EVALUATION OF THE SD BIOLINE HIV/SYPHILIS DUO AND SD BIOLINE SYPHILIS 3.0 TEST KITS FOR HIV/AIDS AND SYPHILIS SCREENING AT KOMFO ANOKYE TEACHING HOSPITAL, KUMASI

By

Bright Opoku-Nketiah BSc (Hons.)

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College of Health Sciences

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DECLARATION

I hereby declare that this submission is my own work towards the award of an MSc. Degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Student Name	……	……
Opoku-Nketiah Bright	Signature	Date
(PG6067211)		

Certified by	……	……
Prof. Yaw Adu-Sarkodie	Signature	Date
(Supervisor)		

Certified by	……	……
Prof. E. H. Frimpong	Signature	Date
(Head of Department)		

DEDICATION

I dedicate this work to my family,

especially my parents and siblings for their financial and spiritual

support towards my education.

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"What do have that you did not receive"? (I Corinthians 4:7b). Blessed be the name of God, who gave me the wisdom and knowledge and the urge and strength to complete this course of study.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Clinic
ART	Anti-Retroviral Therapy
CDC	Centres for Disease Control
CFR	Compliment Fixation Reactions
CHRPE	Committee on Human Research, Publications and Ethics
COV	Cut-Off Value
CSF	Cerebro-Spinal Fluid
DFA-TP	Direct Fluorescent Antibody Test for *T. Pallidum*
EBV	Estein-Baar Virus
ECS	Eliminating Congenital Syphilis
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immuno-Sorbent Assay
FTA	Fluorescent *Treponema* Antibody
FTA-ABS	Fluorescent *Treponema* Antibody
GHS	Ghana Health Service
HIV	Human Immunodeficiency Virus
HSS	HIV Sentinental Survey
KATH	Komfo Anokye Teaching Hospital
KNUST	Kwame Nkrumah University of Science and Technology
LIA	Line Immuno Assay
MHA-TP	Micro Haemagglutination Assay for *T. Pallidum*
MTCT	Mother-to-Child Transmission
NAAT	Nucleic Acid Amplification Test
NPV	Negative Predictive Value

PCR	Polymerase Chain Reaction
PMTCT	Prevention of Mother-To-Child Transmission
PPV	Positive Predictive Value
RDT	Rapid Diagnostic Test
RPR	Rapid Plasma Reagin
RST	Rapid Syphilis Testing
SMS	School Of Medical Sciences
STI	Sexually Transmitted Infection
TMB	Tetramethyl Benzidine
TPHA	*Treponema pallidum* Haemagglutination Assay
TPI	*T. pallidum* Immobilization
TPPA	*T. pallidum* Particle Agglutination Assay
VCT	Voluntary Counselling and Testing
VDRL	Venereal Disease Research Laboratory
WB	Western Blot
WHO	World Health Organisation

ABSTRACT

Diagnostic tests are needed for the control of sexually transmitted infections, as many infected individuals are asymptomatic. Rapid advances in molecular biology have led to the development of sensitive and specific diagnostic tests. New diagnostics tests must be evaluated in the situations in which they will be deployed.

This study evaluated the SD Bioline HIV/syphilis Duo and the SD Bioline syphilis 3.0 test kits in HIV and syphilis testing.

A total of 644 well characterised, archived serum samples were used. IMMUTREP® RPR, IMMUTREP® TPHA, Genscreen ULTRA HIV Ag-Ab and First Response® HIV 1-2-0 were used as gold standards. All 644 samples were assayed for the presence of antibodies to HIV and *T. Pallidum* by the SD Bioline HIV/syphilis Duo, the SD Bioline syphilis 3.0 and the Abbott Determine™ Syphilis TP.

The sensitivity, specificity, positive and negative predictive values of the SD Bioline syphilis 3.0 test kits were 91.67%, 100.00%, 100.00% and 89.29% respectively. The Abbott Determine™ Syphilis TP had 75.69%, 100.00%, 100.00% and 74.07% respectively. In HIV testing, the SD Bioline HIV/syphilis Duo had 100.00%, 100.00%, 100.00% and 100.00% respectively. In syphilis testing, it had 100.00%, 99.33%, 99.60% and 100.00% respectively. The kappa value for SD Bioline syphilis 3.0 and Abbott Determine™ Syphilis TP were 0.90 and 0.72 respectively, whiles the SD Bioline HIV/syphilis Duo had 1.00 and 0.99 in HIV and syphilis testing respectively.

The SD Bioline HIV/syphilis Duo and SD Bioline syphilis 3.0 compared favourably with the gold standards. They are highly specific and very sensitive. They can be performed in clinics and/or hospitals without laboratory training and require no equipment or refrigeration. The SD Bioline HIV/syphilis Duo can be used for simultaneous screening of HIV and syphilis at antenatal clinics in Ghana. This would increase the coverage of HIV and syphilis screening, which will also increase the proportion of cases treated, facilitating the elimination of mother-to-child transmission of HIV and congenital syphilis.

Key words: Gold Standard, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Kappa Coefficient.

CHAPTER ONE

INTRODUCTION

1.1: Background to the study

The development and economy of Ghana is still being challenged by the Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) pandemic (National AIDS Control Programme, 2007). It was estimated in 2010 that there were 230,348 adults and 32,057 children living with HIV and a total of 14,165 new infections, while in 2011 there were 225,478 adults and 30,401 children living with HIV and 12,077 new infections. The estimated annual AIDS deaths for 2010 and 2011 were 17,230 and 15,263 respectively (Ghana AIDS Commission, 2012). It is estimated that about 25% of infected people are not aware of their infection and risk for transmitting HIV. This is due in part to a prolonged asymptomatic stage, and this stage makes early diagnosis important (Department of Health and Human Services Centres for Disease Control, 2006).

The conventional testing scheme for HIV is an enzyme-linked immuno-sorbent assay (ELISA) screening test, followed by a Western blot (WB) or immunofluorescent assay as a confirmatory test (Cunningham *et al.,* 2005). The conventional testing scheme requires patients to present for testing on one day and then return for results. Up to one third of patients presenting for HIV testing never return for their results (Greenwald *et al.,* 2006). Extra equipment such as automatic pipettes, incubators, washers, and readers must be available and these are costly to purchase and maintain, and must be located near clean water and a reliable supply of electricity. The validity of the results obtained by these techniques depends on the skills of the technicians, and their interpretation requires skills training and supervision. These conditions are often lacking in sub-Saharan Africa (Mylonakis *et al.,* 2000).

The CDC therefore recommended that rapid HIV testing be considered in clinics (Department of Health and Human Services Centres for Disease Control, 2006). Studies assessing patient satisfaction with rapid testing show that almost 90% of patients prefer rapid testing and that more than 10% of patients would have deferred testing if same-day results were not available (Greenwald *et al.*, 2006). Additionally, rapid testing, by providing results while patients were already at a clinic, allowed them to get HIV-infected patients into HIV treatment centres more quickly (Kendrick *et al.*, 2005).

Rapid diagnostic tests (RDTs) are simple to use, require little or no equipment, are cheaper than laboratory based HIV antibody tests, can be stored at room temperature, and results are easy to read and interpret. They allow for real-time, on-site HIV testing. RDTs are particularly suited for use in sub-Saharan Africa, where up to 2 weeks or more may be needed for laboratory results to become available (Respess *et al.*, 2001). A second area of research interest in rapid testing is its use during labour in an effort to prevent perinatal infection (Cunningham *et al.*, 2005).

In Ghana, according to the HIV Sentinental Survey (HSS), the median syphilis prevalence for 2009 was 3.7% having recorded 3.8% and 5.1% in 2008 and 2007 respectively (GHS HSS reports, 2006, 2007, 2008 and 2009). An estimated 1 million pregnancies each year are adversely affected by syphilis due to maternal infection, and about half of these pregnancies end in stillbirth or neonatal death (Walker and Walker, 2002). Management of maternal syphilis relies on serological screening in pregnancy (World Health Organization, 2001).

Screening for syphilis has traditionally been performed with non-treponemal tests such as the Rapid Plasma Reagin (RPR) or Venereal Disease Research Laboratory

(VDRL) assays, with positive results confirmed by a treponemal test such as the *Treponema pallidum* haemagglutination assay (TPHA), the *Treponema pallidum* particle agglutination assay (TPPA) or the fluorescent *Treponema* antibody (FTA-ABS) (Van Dyck *et al.*, 1990). However, it is estimated that less than 30% of pregnant women are screened for the disease in sub-Saharan Africa (Schmid, 2004).

One major barrier for syphilis screening is that current screening using a non-treponemal test requires a laboratory with trained personnel and a source of electricity to run a refrigerator to store the RPR reagent, a centrifuge to separate serum from whole blood, and a shaker to perform the serology. Since such facilities are generally not available in primary health care settings, blood or serum samples have to be transported to regional or central facilities for testing. Often results are only available days or weeks after testing (West *et al.*, 2002). Studies have shown that only a small proportion of infected women receive treatment when RPR testing is performed off-site, because women do not return for their results or specimens or results are lost in transit (Fonn, 1996). Even when testing is available at clinical sites, there are technical difficulties associated with maintaining trained personnel and assuring quality standards and supplies of tests and treatment (West *et al.*, 2002).

A rapid syphilis test with immediate results implemented in a field setting has the advantage of allowing women who test positive to be treated on-site at the same visit, avoiding the losses to follow-up associated with return visits and the adverse outcomes associated with delayed treatment (Peeling and Ye, 2004). A number of simple, rapid treponemal tests have recently become commercially available (Fears and Pope, 2001). These tests are simple, robust and affordable and can be stored and transported without need for refrigeration. Evaluations proved that their performance was comparable with the best laboratory-based diagnostics (Fears and Pope, 2001).

1.2: Justification

HIV antibody testing is a critical step that allows the implementation of effective prevention and care interventions in HIV-infected individuals (Dabis and Ekpini, 2002), and patient management (Lackiritz, 1998). The World Health Organisation (WHO) and the CDC recommended the use of simple and rapid assays in resource-limited settings since their operational characteristics make them more suitable than ELISAs (World Health Organization, 1998).

Syphilis has significant long-term morbidity for mothers and can seriously complicate pregnancy. Congenital syphilis results in serious sequelae in live-born infected children. Screening pregnant women for syphilis and treating them appropriately can eliminate complications (Walker, 2001).

To avoid the tragedy of babies escaping HIV but dying of syphilis, Peeling *et al.,* suggested that rapid syphilis screening can be integrated into rapid HIV testing for prevention of mother-to-child transmission (PMTCT) programmes (Peeling *et al.,* 2004). A simple, proven and inexpensive dual test for syphilis and HIV could improve the quality, acceptability and uptake of testing and treatment in rural areas to accelerate elimination of MTCT of syphilis and HIV (World Health Organisation, 2013).

The SD Bioline syphilis 3.0 is a new rapid diagnostic test designed to screen syphilis whiles the SD Bioline HIV/syphilis Duo is a new rapid diagnostic test designed to screen for both syphilis and HIV at the same time.

Data from a number of evaluations suggest that the sensitivity and specificity of RDTs are similar to those of ELISA- and WB-based algorithms (Lien *et al.,* 2000) but more recent studies have reported lower-than-expected sensitivities and specificities of RDTs (Gray *et al.,* 2007).

Due to the contrary effects of false positives and false negatives test results of RDTs, investigators and manufacturers keeps on designing different RDTs (with a range of sensitivities and specificities) for STIs. Whether these tests are useful in a given setting and, if so, which test is most appropriate are questions that can be answered only through evaluations in the appropriate laboratory, clinical or field settings. It is also required that diagnostic tests are evaluated for their technical and operational performances within the population in which they are to be used (Bossuyt *et al.*, 2003).

In this study, the performance and major operational characteristics of the SD Bioline HIV/syphilis Duo and SD Bioline syphilis 3.0 test kits were evaluated using 644 well characterised, archived serum specimens at Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana.

1.3: Aim of Study

To evaluate the SD Bioline HIV/syphilis Duo and SD Bioline syphilis 3.0 test kits.

1.4: Objectives of Study

1. To determine the performance and operational characteristics of the SD Bioline HIV/syphilis Duo and SD Bioline syphilis 3.0 test kits.
2. To compare the performance of the SD Bioline syphilis 3.0 to that of the Abbott Determine^{TM} Syphilis TP test kits.
3. To determine whether or not the SD Bioline HIV/syphilis Duo and SD Bioline syphilis 3.0 in HIV/AIDS and syphilis testing in Ghana.

CHAPTER TWO

LITERATURE REVIEW

2.1: HIV/AIDS

AIDS is an autoimmune deficiency syndrome caused by HIV, which is spread through blood, semen, vaginal secretions, and breast milk. The main means of transmission is unprotected sexual intercourse with an HIV-positive partner. Other routes include transfusion of HIV-infected blood or blood products; tissue or organ transplants; use of contaminated needles and syringes (or other skin-piercing equipment); and MTCT during pregnancy, birth or breastfeeding (International Labour Organization, 2001).

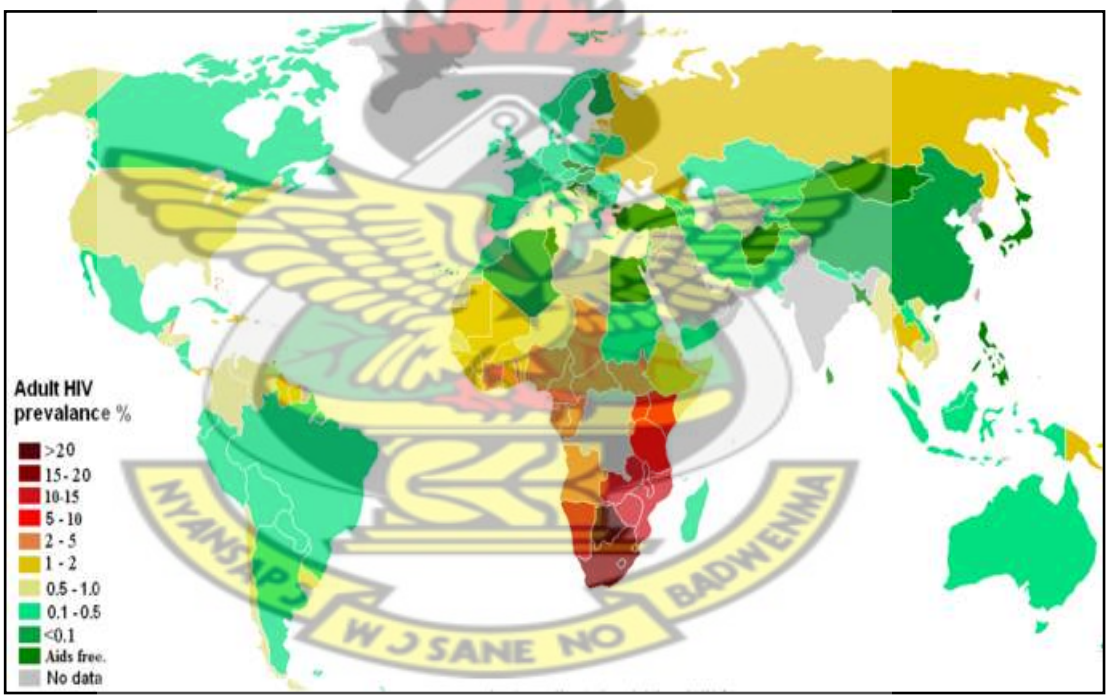

Figure 1: Global prevalence of HIV.
Source: http://www.unaids.org/en/dataanalysis/datatools/aidsinfo/

At the end of 2001, an estimated 40 million people were living with HIV/AIDS. Sub-Saharan Africa contains more than 70% of all HIV infected people (Garrett, 2002). The National HIV prevalence in 2009 was 1.9%, which dropped to 1.5% in 2010 and 2011 (Ghana AIDS Commission, 2012).

2.1.1: HIV/AIDS in women and children

Whereas men were most affected at the beginning of the HIV/AIDS epidemic, women's rates of new infection now surpass men's. At the end of 1998, more than 33 million people were living with the HIV, almost half of whom were women (UNAIDS, 1998). The rising HIV infection rates among women expose children to increased HIV risk even before they are born. In 15 sub-Saharan African countries, at least 5% of pregnant women attending prenatal clinics between 1999 and 2000 were HIV-positive; MTCT rates were as high as 40%. It is estimated that 2.7 million children were living with AIDS at the end of 2001 (USAID, UNICEF, and UNAIDS, 2002) and 11.8 million people between 15 to 24 years were living with HIV/AIDS at the end of 2001 (UNICEF, UNAIDS and WHO, 2002).

