COMPARISON OF SEROLOGICAL AND MOLECULAR *TREPONEMA PALLIDUM* TESTS IN HIV PATIENTS IN KENYA

JAMES WACHIRA WAMBUGU

MASTER OF SCIENCE IN MEDICAL LABORATORY SCIENCES

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2026

Comparison of Serological and Molecular *Treponema pallidum* Tests in HIV Patients in Kenya

James Wachira Wambugu

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Laboratory Sciences of the Jomo Kenyatta University of Agriculture and Technology

2026

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature……..Date……..

James Wachira Wambugu

This thesis has been submitted for examination with our approval as University Supervisors:

Signature……..Date……..

Dr. Michael Kahato, PhD

JKUAT, Kenya

Signature……..Date……..

Dr. James Kigera, PhD

UoN, Kenya

DEDICATION

I dedicate this dissertation work to my dear wife Jackline, and my three children, Abigail, Andrew and Amos, whose words of encouragement and push for tenacity ring in my ears. My brothers Joseph and Dave, my sister Rahab have never left my side and are very special. My parents for their unceasing prayers and encouragement throughout the process.

ACKNOWLEDGEMENT

I sincerely thank my supervisors, Dr. Michael Kahato and Dr. James Kigera, for their love and genuine interest in me and my work. I appreciate their tireless efforts, advice, and constructive criticism, which helped shape this project to reach a master's degree level. I also want to thank all my lecturers who taught me and shared valuable lessons and notes during my studies at JKUAT. I am especially grateful to Dr. Nelson Muriu, the County Director of Health Services in Nyeri, and the Ethics and Research Committee at UON/KNH for their approval. I also thank the Nyeri County Referral Hospital CCC staff for their support while I conducted my research in the clinic. I am deeply grateful to my wife, Jackline, who continuously encouraged me to pursue my studies to better equip myself as a lecturer. Jackline, along with my two children, Abigail and Andrew, has offered me immense support and prayers throughout my time at school. They motivated me to work hard and finish this course, especially during difficult times. I also thank my colleagues at Kenya Medical Training College Nyeri Campus MLS department for their support. Finally, I want to extend my gratitude to everyone who helped me in various ways, whose names I cannot list here due to limited space. A big thank you to all of you—may the Almighty God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABREVIATIONS

3TC	Lamivudine
AFV	Adefovir diphosphate
AIDS	Acquired Immune Deficiency Syndrome
ARVs	Antiretrovirals
CCC	Comprehensive Care Centre
CD4	Cluster of Differentiation 4
DHIS	District Health Information System
DNA	Deoxyribonucleic Acid
DTG	Dolutegravir
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme Immunoassay
FSW	Female Sex Workers
FTA	Fluorescent Treponemal Antibody Test
HIV	Human Immunodeficiency Virus
HTS	HIV Testing Services
INSTI	Integrase Strand Transfer Inhibitor
IQC	International Quality Care System
IQC	International Quality Care

KAIS	Kenya AIDS Indicator Survey
Ke–HMIS	Kenya Health Management Information System
MSW	Male Sex Workers
NCRH	Nyeri County Referral Hospital
NRTI	Nucleoside reverse transcriptase inhibitor
NTTs	Non-Treponemal Tests
PCR	Polymerase Chain Reaction
PrEP	Pre-exposure prophylaxis
RPR	Rapid Plasma Reagin
SD	Standard deviation
STIs	Sexually transmitted infections
TDF	Tenofovir Disoproxil Fumarate
TP	*Treponema pallidum*
TPHA	*Treponema pallidum* Hemagglutination Assay
TPPA	*Treponema pallidum* Particle Agglutination Assay
TTs	Treponemal Tests
VCT	Voluntary counseling and testing
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organization

DEFINITION OF OPERATIONAL TERMS

Adefovir diphosphate (AFV) is the active metabolite of the antiviral drug adefovir dipivoxil, used for the treatment of chronic c infection.

AIDS A group of symptoms and illnesses that occur due to advanced HIV infection, which has damaged the immune system.

Comprehensive Care Clinic is a facility where people living with HIV/AIDS go to receive holistic care and management.

Dolutegravir (DTG) is a second-line integrase strand transfer inhibitor (INSTI) approved for the treatment of HIV infection. DTG is equivalent to or superior to existing treatment regimens in both treatment-naïve and treatment-experienced patients, including those with prior raltegravir or elvitegravir failure.

Endemic syphilis a chronic skin and tissue disease caused by the endemicum subspecies of the spirochete *Treponema pallidum*.

Lamivudine (3TC) is a nucleoside reverse transcriptase inhibitor (NRTI) typically taken in combination with other antiretroviral medications used to treat HIV/AIDS and chronic hepatitis B by inhibiting viral deoxyribonucleic acid (DNA) synthesis.

Serofast A persistent non-treponemal serological response that can be seen in patients with syphilis after treatment.

Tenofovir Disoproxil Fumarate (TDF) is a nucleoside reverse transcriptase inhibitor (NRTI) and a crucial antiviral medication used to treat HIV and chronic hepatitis B, often as part of Pre-exposure prophylaxis (PrEP).

Treponema pallidum: Bacteria that cause syphilis

Venereal syphilis A bacterial infection typically transmitted through sexual contact that begins as a painless sore.

Venereal syphilis A bacterial infection usually spread by sexual contact that starts as a painless sore.

Vertical Transmission Passage of a disease-causing agent from the mother to the baby before birth or after birth.

Vertical Transmission The transfer of a disease-causing agent from mother to baby either before or after birth.

ABSTRACT

Syphilis is a common co-infection among people living with Human Immunodeficiency Virus (HIV), where it has a significant impact. Syphilis is caused by *Treponema pallidum* and is typically diagnosed using two main types of tests: non-treponemal tests, such as rapid plasma reagin (RPR) and venereal disease research laboratory (VDRL), and treponemal tests, like the *Treponema pallidum* hemagglutination assay (TPHA)- considered the gold standard in Kenya. The sensitivity of these serological tests for primary syphilis generally ranges between 70-80%, Currently molecular methods such as Polymerase chain reaction (PCR) offers higher sensitivity and specificity than serological tests. This study, therefore, compared the performance of serological tests (VDRL, RPR, TPHA) against PCR in detecting syphilis among HIV patients attending the Comprehensive Care Clinic (CCC) at Nyeri County Referral Hospital (NCRH). This cross-sectional study consented and recruited a random sample of 177 HIV- positive patients, who were tested with three serological tests (RPR, VDRL, and TPHA) and one molecular method (PCR). A sociodemographic questionnaire was also administered. Key outcomes were syphilis prevalence and test accuracy. Participants had a mean (Standard deviation – SD) age of 48.3 years (SD 11. 07); 40. 1% were 51 or older. Females comprised 60.5%, 58.8% were married, 48.6% had been HIV positive for 1-8 years, 88.1% were on a lamivudine/ tenofovir disoproxil fumarate / dolutegravir / adefovir-diphosphate (3TC/TDF/DTG/AFV) ART regimen, and 7.9% experienced virological failure. Only 1.1% previously tested positive for syphilis. PCR detected a syphilis prevalence of 18.6%. Test performance metrics in this high-prevalence setting (18.6% by PCR) were: RPR—100% sensitivity, 76.4% specificity, and a kappa of 0. 0.546 (moderate agreement); VDRL—100% sensitivity, 55.6% specificity, and a kappa of 0. 0.317 (fair agreement); TPHA—100% sensitivity, 94.4% specificity, and a kappa of 0.864 (near- perfect agreement); RPR/VDRL combined with TPHA—100% sensitivity, 54.2% specificity, and a kappa of 0. 0.306 (fair agreement). Using TPHA as the gold standard, the sensitivity, specificity, and kappa for RPR and VDRL were 97%, 97%, 0.63%, and 97.6%, 58. 1%, 0.377, respectively. Combining RPR/VDRL yielded similar results. Switching the gold standard to TPHA slightly reduced the sensitivity of RPR and VDRL and increased their specificity. Considering weakly reactive TPHA samples as negative improved the sensitivity and specificity of TPHA to 100% against PCR. In summary, neither RPR nor VDRL can be used alone or as confirmatory tests. TPHA, with weakly reactive samples considered negative, had the highest agreement with PCR. Nonetheless, low- positive TPHA results should be interpreted with caution, as they may represent early seroconversion or false positives.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Syphilis is an infection characterized by chancres or lesions that is transmitted sexually and has been a major public health problem worldwide. According to the World Health Organization (WHO, 2025), in 2022, an estimated 8 million adults aged 15–49 years acquired new syphilis infections. Additionally, over twelve million people worldwide acquire new syphilis infections each year (Adler, 2024), with 90% of cases reported in countries with limited resources (Almeida, et al., 2022; Angel-Müller, et al., 2021; Lyngdoh, et al., 2024). In Africa, the number of new syphilis cases annually among people aged 15 to 49 years was 3.4 million (Rosset, et al., 2025). Syphilis is a key sexually transmitted infection due to its infectiousness, prevalence, ability to be transmitted, and its economic impact on infected individuals and health systems. It also facilitates the acquisition and spread of HIV through ulcerative lesions around the genital area. This has increased focus on the infection. Syphilis also has potential for vertical transmission (Fan, et al., 2021; Tsega, et al., 2022).

The risk of acquiring HIV through sexual intercourse increases three to five times more in people infected with syphilis compared to uninfected individuals (Simões, et al., 2022). Incidents of syphilis (primary/secondary) have been increasing annually worldwide due to the HIV pandemic (Ciepłucha, et al., 2025). For example, in Kenya, the prevalence of syphilis infection was 6.4% among HIV-infected individuals, while it was lower (1.6%) among HIV-negative people (Gilbert, et al., 2021).

The diagnosis of primary and secondary syphilis mainly relies on clinical presentation, i.e., signs and symptoms of the individual, followed by a treponemal test for confirmation. In individuals who are HIV positive and have decreased Cluster of Differentiation 4 (CD4+) lymphocyte counts, diagnosing syphilis can be challenging because they may present with abnormal symptoms like herpetiform ulcerations (Ahmed, et al., 2022). Serological screening tests for syphilis include cardiolipin-based tests such as the Venereal Disease Research Laboratory (VDRL) and rapid

plasma reagin (RPR). Treponemal-specific antibody tests include the *Treponema pallidum* hemagglutination assay (TPHA), *T. pallidum* particle agglutination assay (TPPA), fluorescent treponemal antibody test (FTA), and treponemal enzyme immunoassay (EIA), which detect IgG and/or IgM. The sensitivity of these serological tests for primary syphilis is: VDRL/RPR (70-80%), TPHA/TPPA (70-80%), treponemal EIA (85-90%), and FTA-ABS (85-90%) (Rathore, et al., 2024). Seroconversion typically occurs within 7 to 28 days, so a primary chancre is usually already present at clinical examination. At this stage, IgM antibodies can be detected via enzyme immunoassay. However, about 30% of primary syphilis cases may be missed with these serological screenings (Cao, et al., 2023).

The Polymerase Chain Reaction (PCR) for deoxyribonucleic acid (DNA) provides a promising alternative for detecting *T. pallidum* (Cao et al., 2023; Queiroz, et al., 2022; Wong, et al., 2021). These tests are highly specific for pathogenic treponemes, with a specificity of 95-97%. When analyzing specimens from the mouth and rectum, they can achieve a sensitivity of 91 to 95% and detect as few as one to sixty-five microorganisms (Cao et al., 2023; Queiroz et al., 2022). Using PCR techniques targeting unique regions of the DNA polymerase I gene of *T. pallidum* has proven highly effective for detecting treponemes in various clinical samples, including blood, CSF, amniotic fluid, and genital ulcer specimens. With a detection limit of 1 to 65 organisms, this advanced technology is both sensitive—with sensitivity exceeding 90%—and specific —with specificity over 95% (Queiroz et al., 2022).

