling and Ye, 2004). This low frequency is largely due to the control of early-acquired infections of syphilis through mass screening of all pregnant women (Deperthes e*t al*., 2004). However, recent reports suggest a re-emergence of congenital syphilis in several developed countries as a consequence of an upsurge of infectious syphilis in several major European and North American cities (French, 2007). There has been an equally striking increase in congenital syphilis in rural areas of Eastern Europe and Central Asia (Walker and Walker, 2002). There is limited information on the prevalence of syphilis among pregnant women in Asian countries. Studies from China in the 1990s found rates between less than 1% to 5% (Walker and Walker, 2002). High rates of seropositivity (3-8%) have consistently been reported at antenatal clinics in Africa, where congenital syphilis may account for 1% of admission to pediatric wards (Salojee *et al*., 2004).

In African countries, syphilis is the leading cause of perinatal mortality (21%; Myer *et al*., 2003). Women with early syphilis are likely to infect their fetuses (Walker and Walker,

2002). In Ethiopia, it was estimated that 5% of all fetuses each year were lost through syphilis-induced abortion while in Zambia 24% of still births and 30% of perinatal mortality were attributed to congenital syphilis (Salojee *et al*., 2004). Data on the impact of congenital syphilis on health systems originate from better-resourced settings based mostly in developing countries. The cost of hospitalization for infants with congenital syphilis is more than three times higher that of caring for an infant without the disease (Salojee *et al*., 2004).

In Kenya, the World Health Organization (WHO) estimates in 2004 showed that syphilis constituted 3.8% of antenatal clients (Deperthes *et al*., 2004). There is limited data on fetal and neonatal consequences of untreated syphilis. Adverse pregnancy outcomes are 12 times more likely in women with syphilis than in seronegative women (Diaz *et al*., 2004). The World Health Organization also estimates that each year, maternal syphilis is responsible for 460,000 abortions or stillbirths, 270,000 cases of congenital syphilis and the birth of 270,000 low birth weight or premature babies (Finelli *et al*., 1998; Peeling and Ye 2004; Hawkes *et al*., 2004). The toll of maternal syphilis exceeds that of other neonatal infections such as HIV and tetanus, which have attracted global attention (Hawkes *et al*., 2004). In sub-Saharan Africa, an estimated two million or more women with active syphilis become pregnant each year. In an estimated 1,640,000 of them, infection remains undetected during pregnancy (Gloyd *et al*., 2001).

Fig. 1.1: The global distribution of syphilis cases. Regions most affected are circled. (Adapted from WHO, 2001). WHO estimates that 12 million new cases of syphilis occur worldwide each year.

1.3 Statement of the problem and justification of the study

Syphilis is particularly common among pregnant women in sub-Saharan Africa with prevalence rates ranging from 2.5% to 17.4% and it accounts for 26% of stillbirths and 11% of neonatal deaths (Walker and Walker, 2002). Antenatal syphilis as diagnosed by RPR test has a prevalence rate of 3.6% in Nairobi, Kenya (Deperthes *et al*., 2004). Most cases of syphilis are asymptomatic in women (Diaz *et al*., 2004).

Serological diagnosis and treatment of antenatal clinic attendants has been shown to be highly cost effective as a means to reduce infant mortality and morbidity (Montoya *et al*., 2006). The primary diagnostic test in current use in Kenya is the VDRL test. The test is carried out in a laboratory setting and requires a centrifuge as well as skilled personnel to

draw blood and carry out the test. Reagents for the VDRL test can only be stored in refrigerators or cool boxes, which are hardly available in resource poor settings in Kenya. Consequently, treatment rates are poor in settings where testing is centralized due to low return rates and delay in obtaining results (Hawkes *et al*., 2004). In addition doubts have been cast about the accuracy of the VDRL test (Hall and Klausner, 2004). Test specificity can be limited due to the non-specific nature of the cardiolipin antigen as biological false positive results can occur due to viral infections, autoimmune disorders, malaria and pregnancy (West *et al*., 2002).

Although concerted efforts have been made by a number of African countries to improve testing of syphilis on-site by use of the VDRL test, these efforts have been faced with serious logistical problems (Hawkes *et al*., 2004). Results obtained at clinics also differ from those obtained at reference laboratories (West *et al*., 2002). Screening for large numbers of patients using the VDRL test is cumbersome and time consuming due to lack of automation for performance of these tests (Stoner, 2007). A simple, rapid, antibody detection test based on specific treponemal antigen would be useful for screening syphilis in pregnant women. Although several rapid tests have been developed, they have not validated. Hence for these tests to be routinely used, they must be validated in the population where they are to be applied. Moreover, the prevalence rate of syphilis in many parts of Kenya has not been established. The purpose of this study was to validate the ICS test, a new rapid treponemal test kit for adoption for local use in resource poor settings in Kenya in the screening of antenatal syphilis in women and to determine prevalence of antenatal syphilis in Eldoret, Kenya. It is apparent that universal screening for syphilis in antenatal programmes remains one of the most appropriate ways to prevent syphilis and the associated morbidity and mortality (Temmerman *et al*., 2000).

1.4 Research questions

i) What is the prevalence of syphilis in antenatal women in Eldoret municipality?

ii) What is the sensitivity and specificity of ICS test in the diagnosis of antenatal syphilis?

iii) What is the positive predictive value and negative predictive value of ICS test in the diagnosis of antenatal syphilis in women?

1.5 Null hypothesis

There is no significant difference in the performance of the ICS test and the VDRL test in the screening of antenatal syphilis.

1.6 Objectives of the study

1.6.1 General Objective

To evaluate the immunochromatographic strip (ICS) test for rapid diagnosis of antenatal syphilis in women in Eldoret.

1.6.2 Specific Objectives

a) To determine the prevalence of syphilis in antenatal women in Eldoret municipality.

b) To determine the sensitivity and specificity of the ICS test in the detection of antenatal syphilis in women.

c) To determine the positive predictive value and negative predictive value of the ICS test in detection of antenatal syphilis in women.

1.7 Significance of the study

The findings of this study will provide new insights on the performance of the immunochromatographic strip test in the screening of antenatal syphilis in Kenya for possible adoption by policy makers. It will also provide data on prevalence of syphilis in Eldoret.

CHAPTER TWO: LITERATURE REVIEW

2.1 Historical perspective of syphilis

The modern era of syphilis research began with the experimental transmission of the disease by Metchinkoff and Roux in 1903 from men to anthropoid apes (Metchinkoff and Roux, 1903). Schaudin and Hoffman discovered the causative agent of syphilis, *T. pallidum* in lesion exudates in 1905 (Schaudin and Hoffman, 1905). In 1906, Wasserman, Neisser and Bruck detected the presence of lipoidal antibodies in serum of infected individuals (Wasserman *et al*., 1906).

2.2 Classification of *Treponema pallidum*

Treponema pallidum belongs to the class of prokaryotic organisms: nuclear membrane absent, DNA not subdivided into chromosomes, multiplication occurs through cross fission and cell wall contains a mureine-macromolecule (Norris and Larsen, 1995). The genus *Treponema*, order *spirochaetales*, family *spirochaetaceae,* includes human pathogens and human nonpathogens (Rhodes and Luger, 1987). The human pathogens are *T. pallidum* subspecies *pallidum* (venereal syphilis), *T. pallidum* subspecies *endemicum* (endemic syphilis), *T. pallidum* subspecies *carateum* (pinta) and *T. pallidum* subspecies *pertenue* (yaws). These pathogenic treponemes are very closely related to the extent that they are distinguished primarily by their patterns of pathogenesis in humans and experimentally infected animals (Norris and Larsen, 1995).

2.3 Structure of *Treponema pallidum*

The spirochete *Treponema pallidum* is a thin, motile, spiral shaped bacterium (Fig.2.1; Salazar *et al*., 2002). Schaudin and Hoffman discovered *T. pallidum* in 1905 and

demonstrated its presence in the primary lesion and in the adjacent lymph glands of syphilitic patients (Schaudin and Hoffman, 1905). Their spiral cellular shape is approximately 16 to 18 bends consisting of an outer sheath, periplasmic space with periplasmic flagella, and a peptidoglycan layer (Cox *et al*., 1992).

Fig. 2.1: Structure of *Treponema pallidum* (Adapted from French, 2007).

2.4 Transmission of syphilis

Transmission of syphilis occurs through direct lesion contact, sexual contact with infected individuals, oral ingestion of menstrual blood and vaginal secretions in acquired syphilis (Watson-Jones *et al*., 2002). In acquired syphilis, *T. pallidum* is ideally suited for sexual transmission (venereal syphilis) since it requires moisture and tissue medium for survival (Rhodes and Luger, 1987). However, according to Cockayne, (2002), rarely, has syphilis

been acquired by transfusion of infected fresh human blood. This is a serious problem where screening of blood for transfusion is not thorough.

Congenital syphilis results from vertical transmission of *T. pallidum* from the infected mother to the fetus across the placenta *in utero*, but neonates may also be infected during passage through an infected birth canal at delivery (Cockayne, 2002). Early congenital syphilis tends to be manifested when mothers are afflicted with early syphilis during the course of pregnancy resulting in stillbirth or fulminant infection of the newborn while late manifestations of congenital syphilis are the outcome of chronic untreated infection and results in multiple stigmata (Norris and Larsen, 1995).

2.5 Pathogenesis of *Treponema pallidum* infection

Untreated syphilis may be a progressive disease with primary, secondary, latent and tertiary stages (Rhodes and Luger, 1987). *Treponema pallidum* enters tissues by penetration of intact mucosae or abraded skin (Gerbase *et al*., 1998). The bacterium rapidly enters the lymphatics, is widely disseminated via the bloodstream and may lodge in any organ. The exact infectious dose for man is not known but in experimental animals fewer than 10 organisms are sufficient to initiate infection (Cockayne, 2002). The bacteria multiply at the initial entry site, and a chancre, a lesion characteristic of primary syphilis forms after an average incubation period of 3 weeks (Morton, 1992). The chancre is painless and most frequently on the external genitalia but it may occur in the cervix, peri-anal area, in the mouth or anal canal (French, 2007). Chancres usually occur singly, but in immunocompromised individuals such as those with the HIV, multiple or persistent chancres may develop (Katz *et al*., 1993).

The chancre heals spontaneously within 3-6 weeks, and 2-12 weeks later the symptoms of secondary syphilis develop (Rompalo *et al*., 2001a). They are highly variable and widespread but most commonly involve the skin where macular or pustular lesions develop, particularly on the trunk and extremities (Lewis and Young, 2006). The lesions of secondary syphilis are highly infectious and gradually resolve then a period of latent infection is entered, in which no clinical manifestations are evident, but serological evidence of infection persists (Morton, 1992). Relapse of the lesions of secondary syphilis is common, and latent syphilis is classified as early or late (Hutto, 2001). Individuals with latent late syphilis are not generally considered infectious, but may still transmit infection to the fetus during pregnancy and their blood may remain infectious (Tortora *et al*., 1998). Late or tertiary syphilis, which may develop decades after the primary infection, is a slowly progressive, destructive inflammatory disease that may affect any organ (Jackman and Radolf, 1989). The three most common forms of late syphilis are neurosyphilis, cardiovascular syphilis, and gummatous syphilis (Norris and Larsen, 1995). Isolation of *T. pallidum* from patients with late syphilis is usually impossible, and much of the observed pathology may be due to autoimmune phenomena (Cockayne, 2002).