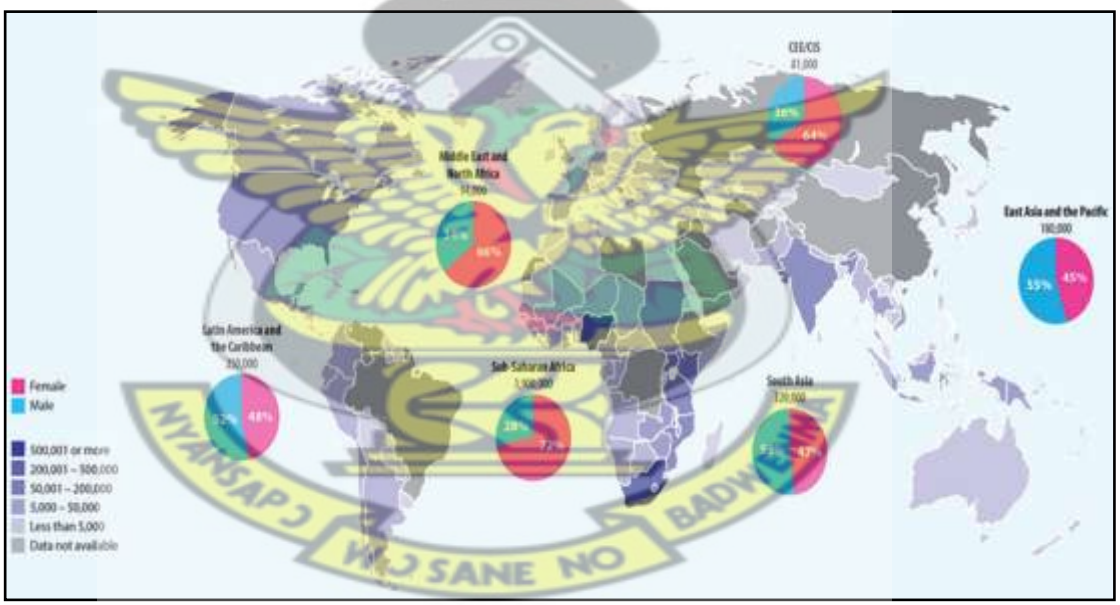

Figure 2: Estimated number of young people aged 15-24 living with HIV, 2009.
Source: http://www.unicef.org/factoftheweek/index_59194.html

2.1.2: HIV/AIDS and pregnancy

With an estimated 1.5 million HIV-positive women becoming pregnant each year, almost 600,000 children will be infected by MTCT (UNAIDS, 1997). HIV infection has been reported to have adverse effects on pregnancy in Africa (McIntyre, 1993;

Minkoff *et al.,* 1990) including complications of both early and late pregnancy. Complications of early pregnancy have been associated with HIV infection in several studies (D'Ubaldo *et al.,* 1998). An American study showed a three-fold increase in early spontaneous abortion in a prospective follow-up study (Shearer *et al.*, 1997). Higher rates of ectopic pregnancy have been reported in HIV-positive women than in uninfected women (Leroy, *et al.,* 1995). Preterm labour may be more common in HIV-positive women, with rates as high as double those rates seen in uninfected women in some reports (Bergstrom, *et al.*, 1995). Low birth weight has been reported in some studies in developing countries (Taha, *et al.,* 1995).

2.1.3: Mother-to-Child Transmission

MTCT is the most common and important source of HIV infection in childhood. In the absence of any intervention, between 30% and 45% of children born to HIV positive mothers will become infected with HIV (De Cock *et al.,* 2000). Reported rates of transmission of HIV from mother to child range from around 15%-25% in Europe and the United States to 25% to 40% in some African and Asian studies (Newell *et al.*, 1997). Transmission of HIV-1 can occur in utero, at the time of labour and delivery, or postnatally through breastfeeding. Transmission is believed to be uncommon during early pregnancy, but the risk increases sharply in late pregnancy and during labour and delivery. Overall, about 15-20% of children who acquire HIV infection from their mothers are infected during the antenatal period, 50% during delivery and 33% through breast feeding (Rashid and Mamatha, 2005).

Determination of HIV status enables appropriate counselling, timely anti-retroviral therapy (ART) and management of HIV positive pregnant women to reduce the risk of vertical transmission (Volmink *et al.,* 2007). In developed countries, infection in

infants has virtually been eliminated. However, in middle and low income countries, only about 23% of HIV positive pregnant women were reached by PMTCT interventions in 2006 (UNICEF/WHO, 2007). Coverage rates are lowest in West and Central Africa, Asia and East and Southern Africa, reaching only 7%, 7% and 30.5% of HIV-infected women respectively (UNICEF, 2007).

According to the Ghana Health Service (GHS), in 2007, only 12% of all antenatal clinic (ANC) registrants were counselled and tested for HIV in Ghana (Reproductive and Child Health Department, 2008). In response to the low HIV testing in pregnancy, the WHO has encouraged countries to adopt the routine offer of HIV testing. By this strategy, the HIV test is voluntarily offered routinely to all pregnant women accessing the ANC (UNAIDS Global Reference Group on HIV/AIDS and Human Rights, 2004). Voluntary testing of pregnant women is offered in many countries (Newell and Thorne, 1997), which has increased the number of identified HIV positive women in many centres (Lewis *et al.*, 1995).

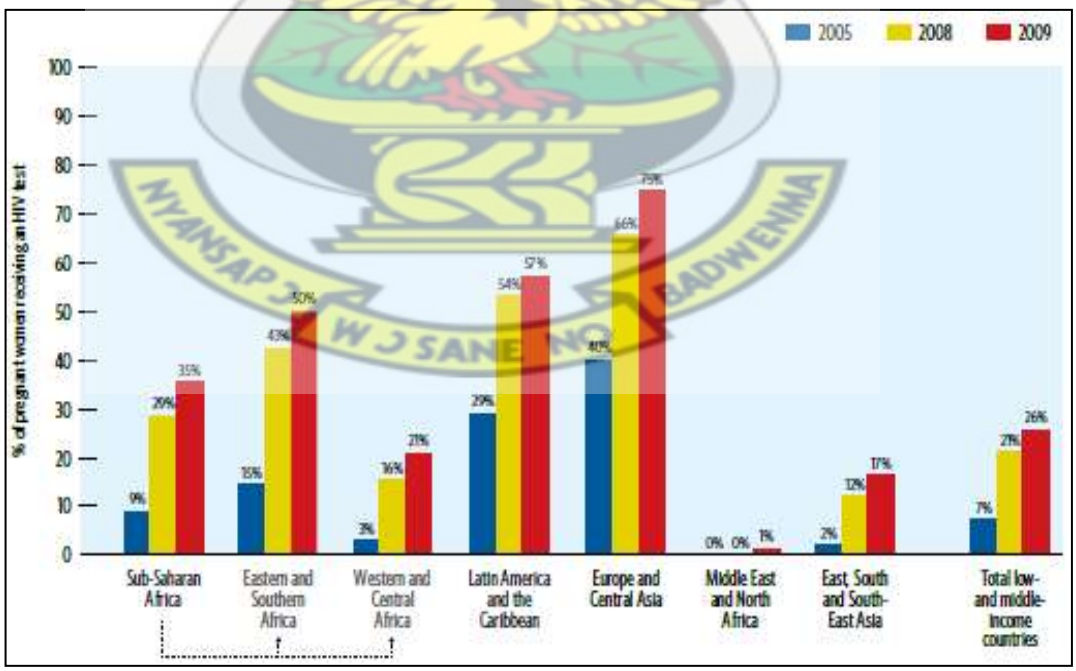

Figure 3: Percentage of pregnant women who received an HIV test in low- and middle-income countries by region, 2005, 2008, and 2009.

Source: Towards Universal Access, Scaling up priority HIV/AIDS interventions in the health sector, Progress report 2010, Page 87.

2.2: Syphilis

Syphilis is an acute and chronic infectious disease caused by the bacterium *Treponema pallidum* subspecies *pallidum* and transmitted either by direct contact, sexual intercourse or congenitally from a pregnant mother to her unborn foetus (Brooks *et al.*, 2004). It is a chronic illness that spans many years and that can be divided into three stages - primary, secondary and tertiary (Musher, 1999). The sexual transmission of syphilis occurs following contact with infectious lesions during the primary and secondary stages, whereas the congenital transmission can occur at any stage of the infection, including the latent stage (Swartz *et al.*, 1999). Congenital syphilis can lead to stillbirth or congenital infections that result in neonatal death or life-long sequelae (Berman, 2004). Globally, there are an estimated 12 million new cases of syphilis each year, the majority of which occur in developing countries (World Health Organisation, 2001).

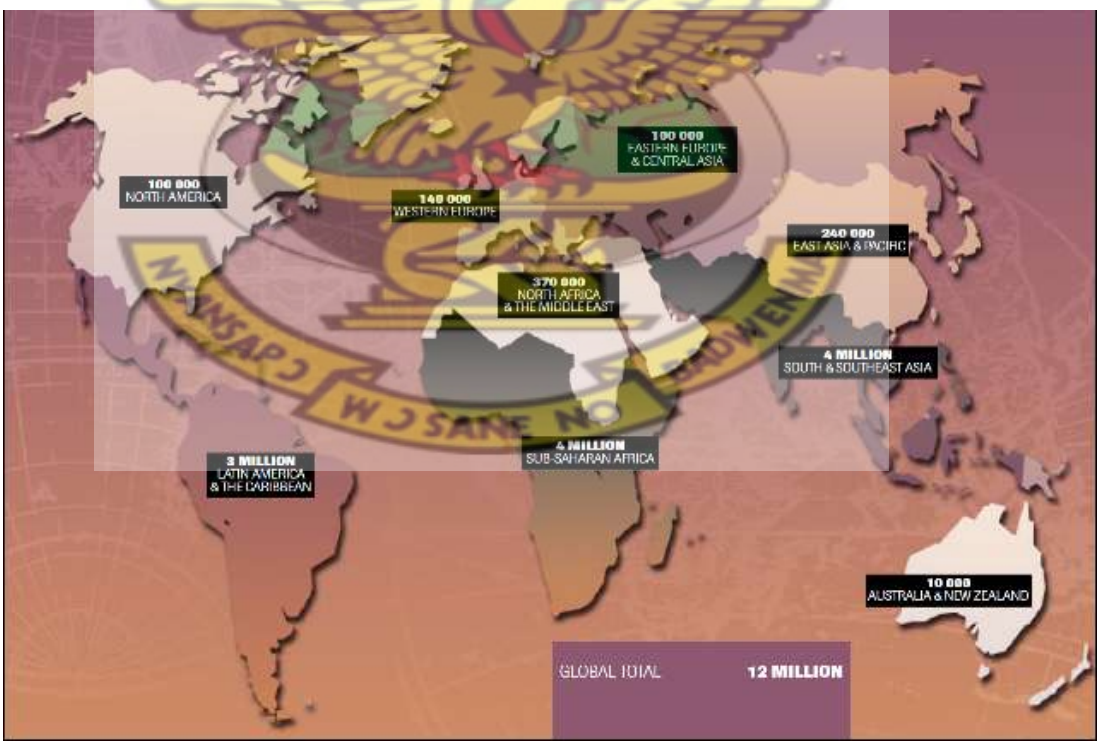

Figure 4: Estimated new cases of syphilis among adults, 1999.
Source: Global prevalence and incidence of selected curable STIs: Overview and estimates, WHO.

2.2.1: Syphilis and pregnancy

In sub-Saharan Africa, the prevalence of syphilis among pregnant women attending ANC ranges from 2.5% to 17% (Mayaud *et al.,* 1998). In Ethiopia, an estimated 5% of all pregnancies are lost each year through syphilis-induced abortions whiles stillbirth among sero-reactive women is 5 times more common than in sero-negative women (Schulz *et al.,* 1990). In rural South Africa, an adverse pregnancy outcome in women with syphilis is 12 times more common than in seronegative women (Wilkinson *et al.,* 1997).

The leading factor accounting for the failure to prevent congenital syphilis is the lack of prenatal care. Routine prenatal screening is the major line of defence against congenital syphilis. Screening in the first trimester with non-treponemal tests combined with confirmation of reactive individuals with treponemal tests is a cost effective strategy (Genç and Ledger, 2000).

2.4: Diagnosis of HIV/AIDS

HIV testing assays can be divided into two categories: screening assays, designed to detect all infected individuals; and confirmatory assays, designed to differentiate those persons who test falsely reactive by screening assays from those who are truly infected (Gürtler, 1996).

2.4.1: Screening assays

A variety of manual and automated test methods are available. However, screening assays are often performed on automated systems, so that large numbers of samples can be tested safely and economically. All antibody assays are based on the principle of a specific antigen-antibody reaction (Weber, 2006).

2.4.1.1: First generation assays

In 1985, first generation assays appeared. These assays employed "whole virus" antigens. Detection of antibodies bound to HIV antigens used an "indirect" approach. The first generation enzyme immunoassays (EIAs) were sensitive, but less effective regarding their specificity. Their capacity to detect early HIV antibodies averaged a bit more than 40 days after infection (Fanales-Belasio *et al.*, 2010).

2.4.1.2: Second generation assays

In 1987, the second generation EIA appeared. They used the same indirect format as the first generation assays, but the difference was the presence of HIV recombinant antigens and peptides, instead of the full viral lysate, bound in solid phase. The introduction of recombinant antigens increased the specificity of the test and, in the same time, ensured a good sensitivity. These tests reduced the window period (Weber, 2006), being able to detect antibodies as early as 33-35 days after infection. In the 1990s, the problem of the huge variability of HIV became progressively evident. EIA kits started to include also antigens from the HIV-2 virus, in order to ensure recognition of antibodies directed against both HIV-1 and HIV-2. In addition, new antigens from viruses of the HIV-1 groups M, N, and O were included. Determination of antibodies directed against the different HIV-1 subtypes from group M was ensured by the proven cross reactivity of antibodies with all group M subtypes (HIV-1 A to K) (Joint United Nations Programme on HIV/AIDS, 1997).

2.4.1.3: Third generation assays

In 1994, third generation EIA were designed on a new format. Recombinant HIV-1 and HIV-2 proteins and/or peptides, bound on the solid phase, react with the patient

serum. Antigen-bound antibody is revealed by the addition of the same viral antigen conjugated with an enzyme molecule. This "sandwich" format ensured higher sensitivity and specificity, since all potential classes of anti-HIV antibodies could be revealed. This generation of tests drastically reduced the "window period", bringing it to about 22 days after infection (Weber, 2006).

2.4.1.4: Fourth generation assays

Recently, fourth generation assays have been introduced. These assays are able to reveal the presence of both the antibodies and the p24 major antigen of HIV. This has further reduced the window period, at almost the levels of the detection of virus RNA (Weber, 2006).

Figure 5: The ELISA/EIA test

Source:http://fivephoton.com/index.php?route=product/product&product_id=138

2.4.2: Confirmatory assays

Most commonly used confirmatory assays are WB and line immuno assays (LIA). For HIV-1, proteins detectable by WB can be divided into three groups: the Env (envelope) glycoproteins (gp41, gp120, and gp160), the Gag or nuclear proteins (p17, p24/25, p55) and the Pol or endonuclease-polymerase proteins (p34, p40, p52, p68). The result of a WB may be either positive or negative or (in case of an

incomplete pattern of visible bands) "indeterminate", which may reflect borderline or non-specific reactivity (Weber, 2006). A great disadvantage of the WB assay is its high cost. In addition, the unavoidable subjectivity when reading and interpreting the result and the uncertainty about the criteria of positivity is, often, a great obstacle for a quick and clear result in HIV testing. Finally, the relatively frequent presence of indeterminate results can greatly delay diagnosis and increase costs. Assays similar to WB, generically called LIA, based on recombinant proteins and/or synthetic peptides capable of detecting antibodies to specific HIV-1 and/or HIV-2 proteins, have been developed. These assays produce fewer indeterminate results as compared to WB, but are equally expensive (Weber, 2006).

2.4.3: Rapid Diagnostic Tests (RDTs)

A number of rapid HIV tests are available. RDTs, which are easy to perform and require little in terms of equipment, can be useful in developing countries (World Health Organization, 2004). These tests are based on one of four immunodiagnostic principles: particle agglutination, immunodot (dipstick), immunofiltration and immune chromatography (Ekwueme *et al.*, 2003). In most cases whole blood or capillary blood can be used, thus avoiding the centrifugation of a venous blood sample obtained through venepuncture, and test results are normally available within fifteen to thirty minutes. Immunoglobulins may also be eluted from blood spots blotted onto filter paper and dried (Lillo *et al.*, 1992). Once completely dry, blood from HIV-infected patients does not constitute a relevant infectious risk and the dried blood is stable over long time periods. Urine or oral fluid may also be employed for some assays (Kagulire *et al.*, 2007). Many rapid tests contain a "built-in" internal control, for example a control band indicating whether the samples and reagents have

been added correctly. At the present, many rapid tests are based on the principles of the second or third generation EIA with antigens from both HIV-1 and HIV-2, and very few are structured as fourth generation tests.

Rapid tests can present problems of sensitivity. It has been recently reported that, in South Africa, a significant proportion of HIV-infected children have been tested as false-negative using rapid tests (Tamashiro and Constantine, 1994). Occurrence of false positive tests by using rapid tests has also been reported, particularly in countries with high prevalence of HIV infection.

In countries with a limited laboratory infrastructure the use of HIV rapid testing algorithms has been more feasible and effective as EIA/WB algorithms (Stetler *et al.,* 1997). In developed countries a large proportion of people who are tested for HIV do not return for their test results (Tao *et al.,* 1999). Many testing and counselling sites have reported an increased demand after rapid testing was introduced, suggesting that many people prefer services where they can receive their test result without delay (Kassler *et al*., 1998). Several reports (Liu *et al.,* 2003) and case studies (UNAIDS, 2002) have indicated that rapid tests improve the acceptability of HIV testing. In comparison with other testing strategies, testing algorithms based on rapid tests have a lower cost per patient (Ekwueme *et al.,* 2003).

