The Utility of a rapid point-of-care test for syphilis in an ART clinic in Tigoni

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A dissertation submitted in part fulfillment for the Postgraduate diploma in Biomedical Research Methodology, a UNITID Programme

DECLARATION

I, Dr. Lucy Wangari Muchiri, declare that this is my original work, and that it has not been submitted in any other institution or previously for examination and is being presented for the first time for the Postgraduate Diploma in Biomedical Research Methodology.

Signed _____ Date: 30/11/07

Dr Lucy W. Muchiri

This work is being presented for examination in part fulfillment for the Postgraduate Diploma in Biomedical Research Methodology and was carried out under my supervision as a University of Nairobi approved Supervisor and Examiner.

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GLOSSARY OF TERMS

ANC	Antenatal Clinic
AIDS	Acquired Immunodeficiency Syndrome
ARVs	Anti-retroviral drugs
ART	Anti-retroviral Therapy
CDC	Center for Disease Control and Prevention
FP	Family Planning
HIV	Human Immunodeficiency Virus
KDSP	Kiambu District Strategic Plan
PCR	Polymerase Chain Reaction
PEPFAR	The US President's Emergency Plan for AIDS Relief
RPR	Rapid Plasma Reagin
SDI	Sexually Transmitted Diseases Diagnostics Initiative
STD	Sexually Transmitted Diseases
STI	Sexually Transmitted Infections
TP	Treponema Pallidum
TPHA	Treponema Antigen Haemaglutination Assay
VCT	Voluntary Counseling and Testing
VDRL	Venereal Diseases Research Laboratory
UNITID	University of Nairobi Institute of Tropical & Infectious Diseases
WHO	World Health Organization

Abstract

Problem Statement: The Sub-Saharan African region has a high prevalence of both HIV infection and syphilis. Access to diagnosis is poor or non-existent in this region. Syphilis can mimic HIV infection and vice versa. HIV can lead to more numerous chancres, accelerated secondary syphilis, and faster progression to late syphilis. Management of Syphilis is important for control and prevention of HIV infection and transmission.

Background: Sexually transmitted infections (STIs) are a major global cause of acute illness, infertility, long term disability and death, with severe medical and psychological consequences for millions of men, women and infants. World Health Organization global estimates indicate that there are about 387 million new cases of curable sexually transmitted infections every year, 80-90% of which are in resource-poor countries where there is poor or no access to diagnosis. Most of these infections occur in men and women aged 15-49 years. WHO further estimates that approximately 12 million new cases of syphilis occur worldwide each year. In developing countries, STIs and their complications are amongst the top five disease categories for which adults seek health care. In women of childbearing age, STIs (excluding HIV) are second only to maternal factors as causes of disease, death and healthy life lost. Apart from being serious diseases in their own right, STIs enhance the sexual transmission of HIV infection. The presence of an untreated STD (ulcerative or non-ulcerative) can increase the risk of both acquisition and transmission of HIV by a factor of up to 10. Moreover, the improvement in the management of STIs can reduce the incidence of HIV-1 infection in the general population by about 40%. STIs prevention and treatment are, therefore, an important component in HIV prevention strategy.

Main Objective

To determine the operational feasibility and diagnostic utility of a rapid point-of-care syphilis test in an ART clinic in Tigoni, Kenya

Specific Objectives:

1. To determine the feasibility and utility of a rapid point-of-care syphilis test in an ART clinic
2. To establish the prevalence of syphilis in adults attending an ART clinic in Tigoni District Hospital
3. To compare the clinical stage of HIV and Syphilis seropositivity

Methods & Materials: The study was conducted in a busy ART clinic in Tigoni District Hospital, Tigoni, Kiambu District in Kenya. All eligible consenting patients were screened for syphilis using a rapid test. Those whose test were positive were counseled, and given a prescription for treatment before they left. Their blood was also taken for a confirmatory test whose results they would be told at the next visit.

Study Design: This was a descriptive cross-sectional survey.

Sample Size and sampling method

About 400 patients were enrolled in the ART clinic in Tigoni Hospital at the time of commencement of the study. The prevalence of syphilis in antenatal clinic in a previous study in Nairobi was 6%. A calculated sample size of 229 was required. A stratified random sampling method was used in order to have about 25% of the sample being men since majority of attendees of this clinic are women.

Results: The clinical presentation of syphilis in HIV is often blurred. The introduction of a rapid cost-effective POC test for syphilis in a busy ART clinic was feasible and enabled treatment of syphilis to be started on the same clinic day. This meant that hitherto

undetected co-infection with syphilis of HIV patients was better managed and the long-term goal of improvement of control and reduction of transmission of HIV was possible. The prevalence of syphilis in a HIV positive adult cohort was 5.5%, somewhat lower than expected in a HIV positive cohort, as well as lower than the WHO estimated sero-prevalence of syphilis for Sub-Saharan Africa. The numbers were too small however, for a conclusive comparison of clinical stage of HIV and syphilis.

BACKGROUND AND LITERATURE REVIEW

Pathology of Syphilis

Syphilis was first recognized in epidemic form in sixteenth-century Europe as the Great Pox and has remained endemic in most parts of the world. Although penicillin and public health measures resulted in major reductions in cases of syphilis from the late 1940s into the late 1970s, there has been significant resurgence of cases of both primary and secondary syphilis in the last two decades (1, 2). Center for Disease Control (CDC) have reported that the number of cases of syphilis infection has markedly increased since 1998 (3). Several studies have also reported a substantial epidemiological correlation between the acquisition and transmission of the HIV and syphilis (3, 4, 5).

Syphilis is a chronic systemic venereal disease caused by *Treponema pallidum* (TP). TP is a spirochete bacterium with an outer envelope and a cytoplasmic membrane (2). TP is a fastidious spirochete whose only natural hosts are humans. The usual source of infection is an active cutaneous or mucosal lesion in a patient with early (primary or secondary) stages of syphilis. The organism is transmitted from such lesions during sexual intercourse across minute breaks in the skin or mucous membranes of the uninfected partner. In cases of congenital syphilis, TP is transmitted across the placenta from the mother to fetus, particularly during the early stages of maternal infection. Once introduced into the body, the microorganism is rapidly disseminated to distant sites by lymphatics and the bloodstream, even before the appearance of the lesions at the primary inoculation site. Two to 6 weeks after the initial infection, a primary lesion, the chancre, appears at the point of entry, usually in the anogenital region. Systemic dissemination of the organisms continues during this period while the host mounts an immune response. Two types of antibodies are formed: non-treponemal antibodies and antibodies to specific treponemal antigens. The detection of these antibodies plays an important role in the diagnosis of syphilis. This acquired immunity, however, fails to eradicate spirochetes introduced during the primary inoculation (2).

The chancre of primary syphilis resolves spontaneously over a period of 4 to 6 weeks and is followed in approximately 25% of untreated patients by the development of secondary syphilis. The manifestations of secondary syphilis include generalized lymphadenopathy and variable mucocutaneous lesions and reflect the presence of organisms disseminated throughout the body during the primary stage of the disease. The mucocutaneous lesions of both primary and secondary syphilis are teeming with spirochetes and are highly infectious. Like the chancre, the lesions of secondary syphilis resolve without any specific treatment, at which point patients are said to be in *early latent phase of syphilis*. Mucocutaneous lesions may recur during this phase of the disease. In recent years, the definition of early latent syphilis has been restricted to the period 1 year after the infection (2).