2.6 Immune responses to *Treponema pallidum* infection

Infection with *T. pallidum* provokes a strong humoral and cell-mediated immune response early in the course of infection. Serological tests demonstrate antibodies to *T. pallidum* early in the primary stage of infection which remain readily detectable throughout the course of infection and which are utilized to monitor the response to therapy following treatment (Todd *et al*., 2006). The resolution of both the primary and the secondary manifestations of infection correlate with the development of cellular immune responses both in animal models and in humans (Peeling and Hook, 2006). Despite the presence of these brisk immune

responses, without treatment, *T. pallidum* is able to survive in the human host for several decades and may continue to be transmitted or cause end-organ damage despite this host response (Rompalo *et al*., 2001a).

2.6.1 Protective immunity

Although patients who have been previously treated for syphilis can be re-infected, untreated patients appear to have some degree of immunity to repeated infection (Lewinski *et al*., 1999). In the 19th century in Dublin, Colles (1881) observed that wet nurses who breast-fed infants with congenital syphilis often developed chancres of the nipple, whereas the mothers of such infants did not, implying that they were somehow protected from repeated infection (Peeling and Hook, 2006). Subsequent studies in which prisoner volunteers in the United States were inoculated with *T. pallidum* likewise demonstrated that men with untreated syphilis did not develop chancres at the site of cutaneous inoculation, while those who had been treated for syphilis in the past, as well as those who had not been previously infected, developed infection (Van Voorhis *et al*., 1996). A similar phenomenon, referred to as 'chancre immunity' has been described in the rabbit model. Repeated immunization of rabbits with irradiated *T. pallidum* has been shown to induce complete protective immunity in rabbits (Blanco *et al*., 1990). However, antigens eliciting this protective response, as well as the relative contribution of humoral and cell mediated responses to protective immunity, are not clear (Pavia and Niederbuhl, 1985). The *Treponema pallidum* repeated gene K (Tpr) protein of the Tpr family of polymorphic multi-copy repeat proteins, identified through subtraction hybridization and differential immunological screening of a *T. pallidum* genome library, has been shown to be a target for opsonic antibody (Centurion- Lara *et al*., 1999). Immunization of rabbits with purified Tpr K offered significant, albeit incomplete, protection against infection (Peeling and Hook, 2006).

2.6.2 Cell-mediated immune responses

At all stages of the disease, syphilitic lesions are characterized by vasculopathic changes and local cellular infiltrates consisting of lymphocytes, macrophages, and plasma cells (Engelkens *et al*., 1991). The importance of cellular immune responses in containing the infection, as well as in pathogenesis, is shown by the presence of granulomata, which, in the case of gummatous disease, assume a necrotizing character (Abell *et al*., 1975). In primary chancres, CD4+ T cells and macrophages predominate, whereas in the lesions of secondary syphilis there is a majority of CD8+ cells (Salazar *et al*., 2002). This is surprising, since *T. pallidum* is believed to be an extracellular pathogen.

In lesions of both primary and secondary syphilis, increased expression of the T-helper 1 (Th1) cytokines, interleukin-2 (IL-2) and interferon-gamma (IFN-γ) is seen in humans, as has been observed in the rabbit model (Van Voorhis *et al*., 1996; Podwinskar *et al*., 2000). Circulating T lymphocytes responsive to treponemal antigens can be detected in late primary syphilis and cell-mediated immune responses peak in the secondary stage (Singh and Romanowski, 1999). Increased apoptosis of peripheral blood lymphocytes and CD4+ T cells by a Fas-mediated death pathway in patients with secondary early syphilis could account for the incomplete clearance of *T. pallidum* from the lesions, leading to the establishment of chronic infection (Fan *et al*., 2004).

2.6.3 Humoral immune responses

Circulating antibodies to *T. pallidum* can be found soon after the onset of primary syphilis and reach high titres as the infection disseminates in the secondary stage (Baker-Zander *et al*., 1985; Gerber *et al*., 1996). Human sera containing antibodies can immobilize *T. pallidum*

in the presence of complement - the basis of the old *T. pallidum* immobilization test for diagnosing syphilis and can block attachment of the organism to eukaryotic cells (Peeling and Hook, 2006). The antigens to which these immobilizing antibodies are directed are not clear. Antibody can also confer passive immunity in the rabbit model and enhance phagocytosis of *T. pallidum* in *vitro* (Blanco *et al*., 1990).

2.6.4 Innate immunity

Treponema pallidum does not contain lipopolysaccharide, but the lipoproteins present under the outer membrane are strongly immunogenic and have been shown to activate the innate inflammatory response via the toll like receptor-4 (TLR4; Radolf *et al*., 1995). These proteins are not surface-exposed, and live *T. pallidum* elicits a much less marked inflammatory response than *T. pallidum* lysates (Brightbill *et al*., 1999). In *vivo*, lipoproteins of *T. pallidum* are thought to gain access to TLRs on the surface of macrophages following degradation of organisms in phagolysosomal vacuoles (Peeling and Hook, 2006).

2.7 Clinical features of syphilis

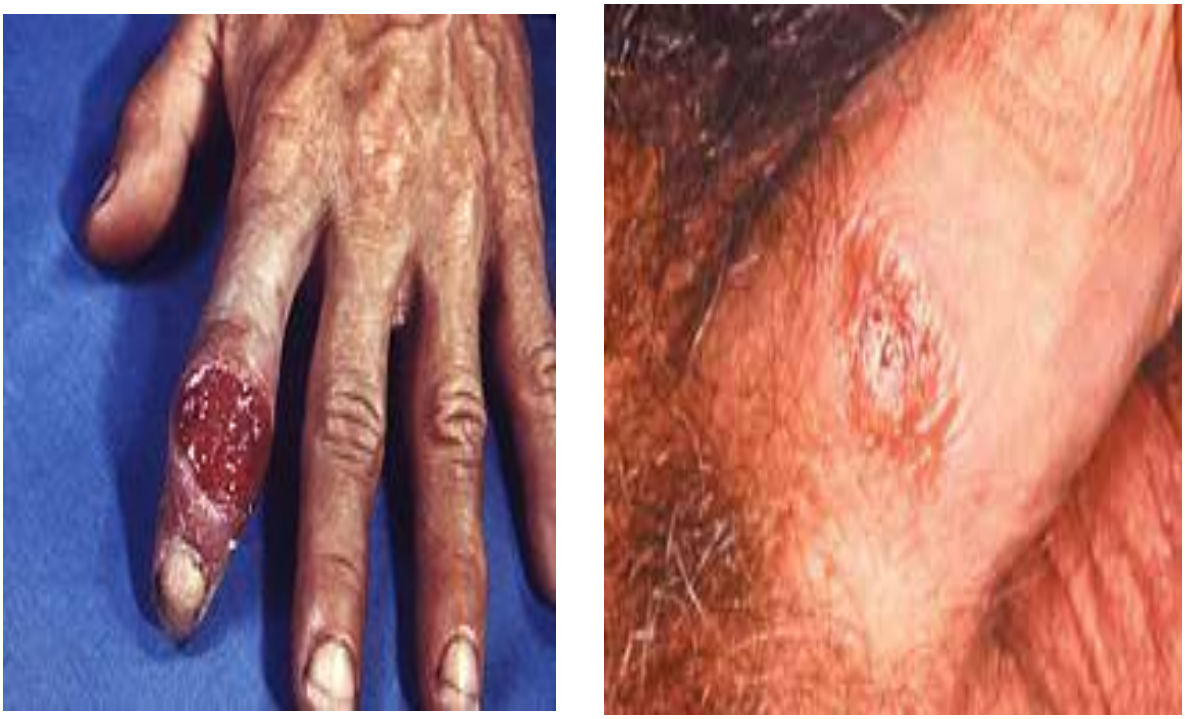
Syphilis is a variable chronic infection with many diverse clinical manifestations that occur in distinct stages (Swartz *et al*., 1999). It has a myriad of presentations and can mimic many other infections and immune-mediated processes in advanced stages. Hence, it has earned the nickname “the great imposter” (Singh and Romanowski, 1999). The complex and variable manifestations of the disease prompted Sir William Osler to remark that, “The physician who knows syphilis knows medicine” (Todd *et al*., 2006). It is a multisystem infection, which can be spread venereally or vertically. Syphilis is classified as acquired or congenital (Gerbase *et al*., 1998).

2.7.1 Acquired syphilis

Acquired syphilis is divided into early (primary, secondary and early latent < 2 years of infection) and late (late latent > 2 years, tertiary including gummatous, cardiovascular and neurological involvement, the latter two are also sometimes classified as quaternary) syphilis (Larsen *et al*., 1995).

2.7.1.1 Primary syphilis

Between 10 and 90 days after exposure to the pathogen a papule develops at the site of inoculation (Alam *et al*., 2000). It is usually single and painless but can be multiple and painful (French, 2007). This ulcerates to become a painless, firm chancre (Fig. 2.2). There is usually local, non-tender painless regional lymphadenopathy in association (Leao *et al*., 2006). The primary lesion may go unnoticed even without treatment especially if it is on the cervix or within the rectum (Hall and Klausner, 2004). Healing occurs spontaneously within 2-3 weeks (Todd *et al*., 2006).



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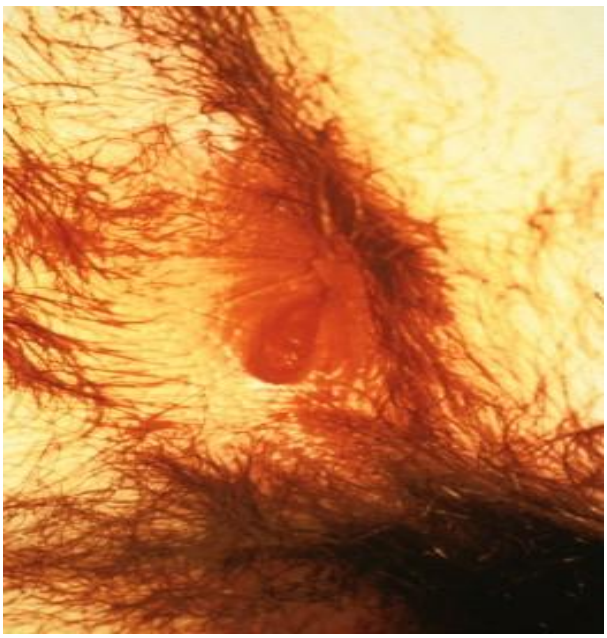
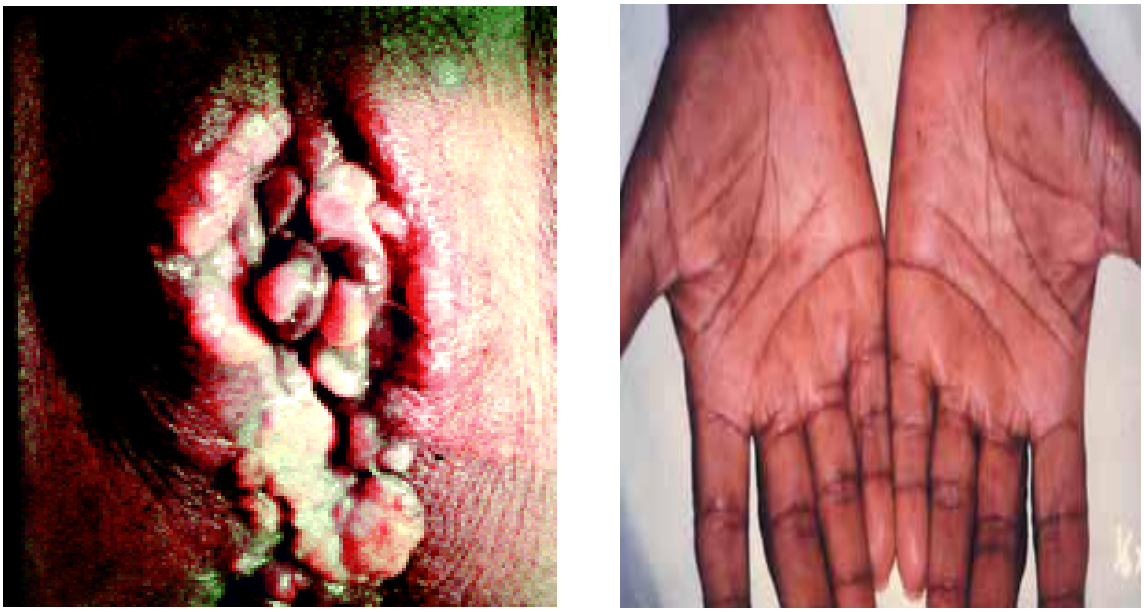


c)

Fig. 2.2: Primary syphilis; a) Chancre on finger b) Primary chancre on penis c) Perianal chancre (Adapted from French, 2007).