There has been variation in the prevalence of syphilis/HIV co-infection based on geographical location, study population, and time period (Kassaw, et al., 2022). Importantly, early and accurate diagnosis and treatment of individuals testing positive for syphilis have been shown to effectively reduce stillbirths, neonatal deaths, congenital infections, and the prevalence of other Sexually transmitted infections (STIs), including HIV (Njau, et al., 2025). These prompt interventions are also estimated to be highly cost-effective, even in developing countries (NACC, 2024a; NACC, 2024b). Currently, however, there is no conclusive data on the epidemiology of syphilis or on the test performance for diagnosing this disease among HIV patients in Nyeri County. This study, therefore aims to evaluate the prevalence and correlates

of syphilis, as well as the performance (sensitivity, specificity, predictive values, and kappa statistics) of three serological tests (RPR, VDRL, and TPHA) and a molecular-based method for detecting syphilis among HIV patients attending the Comprehensive Care Centre (CCC) at Nyeri County Referral Hospital (NCRH).

1.2 Statement of the Problem

Syphilis and HIV infections are STIs that present major public health challenges in developing countries. Syphilis infection has an increased potential for morbidity and mortality due to its higher risk of HIV infection (Geremew & Geremew, 2021). Since they share the same transmission route and are primarily blood-borne illnesses, co-infection with syphilis and HIV remains a significant public health concern, especially in low-income settings. Specifically, syphilis causes genital lesions that ease HIV entry and colonization. It also triggers immune activation and promotes viral replication, speeding up HIV transmission (Ferreira, et al., 2023). The transmission rate for co-infection varies with the prevalence of both infections in the community or population studied, as well as with individual risky behaviors. Studies have shown variation in syphilis-HIV co-infection rates across different settings, including: 2.2% in northern Ethiopia (Ferreira, et al., 2023), 7.3% in southern Ethiopia (Anteneh, Taye, Seyoum, Abuhay, & Cherkose, 2024), 0.3% in Tanzania (Kamori, et al., 2021), and 0.05% reported in Nigeria (Ferreira, et al., 2023). The prevalence of HIV-syphilis co-infection in Kenya has been reported to range from 2.2% in Nairobi to 6.4% in a national survey (Gilbert, et al., 2021; Henninger, Bean, & Lin, 2022). Currently, there is no definitive data on the prevalence of syphilis among HIV-positive individuals in Nyeri County.

1.3 Justification

Laboratory investigation is a crucial aspect of the proper and timely management of syphilis. Syphilis tests can be categorized into PCR-DNA detection and serological tests (Satyaputra, et al., 2021). Immunological diagnosis of syphilis is divided into non-treponemal tests (NTT) and treponemal tests (TT) for detecting antibodies against *T. pallidum* (Alexander, et al., 2024). Nontreponemal assays, such as RPR and VDRL, detect antibodies to reagin, which is produced as the initial immune response to *T. pallidum* infection. These antibodies are nonspecific; when present at high levels in

serum or with elevated titers, they strongly suggest active infection. A rapid decline in antibody titers indicates successful treatment. In untreated individuals, low levels of antibodies are detectable, resulting in reduced but stable titers during late latent and tertiary stages of syphilis. Conversely, treponemal-specific tests, such as TPPA, detect *T. pallidum*-specific antibodies that often remain in circulation for many years, even after effective treatment. Additionally, most diagnostic tests used for screening are based on non-treponemal methods, with some combining both treponemal and non-treponemal principles depending on the individual's history of syphilis infection.

Performing a comparative analysis of VDRL, RPR, and TPHA tests against PCR for detecting *T. pallidum* in HIV patients is justified due to the high likelihood of serological discrepancies (false positives/negatives) caused by HIV-related immune dysfunction, the need for high-sensitivity diagnostics in high-risk populations, and the potential to establish more reliable, cost-effective diagnostic algorithms. HIV patients often produce non-specific antibodies that react in VDRL and RPR (nontreponemal) tests, leading to false-positive results (Negash, Wondmagegn, & Geremew, 2018). Conversely, advanced immunosuppression in HIV can impair antibody production, causing Treponemal tests (TPHA) or VDRL/RPR to miss active syphilis infections. PCR directly detects *T. pallidum* DNA, making it immune to the antibody-related inaccuracies found in VDRL, RPR, and TPHA tests in immunocompromised individuals (Zeng, et al., 2025).

Studies show variable test performance, with sensitivities ranging from 70% to 80% for VDRL/RPR, similar to TPHA/TPPA. In other contexts, PCR tests have demonstrated specificity of 95–97% and sensitivity of 91–95% (Cao, et al., 2023; Queiroz, et al., 2022). Serological tests (RPR/VDRL) are often used as routine, cost-effective screening tools. Validating their performance against PCR at a local level is crucial to determine if they are still fit for purpose in this setting. Studies suggest TPHA (a treponemal test) often shows higher concordance with PCR than RPR or VDRL. This study will help determine the most reliable test combination (e.g., RPR/TPHA) to recommend for local practice

Misdiagnosis causes delayed treatment, which helps the spread of syphilis and raises the risk of congenital syphilis. While TPHA remains positive indefinitely (making it ineffective for determining whether treatment worked), RPR/VDRL titers should decrease. Comparing these trends with PCR-based molecular detection offers a clearer view of active infection versus past exposure in HIV patients.

1.4 Research Questions

1. What is the prevalence of syphilis using three serological/immunological tests (RPR, VDRL, and TPHA) and a PCR-based method to detect syphilis among HIV patients attending CCC at NCRH?
2. What is the test performance of three serological/immunological tests (RPR, VDRL, and TPHA) and a PCR-based method to detect syphilis among HIV patients attending CCC at NCRH?
3. What are the comparisons in the utility of RPR, VDRL, TPHA, and a PCR-based method for diagnosing syphilis among HIV patients attending CCC at NCRH using a Bayesian method with a latent class analysis model?

1.5 Objectives

1.5.1 General Objective

Perform a comparative analysis of VDRL, RPR, and TPHA tests against PCR as the gold standard for detecting *Treponema pallidum* in HIV patients visiting Nyeri County Referral Hospital.

1.5.2 Specific Objectives

1. To determine the prevalence of syphilis using three serological/immunological tests (RPR, VDRL, and TPHA) and a PCR-based method to detect syphilis among HIV patients attending CCC at NCRH.
2. To assess the performance of three serological/immunological tests (RPR, VDRL, and TPHA) against PCR to detect syphilis among HIV patients attending CCC at NCRH.

3. To compare the utility of RPR, VDRL, TPHA, and PCR for diagnosing syphilis among HIV patients attending CCC at NCRH using a Bayesian method with a latent class analysis model.

CHAPTER TWO

LITERATURE REVIEW

2.1 The *Treponema pallidum*

Syphilis is a sexually transmitted infection caused by the pathogenic spirochete Treponema pallidum. The spirochete measures between 6 and 15 μm in length and about 0.2 μm in width. It has a doubling time of 30 to 50 hours. Treponema pallidum is very difficult to culture in vitro (Chaudhry, et al., 2023). Other T. pallidum subspecies cause non-venereal diseases transmitted through non-sexual contact. *Treponema pallidum endemicum* causes endemic syphilis, *Treponema carateum* causes pinta, and *Treponema pertenue* causes yaws. All treponematoses have similar DNA but differ in their geographic distribution and pathogenesis (Ramchandani, Cannon, & Marra, 2023).

2.2 Syphilis Transmission

Venereal syphilis transmission occurs when a person has unprotected sexual contact with someone who is infected. Exudate containing many microorganisms can cause infection. *T. pallidum* enters the body through mucous membranes directly or through broken skin, especially in areas with less keratin, such as the perigenital and perianal regions, compared to other skin areas (Chaudhry et al., 2023). For infection to occur, *T. pallidum* must attach to epithelial cells and extracellular matrix components. Studies show that fibronectin and laminin are the main substances involved in this process (Chen, et al., 2022). Once the organisms penetrate the epithelium, they multiply locally and begin to spread via the bloodstream and lymphatic system. Spirochetes move through the extracellular matrix and between cells with coordinated motions that help adherence, powered by front-to-back waves generated by flagella rotation, likely aided by the proteolytic activity of TP0751 (Nam, 2025). The infection quickly becomes systemic (Tang, et al., 2023). In secondary syphilitic lesions, many spirochetes are found within the epidermis and superficial dermis, enabling transmission through tiny skin lacerations caused by friction during sexual activity. Penetration of the blood-

brain barrier occurs in up to 40% of untreated cases, leading to permanent nervous system damage and other complications (Tang et al., 2023).

2.3 Epidemiology of Syphilis

WHO estimated that approximately 17.6 million people aged 15 to 49 worldwide had venereal syphilis in 2012. About 5.6 million new cases are expected each year (Gulersen, et al., 2023), as shown in Figure 2.1. The incidence and prevalence of syphilis vary greatly across regions, with Africa having the highest rates. More than 60% of new cases occur in economically struggling countries (Gulersen et al., 2023). Maternal syphilis burden is highest in Africa, making up over 60% of the global estimate (Gulersen et al., 2023).

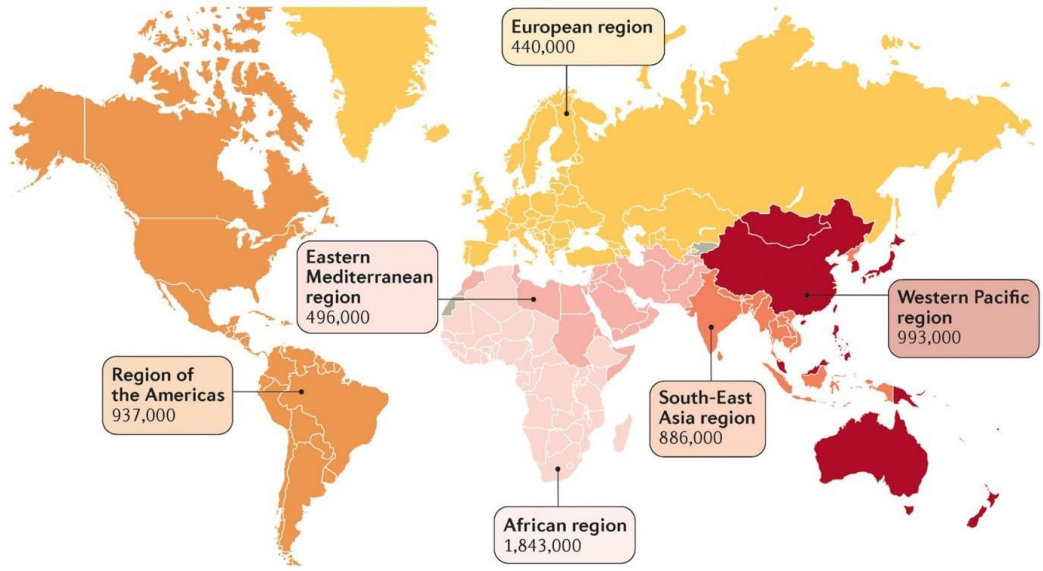

Figure 2.1: Different Geographical Regions Showing 2012 WHO Estimates Of Syphilis Incidence Cases

Source: (Gulersen et al., 2023)

2.3.1 Prevalence and Incidence of Syphilis

In developing countries, the spread of syphilis among heterosexuals has been decreasing in the general population but remains a significant issue in some high-risk groups, such as male sex workers (MSWs) and female sex workers (FSWs). A study of female sex workers in Johannesburg found that 21% of participants showed immunoglobulins indicating a treated or current infection, while 3% had active infections (Kularatne, et al., 2024). Another study in Sudan, conducted across 14 regions, reported a high seroprevalence—median 4.1%—with the highest rate of 8.9% in the eastern zone of the country (Dery, et al., 2024). In Kampala, Uganda, a large study involving over 1,000 FSWs revealed that 10% had active infections and 21% tested seropositive for syphilis (Ssenyonjo, et al., 2024). Studies in countries with developed economies, such as China, indicate that syphilis is rising among wealthy businessmen. In China, the prevalence among FSWs is about 5%, with 3% among male clients, while general population rates remain low (Cai, et al., 2024). The risk of infection varies among female sex workers based on location, with a prevalence of 10% among street-based FSWs and 2% among venue-based FSWs (Wong et al., 2021). In a nationwide survey in Kenya, syphilis prevalence was higher among HIV-infected individuals at 6.4%, compared to 1.6% among HIV-negative individuals (Gilbert et al., 2021).