Patients with untreated syphilis then enter into an asymptomatic, *late latent phase* of the illness. In about one third of cases, subsequent symptomatic lesions may develop over the next 10 to 20 years. This late symptomatic phase, or tertiary syphilis, is marked by the development of lesions of the cardiovascular system, central nervous system, and less frequently, other organs. Spirochetes are much more difficult to demonstrate during the later stages of the disease and patients are less likely to be infectious than are those in the primary or secondary stages of disease (2, 6).

Multiple clinical stages and long periods of latent, asymptomatic infection are characteristic of syphilis. Primary syphilis is defined by the presence of a chancre at the site of inoculation. The antibody response to TP can be detected within 4 to 7 days after the chancre appears. The antibodies remain detectable until the patient receives adequate treatment.

Epidemiology

Globally sexually transmitted infections (STIs) are a major cause of acute illness, infertility, long term disability and death, and are associated with severe medical and psychological consequences for millions of men, women and infants(1,5). World Health

Organization (WHO) global estimates indicate that there are about 387 million new cases of curable sexually transmitted infections every year, 80-90% of which are in resource-poor countries where there is poor or no access to diagnosis (1). Most of these infections, which include syphilis, gonorrhoea, chlamydia and trichomoniasis, are reported in men and women aged 15-49 years (1, 4).

Although based on a comprehensive survey of the available information, the WHO estimates are affected by the quantity and quality of prevalence and incidence data from the different regions, as well as our knowledge of the duration of infection (1, 4).
Interpreting the data from prevalence studies and comparing results is further complicated by the nature of the populations studied. Few studies are community-based and the majority of data come from studies carried out in specific populations, such as STI or antenatal clinic attendees. Other limitations include small samples sizes, the different diagnostic methods, and study designs used. Epidemiological survey data show that within countries and between countries in the same region, the prevalence and incidence of STIs may vary widely between urban and rural population, and even in similar population groups. These differences reflect among others a variety of social, cultural, and economic factors, as illustrated by the HIV epidemic, and may also be attributable in part to differences in the access to appropriate treatment. In general, the prevalence of STIs tends to be higher among urban residents, unmarried individuals, and in young adults. STIs tend to occur at a younger age in females than in males, which may be explained by differences in patterns of sexual activity and in the relative rates of transmission from one sex to the other (5).

The exact magnitude of the STIs burden is frequently unknown. Although passive STIs surveillance systems exist in many countries, the data is not always reliable or complete (1,5). The quality and completeness of the available data and estimates depend on the quality of STIs services, the extent to which patients seek health care, the intensity of case finding and diagnosis and the quality of reporting. The completeness is further affected by the STIs natural history, since a large number of infections are asymptomatic. Moreover, only part of the symptomatic population seeks health care and even a smaller

number of cases are reported. The social stigma that usually is associated with STIs may result in people seeking care from alternative providers or not seeking care at all. As a result, report-based STI surveillance systems tend to underestimate substantially the total number of new cases (1, 6).

A large proportion of STIs present with little or no symptoms, but undiagnosed and untreated infection often leads to serious sequelae (1, 6). STIs diagnostics are especially needed in areas where HIV is endemic as studies in Sub-Saharan Africa have shown that STIs are important co-factors in the transmission of HIV infection (5). A high incidence rate of syphilis was observed among STI clinic attendees in a clinic in India and according to Reynolds the elevated risk of HIV-1 infection observed among participants with incident syphilis supports the hypothesis that syphilis enhances the sexual transmission of HIV-1 and highlights the importance of early diagnosis and treatment of syphilis (7).

Curable STIs are not only a concern due to the discomfort resulting from the acute infection but because both symptomatic and asymptomatic infections can lead to the development of serious complications with severe consequences for the individuals and for the community. The most serious complications and long term consequences of untreated STIs tend to be in women and newborn babies (5, 8).

In developing countries, laboratory services for sexually transmitted infections (STIs) are either not available, or where limited services are available, patients may not be able to pay for or physically access those services (4). Despite the existence of national policies in many of these countries for antenatal screening to prevent congenital syphilis and substantial evidence that antenatal screening is cost-effective, implementation of syphilis screening programmes remains unacceptably low because of lack of screening tools that can be used in primary health care settings, as well as other logistical difficulties. (4, 5, 8, 9). Even when testing is available at clinical sites, there are technical difficulties associated with maintaining trained staff and assuring quality standards and supplies of tests and treatment (4).

Laboratory Diagnosis of Syphilis

Although polymerase chain reaction (PCR)-based testing for syphilis has been developed, serologic testing remains the mainstay of diagnosis (10). Serologic tests for syphilis include both non-treponemal antibody tests and anti-treponemal antibody tests. Non-treponemal tests measure antibody to cardiolipin, an antigen that is present in both host tissues and the treponemal cell wall. These antibodies are detected by the rapid plasma reagin (RPR) and the Venereal Disease Research Laboratory (VDRL) tests. Non-treponemal antibody tests begin to become positive 1 to 2 weeks after infection and are usually positive for about 4 to 6 weeks. Titers of these antibodies usually begin to fall after successful treatment. The VDRL and RPR tests are widely used as screening tests for syphilis and also to monitor the results of therapy. They may be negative however, in late latent or tertiary phase of the disease. Non-treponemal antibodies may persist in some patients even after successful treatment. Two points about non-treponemal antibody tests deserve mention:

- *Non-treponemal antibody tests are often negative during early stages of disease, even in the presence of a primary chancre.* Hence dark field microscopy should, where possible, be performed in the evaluation of a suspected chancre, even if serologic tests for syphilis are negative.
- Up to 15% of positive VDRL tests represent biologic false-positive results. These false-positives, which may be acute (transient) or chronic (persistent), increase in frequency with age. Conditions associated with false positives include certain acute infections, collagen vascular diseases (e.g. SLE), drug addition, pregnancy, hypergammaglobulinemia of any cause, and lepromatous leprosy (10).

Treponemal antibodies include the fluorescent treponemal antibody absorption test (FTA-Abs) and the microhaemagglutination assay for *Treponema pallidum* antibodies (MHATP). These tests also become positive within 4 to 6 weeks after an infection, but unlike non-treponemal antibody tests, they remain positive indefinitely, even after successful treatment. They are not recommended as screening tests, because they are

significantly more expensive than non-treponemal tests, and they also remain positive after treatment, and up to 2% of the population have a false-positive test result (2,10).

Serologic response may be delayed, exaggerated (false-positive results), or even absent in some patients with syphilis and co-existent HIV infection. However, in most cases, these tests remain extremely useful in the diagnosis and management of syphilis in patients with acquired immunodeficiency syndrome.

The World Health Organization Sexually Transmitted Diseases Diagnostics Initiative (SDI) has developed the ASSURED criteria as a benchmark to decide if tests address disease control needs: **A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free and **D**eliverable to end-users. Rapid syphilis tests that can be used with whole blood approach the ASSURED criteria and can now be deployed in areas where no previous screening has been possible. The way forward for STI diagnostics requires a Continuous quest for ASSURED tests, the development of a road map for test introduction, sustainable programmes for quality assurance, and the creation of a robust infrastructure linked to HIV prevention that ensures sustainability of STI control efforts that include viral STIs (9).

Table 1 Barriers to effective syphilis screening using rapid plasma regain (RPR) tests (9)

Health Seeker	Health Provider
Distance to clinic Failure to return for test results & treatment	Procurement – limited quantities due to need for refrigeration Limited facilities for cold chain transport & storage Clinic constraints • Few trained personnel • Facilities inadequate: no refrigerator, rotator or centrifuge; poor lighting • Organization of services – drugs & diagnostics stock-outs

The rapid plasma reagin (RPR) 18mm circle card test for syphilis is used as a screening test in many antenatal clinics and health facilities in developing countries (11). It is easy to perform and inexpensive but has certain disadvantages: it may be difficult to interpret, and requires training of personnel to ensure test is carried out and results read correctly; specificity may be limited owing to the non-specific nature of the cardiolipin antigen as biological false positives occur due to viral infections, malaria, and pregnancy among others (2,12).