2.7.1.2 Secondary syphilis

This is a stage of infection that develops about 4-10 weeks after the appearance of the primary lesion and has a wide range of presentations. It is characterized by nonpruritic, maculopapular rash involving the entire body, including the scalp, palms and soles of feet, often with generalized lymphadenopathy (Fig. 2.3b; Lewis and Young, 2006). These manifestations are termed the dermatitis-arthritis syndrome. In moist areas of the body, the rash becomes flat broad whitish lesions known as condylomata lata (wart-like plaques; Fig. 2.3a; Hyman, 2006). Mucous patches may also appear on the genitals or in the mouth (Rompalo *et al*., 2001a; Golden *et al*., 2003). All of these lesions are infectious and harbor active treponeme organisms (Rolfs *et al*., 1997). Other symptoms common at this stage include flu-like illness characterized by fever, swollen lymph glands, sore throat, patchy hair loss, headaches, weight loss, muscle aches, and tiredness (Rompalo *et al*., 2001b). Rare manifestations include an acute meningitis that occurs in about 2% of patients, hepatitis, renal disease, hypertrophy gastritis, patchy proctitis, ulcerative colitis, rectosigmoid mass, arthritis, periostitis, optic neuritis, interstitial keratitis, iritis, and uveitis (Hutto, 2001). During secondary infection, the immune reaction is at its peak and antibody titers are high (Todd *et al*., 2006). However, the primary chancre may still be present.



a) b)

Fig. 2.3: Secondary syphilis a) Condylomata lata b) Rash on palms (Adapted from French, 2007).

2.7.1.3 Latent syphilis

Latent syphilis is defined as having serologic proof of infection without signs or symptoms of disease (Stoner, 2007). Latent syphilis is further described as either early or late (CDC, 2006). Early latent syphilis is defined as having syphilis for two years or less from the time of initial infection without signs or symptoms of disease (CDC, 2002; Todd *et al*., 2006). Late latent syphilis is infection for greater than two years but without clinical evidence of disease (Ho, 2002). The distinction is important for both therapy and risk for transmission (Schulz *et a1*., 1987). Without treatment, the infected person still has syphilis even though there are no signs or symptoms. The disease remains in the body, and it may begin to damage the internal organs, including the brain, nerves, eyes, heart, blood vessels, liver, bones and joints. This internal damage may show up many years later in the late or tertiary stage of syphilis (Peeling and Hook 2006).

2.7.1.4 Tertiary syphilis

Tertiary syphilis usually occurs 1-10 years after the initial infection, though in some cases it can take up to 50 years (Hibbs and Gunn, 1991). This stage is characterized by the formation of "gummas" which are soft, tumor-like balls of inflammation known as granulomas (Fig. 2.4 Hall and Klausner, 2004). The granulomas are chronic and represent an inability of the immune system to completely clear the organism. Gummas were once readily seen in the skin and mucous membranes although they tend to occur internally in recent history (Hutto, 2001). They may appear almost anywhere in the body including in the skeleton (Chung *et al*., 1991). The gummas produce a chronic inflammatory state in the body with mass-effects upon the local anatomy (Hook and Peeling, 2004).

Other characteristics of untreated tertiary syphilis include neuropathic joint disease, which is a degeneration of joint surfaces resulting from loss of sensation and fine position sense (Heimberger *et al*., 1993). The more severe manifestations include neurosyphilis and cardiovascular syphilis (Danielsen *et al*., 2004). In one study of untreated syphilis, 10% of patients developed cardiovascular syphilis, 16% had gumma formation, and 7% had neurosyphilis (Swartz et *al*., 1999). Neurological complications at this stage can be diverse. In some patients, manifestations include generalized paresis of the insane which results in personality changes, changes in emotional affect, hyperactive reflexes, and Argyll-Robertson pupil (Simon, 1985). *Tabes dorsalis*, also known as locomotor ataxia, a disorder of the spinal cord, often results in a characteristic shuffling gait (Margo and Hamed, 1992).

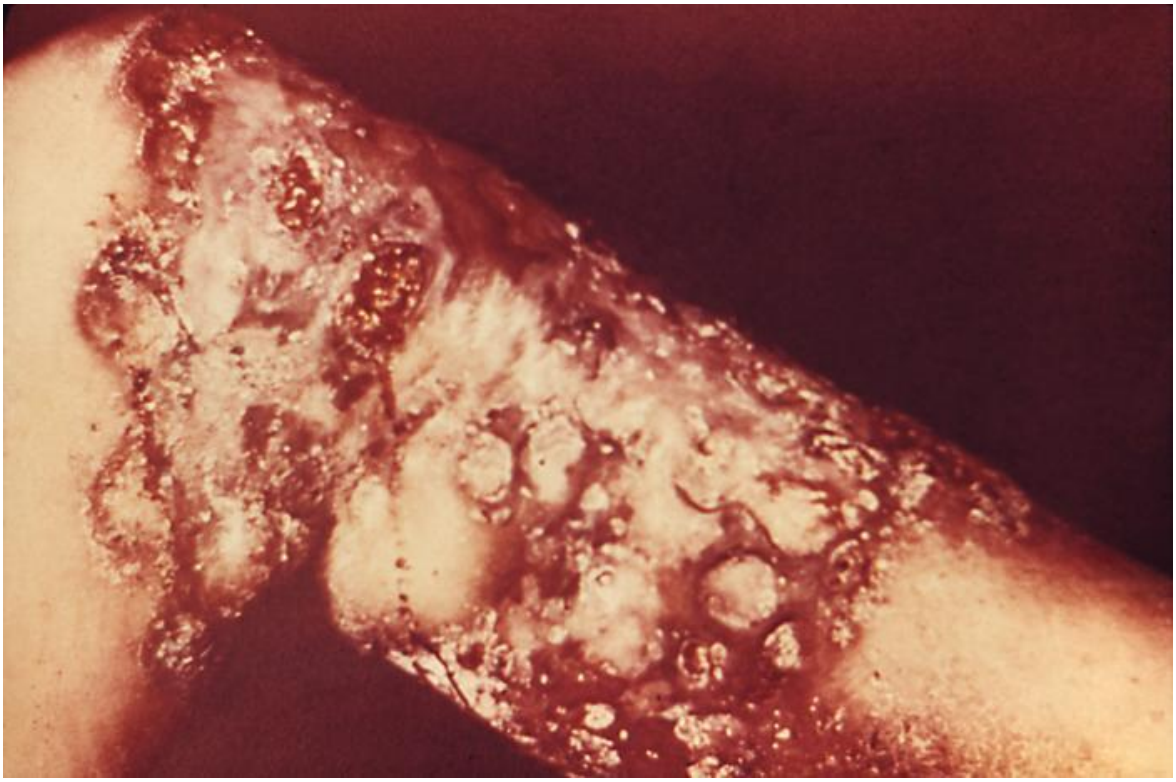


Fig. 2.4: Tertiary syphilis; Gummatous syphilis (Adapted from Hardin, 2008).

2.7.2 Congenital syphilis

Pregnant women who are infected with syphilis can transmit the infection to their fetus, causing congenital syphilis, with serious adverse outcomes for the pregnancy in up to 80% of cases (Rawstron *et al*., 1993). An estimated two million pregnancies are affected annually; approximately 25% of these pregnancies end in stillbirth or spontaneous abortion, and in a further 25% the newborn has a low birth weight or serious infection, both of which are associated with an increased risk of perinatal death (Walker and Walker, 2002). However, there is still a general under appreciation of the burden of congenital syphilis (Saloojee *et al*., 2004). Unlike many neonatal infections, congenital syphilis is a preventable disease, which could be eliminated through effective antenatal screening, and treatment of infected pregnant women. Elimination of congenital syphilis would reduce the numbers of miscarriages, stillbirths, preterm and low-birth-weight infants and perinatal deaths.

Congenital syphilis is classified as either early or late depending on whether it presents before or after 2 years of age (French, 2007). The likelihood of transmission is directly related to the stage of maternal syphilis during pregnancy, or the stage of pregnancy when infection is acquired (WHO, 2007). In early maternal syphilis the maternal–fetal transmission rate can be up to 80%, whereas in late syphilis infectivity decreases (Berman, 2004). The concentration of spirochetes in the blood is highest during the first two years after infection and decreases slowly thereafter as a result of acquired immunity. Thus the risk of infecting sexual partners is highest during the first two years, then virtually ceases, although the risk of maternal-fetal transmission continues. The course of maternal infection does not seem to be altered by pregnancy (Berman, 2004).

About a third of babies born to mothers with early syphilis are born without infection and a third with congenital syphilis; a third of pregnancies will result in miscarriage or stillbirth (WHO, 2007). Congenital syphilis is contracted by transplacental transmission of spirochetes, which can occur at any stage of pregnancy and is more common when mothers have primary and secondary infection rather than latent infection (Hayman, 2006). Almost all cases of congenital syphilis are easily prevented by antenatal screening for syphilis and effective treatment during pregnancy (Bronzan *et al*., 2007). More newborn infants are affected by congenital syphilis than by any other neonatal infection, including HIV infection and tetanus which are currently receiving global attention (Schmid, 2004).