2.5: Diagnosis of syphilis

Diagnosis of syphilis and the choice of the most appropriate laboratory tests should take into consideration the stage of the disease. In primary syphilis and in some secondary stage lesions the diagnosis may be direct, i.e., by demonstrating the presence of *T. pallidum*. Serology may be used as of the second or third week after the chancre appears, when antibodies start to be detected (Azulay and Azulay, 2004).

2.5.1: Direct tests

Direct tests demonstrate the presence of *T. pallidum* and are definitive since they are not subject to interference of crossed mechanisms, that is, false positive results. They are indicated in the initial phase of the illness, when microorganisms are numerous. They can be indicated in primary and secondary syphilis in cases with ulcers, bullous lesions, mucous plaques, and condylomas. The use of material from the oral mucosa should take into account the difficulty in differentiating between the *Treponema* and other saprophyte spirochetes of the mouth, except when direct immunofluorescence methods are used (Azulay and Azulay, 2004).

2.5.1.1: Dark-field microscopy

This test consists of a direct examination of lymph from the exudates. The specimen is seen under the microscope with a dark-field condenser enabling visualization of the live mobile *T. pallidum* with indirect light. It is considered a quick, low-cost, and definitive test. Sensitivity varies from 74 to 86%, and can reach 97% depending on the analyzer's experience (Palmer *et al.*, 2003).

2.5.1.2: Direct examination with stained material

Methods used are those of Fontana-Tribondeau, Burri, Giemsa, and Levaditi. With the Fontana-Tribondeau method, after exudate is collected, it is smeared on a slide with the addition of silver. Silver impregnates the *Treponema* wall and makes it visible. Burri's method uses China ink (India ink). In Giemsa staining, *T. pallidum* colours are very pale and it is difficult to visualize the spirochete; Levaditi's method uses silver in histological slices. All staining methods are inferior to the use of the darkfield microscopy (Rivitti, 1999).

2.5.1.3: Direct immunofluorescence

The direct fluorescent antibody test for *T. pallidum* (DFA-TP) test is another standard microscopic test for syphilis; this test is a practical alternative to the direct darkfield examination when smears cannot be examined immediately because motile organisms are not required. In addition, oral lesions can be examined by DFA-TP because conjugate specific for pathogenic treponemes is used (Romanowski *et al.*, 1987). DFA-TP is the most specific means of diagnosing syphilis in lesion exudates or body fluids (Ito *et al.*, 1992). Immunofluorescence is more sensitive and does not have to be carried out immediately (Goh, 2005).

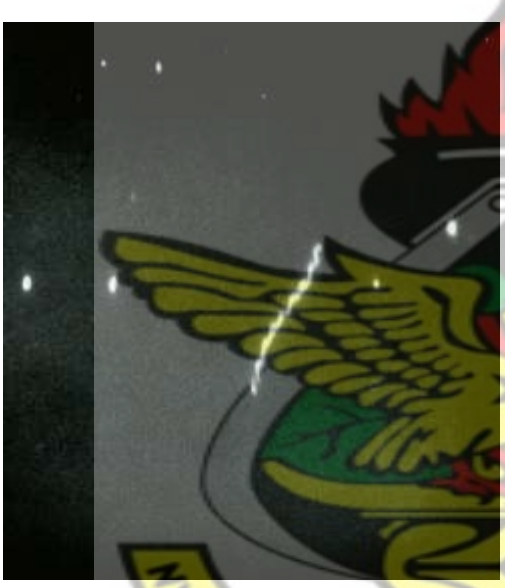

Fig 6: Darkfield View of Spirochetes. **Source:**http://www.brooksidepress.org/Products/OBGYN_101/MyDocuments4/Lab/Darkfield.jpg

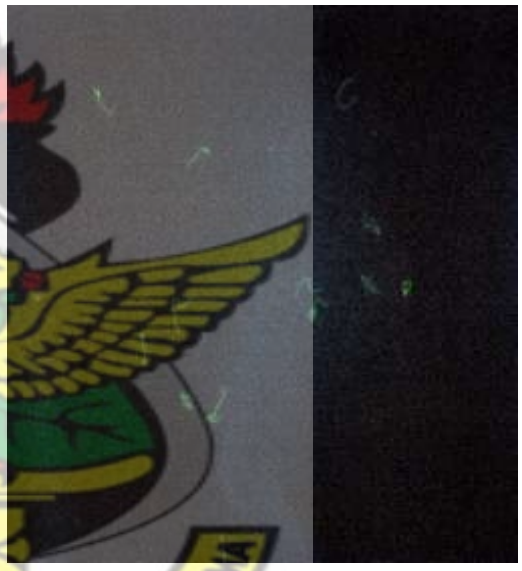

Fig 7: Positive FTA-ABS. **Source:** http://www.brooksidepress.org/Products/OBGYN_101/MyDocuments4/Lab/FTAABS.jpg

While darkfield examination and immunofluorescence provide direct evidence of infection they are not widely available. A failure to find the organism by either of the direct microscopic tests does not exclude a diagnosis of syphilis. Failure to demonstrate *T. pallidum* from typical lesions may be due to a low concentration of organisms or large amounts of debris in the specimen, prior treatment of the patient,

spontaneous healing of the lesion and, most commonly, poor technique (Daniels and Ferneyhough, 1977).

2.5.2: Serologic tests

Syphilis diagnosis continues to rely on serologic assays because *T. pallidum* cannot be cultured in vitro. Furthermore, direct visualization of the spirochete requires lesions and either fluorescent antibodies or a dark-field microscope, neither of which may be readily available. *T. pallidum* nucleic acid amplification tests are not widely available for use by clinical laboratories. Thus, serologic tests are the foundation of syphilis management, and knowledge of their diagnostic limitations is critical for clinicians. Serological tests for syphilis are divided into 2 categories: non-treponemal and treponemal – according to the type of antigen used in the test (Rivitti, 1999).

2.5.2.1: Non-*Treponema* tests

The first tests for syphilis diagnosis were compliment fixation reactions (CFR). The VDRL test turns positive between 5 and 6 weeks after infection and between 2 and 3 weeks after the chancre appears. Consequently, it may be negative in primary syphilis. In secondary syphilis, it proves to be highly sensitive, and in late forms of the disease, its sensitivity diminishes. This reaction is not specific for any particular treponeme, and it can be positive in all treponematoses and in several other situations. Titres are usually high (1:16) in treponematoses, and can even exceed 1:512. False-negative results in secondary syphilis (1% to 2%) are due to an excess of antibodies (prozone effect). These cases could be avoided with the use of greater serum dilutions (Azulay and Azulay, 2004).

Rapid non-*Treponema* tests have a vital significance in syphilis control. RPR, the most widely used and performed by a finger puncture. It provides a result in less than 30 minutes. It is also quantifiable, but not comparable with the titres obtained in the VDRL test. Persistence of low titres in patients who were correctly treated is called a serological scar and can persist for many years (Sanchez, 2003; Rotta, 2005).

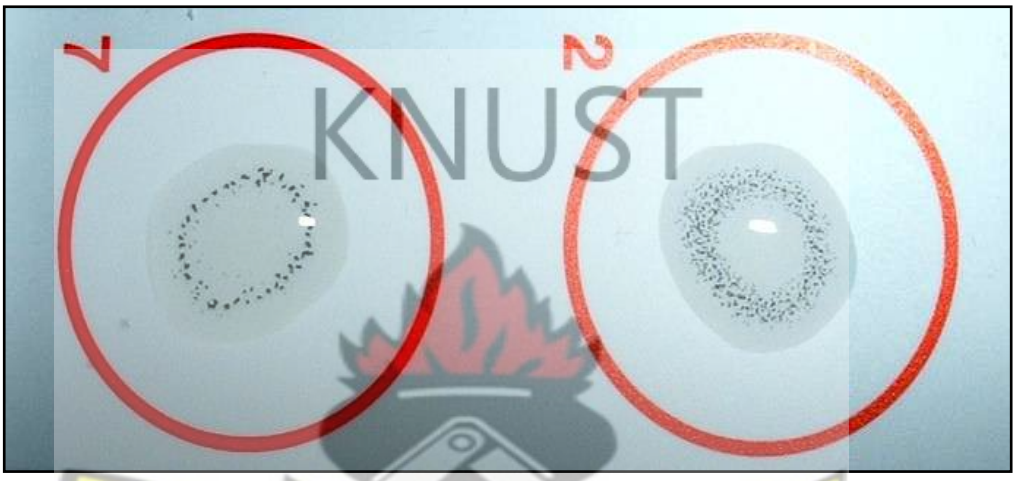

Figure 8: The VDRL test.
Source: http://drugline.org/medic/term/rapid-plasma-reagin-test/

2.5.2.2: *Treponema* tests

Treponema tests use *T. pallidum* as an antigen. They are used to confirm the reactivity of non-*Treponema* tests and in cases where non-*Treponema* tests have low sensitivity, such as in late syphilis. They turn positive a little earlier than non-*Treponema* tests. In 85% of people treated successfully, the results remain reactive for many years or even for their lifetime.

1. The *T. pallidum* immobilization (TPI) test was the first *Treponema* test developed. In spite of being specific, the reaction is difficult and costly to perform, and it is used strictly in research laboratories (Rotta, 2005).
2. The fluorescent *Treponema* antibody (FTA) test has undergone modifications in its dilution and has improved its sensitivity and specificity, resulting in the FTA-

ABS. It is quick and low-cost, but requires a fluorescent microscope. In autoimmune diseases and other Treponematoses there may be false-positive results (Azulay and Azulay, 2004).

3. *T. pallidum* haemagglutination assay (TPHA) and micro haemagglutination assay for *T. pallidum* (MHA-TP) are haemagglutination tests. MHA-TP is based on passive haemagglutination of sensitized erythrocytes of sheep (Larsen *et al.*, 1995). In untreated syphilis, its sensitivity is equal to that of FTA-ABS, except in initial primary syphilis where the latter is more sensitive (Rivitti, 1999).

2.5.3: Confirmatory methods

Treponema EIA and WB tests are confirmatory methods. WB identifies antibodies against IgM and IgG immuno-determinants with molecular masses (15kDa, 17kDa, 44kDa and 47kDa) (Sato *et al.*, 2004). So far, WB or EIA have shown a high level of sensitivity and specificity in all phases of syphilis, but are being used primarily in research projects (Rotta, 2005). In the early 1990s, two polymerase chain reaction (PCR) techniques with high levels of sensitivity and specificity were described and adopted (Orton *et al.*, 2002).

2.5.4: Rapid *Treponema* tests

Rapid *Treponema* tests were developed from agglutination tests and are very important as diagnostic aids in the light of their immediate readings. The immunochromatographic assay is the most effective, and allows visual and qualitative detection of antibodies (IgG, IgM and IgA) against a recombined antigen from 47kDa of the *T. pallidum* in whole blood, serum, and plasma of humans. The blood can be collected by finger puncture. Reading is performed between 5 and 20

minutes. Sensitivity and specificity of the test are 93.7% and 95.2%, respectively, and have proved to be superior to those of RPR in preliminary studies (Sato *et al.,* 2003). Nevertheless, this test should not be used as an exclusive criterion for the *T. pallidum* infection. These tests may substitute rapid non-*Treponema* tests, especially as screening assessments (Montoya *et al.,* 2006).

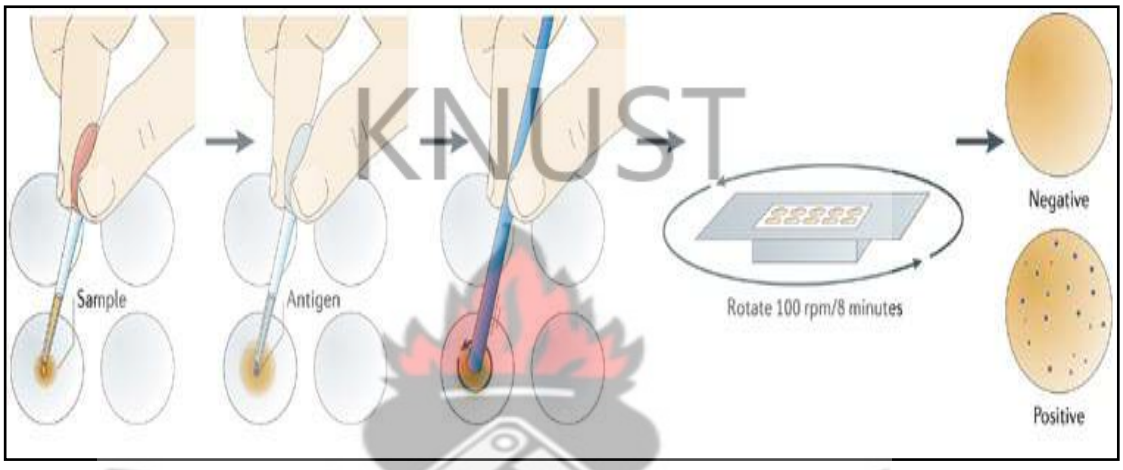

Figure 9: The rapid plasma reagin test.
Source: Peeling *et al.,* 2006

2.6: Ideal test for STIs

A significant barrier to effective healthcare in Africa is the lack of reliable and good quality laboratory services (Petti *et al.,* 2006). As more than 90% of Sexually Transmitted Infections (STIs) occur in settings where laboratory services are limited or not accessible, there is urgent need for simple, cheap, point-of-care (POC) tests for STIs. These tests should meet the 'ASSURED' criteria (Affordable, Sensitive, Specific, User-friendly (simple to perform in a few steps with minimal training), Robust and rapid (results available in less than 30 min), Equipment-free, Deliverable to those who need them) that have been developed by the WHO/SDI (Peeling *et al.,* 2006).

Gift *et al.*, found that the proportion of infected patients who are treated following diagnosis with an RDT with 65% sensitivity that does not require a patient to return to the clinic for the results is higher than the proportion of infected patients who are treated following diagnosis with a nucleic acid amplification test (NAAT) with 90% sensitivity that does require patients to return for treatment. This is because a significant number of infected individuals fail to return for treatment and also because transmission occurs in the interval between testing and treatment (Gift *et al.*, 1999).

2.7: HIV and syphilis screening for pregnant women in Ghana

Complications of pregnancy and childbirth are a major cause of death for women of reproductive age in Ghana. The national maternal mortality rate is estimated at 214 deaths per 100,000. However, rates vary widely by district and region, with some northern districts having a maternal mortality rate as high as 800 per 100,000 live births (Ghana Health Service, 2002). The direct causes include sepsis, haemorrhage, and hypertensive disorders of pregnancy, unsafe abortion and complications of obstructed labour. The indirect causes include malaria, anaemia, malnutrition and opportunistic infections associated with HIV/AIDS (UNFPA and MOH, 2004).

Most clinics tend to be more specialized, offering ANC and other essential services on separate days. For instance, diagnosis and treatment for clients with STI symptoms without referring the pregnant woman elsewhere was a routine component of ANC in just 44% of the facilities. Most hospitals offered a specialized service, with only 18% including STI management in ANC. Voluntary Counselling and Testing (VCT) for HIV/AIDS and syphilis screening are clearly not yet a routine component of ANC in Ghana, with only 8% and 4% of facilities respectively

including VCT and syphilis screening during ANC (Ghana Service Provision Assessment, 2002).

2.8: Dual test for HIV and syphilis

Although effective inexpensive treatment for syphilis exists, congenital syphilis remains a major cause of adverse pregnancy outcomes (World Health Organisation, 2011). 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). A simple, proven and inexpensive dual test for syphilis and HIV could improve the quality, acceptability and uptake of testing and treatment in rural areas to accelerate elimination of MTCT of syphilis and HIV (World Health Organisation, 2013).

The WHO is supporting PMTCT of HIV and syphilis integrated programs (Strasser *et al.,* 2012). The WHO therefore advocates syphilis screening in all pregnant women screened for HIV in countries implementing activities to prevent mother-to-child transmission of HIV (World Health Organisation, 2007). Harmonization of eliminating congenital syphilis (ECS) activities with HIV programmes must take place, since in many countries programmes for ECS and PMTCT of HIV share similar monitoring systems, both HIV and syphilis in pregnancy may affect similar sub-populations, and an incident STI such as a new syphilis infection can be a sign of increased risk of HIV acquisition or transmission (World Health Organisation, 2011). Integrating HIV testing and syphilis screening for pregnant women may enhance PMTCT of HIV, and prevent the adverse pregnancy outcomes of untreated syphilis throughout pregnancy.

Preliminary reports of the use of dual rapid tests for HIV and syphilis screening for same day diagnosis in antenatal clinics suggest that this is an appropriate and acceptable way to provide testing in this setting. The major advantage is that early results enable more women to access antenatal strategies for the PMTCT of HIV and congenital syphilis concurrently (World Health Organisation, 2007).

2.9: Rationale for evaluating rapid diagnostic tests

Although rapid tests continue to improve like EIAs, antigens used for these assays were originally derived from HIV-1 subtype B viruses. Thus, the existence of newly identified aberrant HIV variants in Africa coupled with the high degree of genetic diversity of HIV has historically posed a challenge, especially for persons during early seroconversion. Indeed, some studies have shown a significantly lower sensitivity of some screening assays to detect non-B subtype antibodies during seroconversion (Apetrei *et al.*, 1996). Moreover, several EIAs were withdrawn from circulation when it was shown that some variants of HIV-1 group O viruses were missed by these assays.