A number of simple, rapid treponemal tests are now commercially available. Most of these are 'lateral flow' tests in which antibodies are transported by capillary flow over antigen immobilized on a nitrocellulose membrane strip (also called immunochromatographic strips). Antibodies in the specimen bind to the antigen site in the strip and are revealed by a dye bound to the anti-immunoglobulin. These tests are simple, robust, and affordable and can be stored and transported at room temperature without the need for refrigeration. Evaluations so far suggest that their performance is comparable to the best laboratory-based diagnostics (13, 14, 15, 16)

Table 2 Score card for current rapid STI tests (9)

Test Characteristics	NG/CT	Syphilis
Affordable	US$6-15	US$0 19-3.0
Sensitive	43-65%	85-99%
Specific	98%	93-100%
User-friendly	7-14steps	3-4steps
Rapid/robust	30min/storage at 8-30°C	20min/storage at 8-30°C
Equipment-free	Yes	Yes
Deliverable	?	Negotiated price through WHO bulk procurement Scheme

NG – *Neiserria gonorrhea*
CT – *Chlamydia trachomatis*

The cost effectiveness of screening tests for syphilis will depend on the prevalence in the population and risk groups. While VDRL or RPR test alone is useful for the screening of

infectious syphilis, it will fail to diagnose many primary and late latent /late syphilis as the sensitivity is 44-76% and 70-73% respectively (6). Biological false positives for VDRL/RPR and prozone phenomenon in secondary syphilis causing false negatives caused by using undiluted sera can occur; both may be more common in HIV infection (17, 18, 19, 20, 21)

Management of Syphilis

Treatment guidelines for syphilis from WHO have been widely implemented in many national policy guidelines. Intramuscular benzathine penicillin 2.4 megaunits either as a single dose or weekly in two to three divided doses are the mainstay of treatment in developing countries (24). In patients allergic to penicillin, oral doxycycline 100mg four times daily for 2 weeks or azithromycin 500mg daily for 1 week is recommended. A randomized study by Hook et al suggested that azithromycin 2g as a single dose or as two doses 1 week apart may be as good as benzathine penicillin for the treatment of early syphilis (22). Emergence of azithromycin/macrolide resistance T. pallidum is a cause for concern (23)

STUDY JUSTIFICATION

In developing countries, STIs and their complications are amongst the top five disease categories for which adults seek health care. In women of childbearing age, STIs (excluding HIV) are second only to maternal factors as causes of disease, death and healthy life lost. Apart from being serious diseases in their own right, STIs enhance the sexual transmission of HIV infection. The presence of an untreated STD (ulcerative or non-ulcerative) can increase the risk of both acquisition and transmission of HIV by a factor of up to 10. Moreover, the improvement in the management of STIs can reduce the incidence of HIV-1 infection in the general population by about 40%. STIs prevention and treatment are, therefore, an important component in HIV prevention strategy.

Success of increasing access to syphilis screening depends on level of political commitment, existence of a robust health infrastructure to facilitate implementation, and availability of screening tools (9). In resource-limited settings, health service providers have to prioritize their scarce monetary and human resources to accommodate many competing demands. Instead of setting up new infrastructure, every effort should be made to take advantage of existing initiatives such as Antenatal programmes, Prevention of Mother to Child Transmission programmes for HIV, and HIV Voluntary Counseling and Testing programmes.

Main Objective:

To determine the operational feasibility and diagnostic utility of a rapid point-of-care syphilis test in an ART clinic in Tigoni, Kenya

Specific Objectives:

1. To establish the prevalence of syphilis in adults attending an ART clinic in Tigoni District Hospital
2. To determine operational feasibility and diagnostic utility of a rapid point-of-care syphilis test in an ART clinic
3. To compare the clinical stage of HIV and Syphilis seropositivity

METHODS AND MATERIALS

Study Design:

This was a descriptive cross-sectional survey.

Study Area:

The study was carried out in Tigoni location of Limuru Division of Kiambu District in Central Province of Kenya. Tigoni lies about 33 kilometres north-west of Nairobi, and is served by an all weather road just east of the Trans-Africa Highway.

Kiambu District is located in Central Province and has a total area of 1,323.9 square kilometer divided into five administrative divisions namely Kiambaa, Limuru, Githunguri, Kikuyu and Lari. Tigoni is one of five locations in Limuru division (Table 1).

Kiambu district had a population of 802,625 persons in 2001 with an estimated growth rate of 2.56%. The district population is projected to reach 936,785 persons in 2008. Limuru Division covering an area of 280.7 square kilometres, has a total population of 113, 578 (56,651 males and 56,927 females) in 30,146 households. Tigoni location has a total population of 10, 207 (5,442 males and 4,765 females) with total of 3,484 households. It covers an area of 40.2 square kilometres (24).

Table 2. Demographic indicators

Population (2002)	802,625
Number of Males	398,180
Number of Females	404,445
Female/Male Sex Ratio	1:0.98
Number of Youthful Population (15 - 25) years	186,769
Primary school population (6 - 13) years	153,909
Secondary School Population (14 - 17) years	73,094
Labour Force (15 - 64) years	468,624
Dependency Ratio	100:71
Population Growth Rate	2.56%
Rural Population at start of Plan Period 2002	412,204
Urban Population at start of Plan Period 2002	390,421

Source: District's Statistics Office, Kiambu, 2001

The majority of the population is engaged in farming many of them as labourers in the large-scale farms in tea, coffee, flowers and horticulture, and livestock production. Many of the adults are also engaged in subsistence farming in addition to employment or as a sole means of livelihood.

In the year 2001, Kiambu had over 250 health facilities spread across the district. The doctor/population ratio is about 1:25,000. The average distance to a health facility is 5 Km. The Tigoni District Hospital, upgraded from a sub-district hospital in 2006, is a 75-bed hospital with out-patient services that include Antenatal care/FP, Child health and immunization, a dental clinic, a basic laboratory, an X-ray unit, a pharmacy, theatre, and mortuary. The MCH services also include a VCT that has the capacity to do rapid HIV diagnosis. The ART Programme is part of the reproductive health research activities supported by the University of Nairobi. On-going research at Tigoni indicates that the HIV prevalence is about 9%. The national prevalence of HIV infection was estimated at 14% while that of Kiambu district was 17% (National AIDS Control Programme, 2001) (25). Current national HIV prevalence, 2005, stands at less 9% (26).

The University of Nairobi has been conducting research in the area for more than 40 years. The University has been conducting research in reproductive health in partnership with the hospital for close to ten years now. The ART programme at the hospital is supported by PEPFAR funds through the UNITID support programme for research cohorts and started in April, 2005.

Study Population:

Adults enrolled in the Antiretroviral Therapy Clinic (ART) in Tigoni District Hospital. Women referred from the cervical cancer-screening programme, antenatal clinic and maternity wards. The men are in general partners/spouses of these clients and are significantly fewer. A convenience 'stratified' sampling method was used in order to ascertain that at least 25% of the sample consisted of males.

Sample size calculation

$$\frac{Z^2pq}{d^2} = \frac{(1.65)^2.7x.3}{.0.05^2} = 229$$

Z=confidence interval

p=prevalence

q=1-p

d=statistical significance

Males would number 57 or 25% of the sample size of 229.