Untreated syphilis can profoundly affect pregnancy outcome, resulting in spontaneous abortion, stillbirth, premature delivery, or perinatal death (Wendel, 1988; Wendel *et al*., 1989). Prematurity and low birth weight has been reported for 10 to 40% of infants born to untreated mothers (Mascola *et al*., 1985). Congenital syphilis is also a potentially treatable

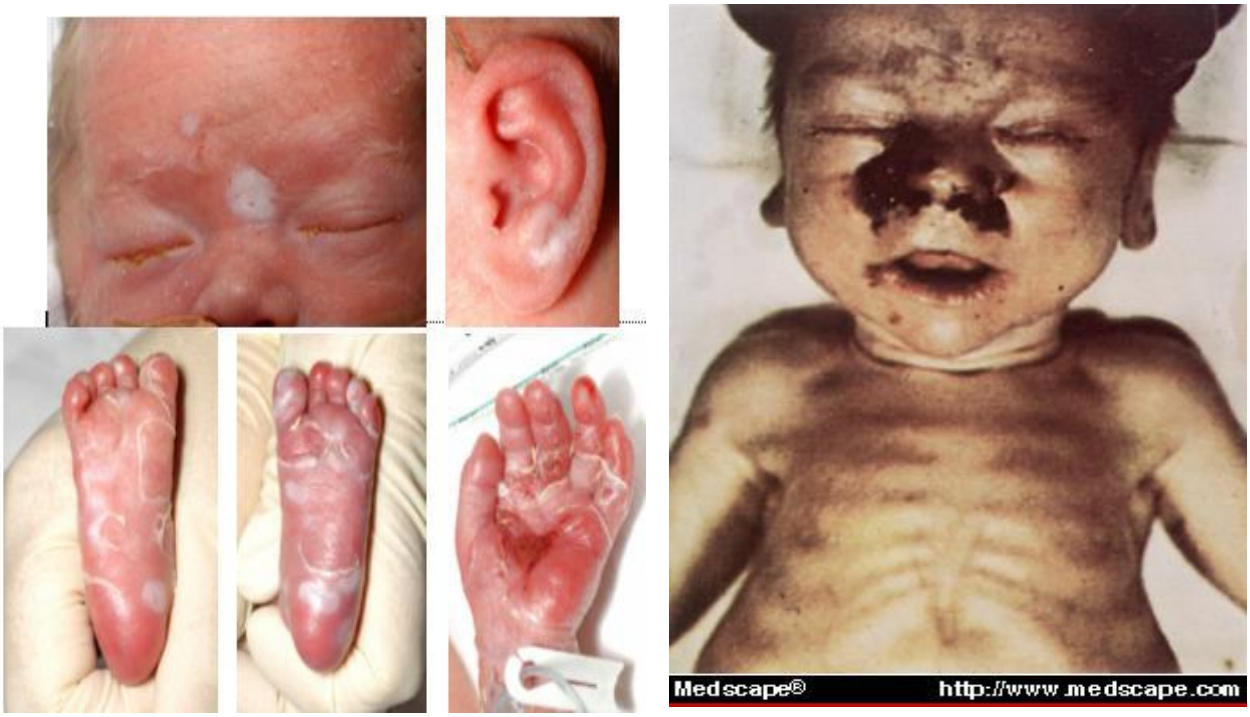
cause of nonimmune hydrops fetalis (Barton *et al*., 1992; El Tabbakh *et al*., 1994). The rate of vertical transmission in untreated women is 70 to 100% for primary syphilis, 40% for early latent syphilis, and of the earliest clinical manifestations, occurring in 4 to 22% of infants. The nasal discharge may be profuse and purulent or blood tinged and is highly infectious. Hepatomegaly with or without splenomegaly (Fig. 2.5b) occurs in 33 to 100% of patients (Satin *et al*., 1992). Glomerulonephritis resulting in nephrotic syndrome may also occur. The infant may have generalized lymphadenopathy with discrete, hard, non-tender nodes (Fig. 2.5a; Delport and Rothberg, 1992). Vesiculobullous lesions and an erythematous maculopapular rash occur in one-third to one-half of patients and frequently involve the palms or soles (Fig. 2.5a).

Asymptomatic central nervous system involvement manifesting as CSF abnormalities of lymphocytosis, elevated protein levels, and positive serologic test occur in up to 80% of infected infants (Berry and Dajan, 1992). In the prepenicillin era, acute syphilitic meningitis occurring between 3 and 6 months of age was seen in 5 to 15% of infected patients, but such cases are now rare (Ghadouane *et al*., 1995). Bone lesions develop within 8 months of birth in early congenital syphilis (Reginato 1993). Osteochondritis (Parrot's pseudoparalysis) is the most common and earliest lesion, affecting mainly the upper limbs and knees. It can result in an asymmetric, painful, flaccid pseudoparalysis (Humphrey and Bradford, 1996). The radiographic appearance demonstrates irregular epiphyseal lines, decalcification of subchondral bone, cupping of the diaphysis, irregularity of the cartilage adjacent to the epiphysis, and periosteal thickening (Rassool and Govender, 1989). Diaphyseal periostitis is asymptomatic and does not produce radiologic signs until after 3 months of age. The bones most often affected are the tibias, followed by the tubular bones of the hands and feet and, less frequently, the clavicles and skull (Reginato, 1993). Osteomyelitis due to gummatous

involvement of the diaphyseal-metaphyseal junction of long limb bones occurs rarely (Delport and Rothberg, 1992).

Late manifestations of congenital syphilis include Hutchinson's riad of interstitial keratitis, peg-shaped upper incisors, and eighth-cranial-nerve deafness (Evans and Frenkel, 1994). The hearing loss is sudden and usually occurs at 8 to 10 years of age. Other characteristic findings include frontal bossing, short maxillae, saddle nose, protruding mandible, high arched palate, mulberry molars, perioral fissures (rhagades), bilateral knee effusions, sternoclavicular thickening, saber shins, flaring scapulas, mental retardation and hydrocephalus (Hayman, 2006). Bilateral hydrarthrosis, or Clutton's joints, involving knees and elbows typically occur between 8 and 15 years of age (Battin and Voss, 2007). As many as one fourth to one-third of patients older than 2 years have asymptomatic neurosyphilis (Jacques and Qureshi, 1992). Symptomatic neurosyphilis develops rarely, with juvenile paresis developing in 1 to 5% of patients with congenital syphilis.

The symptoms of juvenile paresis are usually more severe than those of acquired paresis and the typical onset occurs in puberty (Singh and Romanowski, 1999). *Necrotizing funisitis*, a rare deep-seated inflammatory process of the umbilical cord, was noted to be virtually diagnostic of congenital syphilis and occurs almost exclusively in preterm infants with an average gestational age of 32 weeks (Fojaco *et al*., 1989). The majority of infants with necrotizing funisitis are stillborn and the remainder dies within a few weeks of birth (Battin and Voss, 2007).



a) b)

Fig 2.5: Children suffering from congenital syphilis a) blistered lesions b) hepatomegaly (Adapted from Battin and Voss, 2007)

2.8 Prevention and control of syphilis

The surest way to avoid transmission of sexually transmitted diseases, including syphilis, is to abstain from sexual contact or to be in a long-term monogamous relationship with a partner who was tested and is known to be uninfected (CDC, 1998). Correct and consistent use of latex or polyurethane condoms can reduce the risk of syphilis only when the infected area or site of potential exposure (the sore) is protected (CDC, 2006).

Surgeon General Thomas Parran in 1937 defined a five point syphilis control plan including public education, screening, clinical treatment, partner notification, and prophylactic treatment (Parran, 1937). This framework still forms the basis of syphilis control today. However, given the changes in the epidemiology of syphilis, additional strategies are called

for to improve syphilis control (St. Louis, 1996). The sequencing of the *T. pallidum* genome has also led to renewed hope for the elimination of syphilis (St. Louis and Wasserheit, 1998).

2.8.1 Public Health Education

Educating the general public about the consequences and prevention of syphilis and other sexually transmitted diseases (STIs) is paramount in the primary prevention of these diseases (Stamm *et al*., 1988; Cates *et al*., 1996). Effective education could result in earlier recognition and therefore presentation for medical care with symptoms and signs of disease or in behavior modification strategies, such as safer sex practices (Werdegar *et al*., 1987). Condoms, although clearly effective in preventing the transmission of HIV and other STDs, are not used regularly by persons at risk (Roper *et al*., 1993; Swartz *et al*., 1999). More innovative ways of promoting condom use and provision of condoms at low cost are necessary. Targeted media campaigns, especially in areas or communities with high syphilis rates, are important (Cates *et al*., 1996).

2.8.2 Screening

Screening for syphilis is carried out for several reasons, including prevention of the complications of syphilis, prevention of congenital syphilis, and reduction of the transmission of syphilis, which in turn will reduce the transmission of HIV through genital ulcers (Schmid, 1996; St. Louis, 1996). Screening generally detects patients with noninfectious latent disease and only occasionally detects those with symptoms or signs (Schmid, 1996). The efficiency of case finding can be significantly improved by using epidemiologic features to focus screening efforts; that is, screening of high-risk groups will identify more infectious cases (primary, secondary, and early latent) than will screening of the general population, which will identify more noninfectious (late latent) cases (CDC,

1991). Not only should populations known to be at high risk be targeted for intervention, but also specific strategies should be developed for each group (WHO, 2007).

The concept that a "core" or small subset of persons contributes to a disproportionately large number of transmission events and eventually leads to persistence of syphilis is important (Brunham, 1991). It has been suggested that the syphilis epidemics of the 1980s and 1990s are the result of core transmission (Oxman *et al*., 1996). Therefore, targeting of such core populations for prevention of transmission should, in theory, prevent community transmission and therefore lead to lower syphilis prevalence rates. Targeting the screening and empirical treatment of persons at sites where sex and drugs are sold has been useful in the control of cocaine-related outbreaks (Hibbs and Gunn, 1991). Other high-risk groups in which routine screening is justifiable and may be central to syphilis control efforts include populations in correctional facilities (Heimberger *et al*., 1993; Blank *et al*., 1997), drug users in emergency rooms (Ernst *et al*., 1995), and patients suspected of having STDs other than syphilis (Ernst *et al*., 1991).

To prevent congenital syphilis, the CDC currently recommends that all pregnant women be screened for syphilis (CDC, 1998). This approach, even in low-prevalence situations, has been shown to be cost-effective (Terris-Presthol *et al*., 2003). However, despite the serious consequences of syphilis in pregnancy, syphilis screening in all antenatal clinics is seldom practiced effectively (Hira *et al*., 1990). Similarly, continued screening of blood for antibodies to syphilis is also important but the problem lies on the selectivity of where and when to screen for instance in antenatal clinics and blood for transfusion. Although relative prevalence rates and the risk of transmission are low, syphilis acts a surrogate marker for other STDs, especially HIV (Hutchinson *et al*., 1991).

2.8.3 Clinical Diagnosis and Treatment

Diagnosis and treatment of cases are essential components of syphilis control (Cates *et al*., 1996). Few studies have examined how many patients with syphilis recognize their symptoms or, if they do, how many present for medical attention. In 1990, an estimated 62% of patients with a diagnosis of early syphilis sought clinical services of their own accord (Cates *et al*., 1996). Early detection of disease and prompt treatment will reduce the complications and minimize further disease transmission (Hira *et al*., 1990). Treatment should be inexpensive, safe, simple, and effective (Cates *et al*., 1996). The production, widespread distribution, and compliance with national treatment guidelines will aid in these objectives (CDC, 2006).

2.8.4 Partner Notification

Historically, partner notification with evaluation, treatment, and follow-up has been essential to limit the spread of disease (Goh and Thornton, 2007). However, this method, in which health department personnel visit, inform, and interview all named sexual partners, is expensive, and recent studies have suggested that the yield may be very low (Oxman, 1996). Due to the high cost, this approach will probably have to be used more selectively in the future (St. Louis, 1996).