Many countries are performing evaluations to determine an algorithm of simple rapid tests that can be used at the POC for VCT, PMTCT and surveillance. Due to the number of kits appearing on the market, a preliminary review of available performance data cannot be over emphasized. Evaluating the performance of both current and future tests, their utility in a disease control programme and their acceptability to patients and healthcare providers will improve the diagnosis of infections in primary healthcare settings in developing countries and reduce unnecessary treatment.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1: Study site

The study was carried out at the Serology Unit of the Microbiology Laboratory of Komfo Anokye Teaching Hospital (KATH), the second-largest hospital in Ghana and the only tertiary health institution in the Ashanti Region. The geographical location of the hospital, the road network of the country and commercial nature of Kumasi make the hospital accessible to all the areas that share boundaries with Ashanti Region and others that are further away (Govindaraj *et al.*, 1996).

KATH is located in Kumasi, the Regional Capital of Ashanti Region. The hospital is located between longitudes W 1° 37' 46.8408" and W 1° 37.7806' and latitudes N 6° 41' 48.0746" and N 6° 41.801244' (latitude 6.696687° and longitude 1.629678°) (www.findlatitudeandlongitude.com).

Figure 10: A section of Kumasi Metropolis, showing location of KATH
Source: http://maps.google.com.gh/maps?q=MAP+OF+KUMASI+SHOWING KAT

3.2: Sample size calculation

From the general principles of evaluation of diagnostic tests for infectious diseases by the TDR diagnostics evaluation expert panel, the sample size was calculated according to the formula;

$$n \geq \frac{(1.96)^2 \times P(1-P)}{x^2}$$, where;

P = Sensitivity (or Specificity), *x* = Range within ± *x* to measure the sensitivity (or specificity) and *n*= Number of samples (or sample size)

The test kits were expected to have a sensitivity (or specificity) of 81% (0.81) or more and we wished to measure the sensitivity (or specificity) to within ± 0.05 (95% confidence interval). Thus;

$$n \geq \frac{(1.96)^2 \times 0.81(1-0.81)}{0.05^2}, \quad \text{hence; } n \geq 236.49$$

3.3: Study samples

The test kits were evaluated retrospectively, using 644 well-characterized archived serum specimens. The specimens were collected from pregnant women reporting for routine screening for HIV 1/2 and syphilis infection. 400 samples, consisting of different combination of HIV/AIDS and syphilis (Table 2) were tested with the SD Bioline HIV/Syphilis Duo test kit and the remaining 244 samples, consisting of 144 syphilis positives (quantitated into low (≤1:4), average (>1:4≤1:16) and high (>1:16≤1:64) titres using the IMMUTREP® RPR (Omega Diagnostics Ltd Scotland, UK)) and 100 syphilis negatives (Table 1) were tested with the SD Bioline Syphilis 3.0 and the Abbott Determine™ Syphilis TP.

The samples had been stored at -80ºC for 7 months. Ethical permission was sought from the Committee on Human Research, Publications and Ethics (CHRPE) of the

School of Medical Sciences (SMS), Kwame Nkrumah University of Science and Technology (KNUST) and KATH to use the archived samples for the study.

Table 1: Details of samples tested with the SD Bioline Syphilis 3.0 and the Abbott Determine™ Syphilis TP test kits

SYPHILIS POSITIVES			SYPHILIS NEGATIVES	TOTAL
Low Titres (≤1:4)	Average Titres (>1:4≤1:16)	High Titres (>1:16≤1:64)		
54	68	22	100	244

Table 2: Details of samples tested with the SD Bioline HIV/Syphilis Duo test kit

HIV Positive/ Syphilis Pos.	HIV Positive/ Syphilis Neg.	HIV Negative/ Syphilis Pos.	HIV Negative/ Syphilis Neg.	TOTAL
200	50	50	100	400

3.4: Gold standards

The gold standards which were used to characterise the samples are Genscreen ULTRA HIV Ag-Ab (Bio-Rad Laboratories, USA) and First Response® HIV 1-2-0 (Premier Medical Corporation Limited, India) for HIV testing and IMMUTREP® RPR (Omega Diagnostics Ltd Scotland, UK) and IMMUTREP® TPHA (Omega Diagnostics Ltd Scotland, UK) for syphilis testing.

Genscreen ULTRA HIV Ag-Ab (Bio-Rad Laboratories, USA) has been reported to have a sensitivity of 100% (from 744 HIV Ab positive samples, specimens from acute infected patients and from commercial seroconversion panels and HIV Ag samples) and a specificity of 99.95%, 99.75% and 99.72% from 6038, 409 and 313 samples respectively, tested at different sites. The First Response® HIV 1-2-0 (Premier Medical Corporation Limited, India), when tested with 120 positive and 122 negative samples against leading commercial anti-HIV 1/2 ELISA kit gave 100% and 99.18% sensitivity and specificity respectively. The IMMUTREP® RPR (Omega Diagnostics Ltd Scotland, UK) has been evaluated to give 100% sensitivity

and specificity respectively when 645 negative samples and 30 positive samples were tested at the Syphilis Reference Centre at Bristol Public Health Laboratory in the United Kingdom. The IMMUTREP® TPHA (Omega Diagnostics Ltd Scotland, UK) was evaluated to have a sensitivity and specificity of 98.5% and 99.6% respectively at the European Reference Centre with samples from Antenatal Clinics, Genito-Urinary Medical Clinical and Public Health Laboratories.

A sample was considered HIV positive if it was positive to both Genscreen ULTRA HIV Ag-Ab and First Response® HIV 1-2-0 and a sample was considered syphilis positive if it was positive to both IMMUTREP® RPR and IMMUTREP® TPHA and vice versa. A negative test which is positive by a reference test is considered a false negative and vice versa.

3.5: Confirmation of the gold standard results

To confirm the initial characterisation of the stored samples, they were re-tested using the gold standards. The tests were performed and the results read and interpreted according to the manufacturer's criteria. Results were read and interpreted by two independent readers; a Biomedical Scientist and a Researcher. No discrepant result was obtained.

3.5.1: HIV/AIDS testing using Genscreen ULTRA HIV Ag-Ab

3.5.1.1: Test procedure

25µl of conjugate-1 (R6) was applied to each well. 75µl of HIV Ag positive control (R5) and HIV Ab positive control (R4) were applied to wells A1 and B1. 75µl of negative control (R3) were applied to wells C1 to E1. 75µl of the specimen were applied to the remaining wells. The mixture was homogenised by shaking the

microplate. The microplate was incubated in a thermostat-controlled microplate incubator at 37°C for 1 hour. The wells were washed 4 times with 3.70 ml of washing solution, using an automatic washer. The plate was dried by turning it upside down on an absorbent paper. 100µl of conjugate-2 solution (R7a+R7b) was quickly dispensed into all wells, after been shaken. The plate was incubated for 30 minutes at room temperature (18-30°C).

The wells were washed 4 times with 3.70 ml of washing solution, using an automatic washer. The plate was dried by turning it upside down on an absorbent paper. 80µl of the substrate solution (R8+R9), freshly prepared before use, was quickly dispensed into each well. The reaction was allowed to develop in the dark at room temperature (18-30°C). 100µl of stopping solution (R10) was added to each well. The bottom of the plates were wiped and the optical density of each well was read at 450/620-700 nm, using a plate reader, within 30 minutes of stopping the reaction. The agreement between the spectrophotometric and visual readings and against the plate and sample distribution and identification plans was checked.

3.5.1.2: Interpretation of results

The mean absorbance of the negative control, OD R3 was calculated using the formula;

$$OD\ R3 = \frac{OD\ (C1) + OD\ (D1) + OD\ (E1)}{3}$$

The presence or absence of detectable HIV antigen or antibodies to HIV-1 and/or HIV-2 was determined by comparing the absorbance measured for each sample to the cut-off value (COV). The COV was obtained by the formula;

$$COV = OD\ R3 + 0.200.$$

Samples with absorbance values less than the COV were considered to be negative, whiles those with absorbance values equal to or greater than the COV were considered to be positive.

3.5.2: HIV/AIDS testing using First Response® HIV 1-2-0

3.5.2.1: Test procedure

The test devices were removed from the foil pouches and were placed on a flat, dry surface. 10µl of plasma was added to the sample well using a micropipette. 3 drops of the assay buffer was added to the sample well. The result was interpreted within 5 and 20 minutes at room temperature.

3.5.2.2: Interpretation of results

Appearance of 2 bands in the control and test areas indicated a positive result for HIV-1 and/or HIV-2, respectively. Appearance of the control band (in the control area) only, indicated a negative result. Absence of the control band, even if test band appeared indicated an invalid test.

3.5.3: Syphilis testing using IMMUTREP® TPHA

3.5.3.1: Test procedure (Qualitative)

The diluent was dispensed into the microtitration plate as follows: 25µl in rows 1, 3 and 4 and 100µl in row 2. 25µl of each sample was dispensed into a well in row 1 and mixed. 25µl was transferred from row 1 to row 2 and mixed. 25µl was transferred from row 2 to row 3 and mixed. 25µl from row 3 was discarded. 25µl from row 2 was transferred to row 4 and mixed. 25µl from row 4 was discarded. 75µl of well mixed Control Cells was added to row 3. 75µl of well mixed Test Cells

was added to row 4. The plate was tapped gently to mix. The plate was covered and left to stand at room temperature for 45 minutes. The plate was examined for agglutination patterns.

3.5.3.2: Interpretation of results

Agglutinated cells form an even layer over the bottom of the well. Non-agglutinated cells form a compact button in the centre of the well. Weakly agglutinated cells form a characteristic ring pattern. Agglutination of the Test Cells but not the Control Cells indicated the presence of specific antibody to *T. pallidum*. Absence of agglutination indicated that antibody is below the limit of detection of the system.

3.5.4: Syphilis testing using IMMUTREP® RPR

3.5.4.1: Test procedure (Quantitative)

Using isotonic saline, serial dilutions (50lµl) of the patient's serum (1/2, 1/4, 1/8, 1/16, 1/32, 1/64 etc) were prepared. One drop of each serum dilution was transferred to the test circle on the card. One drop (about 16µl) of the shaken antigen was added. The card was rotated for 8 minutes at 100 revolutions per minute. The result was inspected visually in good light.

3.5.4.2: Interpretation of Results

The titre was the last dilution that produces a reactive result.

3.6: HIV/AIDS-syphilis testing using SD BIOLINE HIV/Syphilis Duo

Out of the 644 serum samples used in the study, 400 (Table 2) were tested by the SD BIOLINE HIV/Syphilis Duo (Standard Diagnostics, INC. Korea). The tests were

performed and the results read and interpreted according to the manufacturer's criteria. Results were read and interpreted by two independent readers; a Biomedical Scientist and a Researcher.

3.6.1: Test Procedure

The test devices were removed from the foil pouches and were placed on a flat, dry surface. 10µl of plasma was added to the sample well using a micropipette. 3 drops (about 100µl) of the assay diluent was added to the sample well. The result was interpreted within 5 and 20 minutes at room temperature.

3.6.2: Result interpretation

The presence of two lines as control line (C) and test line 1(1) within the result window indicated a positive result for HIV-1/2 (Figure 18). The presence of two lines as control line (C) and test line 2 (2) within the result window indicated a positive result for syphilis (Figure 15). The presence of three lines as control line (C), test line 1 (1) and test line 2 (2) within the result window indicated a positive result for HIV-1/2 and syphilis (Figure 19). The presence of only one purple colour band within the result window indicated a negative result (Figure 14). The absence of the purple colour band within the result window after performing the test rendered the test invalid. Such specimens were re-tested.

3.7: Syphilis testing

The remaining 244 samples were tested by the SD BIOLINE Syphilis 3.0 (Standard Diagnostics, INC. Korea) and the Abbott Determine^{TM} Syphilis TP (Abbott Diagnostics, United Kingdom). The tests were performed and the results read and

interpreted according to the manufacturer's criteria. Results were read and interpreted by two independent readers; a Biomedical Scientist and a Researcher. The evaluation of the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP were conducted sequentially. This ensured that results for the tests were interpreted independently (blinding).

3.7.1: Syphilis testing using SD BIOLINE Syphilis 3.0

The test devices were removed from the foil pouches and were placed on a flat, dry surface. 10µl of plasma was added to the sample well using a micropipette. 4 drops (about 120µl) of the assay diluent was added to the sample well. The result was interpreted within 5 and 20 minutes at room temperature.

3.7.1.1: Interpretation of Results

The presence of only one purple colour band within the result window indicated a negative result (Figure 16). The presence of two bands ("T" band and "C" band) within the result window, no matter which band appeared first indicated a positive result for *T. pallidum* antibodies (Figure 17). The absence of the purple colour band within the result window after performing the test rendered the test invalid. Such specimens were re-tested.

3.7.2: Syphilis testing using Abbott Determine™ Syphilis TP

The desired numbers of test units (10-test card each) were arranged and labelled in accordance to those on the specimens. The protective foil covers were removed from each test. Using a precision pipette, 50µL of serum sample was applied to the sample pad. The results were read after 15 minutes.

3.7.2.1: Interpretation of Results

Any visible red colour in the patient and control windows was interpreted as positive, even if the patient bar appeared lighter or darker than the control bar. Appearance of one red bar in the control window of the strip with no red bar in the patient window of the strip was interpreted as a negative test. The absence of a red bar in the control window of the strip, even if a red bar appears in the patient window of the strip indicated an invalid test, and the test was repeated (Figure 13).

3.8: Quality control measures

1. Factors that might limit the generalisability of the results were considered, thus;
    a. The specimens used in the study were collected from a population similar to the population in which the test will be used.
    b. The credibility (performance characteristics) of the gold standards that were available to characterize the specimens was checked.
    c. We checked whether the specimens have been stored appropriately.
    d. We checked whether there are sufficient numbers of positive and negative specimens to provide an adequate sample size.
2. The test kits were inspected for signs of damage caused by heat or humidity.
3. The lot number and expiry date were checked.
4. Correct storage conditions of the test kits, as stated by the manufacturer, were ensured. The test kits were stored in a 2-8°C refrigerator.
5. From the refrigerator, in which the test kits and specimens were stored, they were brought to room temperature approximately 30 minutes before use. Test kits were opened only when they had reached room temperature, and were used immediately after opening.

6. All tests were performed exactly as described in the product insert of the various test kits.
7. National workplace safety guidelines with regard to the safety of clinic and laboratory personnel and the disposal of infectious waste were duly complied with.

3.9: Data analysis

The most commonly used measures of reliability of diagnostic test performance; sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined with Genscreen ULTRA HIV Ag-Ab, First Response® HIV 1-2-0, IMMUTREP® RPR and IMMUTREP® TPHA as the reference, with 95% confidence intervals. A formula for calculation of sensitivity, specificity, PPV and NPV is represented in Table 3.

Table 3: Calculation of sensitivity, specificity, PPV and NPV

	Condition (as determined by Gold Standard)	
	Condition Positive	Condition Negative
Test Outcome Positive	True Positive (a)	False Positive (Type 1 Error (b)
Test Outcome Negative	False Negative (Type 2 Error) (c)	True Negative (d)

Sensitivity	=	True Positive/Condition Positive, $a/(a+c)$
Specificity	=	True Negative/Condition Negative, $d/(b+d)$
PPV	=	True Positive/Test Outcome Positive, $a/(a+b)$
NPV	=	True Negative/Test Outcome Negative, $d/(c+d)$

95% confidence interval, the range at which one can be 95% certain that the interval contains the true values of sensitivity etc was calculated according to the formula;

$$P \pm 1.96\sqrt{\frac{P(1-P)}{n}}$$, where;

P = Sensitivity etc, measured as a proportion and *n* = Number of subjects positive (or negative) by the reference standard.

The McNemar chi square (x^2) for matched data was used to test for significance difference in sensitivity and specificity of the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP. The chi square was calculated according to the formula;

$$x^2 = \frac{\{(c-b)-1\}^2}{(c+b)}$$, refer Table 3 for b and c

A p-value < 0.005 was considered significant.

3.9.1: Kappa Coefficient

Cohen's Kappa (often simply called Kappa) is a measure of agreement between two binary variables, when the individual variables measure the same thing. Two raters classify the objects into categories 1 and 2. Below is the cell probability for a 2 by 2 chart.

		Rater #1		
		1	2	Total
Rater #2	1	P_{11}	P_{12}	$P_{1.}$
	2	P_{21}	P_{22}	$P_{2.}$
	Total	$P_{.1}$	$P_{.2}$	1

To compute Kappa, the observed level of agreement $(P_0=P_{11}+P_{22})$ is calculated. This value is compared to the value that you would expect if the two raters were totally independent $(P_e=P_{.1}xP_{1.}+P_{.2}xP_{2.})$. The value of Kappa, k is defined as;

$$k = \frac{(P_o-P_e)}{(1-P_e)}$$

Kappa is always less than or equal to 1. A value of 1 implies perfect agreement and values less than 1 imply less than perfect agreement. The interpretation of kappa is as follows; less than 0.20 (poor agreement), 0.20 to 0.40 (fair agreement), 0.40 to 0.60 (moderate agreement), 0.60 to 0.80 (good agreement), 0.80 to 1.00 (very good agreement). The kappa values were calculated with 95% confidence intervals.

3.9.2: Performance characteristics

The basic performance characteristics of a test designed to distinguish infected from uninfected individuals are sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

3.9.2.1: Sensitivity

The sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease. A test with 100% sensitivity correctly identifies all patients with the disease. A test with 80% sensitivity detects 80% of patients with the disease (true positives) but 20% with the disease go undetected (false negatives). A high sensitivity is clearly important where the test is used to identify a serious but treatable disease (e.g. HIV/AIDS).