Ethical considerations:

The study was started only after approval by KNH-ERC. Detailed study information was provided to all eligible clients and informed consent obtained. Clients were given a copy of the signed consent form to take home for their own reference, which also had the contact persons they could get in touch in case of any concerns.

All procedures were being done according to good clinical and laboratory practice. Confidentiality of all client information was maintained, and all efforts were be made to minimize risks for all participants. Refusal to participate did not in any way interfere with the rights of participants to care to which they were otherwise entitled at Tigoni District Hospital.

Key ethical principles for this study

- The right of everyone to equitable, affordable and accessible health care;
- Reproductive health rights, as formulated in the Programme of Action adopted at the 1994 International Conference on Population and Development in Cairo (paragraph 7.6);
- The ethical principles of justice, autonomy and beneficence as defined and discussed in the Declaration of Helsinki and the International Ethical Guidelines for Biomedical Research Involving Human Subjects prepared by the Council of International Organizations of Medical Sciences (CIOMS) and WHO;
- A gender-based perspective in reproductive health: sensitivity to gender-related factors that may affect the power balance between men and women, reduce women's power of self-determination, and affect the provision and receipt of services.

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Data Collection

Data collection instruments included structured questionnaires, lab result forms and lab register, and nurse note books. All data were entered into a Spreadsheet database and Stata 9[c] and analyzed by standard statistical methods for comparative analysis.

RESULTS

Study population

Between July 15th and September 30th 2007, a total of 201 (88%) out of the calculated sample size of 229 adults enrolled in the ART clinic at the Tigoni District Hospital were recruited out of which 58 (29%) were males, and 142 (71%) were females. One questionnaire was missing at the time of data entry and was discarded in the analysis.

The mean age for males was 37.24 (range 19-57) and for females was 35.8 (range 21-62) (Table 1). The peak age range for males was 35-39 years, and 30-34 years for women (Table 2).

The mean number of school years completed for males was 9.24 (range 0-18) and for females was 8 1 years (range 0-16) (Table 1). About 95.5% of the study population has had some years of education (60% primary, 33% secondary level, and 2.5% tertiary level education). Only 4.5% of the study population had not had any education

Majority of males were married (67.2%), while majority of those who were widowed were women (80%). Majority of those divorced or separated were also women (85.4%).

About 36.2% of males and 32.4% of females were not on anti-retroviral drugs. The mean period in months of duration of treatment for ARVs was 9.18 months for both sexes (Table 1).

Of those who had been staged for HIV disease, majority, 59.6% were in stage II, while 18.7% were in stage I, 15.8% in stage II, and 5.8% in stage IV. Twenty nine patients (14.5%) had not been staged, some because they were new clients and their work-up had not been completed, or their disease stage was missing from their clinical notes (Table 3). A history of treatment for genital ulcer was elicited in 6.9% in male and 12.7% females with an overall 11% of study population giving a history of genital ulcer disease (Table 3).

Table 1 Baseline characteristics of study population

Variable	Male	Female	Total
Mean age (yr)	37.24	35 8	
	(19-57)	(21-62)	
Level of education			
None	1	8	9 (4 5%)
Primary	32	88	120 (60%)
Secondary	22	44	66 (33%)
Tertiary	3	2	5 (2 5%)
Mean (school years completed)	9 24 (0-18)	8 1 (0-16)	
	SD 3 29	SD 3.23	
Marital status			
Single	8 (13 8)	31 (21 8)	39 (19.5)
Married	39 (67.2)	56 (39.4)	95 (47.5)
Divorced/separated	6 (10 2)	35 (24 6)	41 (20.5)
Widowed	5 (8 6)	20 (14 08)	25 (12.5)
Total	**58 (100%)**	**142 (100%)**	**200 (100%)**
Period on ARVs (mo)			
None	21 (36.2)	46 (32 4)	67 (34)
1-6	16 (27.6)	28 (19 7)	44 (22.3)
7-12	7 (12.1)	19 (13.4)	26 (21.3)
13-24	11 (19)	31 (21 8)	42 (21.3)
25-36	1 (1 7)	14 (9.9)	15 (7 6)
37-48	1 (1 7)	1 ((0 7)	2 (1)
49-59	0	0	0
>60	1 (1.7)	0	1 (0.5)
Total	**58 (100%)**	**139(100%)**	**197 (100%)**
Mean no of months on ARVs	9.18 (95% CI = 7.49-10.86)		

Table 2 Age group distribution by sex

Sex	Age groups										
	<24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	>60	Total	%
Male	**1**	**8**	**13**	**16**	**8**	**9**	**2**	**1**	**0**	**58**	**29.7**
Female	**9**	**17**	**42**	**32**	**27**	**8**	**3**	**1**	**3**	**142**	**70.3**
Total (%)	**10 (20)**	**25 (12.5)**	**55 (27.5)**	**48 (24)**	**35 (17.5)**	**17 (8.5)**	**5 (2.5)**	**2 (1)**	**3 (1.5)**	**200**	**100**

In the initial clinical assessment on enrollment in the ART clinic, only 8% of females and no males had genital ulcer disease, with an overall prevalence of genital ulcers of 5.7% at the time of recruitment Lymphadenopathy was reported for 19 6% of males (n=56) and 14.6% of females (n=137), and overall in 16.1% of the 193 patients in which presence or absence of lymphadenopathy was reported. Skin rash was also reported in 19.6% of

males and 14.6% females in the same group. The only neurological signs reported were hemiplegia and hemiparesis in this group, 3.6% and 9.3% in males respectively, and in 3% and 23.4% in females respectively (Table 3).

Table 3 Baseline disease characteristics of study population

Variable		Male	Female	Total
WHO stage HIV disease				
Stage I		9	23	32 (18.7%)
Stage II		27	75	102 (59.6%)
Stage III		10	17	27 (15.8%)
Stage IV		5	5	10 (5.8%)
Total		51	120	171 (100%)
Clinical presentation				
History of genital ulcer				
	Yes	4 (6.9%)	18 (12.7%)	22 (11%)
	No	54 (93.1%)	124 (87.3%)	178 (89%)
	Total	58 (100%)	142 (100%)	200 (100%)
Presence of genital ulcer disease				
	Absent	56 (100%)	126 (92%)	182 (94.3%)
	Present	0	11 (8%)	11 (5.7%)
	Total	56	137 (100%)	193 (100%)
Lymphadenopathy				
	Absent	45 (80.4%)	117 (85.4%)	162 (83.9%)
	Present	11 (19.6%)	20 (14.6%)	31(16.1%)
	Total	56 (100%)	137 (100%)	193 (100%)
Skin rash				
Absent		45 (80.4%)	94 (68.6%)	139 (72.0%)
Present		11 (19.6%)	43 (31.4%)	54 (28%)
Total		56 (100%)	137 (100%)	193 (100%)
Neurological signs				
Hemiplegia	Absent	54 (96.4%)	133 (97%)	187 (96.9%)
	Present	2 (3.6%)	4 (3%)	6 (3.1)
	Total	56 (100%)	137 (100%)	193 (100%)
Hemiparesis	Absent	49 (90.7%)	105 (76.6%)	154 (79.8%)
	Present	7 (9.3%)	32 (23.4)	39 (20.2%)
	Total	54 (100%)	137 (100%)	193 (100%)

Of the 200 participants screened for syphilis, 11 (5.5%) were positive (Table 4). However, of these, nine had a confirmatory TPHA result. Of these, 8 tested positive, and one was negative, giving a prevalence rate of 4.1% (8/196) (Table 4). Fifteen patients (included as controls) who tested negative with the screening test had a negative TPHA test.