Advising the individual patients to refer their sexual partners has the advantage of reduced cost but is likely to be unreliable and certainly is difficult to evaluate and monitor (Cates *et al*., 1996). In addition, partner notification in certain situations, for example, cocaine-related outbreaks, may not be effective, since cocaine users either do not provide enough information to enable the identification of their sexual partners or may have multiple anonymous partners (Rolfs *et al*., 1990; Greenberg *et al*., 1991). Recent studies have

confirmed the effectiveness of approaches involving cluster interviewing (Engelgau *et al*., 1995). The time frames for partner notification for primary, secondary, and early latent syphilis are 3, 6, and 12 months, respectively, before the development of symptoms in the index case (CDC, 1998). For contacts of patients with late latent syphilis, long-term partners and children should be evaluated. The mother of a patient with congenital syphilis and her sexual partner(s) should also be assessed (Peterman *et al*., 1997).

2.8.5 Prophylactic Treatment

Since it is not possible to predict which contacts of individuals with infectious syphilis will develop the disease, "epidemiologic" or prophylactic treatment is recommended for all contacts (Hutto, 2001). This approach, together with partner notification, has been effective in controlling epidemics of syphilis (Lee *et al*., 1987). "Mass" treatment of populations with a high prevalence of infection has also been effective (CDC, 2006).

2.9 Control of congenital syphilis

According to Schmid, (2004) understanding the causes of congenital syphilis is important because interventions can be made at each step (Schmid, 2004).

2.9.1 Preventing infection in women

Congenital syphilis can be prevented, either through prevention or detection of infection in pregnant women. Programmes promoting safer sex or control of sexually transmitted infection in the community will prevent maternal infection. However, if an infected woman becomes pregnant, only screening programmes can prevent the effects of maternal infection on the fetus; these programmes must be implemented, although not necessarily exclusively, during ANC (Schmid, 2004).

2.9.2 Access to antenatal care

In developing countries, ANC is often either unavailable or not accessed. In a survey covering an estimated 84% of the population of developing countries, 68% of urban and 39% of rural women were estimated to have access to ANC (Bulatao and Ross, 2002), whereas in 1996, WHO estimated that 68% of women received ANC (WHO,1997). In sub-Saharan Africa, an estimated 63-73% of pregnant women received ANC, but this may be an overestimate (Gloyd *et al*., 2001). The reasons for non-attendance for ANC range from fear of medical care to nonexistent services (Southwick *et al*., 2001).

In industrialized countries, women with syphilis often have other problems, for instance drug use, that interferes with access to ANC (Webber *et al*., 1993). In developing countries, women with syphilis are less likely than those in industrialized countries to have risk factors (Webber *et al*., 1993) that distinguish them from women without syphilis (McDermott *et al*., 1993; Lumbiganon *et al*., 2002). But there is ample evidence that women who deliver an infant with congenital syphilis did not access ANC (Swingler *et al*., 1993; Mobley *et al*., 1998) and this is probably the major risk factor for congenital syphilis in the developing world.

2.9.3 Early access to antenatal care

To prevent congenital syphilis, women must access ANC early in pregnancy. Although it was once thought that the fetus was protected from infection until the twentieth week of gestation by an effective placental barrier (Berman, 2004) this has recently been found to be untrue. In developing countries, the first ANC visit generally occurs at 5-6 months of gestation (WHO, 1997), too late to effectively prevent congenital syphilis. In both industrialized and developing countries, accessing ANC for the first time in the second half

of pregnancy is common among women who have infants with congenital syphilis (Warner *et al*., 2001; Gust *et al*., 2002).

2.9.4 Provision of syphilis testing in antenatal care programmes

Many ANC programmes do not provide syphilis testing and there may be no national policy for this. In sub-Saharan Africa, only 17 (77%) of 22 countries have such a policy and most countries lack the practical means to implement it (Gloyd *et al*., 2001). Serological screening for syphilis using standard RPR or VDRL testing requires that a complicated chain of events takes place: equipment and personnel to draw blood, transport of specimens to the laboratory, a properly equipped and functional laboratory, and a system for reporting results back to the clinic (Peeling and Hook, 2006). The inability to maintain a syphilis testing service that requires transportation of blood to a centralized laboratory for testing has been identified in numerous studies as a major obstacle (Deperthes *et al*., 2004). A recent survey of ANC clinics in South Africa found only four (29%) out of 14 had a functioning testing system for syphilis, despite a national policy of screening twice during pregnancy; lack of transport was the single biggest obstacle to testing (Bronzan *et al*., 2002).

2.9.5 Prompt test results

Results must be returned to the clinic and must reach the woman concerned promptly. For this, women must either return to the clinic or there must be a notification system in place; frequently, neither occurs (Schmid, 2004). However, an estimated 90% of pregnant women in developing countries who access ANC once, do so a second time (WHO, 2001), although the second visit may be considerably later than the first. One study in South Africa asked women to return 14 days following blood sampling to receive test results and therapy (Rotchford *et al*., 2000; West *et al*., 2002). The mean number of days between sampling and

initial therapy was 20, and 19% of women were never treated, presumably because they were never notified — it is likely that in a nonstudy situation these figures would be worse. A confidential system to notify women of their results at home would be an attractive alternative.

The logistical problems with the performance of reaginic antibody tests, like RPR or VDRL at centralized laboratories have led to efforts to promote testing at clinics (decentralized testing). There are difficulties in this approach: staff must be willing, have sufficient time, and be trained to provide testing, and materials must be available and stored appropriately (Maggwa *et al*., 2001). Also, results obtained at clinics differ from those obtained at reference laboratories (Delport and van den Berg, 1998; Patel *et al*., 2001). Nevertheless, studies have universally shown that decentralized testing leads to more women with syphilis being detected and treated than centralized testing. Furthermore, it is cost-effective, and nursing staff prefer it (Bronzan *et al*., 2002), making it far more likely to be implemented and sustained. The difficulties with conducting RPR testing at clinics have led to the development of technologically simple ICS tests (Peeling and Ye, 2004). Although (favourable) results of field trials are only now becoming available (West *et al*., 2002), initial results show their simplicity is preferred by nursing staff to reaginic testing (Bronzan *et al*., 2002) and their favourable performance characteristics make them more cost-effective than decentralized reaginic testing (WHO, 2007).

2.9.6 Appropriate treatment

For appropriate treatment, antimicrobials must be available, but this is often not the case (Bulatao and Ross, 2002). The treatment must be available at the ANC clinic, as referral for therapy results in fewer women being treated (only 24.5% of infected women in one study;

Swingler *et al*., 1993). Where antibiotics are available, therapy must be appropriate. Whether one, two or three weekly doses of penicillin are required for optimal prevention of congenital syphilis is uncertain (Rotchford *et al*., 2000; Watson-Jones *et al*., 2002) but, if several doses are needed, many women do not receive them (Donders *et al*., 1997; Wilkinson *et al*., 1997).

2.9.7 Women remaining uninfected during pregnancy

Women who are tested early in pregnancy and found to be seronegative must remain uninfected. Several studies in developing countries have re-tested women who were seronegative earlier in pregnancy at delivery, and found a prevalence of >1% (Schmid, 2004). It is almost certainly cost-effective to determine the prevalence at the time of delivery although the economics of testing at delivery differ from those during pregnancy because stillbirth has been avoided. The cases detected at delivery are likely to be a mixture of incident cases and cases with previously false-negative results. Both occur, and both benefit from treatment of mother and infant (Watson-Jones *et al*., 2002).

Women treated for syphilis at the beginning of pregnancy are probably at a higher risk of reacquiring syphilis during pregnancy than women without syphilis, either because of failure to notify the partner or because these women have sex in sexual networks in which syphilis occurs. Women treated for syphilis benefit from repeat testing and partner notification (Gichangi *et al*., 2000). Several studies have shown that women who fail to attend ANC are at higher risk of having syphilis than women who do (Gust *et al*., 2002). Women who have no history of having accessed ANC (or having had a serological test during pregnancy) should be tested at delivery (Schmid, 2004).

2.10 Diagnosis of syphilis

In early syphilis mucocutaneous symptoms include primary sore and roseola rash. *T. pallidum* can be detected from the primary sore by dark-field examination and direct fluorescent tests but the diagnosis must always be confirmed by serological tests (Wicher *et al*., 1999; Wicher *et al*., 2001). Nonspecific tests, such as the VDRL and RPR tests are commonly used for screening. These tests turn positive 4-5 weeks after transmission and the *Treponema pallidum* haemagglutination assay (TPHA) test slightly later. The VDRL and RPR tests are sensitive, but both transient and persistent false positive reactions may occur. Transient reactions can occur in infections and permanent false positive reactions, usually in low titers (<16), not infrequently occur in patients with autoimmune diseases (Larsen *et al*., 1995). Immunoglobulin M (IgM) antibodies can be measured with the fluorescent treponemal antibody absorption (FTA-ABS) and this test is useful particularly in the diagnosis of congenital syphilis (Egglestone and Turner, 2000).

2.10.1 Serological testing of syphilis

The lack of a method for demonstrating the presence of *T. pallidum* by growth necessitates the use of alternative methods (Fonck *et al*., 2001). Serological tests are currently the mainstay for syphilis diagnosis and management (Nesteroff, 2004). These tests detect the presence of patient antibody against *T. pallidum*. Serologic methods are further divided into two classes. One class, the nontreponemal tests, detects antibodies to lipoidal antigens present in either the host or *T. pallidum*; examples are the VDRL and RPR tests (Kinghorn, 2004). Reactivity in these tests generally indicates host tissue damage that may not be specific for syphilis. Because these tests are easy and inexpensive to perform, they are commonly used for screening and treatment follow-up, and with proper clinical signs they are suggestive of syphilis (Hall and Klausner, 2004).

The other class of test, the treponemal tests, utilizes specific treponemal antigens and oftenly used to confirm the results of the non-treponemal tests (Alam *et al*., 2000). Examples of the treponemal tests are the microhaemagglutination assay for antibody to *Treponema pallidum* (MHA-TP), *Treponema pallidum* haemagglutination assay (TPHA), *T. pallidum* particle agglutination assay (TPPA) and the fluorescent treponemal antibody absorption (FTA-ABS) test. These tests are more expensive and complicated to perform than the nontreponemal tests. On the horizon are a number of direct antigen, enzyme-linked immunosorbent assay, and PCR techniques (Ho, 2002). Several of these techniques have shown promise in clinical trials for the diagnosis of congenital syphilis and neurosyphilis that are presently difficult to diagnose (Grimprel *et al*., 1991).

2.10.1.1 Non-treponemal assays

The non-treponemal tests, sometimes referred to as non-specific, reagin or cardiolipin tests, use an antigen which contains standardized cardiolipin, cholesterol and lecithin. This antigen is a refinement of the antigen developed by Wasserman in 1908, a watery extract from a syphilitic liver (Nesteroff, 2004). It was later shown that the active component in the extract was not *T. pallidum* but lipoidal material present in many mammalian tissues. The majority of nontreponemal tests are flocculation tests and the most commonly used are VDRL and RPR tests (Baron *et al*., 1994). The antibodies to be measured are non-specific treponemal antibodies, based upon the reactivity of both IgM and IgG, of sera from patients with syphilis to non-specific cardiolipin-cholesterol lecithin antigens.