3.9.2.2: Specificity

The specificity of a clinical test refers to the ability of the test to correctly identify those patients without the disease. A test with 100% specificity correctly identifies all patients without the disease. A test with 80% specificity correctly reports 80% of patients without the disease as test negative (true negatives) but 20% patients without the disease are incorrectly identified as test positive (false positives).

3.9.2.3: Positive predictive value

The PPV of a test is defined as the proportion of people with a positive test result who actually have the disease. For example, in a population of 100 people, 8 people with Disease A had a positive result for Test A, and 5 people without disease also tested positive, thus a total of 13 people tested positive. Out of these 13 people, only 8 actually had the disease.

3.9.2.4: Negative predictive value

The NPV of a test is the proportion of people with a negative test result who do not have disease. For example, in a population of 100 people, 85 people who did not have Disease A tested negative, and 2 people who had Disease A also tested negative, thus a total of 87 people tested negative. Out of these 87 people, 85 did not have the disease.

3.10: Operational characteristics

Operational characteristics include the time taken to perform the test, its technical simplicity or ease of use, the ease of interpretation of results, user acceptability and the stability of the test under user conditions.

The ease of use will depend on the ease of acquiring and maintaining the equipment required to perform the test, how difficult it is to train staff to use the test and to interpret the results of the test correctly, and the stability of the test under the expected conditions of use. Some of these characteristics may be qualitative and subjective.

When making decisions on the worthiness of a diagnostic test for a specific area, the assessment of the operational characteristics of the test is very essential. These

characteristics are important for determining the settings in which a diagnostic test can be used and the level of staff training required. Again, information on test stability is crucial for decisions on procurement.

CHAPTER FOUR

RESULTS

4.1: Performance of the SD BIOLINE HIV/Syphilis Duo

The SD BIOLINE HIV/Syphilis Duo detected neither false positive nor false negative against the gold standards in HIV testing, but reported 1 false positive and no false negative against the gold standards in syphilis testing (Tables 4 and 5).

The sensitivity, specificity, PPV and NPV of the SD BIOLINE HIV/Syphilis Duo in HIV/AIDS testing were 100.00% (95% C.I., 100.00%-100.00%), 100.00% (95% C.I., 100.00%-100.00%), 100.00% (95% C.I., 100.00%-100.00%), and 100.00% (95% C.I., 100.00%-100.00%) respectively (Table 10). The sensitivity, specificity, PPV and NPV of the SD BIOLINE HIV/Syphilis Duo in syphilis testing were 100.00% (95% C.I., 100.00%-100.00%), 99.33% (95% C.I., 98.02%-100.64%), 99.60% (95% C.I., 98.82%-100.38%) and 100.00% (95% C.I., 100.00%-100.00%) respectively (Table 10).

Table 4: Performance of the SD BIOLINE HIV/Syphilis Duo (HIV testing)

		Genscreen ULTRA HIV Ag-Ab/First Response® HIV 1-2-0		
		POSITIVE	**NEGATIVE**	**TOTAL**
SD BIOLINE HIV/Syphilis Duo (HIV testing)	**POSITIVE**	250	0	250
	NEGATIVE	0	150	150
	TOTAL	250	150	400

Table 5: Performance of the SD BIOLINE HIV/Syphilis Duo (Syphilis testing)

		IMMUTREP® RPR/IMMUTREP® TPHA		
		POSITIVE	**NEGATIVE**	**TOTAL**
SD BIOLINE HIV/Syphilis Duo (Syphilis testing)	**POSITIVE**	249	1	250
	NEGATIVE	0	150	150
	TOTAL	249	151	400

4.2: Performance of the SD BIOLINE Syphilis 3.0

Whilst the gold standards detected 100 negative and 144 positive, the SD BIOLINE Syphilis 3.0 detected 112 negatives and 132 positives (Table 9). The specificity, sensitivity, PPV and NPV of the SD BIOLINE Syphilis 3.0 were 91.67% (95% C.I., 87.16%-96.18%), 100.00% (95% C.I., 100.00%-100.00%), 100.00% (95% C.I., 100.00%-100.00%) and 89.29% (95% C.I., 83.23%-95.35%) respectively (Table 10). The SD BIOLINE Syphilis 3.0 obtained 12 false negatives but no false positive (Table 6). The 132 positives that were detected by the SD BIOLINE Syphilis 3.0 consisted of 79.63% (43 in 54) low titres, 98.53% (67 in 68) average titres and 100% (22 in 22) high titres (Figure 11).

Table 6: Performance of the SD BIOLINE Syphilis 3.0

	IMMUTREP® RPR/IMMUTREP® TPHA		
SD BIOLINE Syphilis 3.0	**POSITIVE**	**NEGATIVE**	**TOTAL**
POSITIVE	132	0	132
NEGATIVE	12	100	112
TOTAL	144	100	244

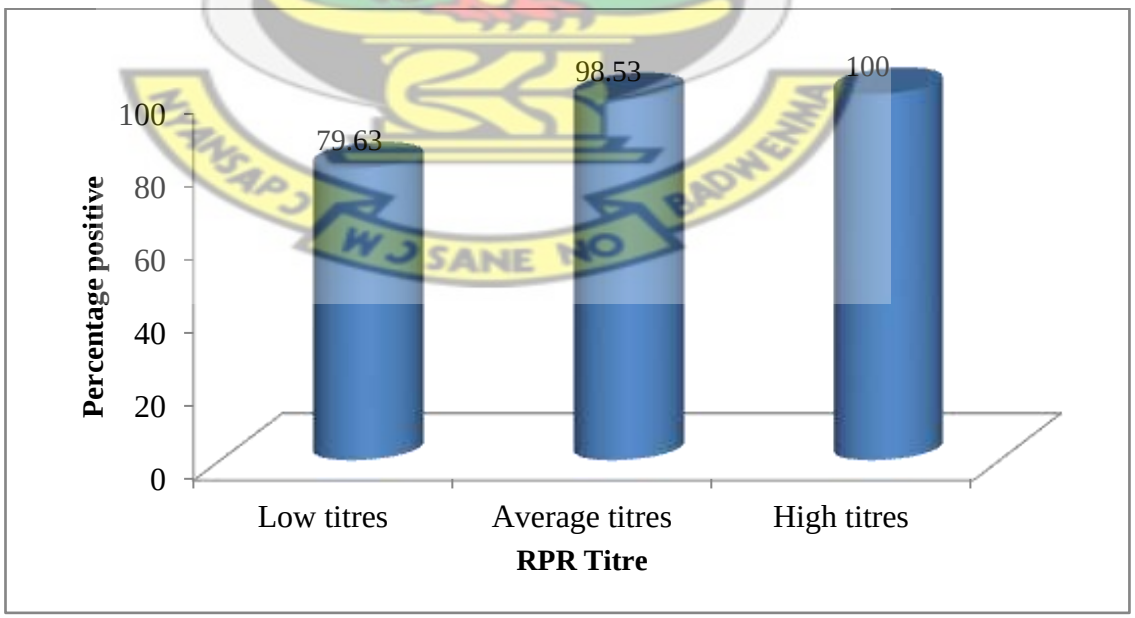

Figure 11: Performances of the SD BIOLINE Syphilis 3.0 by RPR titre

4.3: Performance of the Abbott Determine™ Syphilis TP

Whilst the gold standards detected 100 negative and 144 positive, the Abbott Determine™ Syphilis TP detected 100 negatives and 109 positives (Table 9). The specificity, sensitivity, PPV and NPV of the Abbott Determine™ Syphilis TP were 75.69% (95% C.I., 68.68%-82.70%), 100.00% (95% C.I., 100.00%-100.00%), 100% (95% C.I., 100.00%-100.00%) and 74.07% (95% C.I., 65.48%-82.66%) respectively (Table 10). The Abbott Determine™ Syphilis TP obtained 35 false negatives but no false positive (Table 7). The 109 positives that were detected by the Abbott Determine™ Syphilis TP consisted of 55.56% (30 in 54) low titres, 85.29% (58 in 68) average titres and 95.45% (21 in 22) high titres (Figure 12).

Table 7: Performance of the Abbott Determine™ Syphilis TP

		IMMUTREP® RPR/IMMUTREP® TPHA		
		POSITIVE	**NEGATIVE**	**TOTAL**
Abbott Determine TM Syphilis TP	**POSITIVE**	109	0	109
	NEGATIVE	35	100	135
	TOTAL	144	100	244

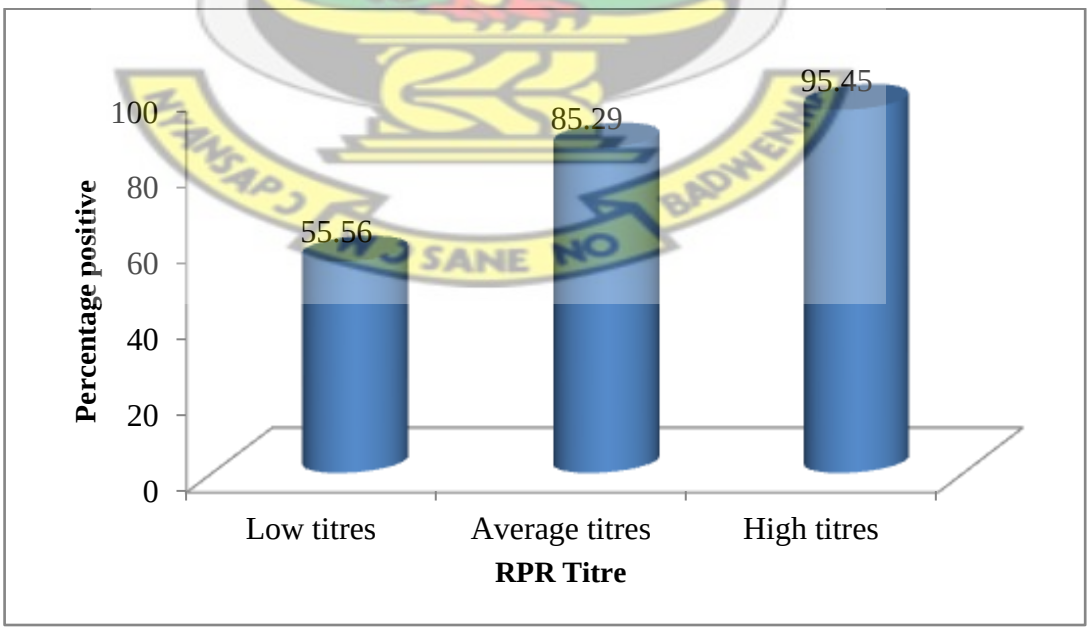

Figure 12: Performances of the Abbott Determine ™ Syphilis TP by RPR titre

Table 8: Summary of test results - SD BIOLINE HIV/Syphilis Duo

	Genscreen ULTRA HIV Ag-Ab/First Response® HIV 1-2-0/IMMUTREP® RPR/ IMMUTREP® TPHA	SD BIOLINE HIV/Syphilis Duo	SD BIOLINE HIV/Syphilis Duo
		(HIV testing)	(Syphilis testing)
Number of positives	250	250	251
Number of negatives	150	150	149
Total	**400**	**400**	**400**

Table 9: Summary of test results - SD BIOLINE Syphilis 3.0 and Abbott Determine™ Syphilis TP

	IMMUTREP® RPR/ IMMUTREP® TPHA	Abbott Determine™ Syphilis TP	SD BIOLINE Syphilis 3.0
Number of positives	144	109	132
Number of negatives	100	135	112
Total	**244**	**244**	**244**

Table 10: Summary of test performance of SD BIOLINE Syphilis 3.0, SD BIOLINE HIV/Syphilis Duo and the Abbott Determine™ Syphilis TP

	Sensitivity (%) (95% C. I.)	Specificity (%) (95% C. I.)	PPV (%) (95% C. I.)	NPV (%) (95% C. I.)
SD BIOLINE Syphilis 3.0	91.67 (87.16-96.18)	100.00 (100-100)	100.00 (100-100)	89.29 83.23-95.35)
Abbott Determine™ Syphilis TP	75.69 (68.68-82.70)	100.00 (100-100)	100.00 (100-100)	74.07 (65.48-82.66)
SD BIOLINE HIV/Syphilis Duo (HIV testing)	100.00 (100-100)	100.00 (100-100)	100.00 (100-100)	100.00 (100-100)
SD BIOLINE HIV/Syphilis Duo (Syphilis testing)	100.00 (100-100)	99.33 (98.02-100.64)	99.60 (98.82-100.38)	100.00 (100-100)

4.4: Kappa statistics

The kappa value was used to estimate the overall performance of the test, that is, the combined correlation of sensitivity and specificity for all tests against the gold standards.

The kappa value of the SD BIOLINE HIV/Syphilis Duo in HIV and syphilis testing were 1.00 (95% C.I., 1.00-1.00) and 0.99 (95% C.I., 0.98-1.00) respectively, whiles that of the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP were 0.90 (95% C.I., 0.86-0.94) and 0.72 (95% C.I., 0.66-0.78) respectively (Table 11).

Table 11: Kappa values of the tests used

Test kit	SD BIOLINE HIV/Syphilis Duo (HIV testing)	SD BIOLINE HIV/Syphilis Duo (Syphilis testing)	SD BIOLINE Syphilis 3.0	Abbott Determine™ Syphilis TP
Kappa value	1.00	0.99	0.90	0.72

4.5: Comparison of the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP

The kappa value was used to determine the agreement between the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP. It was calculated from Table 12 below.

Table 12: Comparison of the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP

		SD BIOLINE Syphilis 3.0		
Abbott Determine TM Syphilis TP		**POSITIVE**	**NEGATIVE**	**TOTAL**
	POSITIVE	109	0	109
	NEGATIVE	23	112	135
	TOTAL	132	112	244

A high degree of agreement with a kappa value 0.81 (95% C. I. 0.76-0.86) was obtained for the two test kits.

The McNemar chi square (x^2) was used to test the significant difference in sensitivity and specificity of the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP. The SPSS output for the calculation of McNemar chi square (x^2) is presented in appendix 4. Chi square value of 25.04 was obtained for the analysis between the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP, with a corresponding p-value of 0.000000561. This indicates that the difference in sensitivity and specificity between the two tests is statistically significant.

4.6: Operational characteristics

The operational characteristics of the tests used in this study are provided in the tables 13 and 14 below.

Table 13: Operational characteristics of the tests kits evaluated in the study

Test kit	**SD BIOLINE HIV/Syphilis Duo**	**SD BIOLINE Syphilis 3.0**	**Abbott Determine™ Syphilis TP**
Manufacturer	Standard Diagnostics, INC. Korea	Standard Diagnostics, INC. Korea	Abbott Diagnostics, USA
Sensitivity	100% (HIV), 99% (Syphilis)	99.3%	100%
Specificity	99.6% (HIV), 99.5% (Syphilis)	99.5%	100%
Storage Conditions	1-30°C at room temperature	Room temperature	2-30°C
Ease of performance	Very easy	Very easy	Very easy
Controls	Procedural control	Procedural control	Procedural control
Procedural steps	One step	One step	One step
Total time required	20-30 minutes	20-30 minutes	20-30 minutes
Source of sample	Whole blood, Plasma, Serum	Whole blood, Plasma, Serum	Whole blood, Plasma, Serum
Antigen/Antibody/ Assay type	HIV 1/2, *Treponema pallidum*	*Treponema pallidum*	*Treponema pallidum*
Reading	Visual	Visual	Visual
Essential items needed but not provided	Disposable cover rips for pipettes, Latex, Timer	Nil	Lancets, EDTA capillary tubes, Pipette, Pipette tips
Ease of interpretation of results	Very easy	Very easy	Very easy
Clarity of kit's instruction	Very easy to read and understand	Very easy to read and understand	Very easy to read and understand

Table 14: Operational characteristics of the gold standards used in the study

Test kit	**Genscreen ULTRA HIV Ag-Ab**	**First Response® HIV 1-2-0**	**IMMUTREP® TPHA**	**IMMUTREP® RPR**
Manufacturer	Bio-Rad Labora-tories, USA	Premier Medical Corporation, India	Omega Diagnostics Ltd Scotland, UK	Omega Diagnostics Ltd Scotland, UK
Sensitivity	100%	100%	98.5%	100%
Specificity	99.71%	99.18%	98.5%	100%
Storage Conditions	2-8°C	2-8°C	2-8°C	2-8°C
Ease of performance	Less easy	Very easy	Easy	Easy
Controls	5	Procedural control	2	None
Procedural steps	Four steps	One step	Four steps	Four steps
Time required	120 minutes	5-20 minutes	45-60 minutes	20-30 minutes
Source of sample	Plasma, Serum	Plasma, Serum, Whole blood	Fresh serum	Plasma, Serum
Antigen/Antibody /Assay type	HIV 1/2	HIV1/2	*Treponema pallidum*	*Treponema pallidum*
Reading	Reading equipment	Visual	Visual	Visual
Essential items needed but not provided	Precision pipettes, Microwell strip reader, Gloves	Micropipette, Timer	Microtitration plates Microtitration droppers	Micropipette, Test tubes, Rotator, Isotonic saline
Ease of interpretation of results	Less easy	Easy	Less easy	Less easy
Clarity of kit's instruction	Less easy to read and understand	Very easy to read and understand	Easy to read and understand	Very easy to read and understand

CHAPTER FIVE

DISCUSSION

Laboratory and point-of-care (POC) tests are potentially powerful contributors to the management and control of STIs through facilitation of prevention of STI transmission and their sequelae (World Health Organisation, 2013). The reasons why prevention and control of STIs in the developing world has not been a success are complex but the common hindrance to effective health care in Africa is the lack of access to reliable, affordable and accurate laboratory services. The use of RDTs provides the opportunity for small or field-based laboratories to deliver results on the same day. RDTs do not require refrigeration as long as temperatures are below 27°C and special equipment, processing of specimens in batches or highly skilled laboratory staff.