Table 4 Test results of the rapid syphilis screening test

	Rapid syphilis screening test		
Sex	Negative	Positive	Total
Male	57	1	58
Female	132	10	142
Total	189 (94.5%)	11 (5.5%)	200

Table 5 shows the characteristics for the 11 patients who had a positive rapid syphilis screening test. One was male, and the other 10 were females. They were aged between 26 and 45 years of age. Of the nine who had been staged for HIV disease, seven were in Stage II, one in Stage I, and one in Stage IV. Of this group 5 (45.5%) were on ARVs . Their baseline CD4 counts ranged from 14 to 938, and only 5 had had a follow-up CD4 count, probably indicating that these were the ones who had been attending the clinic for more than six months. In this group, only two patients gave a history of having been treated for genital ulcer disease (GUD). Of the symptoms and signs elicited at initial clinical assessment, four had none, four had a skin rash, two had lymphadenopathy, and two were reported to have hemiparesis. Only one patient had genital ulcer disease at the time of initial assessment. In the cases which had a positive rapid syphilis test, the test was positive within 10 minutes, and for three, the test was positive when the test strip was re-read at one hour. Four of these patients were staged as Secondary Syphilis, and one as Primary Syphilis based on clinical assessment. The numbers were too small to make any meaningful correlation between clinical stage of syphilis and HIV disease.

Table 5 Characteristics of Patients with positive rapid syphilis test

No	Sex	Age (yr)	HIV stage	Mo on ARVs	CD4 Baseline	CD4 Latest	Treated for GUD	Signs & Symptoms	Rapid syph test + in	TPHA titre	Syph Stage
1.	F	33	II	6	63	74	No	None	+ 1hr	-	-
2.	M	45	II	2	14	-	No	Lymphnode Skin rash	+ 1hr	-	II
3.	F	45	II	24	92	279	No	Skin rash	10min	4+	II
4.	F	35	II	0	36	-	No	Skin rash	10min	4+	II
5.	F	40	IV	11	147	352	No	Hemiparesis	10min	2+	-
6.	F	35	II	0	326	-	No	Hemiparesis	10min	0	-
7.	F	26	II	0	239	177	Yes	GUD	10min	2+	I
8.	F	32	II	0	87	72	No	Lymphnode	+ 1hr	-	II
9.	F	28	I	0	938	-	No	None	10min	3+	-
10.	F	41	-	12	-	-	Yes	None	10min	1+	-
11.	F	39	-	0	-	-	No	None	10min	4+	-

Out of the 25 patients who had both a rapid test for syphilis and the confirmatory or reference test, TPHA, 8 were positive by both tests giving a sensitivity of 100% and the specificity was 94%, with a positive predictive value of 89%, and a negative predictive value of 100%. The positive predictive value (PPV) for the rapid test was 89% and the negative predictive value (NPV) was 100% (Table 6).

Table 6 Comparison of the rapid POC test for syphilis and the reference test

	TPHA test (Reference test)		
Rapid screen syphilis test	Positive	Negative	Total
Positive	8	1	9
Negative	0	16	16
Total	8	17	25

From the records the nurses maintained about duration of performing the tests and giving the client results, reasons for delay in giving the results or difficulties in the test, a total of 195 (97.5%) of the rapid tests carried out were recorded by the nurse performing the test as taking 10 minutes (Table 7). One took 15 minutes and another 3 took 20mins, the reported reason being that the test was repeated for being indeterminate. In one client form, the time it took to perform the test was not recorded. Overall, the two nurses who performed the tests reported that it was easy to do and give timely results to the client

without interruption of the client flow of the very busy clinic. For three tests, the results were negative after 10 minutes, but when Nurse two read the test strip, it was positive.

Table 7 Duration of performing the rapid syphilis test reported by Nurse

Rapid syphilis test result	Duration of test (min)				
	(Missing)	10	15	20	Total
Negative	1	183	1	3	188
Positive	0	12	0	0	12
Total	1 (0.5%)	195(97.5%)	1 (0.5%)	3 (1.5%)	200 (100%)

The two nurses scored the Rapid syphilis screening test as good, on the basis of quality of kit instructions, technical simplicity, and ease of interpretability of the results (Table 8).

Table 8 Operational score of the Rapid Test for Syphilis

Operational characteristic	Reader 1	Reader 2
Quality of kit instruction	2	3
Technical simplicity	3	3
Ease of interpretation of results	3	3
Total score	8	9

Key
1-3 Poor
4-6 Fair
7-9 Good

DISCUSSION

The ART Clinic at Tigoni was started as a support programme for a Cervical Cancer Screening research project that has been going on for the past 4 years and therefore the majority of those enrolled are women. The males enrolled in the clinic are mainly the spouses of the cohort from the reproductive health project. The study set out to recruit at least 25% of the participants as males. Out of 200 participants recruited, 58 (29%) were males, and 142 (79%) females. The mean age for the females was slightly higher for females than males, 35.8 years and 37.2 years respectively. The age group most affected by HIV in Kiambu District is 25-34 years (29). In this study population, the peak age groups for those sampled were 35-39 years for males, and 30-34 years for females. The males were therefore slightly older for this study population. This is consistent with national data that shows HIV affects women at younger age groups than men (24, 29).

A higher number of women were widowed, divorced or separated. This is a reflection of the impact of HIV disease on the social dynamics in our society. The socio-economic impact of HIV/AIDs in the district as reported in the Kiambu District Development Strategic Plan (2005-2010) include the high school drop out rates, female and children headed families, loss of manpower and high mortality and morbidity rates, orphans, and increasing poverty, to name a few (29).

At least 95.5% of the study population reported some level of education (60% primary level, 33% secondary level, and 2.5% tertiary level). School enrollment in Kiambu District is high, at an average of 89% (89.7% for boys and 89% for girls). However, a high dropout rate is also reported at an average of 30% in primary level and 25% for secondary school. The district had a high school enrollment rate of 46.4% for boys and 53.3% for girls. Many children drop out of primary school due to the high cost of education, poverty and the limited number of secondary schools with the boys being affected most (29).

The prevalence of syphilis in this cohort by rapid screening was 5.5% and 4.1% by the reference test. This is somewhat lower than expected since the estimated mean for sero-prevalence of syphilis in Sub-Saharan Africa is 8.3% (9). A possible explanation is that a

HIV positive person is frequently on antibiotics and any syphilis infection is readily treated in a bystander effect. Doxycycline and Benzathine penicillin are widely available in the primary public health care facilities. Doxycycline is also one of the recommended drugs for syndromic treatment of sexually transmitted infections (Min of Health).

The clinical presentation of those who were syphilis seropositive was not significantly different from the rest of the study population, with only one patient reporting history of genital ulcer disease, which was not characterized as a chancre in the patient's clinical notes. The other signs and symptoms elicited lymphadenopathy, skin rash, hemiplegia and hemiparesis were not uncommon in HIV positive syphilis seronegative individuals

Case reports suggest that the unusual clinical manifestations of syphilis may be more common and the course more rapid in patients with HIV infection. Although prospective studies largely have not corroborated these observations, such anecdotal reports have driven the hypotheses that among patients coinfected with HIV and *T pallidum*, cutaneous lesions may be more severe, symptomatic neurosyphilis may be more likely to develop, the latency period before the development of meningovascular syphilis may be shorter, and the efficacy of standard therapy for early syphilis may be reduced (30).