The limitations of non-treponemal serologic tests are lack of sensitivity in early and late syphilis, the possibility of biological false positive reactions associated with increased age, pregnancy, drug addition, malignancy, and autoimmune diseases, such as systemic lupus

erythematosus, as well as with viral (particularly Epstein-Barr virus and hepatitis virus), protozoal, or mycoplasmal infection (Hook and Marra, 1992; Larsen *et al*., 1995) and prozone phenomenon in secondary syphilis (Singh and Romanowski, 1999). Prozone phenomenon, false negative in undiluted serum, occurs in 1 to 2% of patients with secondary syphilis where the antibodies are in excess to block the normal antigen antibody complex formation. However, the advantages include widely available, rapid, inexpensive, mass screening, monitoring the response to treatment or re-infection as positive result is reported as titre of antibodies (Todd *et al*., 2006).

2.10.1.2 Treponemal assays

These are specific antibody tests that use *T. pallidum* or its components as the antigen (Hall and Klausner, 2004). They are used to confirm positive reactions of nontreponemal tests such as VDRL and RPR. The treponemal tests can be positive before the nontreponemal tests in early syphilis (Nesteroff, 2004). Examples of the treponemal tests are the MHA-TP, FTA-ABS, TPHA, TPPA and various ELISA. These tests are more expensive and complicated to perform than the nontreponemal tests. On the horizon are a number of direct antigen, enzyme-linked immunosorbent assay, and PCR techniques. Several of these techniques have shown promise in clinical trials for the diagnosis of early primary syphilis, congenital syphilis and neurosyphilis that are difficult to diagnose but are not readily available for routine clinical use (Grimprel *et al*., 1991). False-positive results are rare but have been found in association with mixed connective tissue and autoimmune disease, viral infections, and pregnancy (Rhodes and Luger, 1987).

2.10.1.3 Rapid treponemal tests

Given multiple incentives to accurately diagnose syphilis at the point of care, including the need to identify those infected and initiate treatment of the index case and partners early, numerous rapid treponemal tests have been developed for field-based use (Hall and Klausner, 2004). A recent review of six such tests by the WHO demonstrated a range of sensitivities from 84.5% to 97.7% and specificities from 92.8% to 98.0% (WHO, 2003). A recent evaluation of three rapid tests by the San Francisco Department of Public Health showed that the Abbott Determine Syphilis TP test (Abbott Laboratories, South Pasadena, CA) had the highest sensitivity, 88% (95% CI 0.81 to 0.96), using whole-blood venipuncture samples, with both 100% sensitivity and specificity on 99 whole-blood finger stick specimens. The test typically takes 15 minutes to perform, does not require sophisticated laboratory equipment, and costs approximately $2 per test.

2.10.2 *Treponema pallidum* identification assays

The diagnosis of syphilis depends on clinical findings; examination of lesion material for treponemes, and/or serologic tests for syphilis (Morse, 2003; Bhat, 2005). Dark-field microscopy is the main diagnostic method for primary syphilis (Larsen *et al*., 1995). Direct fluorescent-antibody testing for *T. pallidum* (DFA-TP) has also been described (Ito *et al*., 1992). However, neither dark-field microscopy nor the DFA-TP can distinguish *T. pallidum* from the other pathogenic species of *Treponema* (Larsen *et al*., 1995). Deoxyribonucleic acid (DNA) PCR has been used to identify *T. pallidum* in clinical specimens but is not readily available for routine clinical use (Grimprel *et al*., 1991). A highly sensitive reverse transcriptase PCR, which is able to detect a single treponeme, has been described (Centurion-Lara *et al.,* 1997).

2.11 Sensitivities and specificities of non-treponemal tests

The most widely used reagin antibody tests are RPR and VDRL (Baron *et al*., 1994). All non-treponemal tests have approximately the same sensitivities and specificities but may differ in reactivity levels as a result of the variation in antigen preparation (Norris and Larsen, 1995). The sensitivity of non-treponemal tests varies with the level of antibodies present during the various stages of syphilis disease (Rhodes and Luger, 1987). In primary untreated syphilis when antibody levels may be too low to detect, results may be uncreative and the sensitivity of the RPR card test is 86% (77 –100%) while that of the VDRL test is 78 % (74-87). The specificities of the RPR card test and the VDRL test are both the same at 98% in primary syphilis (Norris and Larsen, 1995). Antibody levels rise as disease progresses, titres usually peak during untreated secondary syphilis and the sensitivity of non-treponemal tests (both RPR and VDRL) is 100% (Kinghorn, 2004). In late syphilis antibody titres decline (Kinghorn, 2004). The sensitivity of RPR and VDRL are 73% and 71% respectively (Norris and Larsen, 1995).

The VDRL test is the single most useful test available for testing cerebrospinal fluid. The RPR tests are not useful for testing cerebrospinal fluid (Baron *et al*., 1994). The performance of the RPR test has been shown to be lower in field conditions. West *et al.* (2002) evaluated the performance of the RPR in field conditions in the Gambia and found out that the test specificity was 94.1% and the sensitivity 77.5% for active syphilis defined as RPR and TPHA positive (West *et al*., 2002). Similar results were obtained from a study conducted in Mozambique (Montoya *et al*., 2006). Non-treponemal tests are useful for assessing response to treatment since antibody titres decline or revert to normal after successful therapy (Kinghorn, 2004).

Biological false-positive reactions (BFP) can occur in non-treponemal tests. Either acutely (< 6 months duration) as a result of viral infections, malaria, immunization, or pregnancy, or chronically (>6 months duration) as a result of multisystem autoimmune disorders such as systemic lupus erythematosus (SLE) or other conditions including drug addiction, aging, malignancy or leprosy (Cockayne, 2002). The prozone phenomenon occurs in 2% of sera; undiluted sera give negative results because of antibody excess or the presence of blocking antibodies or both (Kinghorn, 2004).

2.12 Sensitivities and specificities of treponemal tests

The first treponemal test, the *T. pallidum* immobilization (TPI) test was introduced in 1949 by Nelson and Mayer (Nelson and Mayer, 1949). The FTA–ABS test is an indirect fluorescent antibody technique (Norris and Larsen, 1995). It is currently the standard confirmatory test (Nesteroff, 2004). At present, the FTA test is the most sensitive serological test in all stages of syphilis rivaled only by the TPHA (Rhodes and Luger, 1987). In untreated syphilis it has sensitivities of 84% (70-100) in primary syphilis, 100% in secondary and latent syphilis, and 96% in late syphilis. It has a specificity of 97% (94-100) (Norris and Larsen, 1995).

False positive FTA-ABS occurs occasionally in healthy persons. They have been associated with the presence of auto- antibodies, genital herpes, and pregnancy. They have also been observed in patients with systemic lupus erythematosus (SLE), alcoholic cirrhosis, scleroderma and mixed connective tissue disease. The TPHA test, first described by Rathlev (1965), has a high sensitivity and specificity each greater than 99.9% (Morton, 1992). However, the TPHA test is less sensitive in primary syphilis than either the FTA–ABS test or VDRL test due to the variable IgM-binding capacity of the reagents. It is also superior to

both FTA–ABS and VDRL tests in secondary syphilis and is as sensitive as the FTA–ABS for latent and late syphilis (Nesteroff, 2004). It has few false positives (0.07%), which have been reported in some patients with infectious mononucleosis, connective tissue disease, leprosy and with intravenous drug users (Rhodes and Luger, 1987).

2.13 Sensitivity and specificity of the treponemal rapid syphilis tests

Few evaluations on the performance of the ICS tests have been done both in laboratory and non-laboratory settings. Lien *et al*. (2000) evaluated a simple rapid 1-step ICS test for syphilis (Determine^{TM} syphilis *T. pallidum* (TP), Abbot, laboratories, Abbot part, IL in Cho Minch City, Vietnam). The sensitivity and specificity was 100% and 98% respectively (Lien e*t al.*, 2000). Determine^{TM} syphilis *T. pallidum* (TP) is a 1-step ICS test for syphilis that detects antibodies to *T. pallidum* through their binding to antigen-selenium colloid that is subsequently captured by immobilized antigen forming a red line on the test strip (Morse, 2003).

Similar evaluations of Determine ^{TM} syphilis *T. pallidum* (TP) have been done in Brazil by Sato *et al*. (2003). The sensitivity and specificity was 93.7% and 95.2% respectively. The positive predictive value and the negative predictive value were 95.2% and 93.7% respectively (Sato *et al*., 2003). In similar studies conducted in Mozambique and the Gambia, the rapid syphilis tests outperformed the RPR test (West *et al*., 2002; Montoya *et al*., 2006). In the Gambia study, the RPR test specificity and sensitivity were 94.1% and 77.5% respectively. The specificity and sensitivity of the rapid syphilis test were 95.2% and 75.0% respectively (West *et al*., 2002). A review of the current diagnostic tests in Kenya is required. Recently introduced tests like the ICS test have not been evaluated in our population setting. Hence, for wide spread application, evaluation of such tests is necessary.

2.14 Treatment of syphilis

Syphilis in adults is easily cured and depending on the stage of infection, treatment may consist of as little as a single dose of penicillin, which is widely available in primary health-care settings (WHO, 2007). The first-choice treatment for all manifestations of syphilis remains penicillin in the form of parenteral penicillin G (CDC, 2006). When provided early in pregnancy Parenteral penicillin G is the only therapy with documented efficacy for syphilis (CDC, 2002). Penicillin remains the drug of choice for all types and stages of syphilis (Hutto, 2001), with no evidence of resistant strains of *T. pallidum* (WHO, 2001). Since the dividing time of *T. pallidum* is slow (days), penicillin G benzathine is the only penicillin effective for single-dose therapy because it is in depo form and levels remain therapeutic in the blood for up to 30 days.

For penicillin-allergic patients, desensitization followed by penicillin therapy is the preferred regimen, although other agents, such as doxycycline, tetracycline, and erythromycin are available. Although neither the tetracycline nor erythromycin has been evaluated as extensively as penicillin G in the treatment of syphilis, some evidence suggests higher treatment failure rates in erythromycin-treated patients. Alternative treatment regimens should be used only in cases of documented penicillin allergy (Todd *et al*., 2006). Primary and secondary syphilis can be treated with a single intramuscular dose (50,000 U/kg) of benzathine penicillin G. Patients who are at high risk (those who are HIV-positive or who have late latent infection) may require three weekly doses (Riedner *et al*., 2005). Neurosyphilis is treated with aqueous crystalline penicillin G administered intravenously for 10 to 14 days (Hayman, 2006).

When provided early in pregnancy, treatment of the mother effectively prevents infection in the fetus (Berman, 2004). Even in women with syphilis of long duration, who themselves would benefit from three weekly doses of penicillin; a single dose of penicillin prevents infection in the fetus (WHO, 2007). Birth outcomes for such women are similar to those for women without syphilis (Watson-Jones *et al.*, 2002). Diagnosis of congenital syphilis in infants and treatment are considerably more difficult than diagnosis and treatment of infected pregnant women. Current treatment regimens for congenital syphilis involve administration of parenteral penicillin every day for 10 days; hospitalization is often indicated to ensure that the infant receives the full course of treatment (WHO, 2007). Clearly, prevention of congenital syphilis by universal screening of women early in pregnancy and treatment, if indicated, is far preferable (Montoya *et al.*, 2006).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study population

One hundred and fifty pregnant women aged between 18 years and 42 years old presenting on their first antenatal visit or follow-up visits without a previous syphilis test during that pregnancy attending West Health Center Eldoret, Kenya who had signed an informed consent form were enrolled into the study between November 2007 and March 2008. Details of trimester and age were included in the study form (Appendix III).