On the contrary, countries with higher incomes showed a decline in the prevalence of syphilis among heterosexuals. However, there has been a notable re-emergence of syphilis infections among MSM (Vargas, et al., 2022). High-risk sexual behaviors influence syphilis transmission, which also facilitates the spread and replication of HIV. Since 1998, in the United States and Western Europe, syphilis cases among MSM have been on an upward trend (Do, et al., 2025). In 2015, case rates of primary and secondary syphilis among men who have sex with men in the United States were 309 per 100,000, which was 221 times higher than the rate in women at 1.4 per 100,000 and 106 times higher than the rate among homosexual men, at 2.9 per 100,000 (Chaudhry et al., 2023). In Canada, for instance, the incidence of syphilis was 300 times higher among MSM who tested positive for HIV compared with reported cases in the general male population (Aho, et al., 2022).

2.4 Burden of HIV in Kenya

Developing countries such as Kenya shoulder the highest impact, with a 4.8% HIV prevalence among adults aged 15 to 49 in 2018 and 1.7 million people in the population living with HIV. The epidemic is widespread and mainly concentrated in high-risk populations. Variations in estimated prevalence by county range from 0.20% to 23.5% HIV burden. Of 47 counties, nine counties account for 65% of new infections. According to the KAIS 2018 survey, heterosexual married couples represent 44% of new HIV infections, while among FSWs, it is 21%.

2.4.1 Burden of HIV in the County of Nyeri

According to NASCOP 2012, HIV prevalence in Nyeri County was estimated at 3.8%. The Nyeri County Integrated Development Plan (2013-2017) indicates that HIV/AIDS was among the leading causes of death in the county's population, with 18,662 people living with HIV and an estimated 1,307 new HIV infections reported annually (Nyeri County, 2021). HIV testing services (HTS) are a key intervention in the prevention and management of HIV/AIDS. However, approximately 30% of the Nyeri County population does not know their HIV status.

2.5 Syphilis - HIV Infection

Syphilis – HIV co-infection has been a major public health concern mainly due to common transmission routes and their synergistic effect in weakening the immune system. The presentation of syphilis infection is the same in both HIV-negative and HIV-positive individuals. In primary syphilis, up to 70% of HIV-infected individuals present with more than one chancre, and the lesions tend to be larger and deeper. Approximately a quarter of HIV-infected persons experience both primary and secondary stage lesions of syphilis at the time of diagnosis (Ahmed et al., 2022). More specifically, syphilis facilitates HIV entry and shedding, with genital ulcers playing a key role. Additionally, *T. pallidum* induces and enhances immune activation, promoting viral replication, which ultimately increases HIV transmissibility. The

median point prevalence of syphilis among HIV–infected individuals was found to be 9.5% after a systematic review of literature from various regions worldwide (Mahmud, et al., 2023).

In syphilis infection, HIV viral load increases progressively while CD4+ lymphocytes decrease. Treatment of the infection corrects this situation. A transient rise in viral load significantly contributes to HIV transmissibility among individuals with syphilitic infection (Eriksen, et al., 2021). HIV may cause a polyclonal expansion of immunoglobulin (Ig), which could lead to diagnostic confusion when considering syphilis in an HIV-infected patient (Dessie, et al., 2021). Syphilis may be more aggressive in patients with HIV; the latency period may be shorter; and benzathine penicillin G may be less effective in co-infected patients.

Syphilis incidence is rising, especially among individuals with HIV. Figures published by Public Health England show that the number of reported syphilis cases has reached the highest level in England since 1949 (UK Health Security Agency, 2025). Among MSMs in Australia, syphilis cases have been increasing since 2000 (Aung, et al., 2021). At the same time, the number of HIV and syphilis co-infections has been on the rise (Yuindartanto, et al., 2022). Between 2010 and 2015 in Australia, syphilis incidence increased by 38% in men who are HIV-positive and by 42% in men who are HIV-negative (Khaw, et al., 2018).

2.6 Syphilis Diagnosis

Clinical diagnosis of syphilis is challenging because its manifestations are usually varied and often subtle. This can lead to missed or misdiagnosed cases. Painless lesions in primary syphilis, especially in hidden sites like the rectum and cervix, can go unnoticed. Additionally, a generalized rash common in secondary syphilis is often mistaken for other conditions such as allergies (Ramchandani et al., 2023). Therefore, diagnosing syphilis typically relies on a combination of a suggestive clinical history and laboratory tests (Tang et al., 2023).

Ensuring the reliability and diagnostic accuracy of syphilis testing is crucial, especially in highly specialized laboratories where most testing occurs (CDC, 2021). Essential

quality assurance protocols for syphilis include training scientists in immunological techniques, test evaluation, internal quality control systems, and the use of commercially available test kits. Additionally, inter-assay standardization should follow a specific schedule (CDC, 2021). Proficiency testing and adequate training must be provided, along with corrective actions, to ensure that technologists and scientists are well skilled and that high-quality tests are maintained in clinical and research laboratories (Maseko, Valashiya, & Kularatne, 2022). The lack of laboratory capacity to accurately diagnose syphilis has made it very difficult to control and eliminate congenital syphilis. Nonetheless, the development of low-cost rapid diagnostic tests that can be used at the point of care has significantly improved the timeliness of diagnosis and antenatal screening, even in resource-limited and remote areas (Ramchandani et al., 2023).

2.6.1 Syphilis Definitive Diagnosis by Direct Detection

In laboratory diagnosis of this infection, the preferred methods depend on the stage of the disease and how it presents clinically (Ramchandani et al., 2023). Condylomata, which are genital lesions of secondary syphilis in individuals with syphilis ulcers and in congenital syphilis lesions, can be directly detected with methods used to make a definitive diagnosis. These methods include PCR, fluorescent antibody staining, darkfield microscopy, and immunohistochemistry. Since these methods require exudate, a swab, or a biopsy from a fresh lesion, they are often insensitive and may require skilled personnel to handle, except for PCR, which is molecular-based (Ramchandani et al., 2023) (Table 1.1).

Since 1920, microscopy has been very common in the direct detection and diagnosis of syphilis infection, but currently its use has drastically decreased. According to a 2014 Caribbean and national reference survey in Latin America and large clinical laboratories, only two of 69 participating facilities, of which half were reference laboratories, still performed direct fluorescent antibody staining (DFA-TP) or darkfield microscopy for *T. pallidum* (Orbe-Orihuela, et al., 2022). Although European guidelines recommend against DFA-TP testing in clinical settings, reagents and materials should still be available (Parczewski, et al., 2025). Meanwhile, the use of

PCR-DNA techniques is on the rise; so far, no internationally approved or commercially available tests for *Treponema pallidum* exist (Ramchandani et al., 2023). There is advancing technology in PCR testing for species and specific subspecies of *T. pallidum*, primarily available in research laboratories, and it is projected that these will become available in the future (Shukla, Pereira, & Pillay, 2022). In diagnosing neurosyphilis, research shows that PCR technology will be very helpful, as it can detect DNA of *T. pallidum* in the cerebrospinal fluid (CSF*)* of individuals infected with HIV (Yang, et al., 2022).

Table 2.1: Different Methods, Samples Used, Advantages, and Disadvantages of *Treponema pallidum* Direct Detection

Method	Sample	Advantages	Disadvantages
Darkfield microscopy	Fresh (<20 minutes) sample from chancres or erosive cutaneous lesions of primary, secondary or congenital syphilis	Diagnosis can be made during the clinical visit by visualization of characteristic motile organisms	1. Insensitive: false negatives in ≤30% of samples 2. Should not be used in oral or rectal specimens due to commensal treponemes 3. Requires specialist equipment 4. Labor-intensive expertise is needed 5. Subjective 6. Rarely used anymore
Direct fluorescent antibody staining for T. pallidum	Sample from chancres or erosive cutaneous lesions of primary, secondary or congenital syphilis	1. Can be used for oral lesions 2. Specific detection of T. pallidum 3. Samples can be saved or shipped for verification	1. Insensitive: negative test does not rule out syphilis 2. Requires specialized equipment and stains that are no longer available 3. Labor-intensive expertise is needed 4. Subjective
Immunohistochemistry	Skin, mucosal or tissue lesions performed on fixed paraffin embedded tissues using commercially available treponemal antibody reagents	1. Samples can be saved or shipped for verification 2. Can use tissue samples from placenta and umbilical cord 3. Useful for unusual forms of syphilis if tissue biopsies are obtained when syphilis is not initially suspected	1. Insensitive: negative test does not rule out syphilis 2. Requires specialized equipment and stains 3. Labour-intensive expertise is needed 4. Subjective
PCR	Skin, mucosal or tissue lesions; not recommended for blood or CSF as few organisms present	Can be used to detect syphilis in oral or rectal lesions either stored or frozen	1. No internationally approved or commercially available test 2. Very good sensitivity and specificity for specimens from genital ulcers 3. Negative test does not rule out syphilis 4. Requires specialized equipment and expertise

2.6.2 Serological Methods for the Detection of Syphilis

To screen individuals with any symptoms of syphilis infection, serodiagnostic tests are commonly used. Additionally, in patients exhibiting apparent signs and symptoms of syphilis, these serological tests are utilized for definitive diagnosis (Ramchandani et al., 2023). Non-treponemal tests (NTTs) and treponemal tests (TTs) are categories of serological tests that detect syphilis antibodies in various specimens.

2.6.2.1 Non-Treponemal Tests (Ntts) for the Detection of Syphilis

The non-treponemal tests are qualitative assays that detect IgG and IgM antibodies against T. pallidum. These antibodies are produced in response to lipoidal material released from damaged, dying host cells and materials shed by the bacteria. Commonly used NTTs, such as the Venereal Disease Research Laboratory (VDRL) test, Toluidine Red Unheated Serum Test (TRUST), and Rapid Plasma Reagin (RPR) test, operate on the principle of precipitation to produce a reactive result. These tests detect immunoglobulin/antibodies to cardiolipin, lecithin, and cholesterol (Ramchandani et al., 2023). They are used in diagnosing active syphilis infection. Typically, syphilis has an incubation period of ten to fifteen days after the primary lesion appears, and NTTs will usually turn positive after seroconversion. This can result in missed cases (25–30%) of primary syphilis (Purwoko, et al., 2021). NTTs are generally simple and inexpensive, but they must be performed manually on plasma or serum and rely mainly on subjective interpretation. They also require skilled laboratory technologists, specialized equipment, and reagents. These factors have prevented them from meeting the ASSURED criteria for those in need, especially at the Point of Care. The acronym ASSURED stands for Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable (Ramchandani et al., 2023).

Regardless of the NTT used, two to five percent of the population can show false-positive results, although this probability heavily depends on the population being studied, making estimates difficult (Kaminiów, Kiołbasa, & Pastuszczak, 2024). In chronic conditions such as Hepatitis C, leprosy, and SLE, low-titer reactions may last longer or be short-lived, depending on factors such as febrile illness, immunization, or pregnancy (Ramchandani et al., 2023). Serum with very high titers can produce false-

negative results, as seen in secondary syphilis. This phenomenon is known as the prozone effect. Such sera must be diluted before testing to ensure optimal antigen-antibody reactions and to correct the prozone effect (Ramchandani et al., 2023).