The aim of this study was to evaluate the technical performance and major operational characteristics of the SD BIOLINE HIV/Syphilis Duo and the SD BIOLINE Syphilis 3.0 test kits.

5.1: False negatives

A relatively low number of 47 false negatives results were obtained. The Abbott Determine™ Syphilis TP detected 35 out of the total 47 whiles the SD BIOLINE Syphilis 3.0 detected the remaining 12. The false negative recorded could be due to the prozone phenomenon. Such false-negatives occur in 1% to 2% of patients, especially in pregnant women and HIV patients (Ratnam, 2005). Declared negative, such individuals may have a sense of false security that could result in continuing transmission of the bacteria to sexual partners and/or foetuses.

5.2: False positives

A relatively very low number of 1 false positive was obtained by the SD BIOLINE HIV/Syphilis Duo in syphilis testing. This could be due to the fact that phospholipid antibodies detected by non-treponemal tests are not only produced in syphilis and other treponemal disease but also in response to a variety of conditions unrelated to syphilis (Ratnam, 2005). Mislabelled or contaminated specimen and cross-reacting antibodies may also be the cause false positive results (Atkins, 2005). False positive results could also occur in individuals with autoimmune disorders, hypergamaglobulinaemias, haemodialysis, haemophilia, acute Estein-Baar virus (EBV), human cytomegalovirus infection or multiple myeloma (Luft *et al.*, 2004). These could not be excluded in the archived samples that were used in the study. The freezing-thawing of the specimens could have affected the quality result obtained by the test kits (UNICEF, UNDP, World Bank, WHO, 2010).

Although relatively low, the false positive results given by the test kit indicates that results from a single test could not be used as a basis for declaring a sample or an individual as positive. It is in line with this that the World Health Organisation and the United States Agency for International Development (WHO/UNAIDS) and the Ghana Health Service (GHS) recommend supplementary testing on initially reactive samples, with specific test, before declaring the sample as positive (GHS, 2004).

5.3: Performance of SD BIOLINE HIV/Syphilis Duo, SD BIOLINE Syphilis 3.0 and Abbott Determine™ Syphilis TP

The SD BIOLINE HIV/Syphilis Duo exhibited excellent sensitivities and specificities of 100.00% (95% C. I. 100.00%-100.00%) and 100.00% (95% C. I. 100.00%-100.00%) respectively for HIV testing and 100.00% (95% C. I. 100.00%-100.00%) and 98.67% (96.84%-100.50%) respectively for syphilis testing. Although not much information is available about the performance of SD BIOLINE HIV/Syphilis Duo, these results compare very well with the 100.00% sensitivity and 99.60% specificity reported for HIV testing against Architect HIV Ag/Ab combo (Abott Laboratories, Inc.) gold standard, using serum samples from 806 patients and 99.00% sensitivity and 99.50% specificity reported for syphilis testing against ASAN TPHA (ASAN Pharmaceutical, Inc.) gold standard using serum samples from 318 patients in Korea. Result of SD BIOLINE HIV/Syphilis Duo tested with serum, plasma and whole blood have 100% agreement, which means the performance of SD BIOLINE HIV/Syphilis Duo in serum, plasma and whole blood are identical (Standard Diagnostics, 2012).

The sensitivity and specificity of SD BIOLINE Syphilis 3.0 in this study was 91.67% (95% C. I. 87.16%-96.18%) and 100.00% (95% C. I. 100.00%-100.00%) respectively. Not much information is available about the performance of SD BIOLINE Syphilis 3.0 in Ghana. However, this compares favourably with the 94.2-95.0% and 94.9%- 97.8% overall sensitivities and specificities reported by Herring *et al.,* using serum samples (Herring *et al.,* 2006). Using whole blood samples in three countries (Tanzania, Brazil and China), Mabey *et al.,* recorded sensitivities ranging from 90.2% to 95.5% and 85.7% to 88.2%, respectively for laboratory and local clinic testing. Specificities were 95.5% to 99.4% and 98.8% to 99.6%, respectively

(Mabey *et al.,* 2006). Montoya *et al.,* found similar results; sensitivities were 96.3% and 86.0%, respectively for laboratory and healthy facility testing, while specificity showed no difference, 96.8% and 96.4% (Montoya *et al.,* 2006).

The sensitivity and specificity of Abbott Determine^{TM} Syphilis TP in this study was 75.69% (95% C. I. 68.68%-82.70%) and 100.00% (95% C. I. 100.00%-100.00%) respectively. This compares quiet well with the sensitivity and specificity of 93.6% and 92.5% respectively obtained by Sato *et al.,* using 125 serum samples from patients with clinical, epidemiological and serological diagnosis of syphilis, patients with sexually transmitted disease other than syphilis, and individuals with negative serology for syphilis in Brazil (Sato *et al.,* 2003). Diaz *et al.,* using stored sera and TPHA as the reference test in Brazil also obtained a sensitivity of 95.6 to 98.4% and specificity of 95.7 to 97.3% (Diaz *et al.,* 2004). For whole blood sample, the sensitivity ranged from 85.9% to95.0%, and specificity was higher than 97.7% (Gianino *et al.,* 2007) when performed in the reference laboratory. Lower sensitivity of 75.6% was found when the assay was performed at local clinic (Li *et al.,* 2009).

5.4: Comparison of the SD BIOLINE Syphilis 3.0 and the Abbott Determine^{TM} Syphilis TP

From Table 12 and result analysis, it could be observed that the sensitivity and specificity (Table 10) of SD BIOLINE Syphilis 3.0 and Abbott Determine^{TM} Syphilis TP were significantly different from each other (p=0.000000561). The kappa value of SD BIOLINE Syphilis 3.0 (0.90 (95% C.I., 0.86-0.94)) was higher than that of the Abbott Determine^{TM} Syphilis TP (0.72 (95% C.I., 0.66-0.78)). This implies that both tests both tests compared favourably in terms of technical performance,

however, the SD BIOLINE Syphilis 3.0 exhibited superiority over the Abbott Determine™ Syphilis TP in terms of sensitivity and NPV.

5.5: Operational characteristics

Table 13 and 14 gives the operational characteristics for the test kits we evaluated and the gold standards respectively. SD BIOLINE HIV/Syphilis Duo, SD BIOLINE Syphilis 3.0, Abbott Determine™ Syphilis TP and First Response® HIV 1-2-0 were easy to perform, rapid and involved just a single step to complete. They are suitable for screening single samples as well as samples in batches and do not require additional instrument or equipment to perform. They do not require any special storage conditions, no refrigeration and no processing, for example, centrifugation. They could be performed by the patient with no or little guidance. These qualities make the SD BIOLINE HIV/Syphilis Duo, the SD BIOLINE Syphilis 3.0 and the Abbott Determine™ Syphilis TP test kits most suitable in rural settings where refrigeration for storage and electricity may not be available and instruments and equipment for a test may be lacking.

However, the Genscreen ULTRA HIV Ag-Ab, the IMMUTREP® RPR and the TPHA Syphilis serodiagnostic test used as the reference standards were time consuming and the result interpretation was difficult. The tests require the use of instruments that are not provided by the manufacturer. The tests involve more steps, more controls and requires more time and high precision to perform. A refrigerator that required constant supply of electricity is also required for the storage of the kits. These features of the Genscreen ULTRA HIV Ag-Ab, the IMMUTREP® RPR and the IMMUTREP® TPHA make it applicable mainly in well-resourced laboratories. Genscreen is ideal and economical for screening large volumes of samples. With its

high sensitivity coupled with its ability to detect p24 antigen, it will be a perfect test for screening blood for transfusion and for organ transplant. It will be the appropriate EIA test to diagnose HIV infections in infants.

5.6: SD BIOLINE HIV/Syphilis Duo – A dual test for HIV and syphilis

The feasibility and acceptability of introducing rapid syphilis testing (RST) in PMTCT of HIV services should be well addressed. Feasibility deals with the implementation of RSTs to improve identification and treatment of maternal syphilis without compromising HIV services and the ability to conduct multiple rapid tests concurrently, while acceptability deals with health workers' satisfaction with and correct and consistent use of RST.

Strasser *et al.* found a significant increase of 97.7% women tested for HIV during the first 5 months of RST implementation in ANCs in Zambia (Strasser *et al.*, 2012). However, conducting multiple rapid tests for HIV and syphilis is time, money and sample consuming. The length of time taken to conduct the tests shall also affect the acceptability of the policy.

The SD Bioline HIV/syphilis Duo which can be used to screen HIV and syphilis simultaneously answers best the question of feasibility and acceptability in the elimination of mother-to-child transmission HIV and congenital syphilis. With the SD Bioline HIV/syphilis Duo, screening for syphilis could be combined with HIV testing. This can potentially overcome many of the current barriers to syphilis screening in ANCs in Ghana. The synergy between a programme for antenatal syphilis testing and a similar programme for HIV testing, would consolidate resources. This is more cost- and time-effective than any one programme because

counselling of patients, sample collection, and testing could be done at the same visit.

However, several studies have described the reluctance of some women to return for their test result (Sorin *et al.,* 1996). But with the SD Bioline HIV/syphilis Duo, results are available in just a few minutes. Therefore, simultaneous on-site testing for HIV and syphilis would be very feasible. Women can be tested and receive treatment at the same visit. This would enable more cases of congenital syphilis and PMTCT of HIV to be prevented. The SD Bioline HIV/syphilis Duo is very accurate, affordable and simple to perform. It can therefore be used at the lowest levels of health-service delivery in the country.

5.7: CONCLUSION

1. This study has found the SD BIOLINE HIV/Syphilis Duo and the SD BIOLINE Syphilis 3.0 test kits to be a reliable diagnostic tool that is very sensitive and specific in diagnosing HIV and syphilis. The test kits were found to be simple, rapid and easy to perform. Results were easy to read and interpret.
2. The tests were safe to conduct, convenient and acceptable, and the tests could be done without additional equipment or reagent. The test could also be performed by patients with very little guidance.
3. The SD BIOLINE HIV/Syphilis Duo and the SD BIOLINE Syphilis 3.0 can therefore be used in resource constrained laboratories. With their high specificities and NPVs, they will be a perfect confirmatory rapid test for HIV and syphilis screening in the rural and the district laboratories of Ghana.

4. The SD BIOLINE HIV/Syphilis Duo, a dual test for HIV and syphilis, was found to be a perfect tool in concurrent HIV and syphilis screening in Ghana to prevent MTCT of HIV and congenital syphilis.

5.8: RECOMMENDATIONS

1. Based on the ASSURED criteria and the need to expand HIV and syphilis diagnostic services as part of a greater framework of health system strengthening within resource-limited settings, the SD BIOLINE HIV/Syphilis Duo and the SD BIOLINE Syphilis 3.0 test kits can be considered as a point-of-care diagnostic device for resource limited endemic areas.
2. Based on the WHOs support for the prevention of MTCT of HIV and congenital syphilis integrated programs, the SD BIOLINE HIV/Syphilis Duo can be used for simultaneous screening of syphilis and HIV at ANC's in Ghana.
3. It is recommended that further studies be carried out to determine the reproducibility of the SD BIOLINE HIV/Syphilis Duo and the SD BIOLINE Syphilis 3.0 test kits and that further evaluation of the test kits be conducted in multi-centre laboratories.

5.9: LIMITATION

Since the specimens have been stored for 7 months, the quality of the specimens could have been affected by the occasional freezing-and-thawing of the specimens. Also, patient information (e.g. age, sex and severity of symptoms) were not available (UNICEF, UNDP, World Bank, WHO, 2010).

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APPENDICES

Appendix 1: Extra information on the test kits used

1.1: Genscreen ULTRA HIV Ag-Ab

1.1.1: Assay Description

The Genscreen ULTRA HIV Ag-Ab kit is a microplate EIA for the detection of HIV antigen or antibodies in human serum and plasma. The kit can be used for screening and simultaneously detects HIV antigens and antibodies against HIV 1 and HIV 2 using the principle of the sandwich technique.

1.1.2: Principle

HIV p24 antibodies and recombinant HIV protein gp160 on the solid phase bind to any p24 antigen or gp160 antibodies present in the sample. The samples are incubated with conjugate 1which binds to any p24 bound to the solid phase antibodies. A second conjugate is added. This labels the antibodies present. A coloured signal is achieved by the addition of tetramethyl benzidine (TMB) substrate. Any positive sample will change colour and are read at a wavelength of 450/620-700 nm.

1.2: SD BIOLINE Syphilis 3.0

1.2.1: Assay Description

The SD BIOLINE Syphilis 3.0 test is a solid phase immunochromatographic assay for the qualitative detection of antibodies to all isotypes (IgG, IgM, IgA) against *T. pallidum* simultaneously in human serum, plasma or whole blood. The test device contains a membrane strip, which is pre-coated with recombinant *T. Pallidum* antigens (17.15 KDa) on the test band region. The device has a letter “T” and “C” as

Test line and Control line on the surface of the case. Both the Test lines and the Control line in the result window are not visible before applying any sample. The Control line is used as a procedural control. It should appear if the test procedure is performed properly and the test reagents of Control line are working.

1.2.2: Test Principle

The recombinant *T. pallidum* antigens – colloid gold conjugate (17.15 KDa), the patient sample and sample diluent move along the membrane chromatographically to the test region (T) and form a visible line as the antigen-antibody-antigen gold particle complex forms. Therefore, the formation of a visible line in the test region (T) indicates a positive result for the detection of *T. pallidum* specific antibodies (IgG, IgM and IgA).

1.3: SD BIOLINE HIV/Syphilis Duo

1.3.1: Assay description

The SD BIOLINE HIV/Syphilis Duo is a solid phase immunochromatographic assay for the qualitative detection of antibodies to all isotypes (IgG, IgM, IgA) specific to HIV-1/2 and/or *T. pallidum* simultaneously in human serum, plasma or whole blood. The test device contains a membrane strip, which is pre-coated with recombinant HIV-1 capture antigen (gp41), recombinant HIV-2 capture antigen (gp36) and recombinant HIV-sub O antigen on test band 1 region and recombinant *T. Pallidum* antigens (17KDa) on test band 2 region respectively. The device has a letter of 1, 2 and C as Test line 1 (HIV-1/2), Test line 2 (Syphilis) and Control line on the surface of the case. Both the Test lines and the Control line in the result window are not visible before applying any sample. The Control line is used as a procedural control.

It should appear if the test procedure is performed properly and the test reagents of Control line are working.

1.3.2: Test Principle

The recombinant HIV-1/2 antigen (gp41, gp36) – colloid gold conjugate, recombinant *T. pallidum* antigens – colloid gold conjugate (17KDa), the specimen sample and sample diluent move along the membrane chromatographically to the test region (T) and form a visible line as the antigen-antibody-antigen gold particle complex forms.

1.4: Abbott Determine ™ Syphilis TP

1.4.1: Assay description

The Abbott Determine™ Syphilis TP is an *in Vitro*, visually red, qualitative immunoassay for the detection of antibodies to *T. pallidum* in human serum, plasma or whole blood. The test is intended as an aid to detect antibodies to *T. Pallidum* from infected individuals. Whole blood or plasma specimens containing anticoagulants other than EDTA may give incorrect results. No test provides absolute assurance that a sample does not contain low levels of antibodies to *T. Pallidum* such as those present at a very early stage of infection. Therefore a negative result at any time does not preclude the possibility of exposure to or infection with syphilis.

1.4.2: Biological Principles of the Procedure

Determine Syphilis TP is an immunochromatographic test for the qualitative detection of antibodies to *Treponema pallidum* antigens. Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and

mixes with the *T. Pallidum* antigen – selenium colloid conjugate. This mixture continues to migrate through the solid phase to the immobilised *T. Pallidum* antigens at the patient window site. If antibodies to *T. Pallidum* are present in the sample, the antibodies bind to the *Treponema pallidum* antigen-selenium colloid and to the *T. Pallidum* antigen at the patient window, forming a red line at the patient window site. If antibodies to *T. Pallidum* are absent, the *T. Pallidum* antigen-selenium colloid flow past the patient window and no red line is formed at the patient window site.

1.5: First Response® HIV 1-2-0

1.5.1: Assay description

The First Response® HIV 1-2-0 Test Strip (Serum/Plasma/Whole Blood) is a rapid chromatographic immunoassay for the qualitative detection of antibody to HIV type-1 and/or type-2 in serum, plasma, or whole blood.

1.5.2: Test principle

The First Response® HIV 1-2-0 Test Strip (Serum/Plasma/Whole Blood) is a qualitative, membrane based immunoassay for the detection of antibody to HIV in serum, plasma, or whole blood. The membrane is coated with recombinant HIV antigens on the test area of the device. When a serum or plasma or whole blood specimen is applied to the test strip, it reacts with recombinant antigen coated coloured particle. The mixture then migrates towards the Result Window of the strip and reacts with the recombinant HIV antigens on the membrane in the test area. If the specimen contains antibodies to HIV-1 or HIV-2, the coloured line will appear in the test area, showing a positive result. The absence of the coloured line indicates that the specimen does not contain the anti-HIV antibodies, showing a negative

result. A coloured line will always appear at the control area if the test has been performed properly.