3.2 Inclusion criteria

Pregnant women aged 18 years and above presenting on their first antenatal visit or follow-up visits but without a previous syphilis test during that pregnancy.

3.3 Exclusion criteria

Pregnant women on follow–up antenatal visits with a previous syphilis test. and pregnant women aged below 18 years were excluded from this study.

3.4 Ethical considerations

Approval was sought from the Medical Officer of Health (MOH) in charge of Eldoret Municipal Council (EMC; Appendix II), and the Board of Postgraduate Studies of Kenyatta University (BPGS; Appendix I). Good Clinical Practices (GCPs) were observed.

3.5 Sampling and sample preparation

The sample size was determined by the method described by Dell *et al.* (2002). The national prevalence rate for antenatal syphilis was used.

log β

$$n = \frac{\beta}{\log p}$$ where, β = probability of committing type II error pegged at 0.05

p = proportion of population not infected

$$n = \frac{\log 0.05}{\log 0.9771} = 130$$

One hundred and fifty antenatal women attending their first or follow-up antenatal visits but without a previous syphilis test were randomly recruited into this study from a population of antenatal women attending West Health Centre Eldoret, Kenya.

3.6 Collection and preparation of samples

Peripheral venous blood (2ml) was drawn by venipuncture directly into vacutainer tubes without anticoagulant. The samples were coded and the blood left to coagulate for 15 minutes. Serum was then obtained by centrifuging coagulated blood at 3000 revolutions per minute (r.p.m.) for 5 minutes.

3.7 Experimental procedures

3.7.1 Venereal Disease Research Laboratory (VDRL) test

Eurotex-VDRL latex slide test for VDRL is based on the principle of agglutination (Cockayne, 2002). The test specimen, serum was mixed with Eurotex-latex reagent and allowed to react (figure 2.1). Briefly, 50µl of serum was dispensed onto one of the reaction circles of a glass slide and one drop of Eurotex-VDRL latex reagent was added. The contents were mixed thoroughly and results were read within six minutes as per the manufacturer's instructions and recorded in the VDRL test worksheet (Appendix IV). Positive and negative controls were included in the tests.

Fig. 2.1: Venereal disease research laboratory (VDRL) test

3.7.2 *Treponema pallidum* Haemagglutination Assay (TPHA) test

The Chronolab (Switzerland) *Treponema pallidum* haemagglutination assay (TPHA) test kit is designed for the detection of antibodies to *T. pallidum* in human serum and plasma by means of an indirect haemagglutination (IHA) method. Preserved avian erythrocytes are coated with antigenic components of pathogenic *T. pallidum* (Nichol's strain). The cells agglutinate in the presence of specific antibodies to *T. pallidum* and show characteristic patterns in microtitration plates. Any non- specific reactions occurring are detected using the control cells, which are avian erythrocytes not coated with *T. pallidum* antigens (West *et al*., 2002). The assay was performed according to the method described by West *et al*. (2002). Briefly, 190 µl of assay diluent and 10 µl of serum was added to the first well of a 96-well microtitre plate. Using a micropipette, 25 µl was transferred to wells two and three. 75 microlitres of control cells was added to well two and 75 µl of test cells to well three. The

plate was tapped gently so as to mix the contents thoroughly and incubated for 45–60 minutes at room temperature after which results were read and recorded in the TPHA test worksheet (Appendix V).

3.7.3 Immunochromatographic strip (ICS) test

Accurate (Ultra rapid strip test; USA); a qualitative assay for detecting anti-*Treponema pallidum* antibodies is based on an immunochromatographic technique, and its reagent Kit is presented in format cards for individual testing (Sato *et al*., 2004). The inert solid support is divided into three regions: region one, with an absorbent support for sample application (sample pad); region two, corresponding to the sample result display (patient window (T) region); and region three, corresponding to the control reaction (control (C) window site) (Sato *et al*., 2004). Briefly, fifty microlitres of serum was applied to the sample pad and the results were read within 15 minutes and recorded in the ICS test worksheet (Appendix VI).

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3.8 Data management and statistical methods

The syphilis status was determined following the CDC/WHO recommended testing strategies (WHO, 2007). All the samples were diagnosed using VDRL, TPHA, and ICS tests. Data processing and statistical analysis was done using SPSS version 13.0. The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) was determined using McNemar's (1947) test as shown below. The PPV measures the probability that a positive result indicates the presence of disease. The NPV measures the probability that a negative result indicates absence of disease. McNemar's (1947) Chi square statistic was used to test the level of agreement between the VDRL and ICS tests at 95% confidence interval (CI). The *P* values of ≤ 0.05 were considered statistically significant.

Table 3.1: McNemar's 2x2 table

METHOD	**STANDARD TEST**			
NEW TEST		**P**	**N**	**TOTAL**
	P	a	b	a + b
	N	c	d	c+ d
	TOTAL	a + c	b + d	N

Where;

Sensitivity = a / (a + c) x100 Specificity = d / (b + d) x 100

PPV = a / (a + b) x 100

NPV = d /(c + d) x 100

Where a = True positives

b = False positives

c = False negatives

d = True negatives

$\chi^2 = (|b - c| - 1)^2 / (b + c)$.

CHAPTER FOUR: RESULTS

4.1 Study population

The study population comprised of pregnant women aged between 18 years and 42 years old (Table 4.1). The antenatal prevalence rate for syphilis in the study population was found to be 3.33% (5/150) using VDRL test as the gold standard and highest among pregnant women of age groups 18-27 (4.7%; Table 4.1). In addition, the 18-22 age groups constituted the highest number of antenatal clients (Table 4.1). It is noted here that majority of the women attended antenatal clinic during the third trimester (Fig. 4.1) and hence late in their pregnancy.

Table 4.1: Syphilis prevalence within the age groups

Age group (years)	Frequency (N)	Syphilis prevalence (%)
18-22	43	4.7
23-27	42	4.7
28-32	41	2.4
33-37	17	0
38- 42	7	0
Mean age 26.92		
Antenatal prevalence rate for syphilis using VDRL		3.33

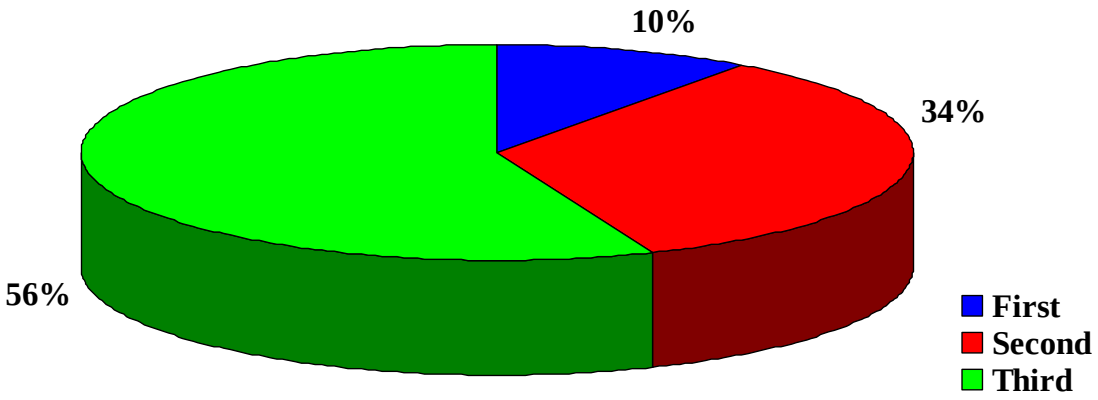

Fig. 4.1: Antenatal clinic attendance per trimester. Different trimesters at which women who were screened for antenatal syphilis attended antenatal clinic.

4.2 Antenatal syphilis screening

Syphilis screening is carried out as a routine test programme for all pregnant women attending antenatal clinics in Kenya. One hundred and fifty pregnant women presenting on their first antenatal visit or follow up visits but without a previous syphilis test during that pregnancy were screened using the three tests VDRL, ICS and TPHA.

4.2.1 Venereal Disease Research Laboratory (VDRL) test results

One hundred and fifty pregnant women presenting on their first antenatal visit or follow up visits but without a previous syphilis test during that pregnancy were screened using the VDRL test. The VDRL test results were scored as reactive (positive) when there was definitive clumping or non-reactive (negative) when no clumping was observed. Five samples out of 150 serum samples were reactive (3.33%).

4.2.2 *Treponema pallidum* Haemagglutination Assay (TPHA) test results

One hundred and fifty pregnant women presenting on their first antenatal visit or follow up visits but without a previous syphilis test during that pregnancy were screened using the

TPHA test. The TPHA test results were scored as positive when there was a definite agglutination or negative when no agglutination was observed. Six samples out of 150 serum samples were detected as positive (4%).

4.2.3 Immunochromatographic strip (ICS) test results

One hundred and fifty pregnant women presenting on their first antenatal visit or follow up visits but without a previous syphilis test during that pregnancy were screened using the ICS test. The ICS test results were scored as positive when two pink lines were observed, at the patient window (T) site and at the control (C) window site or negative when a pink line was observed at the control window (C) site only. Out of 150 serum samples 6 (4%) samples tested positive.

4.3 Determination of sensitivity and specificity of the immunochromatographic strip test using the venereal disease research laboratory (VDRL) test as the gold standard

The ICS test performance in terms of sensitivity and specificity was determined using the already validated VDRL test as the gold standard. Out of 5 samples that were reactive by VDRL test, 1 (20%) was non-reactive by ICS test. Out of the 145 samples that were nonreactive by VDRL test, 2 (1.38%) were reactive by the ICS test. The ICS test results agreed with those of VDRL test with a consistency of 98% (147 out of 150). The sensitivity and specificity of ICS test was 80% and 98.62% respectively (Table 4.2)

Table 4.2: Sensitivity and specificity of immunochromatographic strip (ICS) test using venereal disease research laboratory (VDRL) test as a gold standard. Sensitivity 80% (4/5); Specificity 98.62% (143/145).

METHOD		VDRL TEST		
		P	**N**	**TOTAL**
ICS	**P**	4	2	6
TEST	**N**	1	143	144
	TOTAL	5	145	150

Where: N: Syphilis antibody negative

P: Syphilis antibody positive

When the sensitivity and specificity of the immunochromatographic strip (ICS) and venereal disease research laboratory (VDRL) test were compared there was no significant difference between the two tests ($\chi^2 = 0$; $P>0.05$). The two tests compared favorably with an overall agreement of 98%.

4.4 Determination of the positive predictive value and the negative predictive value of the immunochromatographic strip test using *Treponema pallidum* haemagglutination assay as the confirmatory test.

All the 6 samples that were reactive by TPHA were also reactive by the ICS test. The ICS test results agreed with those of TPHA test with a consistency of 100% (150 out of 150). The sensitivity, specificity, PPV and NPV were all 100% (Table 4.3).

Table 4.3: Positive predictive value (PPV) and the Negative predictive value (NPV) of immunochromatographic strip (ICS) test using *Treponema pallidum* haemagglutination assay (TPHA) as a confirmatory test. Positive predictive value 100% (6/6); Negative predictive value (NPV) 100% (144/144).