Most HIV-infected patients with *T pallidum* infection present with typical dermatologic clinical features of primary and secondary disease, such as chancres and diffuse maculopapular rashes.(30) In a study among patients seen in an STD clinic, however, patients with HIV infection were more likely than HIV-negative individuals to present with signs and symptoms of secondary syphilis and were more likely to have chancres still present at the time of secondary syphilis diagnosis.(31) In a multicenter study of STD clinic patients with early syphilis, HIV-infected patients were more likely to present with multiple chancres, but the size of the chancres, characteristics of the skin rash, and duration of the chancres or rash before presentation did not differ according to HIV status (32). Atypical chancres have been reported,(33) including lesions appearing as fissures or abrasions. Two reports also described gummatous penile ulceration.(34,35) Unusual rashes include papular or nodular eruptions,(36,37) nodular or ulcerative lesions with necrotic centers (ie, lues maligna),(36) and keratoderma.(39) These skin lesions have

been characterized as more aggressive forms of secondary syphilis in HIV-infected persons, yet the same dermatologic presentations are found in HIV-uninfected persons.(40) These isolated findings do not reveal the frequencies of these uncommon cutaneous manifestations, and therefore, whether the clinical course and clinical spectrum of syphilis differ in HIV-infected and HIV-uninfected populations remains unclear. In general, syphilis should be included in the differential diagnosis of mucocutaneous abnormalities in the HIV-infected patient, and diagnostic evaluation and empiric treatment should be given.

In latent syphilis, serologic evidence of infection is found despite absence of symptoms and signs of the primary and secondary stages. Relapses of secondary syphilis symptoms and signs may occur early in the latent stage. Early latent syphilis is defined by CDC as infection less than 12 months in duration, evidenced in the previous year by a negative serologic test, symptoms or signs of primary or secondary syphilis, or contact with a sex partner with early-stage syphilis.(30) If none of these criteria suggest that infection was acquired in the preceding 12 months, then the duration of infection is unclear and the case is referred to as syphilis of unknown duration. In this case, even if the patient had a negative or 4-fold lower non-treponemal serologic test result more than 12 months ago, it is usually impossible to determine the time of acquisition since the previous test, assuming that risk behaviors occurred throughout this time period. When the precise time of acquisition is in doubt because the CDC's early latent criteria are not met, these patients should be treated conservatively as if the infection were present for more than 1 year. These cases require CSF evaluation and treatment with benzathine penicillin G, 7.2 million units divided in 3 weekly doses (ie, 2.4 million units per dose), assuming no evidence of neurosyphilis was found.

None of the patients in this study group was staged as Tertiary syphilis which refers to disease presenting with late manifestations, encompassing cardiovascular features such as aortitis with aneurysm formation, late neurologic sequelae (general paresis, tabes dorsalis), and formation of gummas (indolent but potentially destructive granulomatous lesions that may occur in any organ but chiefly involve skin, bone, and liver). Of note, neurosyphilis is not a stage, but rather a site of infection, where symptoms may manifest

either earlier or later in the course of infection. The conventional staging of syphilis is unaltered by HIV coinfection. Due to the small numbers, the cross-tabulation between HIV and syphilis disease stage was not meaningful.

Rapid Point of Care Testing for Syphilis

The sensitivity of the Acon rapid syphilis test was 100% in this small study with a specificity of 94%, PPV of 89% and a NPV of 100%. The expected manufacturer's sensitivity and specificity was given as 99.7% overall. These were not significantly different from similar studies using a variety of rapid syphilis screening tests (7, 9). This kit performed much better than the widely used RPR which was compared to Abbot Determine ELISA test when compared to TPHA in Bolivia (29). The RPR gave sensitivity of 75.7% (95% CI 70.8-80.2), Specificity of 99.0% (CI 98.9 – 99.3%), PPV of 76.9% (CI 72-81.3%), NPV of 99.0% (CI 98.8-99.2%) (29).

When the performance of the test were scored by the nurses, the rapid test scored very highly, 8 and 9 out of a possible score of nine. The nurses clearly felt that the test instructions were simple to follow, the test easily carried out, and the results easy to interpret. The kits required no refrigeration and no equipment. The only extras required that did not come in the kit were lancets for the finger prick. The test results were ready for 97.5% of the cases in 10minutes. The patients therefore did not have to wait for long and those who were seropositive were given a prescription to pharmacy together with their ARVs. A small percentage (2%) however, had the test repeated because it was invalid. Reasons for an invalid test result (when a control line fails to appear on the test strip) can be due to either insufficient specimen volume or incorrect procedural techniques. The operational scores and the high performance of the test fulfill the basic WHO ASSURED (**A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free and **D**eliverable to end-users) criteria for test that will meet disease control needs (Table 9) (9).

Table 9 Score card for Acon Ultra rapid test for syphilis

Test Characteristics	Acon	Other Syphilis tests (9)
Affordable	US 0 64	US$0 19-3 0
Sensitive	100%	85-99%
Specific	94%	93-100%
User-friendly	4 steps	3-4steps
Rapid/robust	10min/storage at 2-30°C	20min/storage at 8-30°C
Equipment-free	Yes	Yes
Deliverable	Yes	Negotiated price through WHO bulk procurement Scheme

CONCLUSION

Rapid point of care testing for syphilis is feasible and improves care of patients with the co-infection of syphilis and HIV. The rapid test used in this study fulfilled the WHO SDI ASSURED criteria for a test likely to improve disease control. Rapid Point of Care testing for syphilis is a means of expanding syphilis testing to clinics without laboratory facilities, improve case detection and facilitate delivery of treatment.

The clinical diagnosis of syphilis in HIV is often difficult because of the overlap of clinical manifestations of HIV disease itself, and the altered immune status that changes the host immune response to *T. Pallidum* infection.

The prevalence of syphilis in this cross-sectional study was lower than expected. A larger epidemiological study my be useful in establishing the true prevalence of syphilis, particularly in HIV disease, important in continuing HIV care.

CHALLENGES

Adequate time to prepare the logistics and conduct the study was a major challenge since expenditure and ethical approvals took longer than had been allowed for in the planning.

Drug stock-outs for Benzathine penicillin or Doxycycline, the mainstay drugs for treatment of syphilis although an anticipated challenge, were not a problem since these drugs are part of the Ministry of Health Essential drug package. The numbers of syphilis positive patients were also too few to overwhelm the current stocks.

Three of the rapid test strips initially read as negative after the stipulated 10minutes, were definitely positive after one hour, read by the second reader (nurse 2). This resulted in 3 patients who had a positive rapid test not having their blood taken for the confirmatory TPHA test since the patients were released after the first reading. However, their confirmatory test will be done when they return for their next appointment. This however, defeats the objective of same day testing and treatment for a small number of patients.

Incomplete patient files particularly in initial clinical assessment were a significant challenge. In a busy clinic, this becomes common practice unless the attending physician makes a conscious effort to be thorough both in the physical assessment and documentation. If attention to detail is skipped, not only will subtle signs and symptoms of disease, and syphilis in particular are missed, but also the opportunity for laboratory diagnosis and treatment.

RECOMMENDATIONS

1. Introduction of rapid point of care testing for syphilis in HIV care clinics in order to improve case detection and facilitate delivery of care, and reduce HIV transmission.
2. Conduct a larger epidemiological study to establish the prevalence of syphilis in HIV positive subjects.

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APPENDIX I

Client Explanation

My name is Dr. Lucy Muchiri from the Department of Human Pathology, University of Nairobi. I am carrying out a study to determine the utility of a rapid diagnostic test for syphilis among clients enrolled in the ART clinic such as yourself.

Syphilis is an infection caused by a bacterium called *Treponema pallidum* that is transmitted sexually like HIV. It can also be transmitted from a pregnant mother to her unborn baby. It manifests itself as an ulcer in the genital area as well as skin rash. If untreated, it may progress to involve other body organs such as the heart and the brain as well as other parts of the nervous system.