1.6: IMMUTREP® TPHA

1.6.1: Assay description

IMMUTREP TPHA is a specific, sensitive passive haemagglutination test for the detection of antibodies to *T. pallidum* in serum or Cerebro-Spinal Fluid (CSF).

1.6.2: Test principle

IMMUTREP TPHA comprises *T. Pallidum* sensitised formolised tanned fowl erythrocytes; unsensitised formalised tanned fowl erythrocytes, diluent and control sera. When diluted positive samples are mixed with sensitised erythrocytes, antibody to the sensitising antigen causes agglutination of the cells. The cells form a characteristic pattern of cells in the bottom of a microtitration plate well. In the absence of antibody, they form a compact button in the well.

1.7: IMMUTREP® RPR

1.7.1: Assay Description

IMMUTREP® RPR is for use in the non-*Treponema*l flocculation test for the qualitative and semi-quantitative determination of reagin antibodies in serum and plasma. The test device contains carbon particles which improves visual reading of the results. The kit contains an antigen dispensing system comprising a plastic bottle and a blunt ended 20 ga. needle.

1.7.2: Test Principle

When binding occurs between cholesterol/cardiolipin/lecithin in the reagent and the reagin antibodies in the sample, the results can be seen macroscopically in the form of black clumps (visual flocculation indicates a positive result).

Appendix 2: Test results

Figure 13: Abbott Determine ™ Syphilis TP (Negative test result)

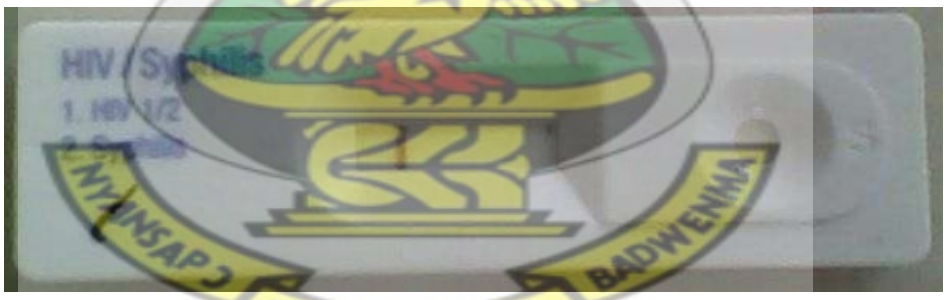

Figure 14: SD BIOLINE HIV/Syphilis Duo (HIV negative, syphilis negative)

Figure 15: SD BIOLINE HIV/Syphilis Duo (HIV negative, syphilis positive)

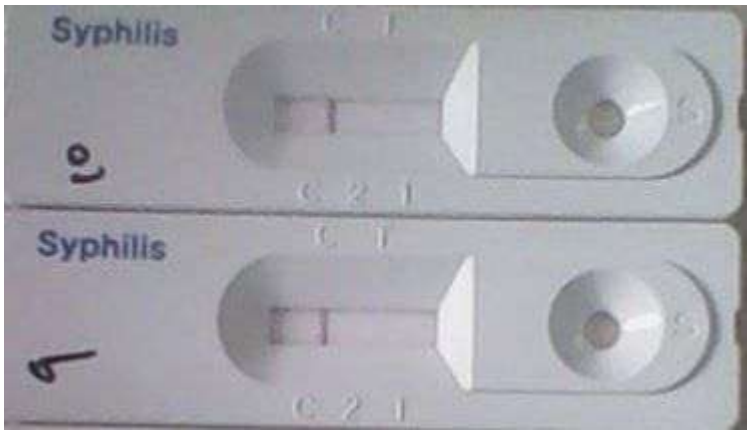

Figure 16: SD BIOLINE Syphilis 3.0 (Negative test result)

Figure 17: SD BIOLINE Syphilis 3.0 (Positive test result)

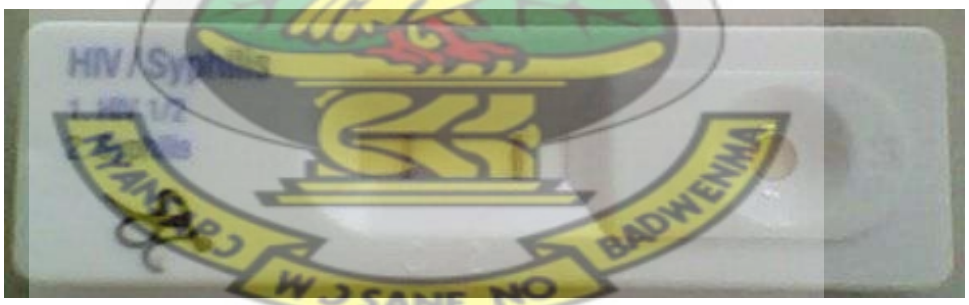

Figure 18: SD BIOLINE HIV/Syphilis Duo (HIV positive, syphilis negative)

Figure 19: SD BIOLINE HIV/Syphilis Duo (HIV positive, syphilis positive)

Appendix 3: Results of tests performed

3.1: HIV-syphilis testing: HIV positive/syphilis positive

S/N	Genscreen ULTRA HIV Ag-Ab/First Response® HIV 1-2-0	IMMUTREP® RPR/IMMUTREP® TPHA	SD BIOLINE HIV/Syphilis Duo	
			HIV/AIDS	SYPHILIS
001	Positive	Positive	Positive	Positive
002	Positive	Positive	Positive	Positive
003	Positive	Positive	Positive	Positive
004	Positive	Positive	Positive	Positive
005	Positive	Positive	Positive	Positive
006	Positive	Positive	Positive	Positive
007	Positive	Positive	Positive	Positive
008	Positive	Positive	Positive	Positive
009	Positive	Positive	Positive	Positive
010	Positive	Positive	Positive	Positive
011	Positive	Positive	Positive	Positive
012	Positive	Positive	Positive	Positive
013	Positive	Positive	Positive	Positive
014	Positive	Positive	Positive	Positive
015	Positive	Positive	Positive	Positive
016	Positive	Positive	Positive	Positive
017	Positive	Positive	Positive	Positive
018	Positive	Positive	Positive	Positive
019	Positive	Positive	Positive	Positive
020	Positive	Positive	Positive	Positive
021	Positive	Positive	Positive	Positive
022	Positive	Positive	Positive	Positive
023	Positive	Positive	Positive	Positive
024	Positive	Positive	Positive	Positive
025	Positive	Positive	Positive	Positive
026	Positive	Positive	Positive	Positive
027	Positive	Positive	Positive	Positive
028	Positive	Positive	Positive	Positive
029	Positive	Positive	Positive	Positive
030	Positive	Positive	Positive	Positive
031	Positive	Positive	Positive	Positive
032	Positive	Positive	Positive	Positive
033	Positive	Positive	Positive	Positive
034	Positive	Positive	Positive	Positive
035	Positive	Positive	Positive	Positive
036	Positive	Positive	Positive	Positive
037	Positive	Positive	Positive	Positive

038	Positive	Positive	Positive	Positive
039	Positive	Positive	Positive	Positive
040	Positive	Positive	Positive	Positive
041	Positive	Positive	Positive	Positive
042	Positive	Positive	Positive	Positive
043	Positive	Positive	Positive	Positive
044	Positive	Positive	Positive	Positive
045	Positive	Positive	Positive	Positive
046	Positive	Positive	Positive	Positive
047	Positive	Positive	Positive	Positive
048	Positive	Positive	Positive	Positive
049	Positive	Positive	Positive	Positive
050	Positive	Positive	Positive	Positive
051	Positive	Positive	Positive	Positive
052	Positive	Positive	Positive	Positive
053	Positive	Positive	Positive	Positive
054	Positive	Positive	Positive	Positive
055	Positive	Positive	Positive	Positive
056	Positive	Positive	Positive	Positive
057	Positive	Positive	Positive	Positive
058	Positive	Positive	Positive	Positive
059	Positive	Positive	Positive	Positive
060	Positive	Positive	Positive	Positive
061	Positive	Positive	Positive	Positive
062	Positive	Positive	Positive	Positive
063	Positive	Positive	Positive	Positive
064	Positive	Positive	Positive	Positive
065	Positive	Positive	Positive	Positive
066	Positive	Positive	Positive	Positive
067	Positive	Positive	Positive	Positive
068	Positive	Positive	Positive	Positive
069	Positive	Positive	Positive	Positive
070	Positive	Positive	Positive	Positive
071	Positive	Positive	Positive	Positive
072	Positive	Positive	Positive	Positive
073	Positive	Positive	Positive	Positive
074	Positive	Positive	Positive	Positive
075	Positive	Positive	Positive	Positive
076	Positive	Positive	Positive	Positive
077	Positive	Positive	Positive	Positive
078	Positive	Positive	Positive	Positive
079	Positive	Positive	Positive	Positive
080	Positive	Positive	Positive	Positive

081	Positive	Positive	Positive	Positive
082	Positive	Positive	Positive	Positive
083	Positive	Positive	Positive	Positive
084	Positive	Positive	Positive	Positive
085	Positive	Positive	Positive	Positive
086	Positive	Positive	Positive	Positive
087	Positive	Positive	Positive	Positive
088	Positive	Positive	Positive	Positive
089	Positive	Positive	Positive	Positive
090	Positive	Positive	Positive	Positive
091	Positive	Positive	Positive	Positive
092	Positive	Positive	Positive	Positive
093	Positive	Positive	Positive	Positive
094	Positive	Positive	Positive	Positive
095	Positive	Positive	Positive	Positive
096	Positive	Positive	Positive	Positive
097	Positive	Positive	Positive	Positive
098	Positive	Positive	Positive	Positive
099	Positive	Positive	Positive	Positive
100	Positive	Positive	Positive	Positive
101	Positive	Positive	Positive	Positive
102	Positive	Positive	Positive	Positive
103	Positive	Positive	Positive	Positive
104	Positive	Positive	Positive	Positive
105	Positive	Positive	Positive	Positive
106	Positive	Positive	Positive	Positive
107	Positive	Positive	Positive	Positive
108	Positive	Positive	Positive	Positive
109	Positive	Positive	Positive	Positive
110	Positive	Positive	Positive	Positive
111	Positive	Positive	Positive	Positive
112	Positive	Positive	Positive	Positive
113	Positive	Positive	Positive	Positive
114	Positive	Positive	Positive	Positive
115	Positive	Positive	Positive	Positive
116	Positive	Positive	Positive	Positive
117	Positive	Positive	Positive	Positive
118	Positive	Positive	Positive	Positive
119	Positive	Positive	Positive	Positive
120	Positive	Positive	Positive	Positive
121	Positive	Positive	Positive	Positive
122	Positive	Positive	Positive	Positive
123	Positive	Positive	Positive	Positive

124	Positive	Positive	Positive	Positive
125	Positive	Positive	Positive	Positive
126	Positive	Positive	Positive	Positive
127	Positive	Positive	Positive	Positive
128	Positive	Positive	Positive	Positive
129	Positive	Positive	Positive	Positive
130	Positive	Positive	Positive	Positive
131	Positive	Positive	Positive	Positive
132	Positive	Positive	Positive	Positive
133	Positive	Positive	Positive	Positive
134	Positive	Positive	Positive	Positive
135	Positive	Positive	Positive	Positive
136	Positive	Positive	Positive	Positive
137	Positive	Positive	Positive	Positive
138	Positive	Positive	Positive	Positive
139	Positive	Positive	Positive	Positive
140	Positive	Positive	Positive	Positive
141	Positive	Positive	Positive	Positive
142	Positive	Positive	Positive	Positive
143	Positive	Positive	Positive	Positive
144	Positive	Positive	Positive	Positive
145	Positive	Positive	Positive	Positive
146	Positive	Positive	Positive	Positive
147	Positive	Positive	Positive	Positive
148	Positive	Positive	Positive	Positive
149	Positive	Positive	Positive	Positive
150	Positive	Positive	Positive	Positive
151	Positive	Positive	Positive	Positive
152	Positive	Positive	Positive	Positive
153	Positive	Positive	Positive	Positive
154	Positive	Positive	Positive	Positive
155	Positive	Positive	Positive	Positive
156	Positive	Positive	Positive	Positive
157	Positive	Positive	Positive	Positive
158	Positive	Positive	Positive	Positive
159	Positive	Positive	Positive	Positive
160	Positive	Positive	Positive	Positive
161	Positive	Positive	Positive	Positive
162	Positive	Positive	Positive	Positive
163	Positive	Positive	Positive	Positive
164	Positive	Positive	Positive	Positive
165	Positive	Positive	Positive	Positive
166	Positive	Positive	Positive	Positive

167	Positive	Positive	Positive	Positive
168	Positive	Positive	Positive	Positive
169	Positive	Positive	Positive	Positive
170	Positive	Positive	Positive	Positive
171	Positive	Positive	Positive	Positive
172	Positive	Positive	Positive	Positive
173	Positive	Positive	Positive	Positive
174	Positive	Positive	Positive	Positive
175	Positive	Positive	Positive	Positive
176	Positive	Positive	Positive	Positive
177	Positive	Positive	Positive	Positive
178	Positive	Positive	Positive	Positive
179	Positive	Positive	Positive	Positive
180	Positive	Positive	Positive	Positive
181	Positive	Positive	Positive	Positive
182	Positive	Positive	Positive	Positive
183	Positive	Positive	Positive	Positive
184	Positive	Positive	Positive	Positive
185	Positive	Positive	Positive	Positive
186	Positive	Positive	Positive	Positive
187	Positive	Positive	Positive	Positive
188	Positive	Positive	Positive	Positive
189	Positive	Positive	Positive	Positive
190	Positive	Positive	Positive	Positive
191	Positive	Positive	Positive	Positive
192	Positive	Positive	Positive	Positive
193	Positive	Positive	Positive	Positive
194	Positive	Positive	Positive	Positive
195	Positive	Positive	Positive	Positive
196	Positive	Positive	Positive	Positive
197	Positive	Positive	Positive	Positive
198	Positive	Positive	Positive	Positive
199	Positive	Positive	Positive	Positive
200	Positive	Positive	Positive	Positive

3.2: HIV-syphilis testing: HIV negative/syphilis negative

S/N	Genscreen ULTRA HIV Ag-Ab/First Response® HIV 1-2-0	IMMUTREP® RPR/IMMUTREP® TPHA	SD BIOLINE HIV/Syphilis Duo	
			HIV/AIDS	SYPHILIS
001	Negative	Negative	Negative	Negative
002	Negative	Negative	Negative	Negative
003	Negative	Negative	Negative	Negative
004	Negative	Negative	Negative	Negative
005	Negative	Negative	Negative	Negative
006	Negative	Negative	Negative	Negative
007	Negative	Negative	Negative	Negative
008	Negative	Negative	Negative	Negative
009	Negative	Negative	Negative	Negative
010	Negative	Negative	Negative	Negative
011	Negative	Negative	Negative	Negative
012	Negative	Negative	Negative	Negative
013	Negative	Negative	Negative	Negative
014	Negative	Negative	Negative	Negative
015	Negative	Negative	Negative	Negative
016	Negative	Negative	Negative	Negative
017	Negative	Negative	Negative	Negative
018	Negative	Negative	Negative	Negative
019	Negative	Negative	Negative	Negative
020	Negative	Negative	Negative	Negative
021	Negative	Negative	Negative	Negative
022	Negative	Negative	Negative	Negative
023	Negative	Negative	Negative	Negative
024	Negative	Negative	Negative	Negative
025	Negative	Negative	Negative	Negative
026	Negative	Negative	Negative	Negative
027	Negative	Negative	Negative	Negative
028	Negative	Negative	Negative	Negative
029	Negative	Negative	Negative	Negative
030	Negative	Negative	Negative	Negative
031	Negative	Negative	Negative	Negative
032	Negative	Negative	Negative	Negative
033	Negative	Negative	Negative	Negative
034	Negative	Negative	Negative	Negative
035	Negative	Negative	Negative	Negative
036	Negative	Negative	Negative	Negative
037	Negative	Negative	Negative	Negative
038	Negative	Negative	Negative	Negative
039	Negative	Negative	Negative	Negative

040	Negative	Negative	Negative	Negative
041	Negative	Negative	Negative	Negative
042	Negative	Negative	Negative	Negative
043	Negative	Negative	Negative	Negative
044	Negative	Negative	Negative	Negative
045	Negative	Negative	Negative	Negative
046	Negative	Negative	Negative	Negative
047	Negative	Negative	Negative	Negative
048	Negative	Negative	Negative	Negative
049	Negative	Negative	Negative	Negative
050	Negative	Negative	Negative	Negative
051	Negative	Negative	Negative	Negative
052	Negative	Negative	Negative	Negative
053	Negative	Negative	Negative	Negative
054	Negative	Negative	Negative	Negative
055	Negative	Negative	Negative	Negative
056	Negative	Negative	Negative	Negative
057	Negative	Negative	Negative	Negative
058	Negative	Negative	Negative	Negative
059	Negative	Negative	Negative	Negative
060	Negative	Negative	Negative	Negative
061	Negative	Negative	Negative	Negative
062	Negative	Negative	Negative	Negative
063	Negative	Negative	Negative	Negative
064	Negative	Negative	Negative	Negative
065	Negative	Negative	Negative	Negative
066	Negative	Negative	Negative	Negative
067	Negative	Negative	Negative	Negative
068	Negative	Negative	Negative	Negative
069	Negative	Negative	Negative	Negative
070	Negative	Negative	Negative	Negative
071	Negative	Negative	Negative	Negative
072	Negative	Negative	Negative	Negative
073	Negative	Negative	Negative	Negative
074	Negative	Negative	Negative	Negative
075	Negative	Negative	Negative	Negative
076	Negative	Negative	Negative	Negative
077	Negative	Negative	Negative	Negative
078	Negative	Negative	Negative	Negative
079	Negative	Negative	Negative	Negative
080	Negative	Negative	Negative	Negative
081	Negative	Negative	Negative	Negative
082	Negative	Negative	Negative	Negative