METHOD		TPHA TEST		
		P	**N**	**TOTAL**
ICS	P	6	0	6
TEST	N	0	144	144
	TOTAL	6	144	150

Where: N: Syphilis antibody negative

P: Syphilis antibody positive

4.5 Determination of the sensitivity, specificity, positive predictive value and the negative predictive value of the venereal disease research laboratory (VDRL) test using *Treponema pallidum* haemagglutination assay (TPHA) as the confirmatory test

Out of the 6 samples that were positive by TPHA test 2 (33.3%) were negative by VDRL test. Out of 144 samples that were TPHA negative 1 (0.69%) were reactive by VDRL test. The VDRL test results agreed with those of TPHA test with a consistency of 98% (147 out of 150). The sensitivity and specificity of VDRL test was 66.67% and 99.3% respectively, while the PPV and NPV were 80% and 98.6% respectively (Table 4.4).

Table 4.4: Sensitivity and specificity of venereal disease research laboratory (VDRL) test using *Treponema pallidum* haemagglutination assay (TPHA) as a confirmatory test. Sensitivity 66.67% (4/6); Specificity: 99.3% (143/144); Positive predictive value 80% (4/5); Negative predictive value 98.6% (143/145).

METHOD		TPHA TEST		
		P	**N**	**TOTAL**
VDRL	**P**	4	1	5
TEST	**N**	2	143	145
	TOTAL	6	144	150

Where: N: Syphilis antibody negative

P: Syphilis antibody positive

CHAPTER FIVE: DISCUSSION

5.1 Characteristics of the study population

The study population comprised of pregnant women aged between 18 years and 42 years old on their first antenatal visit or follow up antenatal visits without a previous syphilis test during that pregnancy in Eldoret West health centre. The majority (56%) of the antenatal women in this study registered for their antenatal clinic attendance while in their third trimester of pregnancy, implying that screening and treatment will not be effective even if they were treated on the day of the first visit (Gloyd *et al*., 2007). Using the VDRL test as the gold standard the prevalence rate of antenatal syphilis in this group was 3.33%. Although this figure is within the sub-Saharan Africa range (WHO, 2007) but slightly higher than the overall national maternal prevalence which is currently standing at 2.29% (WHO, 2007). The mean age of the antenatal clinic attendants was 26.92 with the lowest being 18 and the highest 42 years respectively.

5.2 Comparison of immunochromatographic strip (ICS) test and venereal disease research laboratory (VDRL) test

The sensitivity and specificity of ICS test was 80% and 98.62% respectively using VDRL as the gold standard. There was an overall concordance of 98% between the two tests. In a similar study conducted in Mozambique, the sensitivity and specificity ranged between 75.5-82.6% and 98.7-99.4% respectively (Montoya *et al*., 2006). West *et al*. (2002) reported a sensitivity and specificity of 75% and 95.2% respectively and reports that the low prevalence rate of syphilis (3%) hampered performance of the ICS test. However, Bronzan *et al*. (2007) demonstrated that an on-site treponemal ICS test had high sensitivity (89.4%) and specificity (92.9%), in rural South Africa after giving additional training to the testing personnel.

In this study some discrepancies were found between the ICS and VDRL tests. There was 1 (20%) sample that was reactive by VDRL but non-reactive by ICS which could be a biological false negative result. Previous studies have demonstrated that the ICS test has a lower sensitivity in early primary syphilis when antibody level may be too low to detect (Sato *et al*., 2003; Baron *et al*., 2004; Nesteroff, 2004). Out of the 145 samples that were nonreactive by VDRL test, 2 (1.38%) were reactive by the ICS test. In one of the two negative samples the antenatal client had disseminated secondary lesions all over the body. This could be biological false negative result due the prozone phenomenon which occurs in 2% of sera; undiluted sera give negative results because of antibody excess or the presence of blocking antibodies or both (Kinghorn, 2004; Hall and Klausner, 2004). In the second sample, however, the patient had no clinical signs of disease and this could be explained as either old (latent) or treated case as the treponemal tests remain positive even after successful therapy (Gloyd *et al*., 2007; WHO, 2007). However, for all the positive cases a single dose of 2.4 million units of benzathine penicillin was recommended.

Interestingly, when the 3 samples whose results did not agree between VDRL and ICS were tested by TPHA test, the positive sample by VDRL tested negative and the 2 negative samples were both positive by TPHA test. This suggests that results by VDRL must be confirmed by another test. The WHO recommends that if a non treponemal test is positive, the serum is then tested by a confirmatory treponemal test, using an antigen of *T. pallidum*; examples include the *T. pallidum* haemagglutination assay (TPHA) and the *T. pallidum* particle agglutination assay (TPPA; WHO, 2007; Stoner, 2007).

The non-treponemal tests have the advantages of being inexpensive and sensitive especially in early infection; however, these tests cannot be done on whole blood, they require a

microscope or rotator for processing, and misinterpretation is common by inexperienced laboratory technicians because reading of the result is subjective. Studies have shown a wide variation in the reliability of screening results from non-treponemal tests (WHO, 2007). Nontreponemal tests can be cumbersome and time consuming when performed on large samples, because of the lack of automation for performance of these tests (Stoner, 2007). In treponemal tests, while theoretically more specific than non-treponemal tests, may also give false-positive results. Moreover, they cannot differentiate between individuals with active (untreated) syphilis and those who have previously been successfully treated for infection (Sato *et al*., 2003). In both cases, the treponemal test result will be positive. Non-treponemal tests, on the other hand, can distinguish current or recent infections from old, treated infections (Diaz *et al*., 2004).

The ICS test requires no specialized equipment and takes 8 minutes to perform (Zarakolu *et al*., 2002). Most importantly, the ICS offers an affordable, sensitive, and simple on-site screening option for rural health clinics that do not have ready access to laboratories (Conway, 2007).

5.3 Comparison of immunochromatographic strip (ICS) test and *Treponema pallidum* haemagglutination assay (TPHA)

The ICS test results agreed with those of TPHA test with a consistency of 100% (150 out of 150). The sensitivity, specificity, PPV and NPV were all 100%. A similar study conducted by Zarakolu *et al*. (2002) had similar results when compared to FTA-ABS test results. In a clinical setting in San Francisco, 100% sensitivity and specificity was obtained using Determine Syphilis TP on whole blood collected by finger stick in heparinised capillary tubes (Siedner *et al*., 2004). Lewis *et al*. (2001) reported a sensitivity of 89.5% and specificity of 100% among antenatal women when compared to TPPA test results.

In this study the sensitivity and specificity of the ICS test was 80% and 98.62% respectively. In a similar study conducted in Mozambique, a sensitivity and specificity of 86% and 97% respectively was reported (Gloyd *et al*., 2007). Similarly, Bronzan *et al.* (2007) reported a sensitivity and specificity of 89.4% and 92.9% respectively in rural South Africa. Galvan *et al*. (2001) reported a sensitivity and specificity of 100% and 87% respectively in Peru when ICS test was compared with microhaemagglutination assay for antibody to *Treponema pallidum* (MHA-TP) test. In this study the ICS test had a PPV of 100% when using TPHA test as the reference test. This is very important in antenatal syphilis screening since the PPV measures the probability that a positive result indicates the presence of disease. Specificities and sensitivities reported here for the ICS test show that it could be used as a confirmatory test.

The affordability, convenience and practicality of rapid treponemal tests make them attractive tools, not only as confirmatory assays but also as on-site screening tests in primary health-care settings or in areas where laboratory services are not available. However, since treponemal antibodies persist for years, whether patients are treated or not, these rapid treponemal tests cannot be used to monitor effectiveness of treatment or to distinguish active infection from past treated infection (WHO, 2007). In areas with a low prevalence of syphilis such as antenatal or family planning clinics, or where screening has not previously been available, presumptive treatment should be considered for anyone with a positive rapid treponemal test result. Rapid treponemal tests are less useful as screening tests in areas where the prevalence of syphilis is high, since a high proportion of individuals will have antibodies as a result of past treated infection. It would be preferable, however to treat women who test positive rather than risk missing a maternal infection (WHO, 2007). In any case, whatever the prevalence rate, the tests are very helpful in identifying women without syphilis. Rapid

syphilis tests can be used as a screening, diagnostic or confirmatory test. They can be used in research studies under the correct circumstances. In Mozambique and Bolivia, a program using the rapid ICS tests have been implemented (Peeling and Hook 2006).

The results of this study have great relevance to the control of syphilis on the overall and congenital syphilis in particular in Kenya. These studies demonstrate that there was 98% concordance between the ICS test and the VDRL test that is currently used as a primary screening test for syphilis. Based on these findings, the ICS test should be considered for incorporation as a primary screening test for syphilis in antenatal care programme in Kenya and in low-resource settings, where laboratory facilities are not always available, and in non-clinical settings where individuals who do not access standard health care services can be reached.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

a) The prevalence rate of antenatal syphilis in Eldoret municipality was 3.33%.

b) The ICS test is a rapid and reproducible test and it takes 15 minutes to perform.

c) The sensitivity and specificity of the ICS test were 80% and 98.62% respectively while the PPV and NPV were 100% each.

6.2 RECOMMENDATIONS

a) The ICS test is easy to perform and could be adopted as a screening and confirmatory test in health centers with or without laboratories.

b) The use of the ICS test as screening test in antenatal clinics in Kenya would result in a significant improvement of the ANC syphilis screening and treatment program thus reducing maternal and infant mortality and morbidity. In addition this rapid test may be a helpful tool for laboratory diagnosis in the attempt to break off the transmission chain of congenital syphilis.

c) The conventional serological tests for syphilis are usually time consuming, and commonly the mother and her newborn leave hospital even before the results of serological tests are reported .A rapid test like the ICS test may be useful to overcome these limitations.

d) The development of rapid ICS diagnostic tests for syphilis presents an opportunity to implement screening and treatment services in low-resource settings, where laboratory facilities are not always available, and in non-clinical settings where individuals who do not access standard health care services can be reached.

e) Evaluation of the ICS test is also needed to determine the rate of conversion of the ICS test from positive to negative in syphilis infected patients after receiving appropriate treatment.

f) Further studies are needed to evaluate the practical applications of the ICS test, specifically to clarify their sensitivity and specificity with whole blood and to explore their feasibility, acceptability, and cost effectiveness in resource poor settings in Kenya.

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APPENDIX I

ETHICAL CLEARANCE BOARD OF POSTGRADUATE STUDIES OF KENYATTA UNIVERSITY

APPENDIX II

ETHICAL CLEARANCE ELDORET MUNICIPAL COUNCIL

APPENDIX III

SAMPLE COLLECTION (SC) STUDY FORM

Patient No----------

Age----------

Date----------

Trimester----------

APPENDIX IV

VENEREAL DISEASE RESEARCH LABORATORY (VDRL) TEST WORKSHEET

Date----------

Done by----------

Sample ID	Kit Batch No	Result	Comment

APPENDIX V

Treponema pallidum HAEMAGGLUTINATION ASSAY (TPHA) TEST WORKSHEET

Date----------

Done by----------

Sample ID	Kit Batch No	Result	Comment

APPENDIX VI

IMMUNOCHROMATOGRAPHIC STRIP (ICS) TEST WORKSHEET

Date----------

Done by----------

Sample ID	Strip Batch No	Result	Comment