It has been shown in studies elsewhere that co-infection of syphilis and HIV is not uncommon, and that HIV infection can change the clinical presentation of syphilis so that unless a lab test is done, the signs and symptoms of syphilis can be missed or mistaken for some other infection. It has also been shown in other studies that treatment of syphilis improves the control of HIV disease and decreases transmission. In carrying out this study, we hope to find out how many people have both infections of syphilis and HIV so that both can be treated. We also to determine whether the diagnostic test can be done easily and quickly in the clinic and obtain results before you leave the clinic so that if it is positive, you are given the right medicine on the same clinic day.

How many people will participate and who are eligible?

About 230 clients will be recruited to participate in this study. All adults attending this clinic are eligible to participate as long as they are willing and able to give consent.

Participation is voluntary and even if you decline to participate, you will receive all the benefits to which you are entitled in this clinic and hospital. You can also withdraw at anytime should you change your mind again without penalty.

Confidentiality

All your test results will be kept confidential and will only be revealed to the doctors who are taking care of you in this clinic so that you can be given the appropriate care. You will be encouraged to discuss your results with your partner so that in the event you are positive, they too can be tested and receive treatment. Although results of the study will most likely be published, your names will not appear in any publications or project reports.

Benefits and other rewards

You will not receive any financial rewards for participating but you will be reimbursed bus-fare to a maximum of Ksh.100. You will benefit from this study, as you will have a free syphilis test and if positive, you will receive specific and effective treatment. The drugs are available in the hospital pharmacy at the usual rate of Ksh. 40/=. This study will also benefit others since it will provide us with information on the prevalence of syphilis in the clients attending this clinic as well as provide us with information that may assist us in better planning of services for this clinic. The information will also help the government and other providers in improving services.

Harmful effects or risks

You will be asked a few questions of general and personal nature. If there are any questions with which you are uncomfortable, you are not obliged to answer.

The procedure of taking blood will be similar to what you have experienced previously when blood was taken for HIV investigation in this clinic. Blood amounting to 2ml will be drawn from your arm and there will be a little discomfort. Rarely a blood clot may form under the skin at the site of injection but applying pressure as soon as the needle is withdrawn easily prevents this. You will then be required to wait for about 20 minutes in the waiting room for the test to be done and your results entered in your file. The doctor will then see you and discuss your results and any medication you may require depending on the outcome of the results. The short waiting period may cause you some anxiety, but please feel free to discuss this with the nurse/counselor or the doctor attending you.

UTILITY OF A RAPID POINT OF CARE TEST FOR SYPHILIS STUDY

Consent Form

I _____ have read or have had read to me and understood the details of this study. I have had the opportunity to ask questions and they were answered satisfactorily. I understand that participation is completely voluntary and that I can withdraw at anytime without loss of health benefits to which I am entitled in this clinic or hospital. I understand that there will be no financial rewards for my participation.

I willingly consent to participate in this study.

_____ _____

Signed Date

Independent Witness

_____ _____

Signed Date

Contact persons for any queries regarding this study:

Prof. K.M. Bhatt
Chairman, ERC-KNH
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Or 0722 703364

1 Copy of this form is given to the client to take home

APPENDIX II

QUESTIONAIRE

1. Study Number _____ Sex: Male 1 Female 2

2. Last Name_____

3. Other names_____

4. Date of birth_____

5. Age (in years)_____

6. Residence (location, Division)_____

7. Marital status;
   - 0 Single
   - 1 Married
   - 2 Divorced/separated
   - 3 Widowed

8. Level of education
   - 0 None
   - 1 Primary
   - 2 Secondary
   - 3 Tertiary

9. Number of years of schooling completed _____

10. Number of children _____

11. Year diagnosed with HIV_____

12. Period on ARVs in months_____

13. Have you ever been treated for genital ulcers?
   - 0 No
   - 1 Yes

14. If yes, was it in the last

   - 0 None
   - 1 Six months
   - 2 12 months
   - 3 2 years

From client file

15. HIV first diagnosed_____

16. Stage of HIV disease_____

0 Stage I
1 Stage II
2 Stage III
3 Stage IV

17. Baseline CD4 count_____

18. Latest CD4 count_____

Physical Findings (current visit)	Present/Abnormal (1)	Absent/Normal (0)
19. Lymphadenopathy		
20. Genital ulcer disease		
21. Skin rash		
22. Cardiac Heart rate & rhythm		
23. Cardiac auscultation findings		
24. Neurological		
Orientation in Time, place & person		
Speech		
Neck stiffness		
Blindness		
Hemiplegia		
Hemiparesis		
Numbness of extremities		
Other findings		

LABORATORY RESULTS

Test	Negative (0)	Positive (1)
25 Acon Ultra Syphilis Test		
26 TPHA Qualitative		
27 TPHA Quantitative (Titre)		

28 Clinical Diagnosis

0 Syphilis negative
1 Syphilis positive

29 Syphilis stage

0 Negative
1 Primary
2 Secondary
3 Tertiary

0

APPENDIX III SYPHILIS RAPID POINT OF CARE TEST SCORE CARD

Determining inter-reader variability and stability of test results

In many clinic settings, staff may be unable to read the test at the designated time of 15-20 minutes after the specimen was added. It is therefore of interest to determine if test results are stable after one hour

1. Each test is performed and read by nurse1 according to the instructions described and the results recorded in a laboratory record book
2. The test is then placed in a numbered folder and handed to nurse 2
3. Nurse 2 interprets the test result independently immediately on receipt of the folder and repeats the reading after one hour
4. Nurse 2 records the results in a separate laboratory record book
5. At the conclusion of the evaluation, results are entered into the data collection form as rapid test results under "reader 1, reader 2 designated time, and reader 2 designated time + 1 hour.

Handling of indeterminate results

Results that are not clearly positive or negative are recorded as indeterminate. A repeat test should be carried out, and blood taken for the reference test.

Assessing operational characteristics

Each rapid test was be assessed for the following operational characteristics by nurse 1 after completing the testing of the first 25 specimens and by nurse 2 for the next 25 specimens on the evaluation score card:

- Clarity of kit instructions (maximum possible score of 3)
- Technical complexity or ease of use (maximum possible score of 3)
- Ease of interpretation of results (maximum possible score of 3)

The total possible score is 9. The higher the score, the more suitable the test is for use in primary health care settings in developing countries

Test reproducibility and **Quality Control**

The objectives of this testing was to answer the following questions:

- Operator-to-operator variability: will the test give the same results on the same specimen if two different operators in the clinic and the technician in the reference laboratory perform it? All positive tests by the rapid test and 10% of the negatives will be re-run by the reference lab technician.

Data analyses

The reference or "gold" standard was the TPHA results obtained for each serum specimen at the reference laboratory which was be the research laboratories in the Department of Microbiology, University of Nairobi.

Diagnostic Utility of the rapid POC test for syphilis

Sensitivity and specificity:

For the rapid test compared to the validated reference test results obtained at clinic site was analyzed as follows:

		Reference test results	
	+		
Rapid test results	+	a	b
	-	c	d
		a+c	b+d

Rapid test sensitivity = a/(a+c)
Rapid test specificity = d/(b+d)

a = true-positive result c = false-negative result
b = false-positive result d = true-negative result

Operational Characteristics of the Rapid Diagnostic Test for Syphilis

Operational characteristic	Reader 1	Reader 2
Quality of kit instruction		
Technical complexity		
Ease of interpretation of results		
Total score		

APPENDIX IV ACON[R] ULTRA RAPID TEST STRIP FOR SYPHILIS

Acon[R] is a rapid test for syphilis to detect antibodies (IgG and IgM) to Treponema Pallidum (TP) qualitatively in whole blood, serum or plasma.