2.6.2.2 Treponemal Tests (TTs) for the Detection of Syphilis

Treponemal tests detect the presence of antibodies produced in response to proteins from *T. pallidum* and are highly sensitive and specific. These TTs cannot distinguish between an active infection and a previous one because these antibodies remain in the blood for life. Therefore, they are not used to assess treatment efficacy in patients infected with syphilis (Ramchandani et al., 2023). After a positive NTT result, TTs are useful for confirming the result.

Five weeks after infection, a primary chancre appears. This is followed by a positive Treponemal test (TT) 6–14 days later. Therefore, this makes it very useful for diagnosing early syphilis, which can be easily missed by NTTs. These Treponemal tests include: *T. pallidum* hemagglutination (TPHA) assays, *T. pallidum* passive particle agglutination (TPPA), fluorescent treponemal antibody absorbed (FTA-ABS) test, and micro-hemagglutination assay for antibodies to *T. pallidum* (MHA-TP) (Table 2). To perform these tests, trained personnel with good skills are required due to their technical complexity. They are more expensive than NTTs and involve the use of specialized equipment. This makes TTs less widely used in most clinical laboratories in developing countries, thereby limiting their use as confirmatory tests for positive NTTs (Ramchandani et al., 2023).

Chemiluminescence and recombinant *T. pallidum* antigens (TTs) are available on the market for large-scale screening. These assay methods are useful because they are fully automated or semi-automated, and results are read using a spectrophotometer, making the interpretation non-subjective (Lyngdoh et al., 2024). In developed economies, most health facilities rely on high-throughput syphilis testing and screening and have adopted ‘reverse’ algorithms. This algorithm screens with an automated treponemal CIA/EIA and then confirms results with NTTs rather than the usual approach (WHO, 2023).

2.6.2.3 Rapid Test Point of Care tests for the Detection of Syphilis

The point-of-care (POC) rapid tests are very useful for on-site screening and treatment. They use the latest technology and are especially important in settings with limited laboratory space. These diagnostic kits for syphilis use whole capillary blood from a finger prick, serum, or plasma. They are typically rapid immunochromatographic immunoassays for qualitative detection of IgG and IgM antibodies to *T. pallidum*. They require no equipment, specialized storage, or training, and provide results in 20 minutes (Ramchandani et al., 2023) (Table 2.2). Rapid tests for diagnosing syphilis have been evaluated in intra-assay and inter-assay conditions, demonstrating they meet the assured criteria in both clinical and community settings (Ramchandani, et al., 2023; Wall, et al., 2025). Limitations of rapid diagnostic tests include their inability to distinguish between recent infections and previously treated ones, which can result in unnecessary prolonged treatment. Patients with a reactive POC TT should be confirmed with a NTT to guide treatment decisions; however, this is often not feasible in settings lacking sufficient laboratory capacity. Rapid test strips are the best option when there are delays in diagnosis, as such delays could negatively impact monitoring the individual's health, especially in pregnant women (Liu, et al., 2022).

The POC single devices have been developed that can detect treponemal and non-treponemal antibodies simultaneously. Additionally, syphilis/HIV dual immunochromatographic rapid tests are now available to screen for treponemal antibodies and HIV using a single lateral flow principle. The importance of these tests is significant in the global effort to eliminate HIV and syphilis, especially in the context of MTCT in settings with limited laboratory resources (Pham, et al., 2022).

Table 2.2: Different *Treponema pallidum* Detection Methods

Method	Sample	Advantages	Disadvantages
Treponemal tests (NTTs)			
Venereal Disease Research Laboratory (VDRL) slide test	Serum, plasma or CSF	1. Can be used to monitor treatment efficacy 2. Only test suitable for diagnosis of neurosyphilis using CSF 3. Inexpensive and relatively simple 4. Results available in <15 minutes 5. Sensitivity 71–100% depending on stage 6. Specificity 98%	1. False positives due to cross-reactivity 2. Must be done manually 3. Subjective 4. Requires microscope 5. Fresh antigen required daily 6. Cannot be used on whole blood; requires centrifuge
Rapid Plasma Reagin (RPR) / TRUST	Serum or plasma	1. Monitor treatment efficacy 2. Inexpensive 3. No microscope required 4. Stable antigen 5. Results in <15 minutes 6. Sensitivity 73–100% 7. Specificity 98%	1. False positives in some conditions 2. False negatives early/prozone 3. Manual 4. Subjective 5. Cards cannot be reused 6. Cannot use whole blood
Treatment tests (TTs)			
T. pallidum Particle Agglutination (TPPA)	Serum or plasma	1. Widely available 2. Sensitivity 82–100% 3. Specificity 99%	Manual and subjective
TPHA / MHA-TP	Serum or plasma	1. Less widely available 2. Sensitivity 82–100% 3. Specificity 99%	Manual and subjective
Treponemal Enzyme Immunoassay (EIA)	Serum	1. Can be automated 2. Suitable for screening 3. Sensitivity 82–100% 4. Specificity 99%	Expensive, specialized equipment
Rapid Test			
Treponemal	Whole blood, plasma or serum	1. Presumptive diagnosis at clinic 2. Inexpensive 3. Sensitivity ~84–86%	Cannot distinguish past vs current infection
Treponemal/non-treponemal test	Whole blood, plasma or serum	1. Rapid and easy 2. Distinguishes new vs treated 3. Performance ~88%	More costly per test
Dual syphilis/HIV tests		1. Detects both infections 2. HIV agreement ~98% 3. Syphilis agreement ~85%	Lower sensitivity for syphilis vs HIV

2.7 Management and Treatment of Syphilis

Effective management and treatment of syphilis are crucial to prevent complications, transmission, and adverse pregnancy outcomes. The main treatment for syphilis remains antibiotic therapy, with benzathine penicillin G regarded as the gold standard for all disease stages. According to the World Health Organization, a single intramuscular dose of 2.4 million units of benzathine penicillin G is recommended for early syphilis, including primary, secondary, and early latent stages. For late latent syphilis or syphilis of unknown duration, three doses of benzathine penicillin G given weekly are advised (WHO, 2023).

For individuals allergic to penicillin, alternative antibiotics like doxycycline or tetracycline may be used in non-pregnant adults. However, penicillin remains the only recommended treatment for pregnant women, as it effectively prevents congenital syphilis. In cases where pregnant women are allergic to penicillin, desensitization followed by penicillin treatment is advised (Centers for Disease Control and Prevention, 2024).

Managing syphilis also includes screening, partner notification, and follow-up testing. Sexual partners of infected individuals should be assessed and treated quickly to prevent reinfection and further spread of the disease. Regular follow-up serological testing, often using non-treponemal tests like the Rapid Plasma Reagin Test or Venereal Disease Research Laboratory Test, is recommended to track treatment effectiveness and confirm decreasing antibody levels (Centers for Disease Control and Prevention, 2024).

Additionally, public health strategies such as routine screening for high-risk groups, antenatal screening during pregnancy, and community awareness programs are essential for controlling syphilis. Early diagnosis and prompt treatment greatly decrease complications like neurosyphilis, cardiovascular syphilis, and adverse pregnancy outcomes (WHO, 2023). Overall, effective management of syphilis depends on accurate diagnosis, appropriate antibiotic treatment, partner management, and ongoing surveillance to reduce transmission and improve sexual and reproductive health outcomes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The Nyeri County Referral Hospital (NCRH) is located in Nyeri South, Central, Nyeri Town. The hospital offers services such as curative outpatient services, therapy, cesarean section, obstetrics and gynecology services, prevention of mother-to-child transmission of HIV, inpatient services, family planning, growth monitoring and promotion, HIV voluntary counseling and testing (VCT) services, radiological services, antenatal care, ART management, vaccinations, integrated management of childhood illnesses, tuberculosis treatment, among other services. The NCRH-CCC began operations in 2004, and during this study in 2018, the CCC had 7,624 registered patients, of whom 2,373 were actively receiving ART management (Nyeri County, 2021). Outpatients and inpatients are usually tested by an HIV Testing Services (HTS) counselor and referred to the CCC. The clinic uses the International Quality Care-Kenya Health Management Information System (IQC–Ke–HMIS) to log and manage this data, and any transfers to other facilities are reported to the Medical Superintendent, Sub-County, or directly to the District Health Information System (DHIS). (Nyeri County, 2021). The CCC includes one nutrition room, four clinical rooms, one family planning room, one records office, and one psychosocial room. The pharmacy and laboratory are located outside the main clinic building.

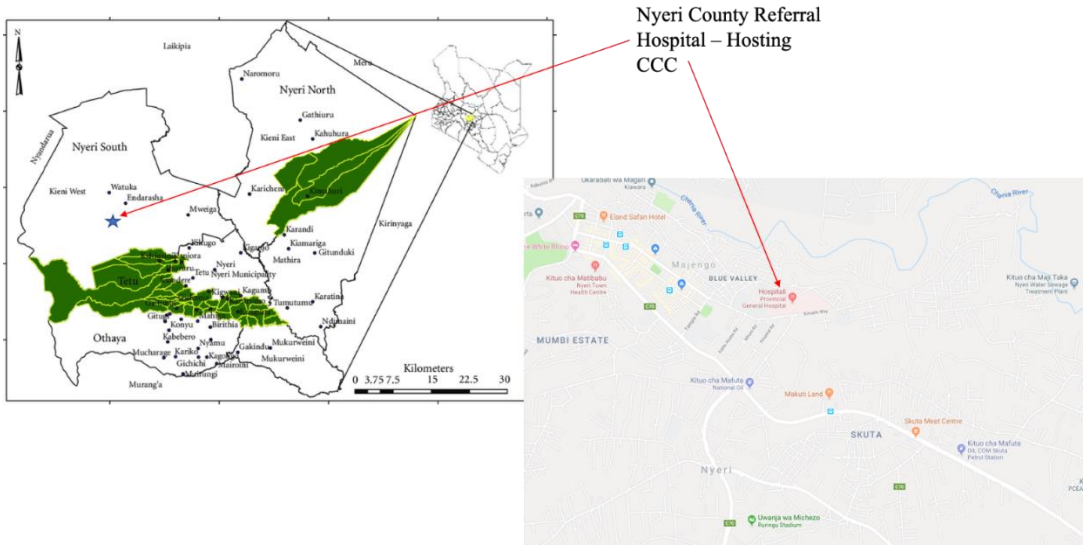

Figure 3.1: Map Showing the Location of the Study Site

Sources: (Google Maps)

3.2 Study Setting

The study setting included patients who regularly attended the CCC clinics for ARV treatment and care.

3.3 Study Population

This study recruited HIV-infected patients who were receiving antiretroviral (ARV) therapy.

3.3.1 Inclusion Criteria

1. HIV-infected adults (aged 18 and above), both males and females, attending the Nyeri CCC HIV treatment program.
2. Those willing to provide written informed consent voluntarily.
3. Those on Anti-retroviral treatment.
4. Patients with HIV viral load results available.

3.3.2 Exclusion Criteria

Pregnant women

3.3.3 Recruitment of Study Participants

Potential participants gathered at the CCC waiting bay, where the purpose and details of the study were explained. They were then directed to a clinical room, where each was given a consent form in their preferred language, either English or Kiswahili. For those who did not understand either language, an interpreter explained the contents of the consent form to ensure full understanding. Those who agreed to participate provided written informed consent by signing the form. They were then given a questionnaire to complete, after which approximately 4 mL of blood was drawn from each participant.

3.4 Study Design

A cross-sectional study was conducted among patients attending the HIV treatment program at the CCC in Nyeri County Referral Hospital.