083	Negative	Negative	Negative	Negative
084	Negative	Negative	Negative	Negative
085	Negative	Negative	Negative	Negative
086	Negative	Negative	Negative	Negative
087	Negative	Negative	Negative	Negative
088	Negative	Negative	Negative	Negative
089	Negative	Negative	Negative	Negative
090	Negative	Negative	Negative	Negative
091	Negative	Negative	Negative	Negative
092	Negative	Negative	Negative	Negative
093	Negative	Negative	Negative	Negative
094	Negative	Negative	Negative	Negative
095	Negative	Negative	Negative	Negative
096	Negative	Negative	Negative	Negative
097	Negative	Negative	Negative	Negative
098	Negative	Negative	Negative	Negative
099	Negative	Negative	Negative	Negative
100	Negative	Negative	Negative	Negative

3.3: HIV-syphilis testing: HIV negative/syphilis positive

S/N	Genscreen ULTRA HIV Ag-Ab/First Response® HIV 1-2-0	IMMUTREP® RPR/IMMUTREP® TPHA	SD BIOLINE HIV/Syphilis Duo	
			HIV/AIDS	SYPHILIS
001	Negative	Positive	Negative	Positive
002	Negative	Positive	Negative	Positive
003	Negative	Positive	Negative	Positive
004	Negative	Positive	Negative	Positive
005	Negative	Positive	Negative	Positive
006	Negative	Positive	Negative	Positive
007	Negative	Positive	Negative	Positive
008	Negative	Positive	Negative	Positive
009	Negative	Positive	Negative	Positive
010	Negative	Positive	Negative	Positive
011	Negative	Positive	Negative	Positive
012	Negative	Positive	Negative	Positive
013	Negative	Positive	Negative	Positive
014	Negative	Positive	Negative	Positive
015	Negative	Positive	Negative	Positive
016	Negative	Positive	Negative	Positive
017	Negative	Positive	Negative	Positive
018	Negative	Positive	Negative	Positive
019	Negative	Positive	Negative	Positive

020	Negative	Positive	Negative	Positive
021	Negative	Positive	Negative	Positive
022	Negative	Positive	Negative	Positive
023	Negative	Positive	Negative	Positive
024	Negative	Positive	Negative	Positive
025	Negative	Positive	Negative	Positive
026	Negative	Positive	Negative	Positive
027	Negative	Positive	Negative	Positive
028	Negative	Positive	Negative	Positive
029	Negative	Positive	Negative	Positive
030	Negative	Positive	Negative	Positive
031	Negative	Positive	Negative	Positive
032	Negative	Positive	Negative	Positive
033	Negative	Positive	Negative	Positive
034	Negative	Positive	Negative	Positive
035	Negative	Positive	Negative	Positive
036	Negative	Positive	Negative	Positive
037	Negative	Positive	Negative	Positive
038	Negative	Positive	Negative	Positive
039	Negative	Positive	Negative	Positive
040	Negative	Positive	Negative	Positive
041	Negative	Positive	Negative	Positive
042	Negative	Positive	Negative	Positive
043	Negative	Positive	Negative	Positive
044	Negative	Positive	Negative	Positive
045	Negative	Positive	Negative	Positive
046	Negative	Positive	Negative	Positive
047	Negative	Positive	Negative	Positive
048	Negative	Positive	Negative	Positive
049	Negative	Positive	Negative	Positive
050	Negative	Positive	Negative	Positive

3.4: HIV-syphilis testing: HIV positive/syphilis negative

S/N	Genscreen ULTRA HIV Ag-Ab/First Response® HIV 1-2-0	IMMUTREP® RPR/IMMUTREP® TPHA	SD BIOLINE HIV/Syphilis Duo	
			HIV/AIDS	SYPHILIS
001	Positive	Negative	Positive	Negative
002	Positive	Negative	Positive	Negative
003	Positive	Negative	Positive	Negative
004	Positive	Negative	Positive	Negative
005	Positive	Negative	Positive	Negative
006	Positive	Negative	Positive	Negative
007	Positive	Negative	Positive	Negative
008	Positive	Negative	Positive	Negative
009	Positive	Negative	Positive	Negative
010	Positive	Negative	Positive	Negative
011	Positive	Negative	Positive	Negative
012	Positive	Negative	Positive	Negative
013	Positive	Negative	Positive	Negative
014	Positive	Negative	Positive	Negative
015	Positive	Negative	Positive	Negative
016	Positive	Negative	Positive	Positive
017	Positive	Negative	Positive	Negative
018	Positive	Negative	Positive	Negative
019	Positive	Negative	Positive	Negative
020	Positive	Negative	Positive	Negative
021	Positive	Negative	Positive	Negative
022	Positive	Negative	Positive	Negative
023	Positive	Negative	Positive	Negative
024	Positive	Negative	Positive	Negative
025	Positive	Negative	Positive	Negative
026	Positive	Negative	Positive	Negative
027	Positive	Negative	Positive	Negative
028	Positive	Negative	Positive	Negative
029	Positive	Negative	Positive	Negative
030	Positive	Negative	Positive	Negative
031	Positive	Negative	Positive	Negative
032	Positive	Negative	Positive	Negative
033	Positive	Negative	Positive	Negative
034	Positive	Negative	Positive	Negative
035	Positive	Negative	Positive	Negative
036	Positive	Negative	Positive	Negative
037	Positive	Negative	Positive	Negative
038	Positive	Negative	Positive	Negative
039	Positive	Negative	Positive	Negative

040	Positive	Negative	Positive	Negative
041	Positive	Negative	Positive	Negative
042	Positive	Negative	Positive	Negative
043	Positive	Negative	Positive	Negative
044	Positive	Negative	Positive	Negative
045	Positive	Negative	Positive	Negative
046	Positive	Negative	Positive	Negative
047	Positive	Negative	Positive	Positive
048	Positive	Negative	Positive	Negative
049	Positive	Negative	Positive	Negative
050	Positive	Negative	Positive	Negative

3.5: Syphilis testing: IMMUTREP® RPR/IMMUTREP® TPHApositive

3.5.1: Low titre (1:2 to 1:4)

S/N	IMMUTREP® RPR/IMMUTREP® TPHA	SD BIOLINE Syphilis 3.0	Abbott Determine™ Syphilis TP
001	Positive	Negative	Negative
002	Positive	Negative	Negative
003	Positive	Positive	Positive
004	Positive	Negative	Negative
005	Positive	Negative	Negative
006	Positive	Negative	Negative
007	Positive	Negative	Negative
008	Positive	Negative	Negative
009	Positive	Positive	Positive
010	Positive	Positive	Positive
011	Positive	Positive	Positive
012	Positive	Positive	Negative
013	Positive	Negative	Negative
014	Positive	Negative	Negative
015	Positive	Negative	Negative
016	Positive	Negative	Negative
017	Positive	Positive	Positive
018	Positive	Positive	Positive
019	Positive	Negative	Negative
020	Positive	Negative	Negative
021	Positive	Positive	Positive
022	Positive	Positive	Positive
023	Positive	Negative	Negative
024	Positive	Positive	Positive
025	Positive	Positive	Positive
026	Positive	Positive	Positive

027	Positive	Positive	Positive
028	Positive	Negative	Negative
029	Positive	Positive	Positive
030	Positive	Positive	Positive
031	Positive	Positive	Positive
032	Positive	Negative	Negative
033	Positive	Positive	Positive
034	Positive	Positive	Positive
035	Positive	Positive	Positive
036	Positive	Positive	Positive
037	Positive	Positive	Positive
038	Positive	Positive	Positive
039	Positive	Negative	Negative
040	Positive	Negative	Negative
041	Positive	Negative	Negative
042	Positive	Positive	Positive
043	Positive	Negative	Negative
044	Positive	Positive	Positive
045	Positive	Positive	Positive
046	Positive	Negative	Negative
047	Positive	Positive	Positive
048	Positive	Negative	Negative
049	Positive	Positive	Positive
050	Positive	Negative	Negative
051	Positive	Positive	Positive
052	Positive	Positive	Positive
053	Positive	Positive	Positive
054	Positive	Positive	Positive

3.5.2: Average titre (1:8 to 1:16)

S/N	**IMMUTREP® RPR/IMMUTREP® TPHA**	**SD BIOLINE Syphilis 3.0**	**Abbott Determine™ Syphilis TP**
055	Positive	Positive	Positive
056	Positive	Positive	Positive
057	Positive	Negative	Negative
058	Positive	Positive	Positive
059	Positive	Negative	Negative
060	Positive	Negative	Positive
061	Positive	Positive	Positive
062	Positive	Negative	Positive
063	Positive	Positive	Positive
064	Positive	Positive	Positive
065	Positive	Positive	Positive
066	Positive	Positive	Positive

067	Positive	Positive	Positive
068	Positive	Positive	Positive
069	Positive	Negative	Negative
070	Positive	Negative	Negative
071	Positive	Negative	Negative
072	Positive	Positive	Positive
073	Positive	Positive	Positive
074	Positive	Positive	Positive
075	Positive	Negative	Positive
076	Positive	Positive	Positive
077	Positive	Positive	Positive
078	Positive	Positive	Positive
079	Positive	Positive	Positive
080	Positive	Negative	Negative
081	Positive	Positive	Positive
082	Positive	Positive	Positive
083	Positive	Positive	Positive
084	Positive	Positive	Positive
085	Positive	Positive	Positive
086	Positive	Positive	Positive
087	Positive	Positive	Positive
088	Positive	Positive	Positive
089	Positive	Positive	Positive
090	Positive	Positive	Positive
091	Positive	Positive	Positive
092	Positive	Positive	Positive
093	Positive	Positive	Positive
094	Positive	Positive	Positive
095	Positive	Positive	Positive
096	Positive	Positive	Positive
097	Positive	Positive	Positive
098	Positive	Positive	Positive
099	Positive	Positive	Positive
100	Positive	Positive	Positive
101	Positive	Positive	Positive
102	Positive	Positive	Positive
103	Positive	Positive	Positive
104	Positive	Positive	Positive
105	Positive	Positive	Positive
106	Positive	Positive	Positive
107	Positive	Positive	Positive
108	Positive	Positive	Positive
109	Positive	Positive	Positive
110	Positive	Positive	Positive
111	Positive	Negative	Negative

112	Positive	Positive	Positive
113	Positive	Positive	Negative
114	Positive	Negative	Negative
115	Positive	Negative	Negative
116	Positive	Positive	Positive
117	Positive	Positive	Positive
118	Positive	Positive	Positive
119	Positive	Positive	Positive
120	Positive	Positive	Positive
121	Positive	Positive	Positive
122	Positive	Positive	Positive

3.5.3: High titre (1:32 to 1:64)

S/N	IMMUTREP® RPR/IMMUTREP® TPHA	SD BIOLINE Syphilis 3.0	Abbott Determine™ Syphilis TP
123	Positive	Positive	Positive
124	Positive	Positive	Positive
125	Positive	Positive	Positive
126	Positive	Positive	Positive
127	Positive	Positive	Positive
128	Positive	Positive	Positive
129	Positive	Positive	Positive
130	Positive	Positive	Positive
131	Positive	Positive	Positive
132	Positive	Positive	Positive
133	Positive	Positive	Negative
134	Positive	Positive	Positive
135	Positive	Positive	Positive
136	Positive	Positive	Positive
137	Positive	Positive	Positive
138	Positive	Positive	Positive
139	Positive	Positive	Positive
140	Positive	Positive	Positive
141	Positive	Positive	Positive
142	Positive	Positive	Positive
143	Positive	Positive	Positive
144	Positive	Positive	Positive

3.6: Syphilis testing: IMMUTREP® RPR/IMMUTREP® TPHAnegative

S/N	IMMUTREP® RPR/IMMUTREP® TPHA	SD BIOLINE Syphilis 3.0	Abbott Determine™ Syphilis TP
001	Negative	Negative	Negative
002	Negative	Negative	Negative
003	Negative	Negative	Negative
004	Negative	Negative	Negative
005	Negative	Negative	Negative
006	Negative	Negative	Negative
007	Negative	Negative	Negative
008	Negative	Negative	Negative
009	Negative	Negative	Negative
010	Negative	Negative	Negative
011	Negative	Negative	Negative
012	Negative	Negative	Negative
013	Negative	Negative	Negative
014	Negative	Negative	Negative
015	Negative	Negative	Negative
016	Negative	Negative	Negative
017	Negative	Negative	Negative
018	Negative	Negative	Negative
019	Negative	Negative	Negative
020	Negative	Negative	Negative
021	Negative	Negative	Negative
022	Negative	Negative	Negative
023	Negative	Negative	Negative
024	Negative	Negative	Negative
025	Negative	Negative	Negative
026	Negative	Negative	Negative
027	Negative	Negative	Negative
028	Negative	Negative	Negative
029	Negative	Negative	Negative
030	Negative	Negative	Negative
031	Negative	Negative	Negative
032	Negative	Negative	Negative
033	Negative	Negative	Negative
034	Negative	Negative	Negative
035	Negative	Negative	Negative
036	Negative	Negative	Negative
037	Negative	Negative	Negative
038	Negative	Negative	Negative
039	Negative	Negative	Negative
040	Negative	Negative	Negative
041	Negative	Negative	Negative

042	Negative	Negative	Negative
043	Negative	Negative	Negative
044	Negative	Negative	Negative
045	Negative	Negative	Negative
046	Negative	Negative	Negative
047	Negative	Negative	Negative
048	Negative	Negative	Negative
049	Negative	Negative	Negative
050	Negative	Negative	Negative
051	Negative	Negative	Negative
052	Negative	Negative	Negative
053	Negative	Negative	Negative
054	Negative	Negative	Negative
055	Negative	Negative	Negative
056	Negative	Negative	Negative
057	Negative	Negative	Negative
058	Negative	Negative	Negative
059	Negative	Negative	Negative
060	Negative	Negative	Negative
061	Negative	Negative	Negative
062	Negative	Negative	Negative
063	Negative	Negative	Negative
064	Negative	Negative	Negative
065	Negative	Negative	Negative
066	Negative	Negative	Negative
067	Negative	Negative	Negative
068	Negative	Negative	Negative
069	Negative	Negative	Negative
070	Negative	Negative	Negative
071	Negative	Negative	Negative
072	Negative	Negative	Negative
073	Negative	Negative	Negative
074	Negative	Negative	Negative
075	Negative	Negative	Negative
076	Negative	Negative	Negative
077	Negative	Negative	Negative
078	Negative	Negative	Negative
079	Negative	Negative	Negative
080	Negative	Negative	Negative
081	Negative	Negative	Negative
082	Negative	Negative	Negative
083	Negative	Negative	Negative
084	Negative	Negative	Negative
085	Negative	Negative	Negative
086	Negative	Negative	Negative

087	Negative	Negative	Negative
088	Negative	Negative	Negative
089	Negative	Negative	Negative
090	Negative	Negative	Negative
091	Negative	Negative	Negative
092	Negative	Negative	Negative
093	Negative	Negative	Negative
094	Negative	Negative	Negative
095	Negative	Negative	Negative
096	Negative	Negative	Negative
097	Negative	Negative	Negative
098	Negative	Negative	Negative
099	Negative	Negative	Negative
100	Negative	Negative	Negative

Appendix 4: SPSS output
Crosstabs

Notes		
Output Created		14-Jun-2013 19:24:53
Comments		
Input	Data	I:\Study\Misc\Stats\Project 2.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	248
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=DetTP BY SDBiol /FORMAT=AVALUE TABLES /STATISTICS=CHISQ KAPPA MCNEMAR /CELLS=COUNT /COUNT ROUND CELL.
Resources	Processor Time	00:00:00.032
	Elapsed Time	00:00:00.093
	Dimensions Requested	2
	Cells Available	174762

[DataSet1] I:\Study\Misc\Stats\Project 2.sav

Case Processing Summary						
	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
DetTP *SDBiol	244	98.4%	4	1.6%	248	100.0%

DetTP * SDBiol Cross tabulation				
Count				
		SDBiol		Total
		Positive	Negative	
DetTP	Positive	109	0	109
	Negative	23	112	135
Total		132	112	244

Chi-Square Tests					
	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	167.158[a]	1	.000		
Continuity Correction[b]	163.834	1	.000		
Likelihood Ratio	213.367	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	166.473	1	.000		
McNemar Test				.000[c]	
N of Valid Cases	244				
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 50.03.					
b. Computed only for a 2x2 table					
c. Binomial distribution used.					

Symmetric Measures					
		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.813	.036	12.929	.000
N of Valid Cases		244			
a. Not assuming the null hypothesis.					
b. Using the asymptotic standard error assuming the null hypothesis.					