(Acon[R] Syphilis Ultra Test Strip (Whole blood/Serum/Plasma) is manufactured by *Acon* Laboratories, Inc 4108 Sorrento Valley Boulevard, San Diego, CA. 92121, USA)

Principle

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

Reagents

The test contains Syphilis antigen coated particles and syphilis antigen coated on the membrane.

Precautions and storage

As detailed in Manufacturers instructions

The test package can be stored either at room temperature or refrigerated at 2-30^{0}C.

Specimen

Whole blood from venipuncture or fingerstick, serum or plasma

Materials

Materials provided: Test strips, Test cards, Buffer, Package insert, droppers

Materials not provided in package: Specimen collection containers, lancets (for fingerstick whole blood only), Timer, centrifuge, Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

Procedure

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30^{0}C) if refrigerated before, prior to testing.

1. Remove the test strip from sealed foil pouch and use as soon as possible. Best results will be obtained if the assay is performed within one hour
2. Peel off the tape from the test card, and stick the test strip in the middle of the test card with arrows pointing downwards as illustrated in the insert.
3. Follow instructions for whole blood, serum or plasma as detailed in the instructions by manufacturer.
4. Wait for the coloured line(s) to appear. Read results in 10 minutes. Do not interpret results after 30mins.

Interpretation of results

POSITIVE: Two lines appear, one coloured line should be in the control line region (C), and another apparent coloured line should be in the test line region (T). Note: The intensity of the colour of the test line will vary depending on the amount of TP antibodies present in the specimen. Therefore any shade of colour in the test line region (T) should be considered as positive.

NEGATIVE: One coloured line appears in the control line region (C). No line appears in the test line region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test. If the problem persists, discontinue using the test kit immediately.

Quality Control

A procedural control is included in the test. A coloured line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit. However, it is recommended that a positive and negative control be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations

1. The Syphilis Ultra Test Strip (Whole blood/Serum/Plasma) is for in vitro diagnostic use only. Neither the quantitative value nor the rate of increase in TP antibodies can be determined by this qualitative test.
2. The Syphilis Ultra Rapid Test Strip (Whole blood/Serum/Plasma) will only indicate the presence of TP antibodies in the specimen and should not be used as the sole criteria for the diagnosis of TP infection
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

If the test result is negative and clinical symptoms persist, additional testing using other methods is recommended. A negative result does not at any time exclude the possibility of TP infection.

Expected values

The Syphilis Ultra Test Strip (Whole blood/Serum/Plasma) has correctly identified specimens of sero-conversion panel and has been compared to commercial TPHA Syphilis test, demonstrating an overall accuracy greater or equal to 99.7%.

Performance Characteristics

The Syphilis Ultra Test Strip (Whole blood/Serum/Plasma) when compared to a commercially available TPHA test using clinical specimens show that Syphilis Ultra Test Strip (Whole blood/Serum/Plasma) has a relative sensitivity of 99.7% and a relative specificity of 99.6%.

APPENDIX V SYPHILIS (SYP-TPHA)

Treponema Pallidum Haemagglutination Assay Manual Method

(Randox Kit By Randox Laboratories Ltd, Ardmore, Diamond Road, Crumin, Co Antrim, United Kingdom, BT29 4QY)

Principle

The syphilis TPHA test is an indirect haemagglutination test for the detection and titration of specific antibodies against *Treponema pallidum*. Avian erythrocytes are sensitized with antigens of the Nichol's strain of *Treponema pallidum*. In the presence of syphilitic antibodies, these test cells aggregate to form characteristic patterns on the surface of the microplate wells. Antibodies directed against other nonpathogenic treponemes are absorbed by an extracts of Teiter's treponemes present in the cell suspensions, thus greatly reducing false positives. Other non-specific reactions can be detected and eliminated with the non-sensitised control cells.

Samples

Dilute serum or EDTA plasma 1/20 and use on the same day. This should be performed by mixing 10µl of sample with 190µl of diluent buffer in a single microtitre well. The samples should be free from contamination and non-heamolysed. Fresh serum or EDTA plasma samples may be stored for 24 hours at +2 to 8^0C or 4 weeks at -20^0C.

Stability & Preparation of reagents

All reagents are ready for use, however both test and control cells should be thoroughly resuspended prior to use. The reagents should be stored at +2 to $+8^0$C in an upright position and are stable until expiry date. Reagents should not be interchanged with those of other batches.

Materials provided

Test cells

Control cells

Diluent Buffer

Positive control

Negative control

Materials required but not provided

1. Variable pipettor(s) to deliver 10µl, 25µl, 75µl and 190µl
2. U-well microtitration plates

Procedure

A. <u>Qualitative Test</u>

1. Bring the test reagents and samples to room temperature
2. Dispense into adjacent wells of a microtitration plate

	Control well	Test well
Sample (1/20) or control	25µl	25µl
Control cells	75µl	-
Test cells	-	75µl

3. Gently tap all four sides of the plate to ensure the contents of each well are thoroughly mixed.
4. Cover the plate and place on a flat, white surface away from vibration and direct sunlight. Leave for 45-60 minutes at room temperature or overnight before reading results.

Interpretation of results

Results are read visually with positive and negative control sera. Readings are scored by the degree of haemagglutination and reported as +4, +3, 2+, 1+, +/- or negative.

The final diagnosis should be based on a correlation of the test results with patient's clinical history.

Degree of Heamagglutination	Reading	Report
Uniform mat of cells covering entire base of well sometimes with folded edges	4+	Reactive
Uniform mat of cells partially covering base of well	3+	Reactive
Smaller mat surrounded by a ring of cells	2+	Reactive
Smaller mat surrounded by a smaller more distinct ring of cells	1+	Reactive
Well defined dense ring with a hole in the centre	+/-	Indeterminate Retest
Definite button of non-agglutinated cells sometimes with a small hole in the centre	Negative	Non-reactive

The negative control must show a non-agglutinated pattern with both test and control cells. The positive control must show agglutination with test cells but not with control cells. Sera or plasma samples showing agglutination with control cells indicates the presence of non-specific agglutinins and should be retested after absorption.

Positive samples should be retested by quantitative test i.e. titrated.

B. Quantitative Test

1. Starting with 1/20 dilution of sample make serial twofold dilutions in diluent, testing each one as described by steps 2-4 in the qualitative test.

   A sample showing haemaggluttination in the test wells should be reported as a positive result provided that no haemagglutination takes place in the control well. The titre is defined as the final dilution that shows a positive result.

Limitations of the test

Despite TPHA's high specificity, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and some autoimmune diseases. Syphilis antibodies persist after a successful course treatment. Therefore a positive result with the TPHA test may indicate a past or current infection.

Performing the quantitative test on successive samples taken over a period of time can be used to indicate a fresh infection, which is characterized by at least a fourfold increase in antibody titre.

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25th June 2007

Dr. Lucy Muchiri
Dept. of Human Pathology
University of Nairobi

Dear Dr Muchiri

RESEARCH PROPOSAL: "THE UTILITY OF A RAPID POINT-OF-CARE FOR SYPHILIS IN AN *ART* CLINIC IN TIGONI" (P126/5/2007)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your above cited research proposal for the period 25th June 2007 – 24th June 2008.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

Prof. A.N. Guantai
SECRETARY, KNH-ERC

c.c The Deputy Director CS, KNH
Prof. K.M. Bhatt, Chairperson, KNH-ERC
Supervisor: Dr. Mark Joshi, Dept of Medicine, UON