3.5 Sampling

3.5.1 Sample Size Determination

Sample size calculation was based on Borderer's formula, where the required absolute precision levels for sensitivity and specificity were determined.

Sample size (n) based on specificity $= Z^2 1-\alpha/2 \text{ X Sp X } (1-\text{Sp}) / L^2 \text{ X } (1\text{-P})$

where

n = required sample size,
S_P = expected specificity,
α = size of the critical region ($1 - \alpha$ is the confidence level),
$z_{1-\alpha/2}$ = standard normal deviate corresponding to the specified size of the critical region (α), and

L = the exact margin of error (half-width of the confidence interval) for sensitivity or specificity.

P = Disease prevalence

Setting the following parameters:

S_P = 0.95

$z_{1-\alpha/2}$ = z = 1.96 for 95% confidence level (two-tailed),

L = 0.05

P = 0.064, the prevalence of syphilis among HIV patients in Kenya (Gilbert et al., 2021)

(Substitution yields)

n = {1.96X1.96X 0.9X 0.1)} / (0.05 X 0.05X0.936)

n = 148.

An extra 30% of participants were included to account for potentially hemolyzed or lost samples. For the prevalence determination of syphilis and the evaluation of the test performance of RPR, VDRL, TPHA, and PCR, a total of 193 HIV patients meeting the inclusion criteria consented and enrolled in this study.

3.5.2 Sampling Method

This study recruited patients infected with HIV at the CCC whose viral load and CD4 count are available at 12 months since they started using ARV for treatment and who meet the recruitment criteria. Then, a consecutive sampling technique was used to recruit every subject meeting the criteria until the sample size of 193 was reached.

3.6 Data, Sample Collection, and Testing

From each of the 193 participants enrolled in this study, the first part of data collection involved assessing them and conducting interviews during blood draws. The second part involved reviewing participant records to determine baseline and current laboratory outcomes.

3.6.1 Participants Interviews

The study subjects participated in a brief face-to-face interview using structured questionnaires to gather information such as demographic characteristics, healthcare access, and other areas (Appendix 2).

3.6.2 Sample Collection, Transportation, and Storage

From each enrolled participant, approximately 4 mL of blood was drawn from the antecubital vein using a 21-gauge hypodermic needle and a 5-cc syringe into an Ethylenediaminetetraacetic acid (EDTA) tube. The median cubital vein was preferred for venipuncture because it is large, visible, well-anchored, and less prone to bruising. After identifying a suitable vein that was visible and straight (ideally visible without applying the tourniquet), a tourniquet was applied about 4–5 finger widths above the puncture site. The area was disinfected with a 70% alcohol swab for 30 seconds, then allowed to air-dry completely. Venipuncture was then performed by anchoring the vein with a thumb placed below the puncture site while the participant made a fist to make the veins more prominent. The needle was inserted smoothly at an angle of approximately 30 degrees or less. Once the required blood volume was collected, the tourniquet was released before withdrawing the needle. Gentle pressure was applied to the puncture site using a dry cotton ball, which the participant was instructed to hold in place with the arm extended and raised. The collected blood was immediately transferred into an EDTA tube and gently mixed. It was then centrifuged using a Hettich centrifuge (Model EBA 280, Serial No. 71451). Plasma was separated and transferred into microvials, which were stored in a freezer (Make FCRQ 29900) at –80°C before being shipped to the laboratory for further testing.

3.6.3 Storage and Transport of Samples to KEMRI

After separating plasma from whole blood, the plasma was stored at -80 degrees in a deep freezer at Nyeri County Referral Laboratory until the desired sample size was reached. The principal investigator then packed the samples in a battery-powered cool box with ice packs and transported them to KEMRI (KNH). After analysis, the samples were preserved for 30 days before disposal.

3.7 Laboratory Procedures

3.7.1 RPR Test

The RPR was performed using the ASI RPR card test for syphilis (Arlington Scientific, Inc., Springville, UT, USA) following the manufacturer's instructions. Briefly, a stirrer pipette was used to drop a single free-falling drop, approximately 0.05 ml, of each serum or plasma sample onto a separate circle on the test card. For each sample, a new stirrer pipette was used. This process was repeated by adding one free-falling drop of reactive, weak reactive, or nonreactive control from the provided dropper vials. Using the flat end of the stirrer pipette, the sample was spread over the entire area of the test circle. The carbon antigen suspension was mixed thoroughly, and one free-falling drop of the antigen suspension was dispensed onto each sample while holding the bottle vertically. The card was then placed on an automatic rotator and covered to maintain humidity. It was rotated at 100 ± 5 rpm for 8 minutes. After rotation, a brief manual rotation and tilting of the card at least 3–4 times were performed to help distinguish minimally reactive from nonreactive results. The results were read immediately in the macroscopic “wet” state under a high-intensity light source.

3.7.1.1 .Interpretation of RPR Results

The presence of aggregates in the center or edges of the test circle indicated a reactive result, which could range from slight to severe and intense. A nonreactive result appeared as a smooth gray area within the test circle or as a cluster of non-aggregate carbon particles in the middle, showing no clumping characteristic of a reactive result. Results for the ASI RPR Card Test were reported only as reactive or nonreactive, regardless of the reactivity level. Minimal to moderate reactivity was classified as reactive. Slightly granular or “rough” reactions were retested using an alternative method. See “Limitations of the Procedure” section.

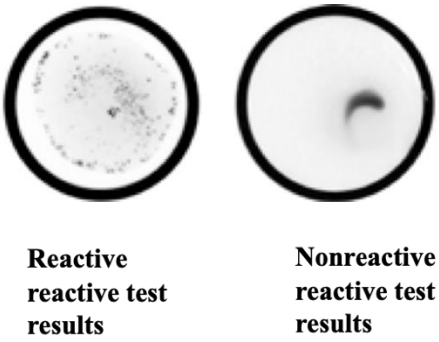

Figure 3.2: RPR Test Interpretation

Source: (ELITech Group available at https://www.elitechgroup.com/product/tpha)

3.7.2 VDRL Test

The VDRL was performed using the Syphilis Ab Rapid Test strip (Laborex IVD Italiano SRL-Italy) according to the manufacturer's instructions. This immunochromatographic assay was used to qualitatively detect antibodies in serum. All specimens and strips were brought to a temperature of 24°C. The strip was removed from the foil pouch and used within an hour. It was placed on a clean, level surface. Using a dropper, two drops (60 µL) of plasma were dispensed onto the sample pad on the strip. The reaction was allowed to proceed for 15 minutes, and the results were read.

3.7.2.1 Interpretation of VDRL Results

Positive results are shown by two distinct lines, one in the control (C) region and another in the test (T) region.

Negative results are indicated by one line appearing in the control (C) region, and no visible red or pink line appears in the test (T) region.

3.7.3 TPHA Test

The TPHA was performed using the ASI TPHA test kit (Arlington Scientific, Inc., Springville, UT, USA) according to the manufacturer's instructions. Briefly, the qualitative screening was conducted. All reagents and samples were brought to room temperature. About 190 µL of diluent was added to well 1. Then, 10 µL of serum was dispensed into well 1 and mixed. 150 µL was discarded from well 1, and 25 µL of the mixture was transferred from well 1 to well 2. Approximately 75 µL of re-suspended Test Cells (CT) was added to well 1, and 75 µL of re-suspended Control Cells (CC) was added to well 2. The plate was gently tapped to mix the contents thoroughly, then covered and left to stand for 45 to 60 minutes at room temperature without moving.

3.7.3.1 TPHA Test Interpretation

The reading was performed at the end of incubation. The images below show reactions conducted in U-well microtiter plates TPHA-0004. Different plates may produce different images.Results are expressed based on the agglutination intensity (- to 4+). All samples showing a positive or weakly positive result (+/- to 4) were tested with a quantitative method.

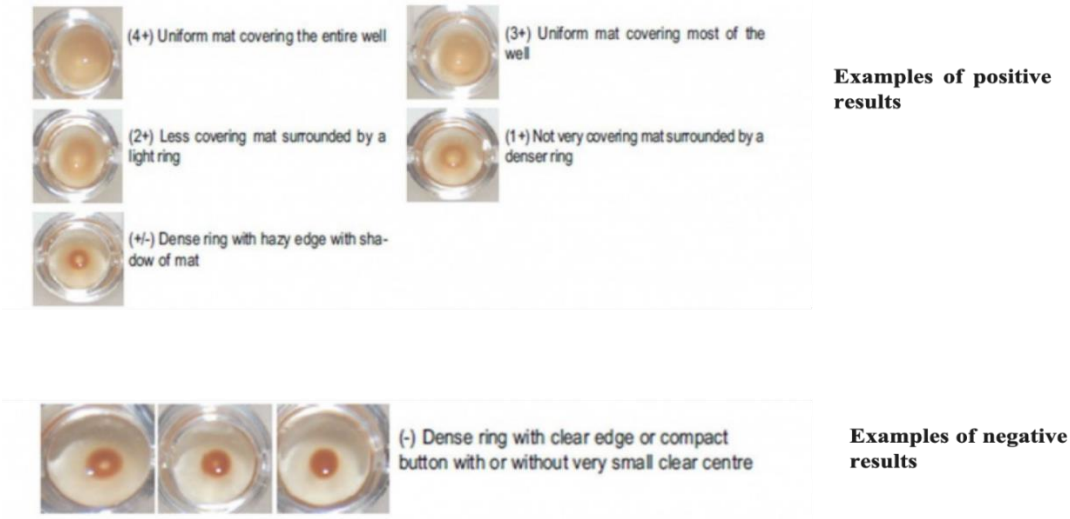

Figure 3.3: TPHA Test Interpretation

Source: (ELITechGroup available at https://www.elitechgroup.com/product/tpha)

3.8 Polymerase Chain Reaction (PCR) Analysis

This was done at CMR KEMRI

3.8.1 DNA Extraction

The *T. pallidum* DNA was extracted from 1 mL of plasma containing EDTA anticoagulant using the QIAamp DNA Mini Blood Kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions. Briefly, all tubes were appropriately labeled. About 20 µL of Qiagen Proteinase K was pipetted into the bottom of a 1.5 mL microcentrifuge tube, and 200 µL of the sample was added. Then, 200 µL of Buffer AL was added to the tube and vortexed for 15 seconds. The mixture was incubated in a 56°C water bath for 10 minutes and briefly centrifuged to remove any droplets from the side. To adsorb DNA to the QIAamp column, 200 µL of absolute ethanol was added and vortexed for 15 seconds. The column was briefly centrifuged, and the entire mixture was added to a QIAamp spin column and centrifuged at 13,200 rpm for 1 minute at room temperature. The eluted DNA was either used immediately or stored at -20°C until ready for use.

3.8.2 Polymerase Chain Reaction Detection

The PCR primers and thermal cycling conditions used to detect T. pallidum were as described by Palmer et al., (2003). This method amplified a 260bp region of the 47 kDa integral membrane lipoprotein gene. Briefly, in a 1.5 mL tube, the following were added: 0.5 mM of primers KO3A (5' GAAGTTTGTCCCAGTTGCGGTT) and KO4 (5' CAGAGCCATCAGCCCTTTTCA), 1 X PCR buffer (50 mM KCl, 20 mM TRIS-HCl pH 8.4), 1.5 mM MgCl2, 0.2 mM dNTPs, and 1.25 units of Platinum Taq DNA polymerase (Invitrogen Life Technologies, Paisley, UK). Each PCR run included a negative control sample (25 µL distilled water), a positive control sample (distilled water containing 100 pg T. pallidum DNA), and an inhibition control for each sample (25 µL sample spiked with 100 pg T. pallidum DNA). Using the Perkin Elmer Applied Biosystems GeneAmp PCR System 9700, the thermal cycling conditions were set as follows: 95°C for 2 minutes, then 35 cycles of 95°C for 20 seconds, 62°C for 20

seconds, and 72°C for 20 seconds. Amplification was performed using a 2% agarose gel electrophoresis and visualized under UV.

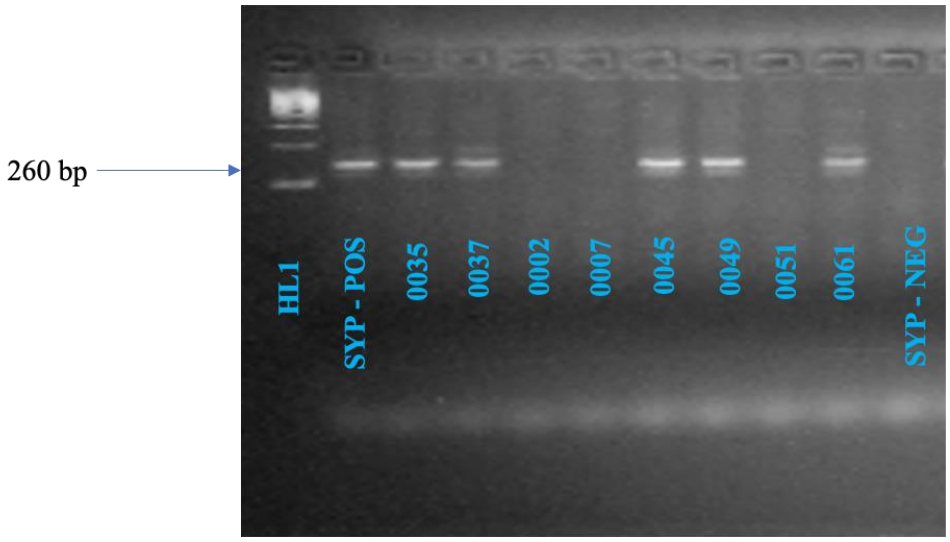

Figure 3.4: 2% Agarose Gel Illustrating Detection of the *T. pallidum* tp47 Gene through PCR Amplification with KO3A and KO4 Primers

Samples 0035, 0037, 0045, 0049, and 0061 amplified at 260bp and were deemed positive based on the testing algorithm.

3.9 Data Management

All patient information and biological samples were assigned a unique identification number (UID). All data entered into the study databases was de-identified and linked only to a UID in password-protected files. A double-entry system for the data was maintained. All paper research records were stored in a password-protected, locked filing cabinet in a restricted-access room at the research station. Data entry, cleaning, and validation were performed to ensure a clean dataset.

3.10 Statistical Analysis

Descriptive statistics, including frequency (%), mean, and standard deviation, were used to describe the patients' characteristics and laboratory parameters. This approach is important for describing the population and applicable to the objective. For objective two, test performance (sensitivity, specificity, predictive values, and kappa statistics)

was analyzed as follows: Sensitivity was calculated using the formula; Sensitivity = number of true positives (TP) / (TP + false negatives (FN)). Specificity was calculated as; Specificity = number of true negatives (TN) / (TN + false positives (FP)). The positive predictive value (PPV), which is the proportion of patients with positive test results who were correctly diagnosed, was determined as; PPV = TP / (TP + FP). The negative predictive value (NPV), defined as the proportion of patients with negative test results who are correctly diagnosed, was calculated as; NPV = TN / (TN + FN). The 95% confidence interval for test performance was also calculated. Cohen's kappa coefficient (k) was used to assess the agreement between tests against a reference standard. For objective three, test performance metrics (sensitivity, specificity, NPV, and PPV) were calculated using R software version 3.2.2 with the Bayesian method and Latent Class Analysis model. This software can determine sensitivity, specificity, negative predictive value, and positive predictive value in the absence of a gold or reference standard test (Salgadu et al., 2025). All statistics were reported with their 95% confidence intervals. Statistical analyses were performed using STATA version 13 (StataCorp LP, College Station, TX, USA) with a significance level set at $P \leq 0.05$.

3.11 Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guideline on Good Clinical Practice (ICH-GCP). The protocol and informed consent form were reviewed and approved by the UON/KNH Ethical Review Committee before any protocol-related procedures were carried out (Appendix 1). Additional approval was obtained from the JKUAT Ethical Review Committee and Nyeri County Referral Hospital. The investigator consistently informed the ERC of the study's progress as required. Written informed consent was obtained from each participant before any protocol-specified procedures were performed. To ensure confidentiality, initials and coded numbers were used to identify participants' source documents and study reports. All study records were securely stored within the CCC. Participant information was not obtained or disclosed without written permission from the participant or their legally authorized representative, except as necessary for monitoring the study. Participation was entirely voluntary.

3.12 Study Limitations

The study limitations identified include: First, it takes between seven and twenty-eight days to produce detectable antibody levels. Therefore, serology tests like PRP, TPHA, and VDRL may fail to detect 30% of primary syphilis infections (Cao et al., 2023). Second, although PCR can detect bacteria directly, its costs and the expertise needed limit its routine use in many clinical settings.

CHAPTER FOUR:

RESULTS

4.1 Baseline Characteristics of the Study Population

4.1.1 Social-Demographic Characteristics

Using the Borderer's formula for sample size calculation, a total of 177 patients out of the 194 (91.2% response rate) with all the required data were analyzed. The mean age of the study patients was 48.3 years (SD 11.07), with the majority being female and aged 51 years and above. This was followed by 35% of patients aged 41 to 50 years, 19.2% aged 31 to 40 years, and the smallest group, 5.7%, aged 20 to 30 years (Figure 4.1).

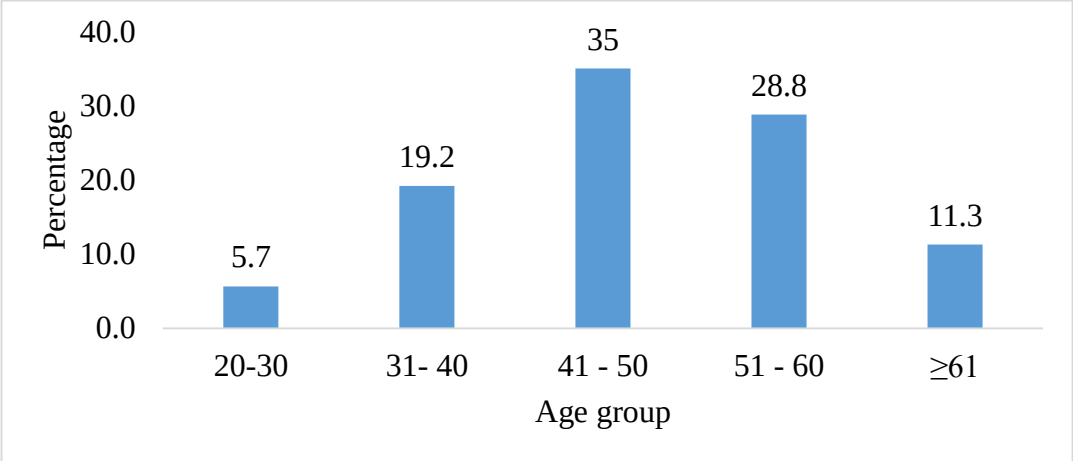

Figure 4.1: The Distribution of Patients by Age Group

Regarding marital status, the majority of patients, 104 (59.1%), were married. This was followed by 27 (15.3%) divorced/separated, 24 (13.6%) widowed, and 21 (11.9%) single (never married), respectively (Figure 4.2).

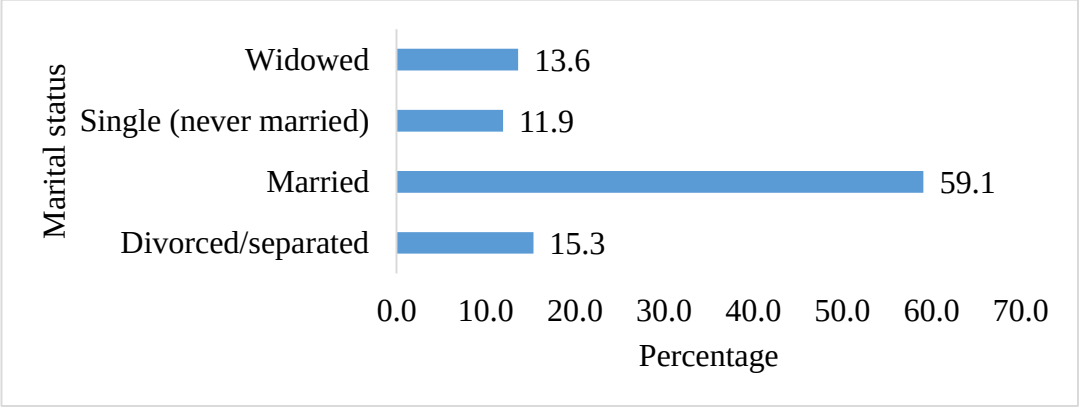

Figure 4.2: The Distribution of Patients by Marital Status

Table 4.1 summarizes additional patients' socio-demographic characteristics. There were 107 (60.5%) females versus 70(39.5%) males. Among the male patients, only 4(5.5%) were not circumcised compared to 69 (94.5%) who were circumcised. Additionally, all 107(100%) female patients were not currently pregnant (Table 4.1).

Table 4.1: Socio-Demographic Characteristics of the Study Population

Variable	Frequency	Percent
Gender		
Female	107	60.5
Male	70	39.5
Age Group		
Mean (± SD)	48.3 (± 11.1)	
Range	57 (20–77)	
20–30	10	5.7
31–40	34	19.2
41–50	62	35
51–60	51	28.8
≥61	20	11.3
Marital status		
Divorced/separated	27	15.3
Married	104	59.1
Single (never married)	21	11.9
Widowed	24	13.6
Circumcised (Men only)		
Yes	69	94.5
No	4	5.5
Currently pregnant (Women only)		
Yes	0	0
No	107	100

SD = Standard Deviation; ≥ = Greater than or equal to

4.1.2 Clinical Characteristics of the Study Population

Regarding the duration of living with HIV (years), there were two peaks, each with 43 (24.3%) patients infected within the last four years and between four to eight years. This was followed by 42 (23.7%) patients living with HIV for a period of 8 to 12 years, 39 (22%) for 12 to 16 years, and 10 (5.7%) for more than 16 years (Figure 4.3).

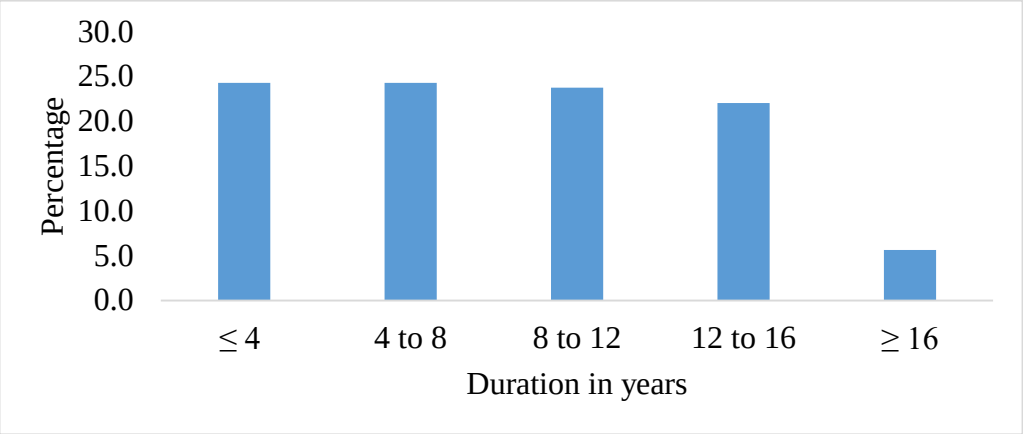

Figure 4.3: Distribution of Patients' Duration Living with HIV Infection

Table 4.2 summarizes additional patients' clinical characteristics. There were 16 (9.1%) patients who reported previous STI infections other than HIV, compared to 161(90.9%) who did not have any previous STI. The previous STIs included 13(81.3%) cases of gonorrhea, 2(12.4%) cases of syphilis, and 1(6.3%) case of co-infection with gonorrhea and trichomoniasis. Three (1.7%) patients reported the presence of chancre or lesions, six (3.4%) had received a previous blood transfusion, while none (100%) reported a previous organ transplant (Table 4.2).

Table 4.2: Clinical Characteristics of the Study Population

Variable	Frequency	Percent
Duration with HIV (Years)		
≤ 4	43	24.3
4 to 8	43	24.3
8 to 12	42	23.7
12 to 16	39	22
≥ 16	10	5.7
Previous STI infection		
Yes	16	9.1
No	161	90.9
Specific previous STI infection		
Gonorrhea	13	81.3
Gonorrhea and Trichomoniasis	1	6.3
Syphilis	2	12.4
Any other Medical conditions		
No	156	88.1
Yes	21	11.9
Presence of chance /lesion		
Yes	3	1.7
No	174	98.3
Previous blood transfusion		
Yes	6	3.4
No	171	96.6
Previous organ transplant		
Yes	0	0
No	177	100

Key: STI – Sexually transmitted infection
≥ - Greater than or equal to
≤ - Less than or equal to

4.1.3 HIV Treatment and Laboratory Outcomes

Most patients, 92.7% (n = 164), were on the currently recommended ART regimen of Tenofovir/Lamivudine/Dolutegravir or Adefovir (TDF + 3TC + DTG/AFV), while only 7.3% (n = 13) were on the older ART regimen of Zidovudine/ Lamivudine/Entecavir (AZT + 3TC + ETV), as shown in Figure 4.4.

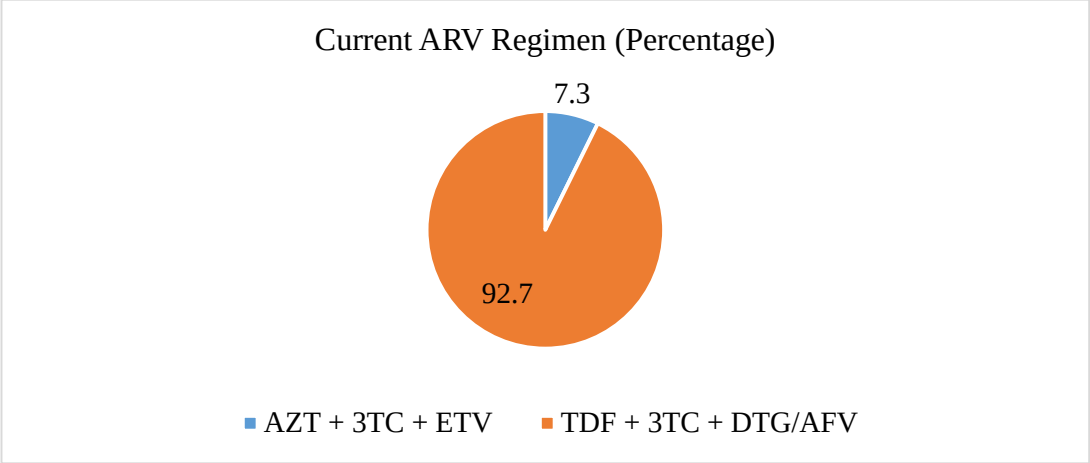

Figure 4.4. The Distribution of Patients by Current ART Regimen Type

Table 4.3 summarizes additional patients' HIV treatment and laboratory outcomes. The majority of the patients, 88.7%, had changed their previous ART regimen. Most of the patients, 90.5%, had their ART changed for optimization, with a few, 7%, due to virological failure and 2.5% due to clinical failure. The majority of patients, 92.1%, had an undetectable viral load, while some, 7.9%, had an HIV viral load of 1000 copies/ml, indicating treatment failure. Additionally, 1.1% of patients had a previous syphilis-positive result (Table 4.3).

Table 4.3: HIV Treatment and Laboratory Outcome Attributes of the Study Population

Variable	Frequency	Percent
Current ARV Regimen		
AZT + 3TC + ETV	13	7.3
TDF + 3TC + DTG/AFV	164	92.7
Changed ART regimen		
Yes	157	88.7
No	20	11.3
Reasons for ART regimen change		
Clinical failure	4	2.5
Optimization	142	90.4
Virological failure	11	7.1
Viral load (Copies/ml)		
1000	14	7.9
Undetectable	163	92.1
Previous syphilis test		
Yes	77	43.5
No	100	56.5
Previous syphilis test results		
Positive	2	2.5
Negative	74	97.5

Key:
AZT - Zidovudine
3TC - Lamivudine
ETV - Entecavir
TDF - Tenofovir
DTG - Dolutegravir
AFV – Adefovir; Ml - milliliter

4.2 Prevalence of Syphilis among the Study Population

The prevalence of syphilis was assessed using three serological/immunological tests (RPR, VDRL, and TPHA) and a PCR-based method among HIV patients attending CCC at NCRH. The prevalence varied by test: 97 (54.8%) by VDRL, 67 (37.9%) by RPR, 41 (23.3%) by TPHA, and 33 (18.6%) by PCR (Figure 4.5).

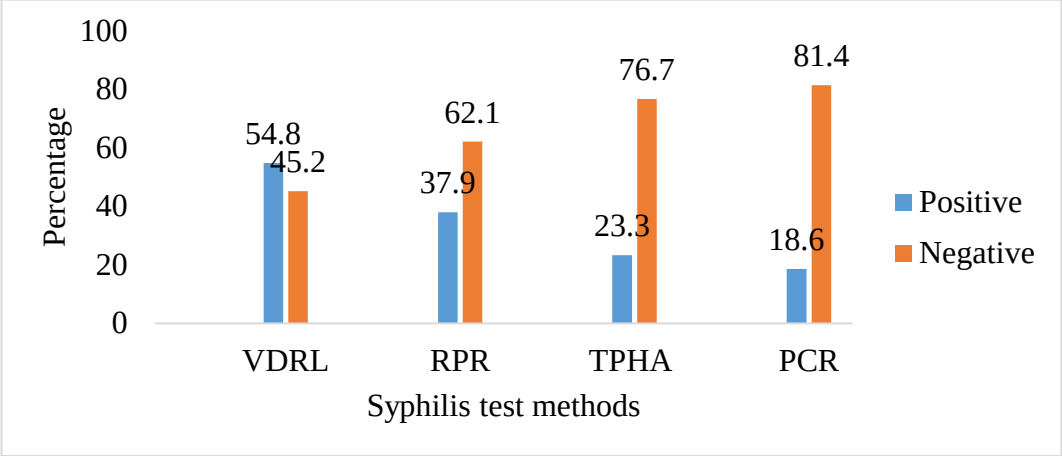

Figure 4.5. The Prevalence of Syphilis by Test Methods

4.3 Performance of Syphilis Diagnostic Methods using PCR as the Gold Standard

Data were used for performance analyses only when the results were definitive. Results consistent with those of the PCR score were obtained in 143/177 (80.8%; 95% CI 74.4% – 85.9%) patients by RPR, 113/177 (63.8%; 95% CI 56.5% – 70.6%) by VDRL, and 169/177 (84.2%; 95% CI 78.1% – 88.8%) by TPHA. The kappa statistic, which measures the level of agreement, showed

The level of agreement between RPR and PCR was kappa (0.546 - moderate agreement); kappa (0.317 - fair agreement) between VDRL and PCR, and kappa (0.864 – near perfect agreement) between TPHA and PCR (Table 4.4).

Based on PCR test results as the gold standard, the test sensitivities were as follows: RPR criteria (100%; 95% CI 89.6% - 100%) for the 33 true syphilis-positive results by PCR, VDRL 33 (100%; 95% CI 89.6% - 100%) for the 33 true syphilis-positive results by PCR, and 33 (100%; 95% CI 89.6% - 100%) by TPHA of the 33 true positive samples by PCR (Table 4.4).

The specifics of each test were: 76.4% (95% CI 68.8% - 82.6%) of the 144 true negative samples by PCR, 55.6% (95% CI 47.4% – 63.4%) by VDRL, and 94.4% (95% CI 89.4% – 97.2%) by TPHA test out of 144 true negative results by PCR (Table 4.4).

The positive predictive values (PPV) of the three tests ranged from 33 (34.1%) for VDRL out of 97 by PCR, followed by 33 (49.3%) for RPR out of 67 by PCR, and 33 (80.5%) for TPHA out of 41 by PCR (Table 4.4).

The negative predictive values (NPV) for all three tests—RPR, VDRL, and TPHA—were 100% each against PCR as the gold standard (Table 4.4).

The common practice in Kenya is to test for syphilis using either the RPR or VDRL tests, with confirmation through TPHA. When using PCR as the gold standard, we combined the results of all three tests—RPR, VDRL, and TPHA—and compared their performance against PCR. This combination's test concordance slightly decreased to 111/177 (62.7%; 95% CI 55.4% – 69.5%), with a kappa of 0.306, indicating fair agreement. The sensitivity of this combination remained at 100% (95% CI 89.6% – 100%) and did not improve over individual tests, while specificity dropped to 54.2% (95% CI 46.1% – 62.1%). The NPV of this combination was also unchanged at 100%, but the positive predictive value fell to 34.1% (Table 4.4).

Table 4.4: Performance of RPR, VDRL, TPHA, and Combined RPR/VDRL/TPHA Tests Compared to PCR

		PCR standard						
Test	**N**	**Concordant results (%) 95% CI**	**Sensitivity (%) 95% CI**	**Specificity (%) 95% CI**	**NPV (%) 95% CI**	**PPV (%) 95% CI**	**Kappa**	**Agreement**
RPR	177	84.2(78.1 - 88.8)	97.6(87.4 - 99.6)	80.1(72.7 - 86)	99.1(95 - 99.8)	59.7(47.7 - 70.6)	0.636	Substantial
VDRL	177	67.8(60.6 - 74.2)	97.6(87.4 - 99.6)	58.1(49.7 - 66)	98.8(93.3 - 99.8)	41.2(32 - 51.2)	0.377	Fair
RPR/VDRL	177	66.7(59.4 - 73.2)	97.6(87.4 - 99.6)	57.4(49 - 65.4)	98.2(93.2 - 99.8)	40.8(31.6 - 50.7)	0.369	Fair

N- number, NPV-negative predictive valus, PPV – positive predictive valus, CI- Confidence interval

4.4 Performance of Syphilis Diagnostic Methods Using TPHA as the Gold Standard

Because PCR is routinely unavailable in Kenya, using TPHA as the gold standard, the sensitivity, specificity, and kappa of tests in this population were: RPR: 97.6%, 80.1%, and kappa (0.636 - substantial agreement); VDRL: 97.6%, 58.1%, and kappa (0.377 - fair agreement); and RPR/VDRL combined: 97.6%, 57.4%, and kappa (0.369 - fair agreement). Using TPHA as the gold standard decreases the sensitivity of RPR from 100% to 97.6% and that of VDRL from 100% to 97.6%, but increases the specificity of RPR from 76.4% to 80.1% and VDRL from 55.6% to 58.1%. Considering all the TPHA weakly reactive samples as negative improves both the sensitivity and specificity of TPHA against PCR as the gold standard, each to 100%. Conversely, when all weakly reactive TPHA samples are considered negative, both the sensitivity and specificity of RPR and VDRL with TPHA as the gold standard decrease (Table 4.5)

Table 4.5: Performance of RPR, VDRL and RPR/VDRL Combined Tests against TPHA Results as the Gold Standard

		TPHA (Gold standard)						
Test	**N**	**Concordant results (%) 95% CI**	**Sensitivity (%) 95% CI**	**Specificity (%) 95% CI**	**NPV (%) 95% CI**	**PPV (%) 95% CI**	**Kappa**	**Agreement**
RPR	177	84.2(78.1 - 88.8)	97.6(87.4 - 99.6)	80.1(72.7 - 86)	99.1(95 - 99.8)	59.7(47.7 - 70.6)	0.636	Substantial
VDRL	177	67.8(60.6 - 74.2)	97.6(87.4 - 99.6)	58.1(49.7 - 66)	98.8(93.3 - 99.8)	41.2(32 - 51.2)	0.377	Fair
RPR/VDRL	177	66.7(59.4 - 73.2)	97.6(87.4 - 99.6)	57.4(49 - 65.4)	98.2(93.2 - 99.8)	40.8(31.6 - 50.7)	0.369	Fair

N - number; % = Percentage; CI - Confidence Interval; PPV - Positive Predictive Value; NPV - Negative Predictive Value

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of Syphilis

The goal of this study was to identify the most accurate test or combination of tests for detecting syphilis. Serum testing for syphilis was conducted in a population with a higher prevalence of syphilis infection among HIV-infected individuals receiving treatment. The prevalence of syphilis in this population, as determined by PCR, was 18.6%, which exceeds previous studies in Kenya, including a prevalence of 9.6% among high-risk fishermen. The estimated prevalence and incidence of syphilis vary significantly by region or country, with the highest rates in Africa and over 60% of new cases occurring in low- and middle-income countries (Gulersen et al., 2023). The greatest burden of maternal syphilis is found in Africa, accounting for over 60% of the global estimate (Gulersen et al., 2023). Although in low- and middle-income countries, the prevalence of syphilis has decreased in the general population, the infection remains a problem in certain high-risk groups, such as female sex workers and their male clients, with prevalence rates of 21% in South Africa (Kularatne et al., 2024), 21% in Uganda (Ssenyonjo et al., 2024), and 8.9% in Sudan (Dery et al., 2024). Syphilis among men who have sex with men (MSM) is several hundred times higher than in the general population. Additionally, the incidence continues to rise as condom use declines and the use of pre-exposure prophylactic antiretroviral medications for HIV increases (Barbosa et al., 2024). Indeed, with broader HIV treatment coverage in recent years and HIV no longer seen as a ‘death sentence’, there has been a decrease in safe sex practices and an increase in risk-taking behaviors.

5.1.2 Test Performance

The manifestation of syphilis varies and often makes clinical diagnosis difficult, leading to many infections going unrecognized (Ramchandani et al., 2023). The classically painless lesions of primary syphilis can be missed, especially in hidden sites of exposure such as the rectum. Often, symptoms of secondary syphilis, like rash and others, can be faint or mistaken for other conditions (Ramchandani et al., 2023). Serological testing has therefore become the most common method to diagnose syphilis, whether in individuals with symptoms or in those without symptoms who are detected through screening. This study therefore evaluated the test performance of syphilis diagnostic methods that combined serological and immunological methods: the qualitative non-treponemal test (NTT), the treponemal test (TT), and PCR (Satyaputra et al., 2021). The diagnostic performance, or accuracy, of the test in differentiating between seropositive and seronegative cases, compared to PCR, was also measured using the kappa statistic.

The results showed that TPHA (a treponemal-based test) had higher overall accuracy for detecting syphilis in this population compared to the two non-treponemal tests (RPR and VDRL), based on sensitivity and specificity calculations (100%, 94.4%; 100%, 76.4%; and 100%, 55.6%, respectively). Because PCR is routinely unavailable in Kenya, it was used for research purposes. Using TPHA, which is generally considered the gold standard, lowered the sensitivity of both RPR and VDRL from 100% to 97.6%, but increased the specificity of RPR and VDRL from 76.4% to 80.1% and 55.6% to 58.1%, respectively. Previous studies have reported varying sensitivity for primary syphilis using VDRL/RPR tests, ranging between 70% and 80%, while TPHA/TPPA tests showed similar sensitivity ranges (Rathore et al., 2024; Cao et al., 2023). Caution has been advised regarding the performance of NTTs, especially among early primary and previously treated infections, which can be missed due to their lower sensitivity (Ramchandani et al., 2023). Additionally, false-negative NTT results can occur due to the prozone effect, which happens when there is an excess of antibodies (Ramchandani et al., 2023).

PCR-DNA offers a promising alternative for detecting *T. pallidum* (Cao et al., 2023; Wong et al., 2021). These tests are highly specific to pathogenic treponemes, with a specificity of 95 to 97%. When analyzing specimens from the mouth and rectum, they can achieve a sensitivity of 91 to 95% and detect as few as one to sixty-five microorganisms (Cao et al., 2023; Queiroz et al., 2022). The PCR technique targeting unique regions of the DNA polymerase I gene of *T. pallidum* has proven highly effective for detecting treponemes in multiple clinical samples, including blood, CSF, amniotic fluid, and genital ulcer specimens. With a reported detection limit of 1 to 65 organisms, this advanced technology has demonstrated high sensitivity (over 90%) and high specificity (over 95%) (Queiroz et al., 2022).

This study had three major limitations. First, due to its cross-sectional design, the study lacked clinical follow-up data to help predict the stages of syphilis infection among those found infected. Without this information, the impact of disease stage on test performance cannot be completely ruled out. The second limitation is that the subjects were from a single region of Kenya. Tests may perform differently in various geographic areas. Without more extensive sampling, it is not possible to generalize the results of this study to other populations, such as women or individuals from different regions. Third, using PCR as the gold standard was based on its performance with samples collected from genital ulcer specimens. The influence of blood samples as a source of the DNA polymerase I gene of *T. pallidum* used during PCR was not fully evaluated. The utility of the PCR technique, which targets unique regions of the DNA polymerase I gene of *T. pallidum, in* detecting treponemes in multiple clinical samples (such as blood, CSF, amniotic fluid, and genital ulcer specimens), mitigates this limitation.

5.2 Conclusion

i. This study found that the prevalence of Treponema pallidum infection among the study population, as detected by Polymerase Chain Reaction (PCR), was 18.6%. This rate is significantly higher than previous figures reported in Kenya, including the 9.6% observed among high-risk fishermen, highlighting an under-recognized burden of syphilis and the urgent need for targeted public

health interventions. The high prevalence indicates ongoing transmission, potential reinfections, and gaps in current screening and treatment strategies within this group.

ii. Regarding test performance, the Treponema Pallidum Hemagglutination Assay (TPHA) showed the highest sensitivity (100%) and specificity (94.4%) compared to PCR, with a near-perfect agreement (Kappa = 0.864). This finding suggests that TPHA remains a reliable, accurate, and cost-effective diagnostic option, capable of serving as either a confirmatory or a standalone test in settings with limited molecular diagnostic capacity. In contrast, the Rapid Plasma Reagin (RPR) and Venereal Disease Research Laboratory (VDRL) tests demonstrated lower sensitivity and specificity, which limits their usefulness in both screening and confirming syphilis infections in high-prevalence populations.

iii. The strong agreement between TPHA and PCR highlights the clinical reliability of treponemal-based tests for accurate case detection. The study emphasizes the importance of moving away from non-treponemal tests toward treponemal immunoassays in diagnosing syphilis. Overall, the findings show an urgent need to improve laboratory diagnostic systems, increase community awareness, and incorporate syphilis screening into broader sexual and reproductive health programs, especially among populations at risk of co-infection with HIV.

5.3 Recommendations

i. Based on the findings, it is recommended that comprehensive interventions targeting syphilis and HIV co-infection be prioritized in this population. The high prevalence of syphilis detected by PCR highlights the importance of integrating syphilis screening, prevention, and treatment into existing HIV/AIDS care and reproductive health services. Strengthening this integration will improve early case detection, reduce vertical transmission, and decrease adverse maternal and neonatal outcomes related to untreated syphilis.

ii. Health facilities should adopt treponemal immunoassays such as the Enzyme Immunoassay (EIA), Chemiluminescence Immunoassay (CIA), and

Microbead Immunoassay (MBIA) for routine screening. These tests can be automated, enhancing efficiency, reducing turnaround time, and minimizing human error compared to manual techniques. Given TPHA's proven accuracy and strong agreement with PCR, it can be used as a confirmatory or stand-alone test, especially in resource-limited settings where molecular testing is unavailable. However, further evaluation of automated treponemal platforms is encouraged to improve diagnostic workflows.

iii. At the research level, there is a vital need to develop and validate biomarkers that can distinguish between past, treated, and active infections, as well as identify reinfections. This progress will improve clinical decision-making and patient management. Additionally, studies should examine the cost-effectiveness and feasibility of integrating molecular diagnostics such as PCR into national surveillance systems for sexually transmitted infections (STIs). Policymakers and healthcare planners need to strengthen laboratory infrastructure, ensure ongoing training for laboratory staff, and expand access to high-quality diagnostic reagents. Community-based health education and behavior change programs are equally important to reduce stigma, promote testing, and support treatment adherence. By following these recommendations, Kenya can greatly advance syphilis control efforts and enhance sexual and reproductive health outcomes among vulnerable populations.

REFERENCES

Adler, M. (2024). Sexually transmitted infections from the 20th to the 21st century. *Clinics in Dermatology, 42*(2), 108-109. doi:10.1016/j.clindermatol.2023.12.008

Ahmed, J., Rawre, J., Dhawan, N., Dudani, P., Khanna, N., & Dhawan, B. (2022). Genital ulcer disease: A review. *Journal of Family Medicine and Primary Care, 11*(8), 4255-4262. doi:10.4103/jfmpc.jfmpc_2111_21

Aho, J., Lybeck, C., Tetteh, A., Issa, C., Kouyoumdjian, F., Wong, J., . . . Popovic, N. (2022). Rising syphilis rates in Canada, 2011-2020. *Can Commun Dis Rep, 48*(3), 52-60. doi:10.14745/ccdr.v48i23a01

Alexander, D. C., Morshed, M., Stein, D., Bullard, J., MacKenzie, K., & Tsang, R. S. (2024). An Update on the Status of Direct Testing for Treponema Pallidum Subspecies Pallidum for the Laboratory Diagnosis of Syphilis in Canada. *J Assoc Med Microbiol Infect Dis Can., 9*(2), 95-103. doi:10.3138/jammi-2023-0032

Almeida, M. C., Cordeiro, A. M., Cunha-Oliveira, A., Barros, D. M., Santos, D. G., Lima, T. S., & Valentim, R. A. (2022). Syphilis response policies and their assessments: A scoping review. *Frontiers in Public Health, 10*, 1002245. doi:10.3389/fpubh.2022.1002245

Angel-Müller, E., Grillo-Ardila, C. F., Amaya-Guio, J., & Torres-Montañez, N. (2021). Diagnostic accuracy of rapid point-of-care tests for detecting active syphilis: a systematic review and meta-analysis. *Sex Transm Dis., 48*(12), e202-e208. doi:10.1097/OLQ.0000000000001498

Anteneh, D. E., Taye, E. B., Seyoum, A. T., Abuhay, A. E., & Cherkose, E. A. (2024). Seroprevalence of HIV, HBV, and syphilis co-infections and associated factors among pregnant women attending antenatal care in Amhara regional state, northern Ethiopia: A hospital-based cross-sectional study. *PLoS One, 19*(8), e0308634. doi:10.1371/journal.pone.0308634

Aung, E. T., Chen, M. Y., Fairley, C. K., Higgins, N., Williamson, D. A., Tomnay, J. E., . . . Chow, E. P. (2021). Spatial and temporal epidemiology of infectious syphilis in Victoria, Australia, 2015–2018. *Sexually Transmitted Diseases, 48*(12), e178-e182. doi:10.1097/OLQ.0000000000001438

Cai, G., Liu, Y., Zhuang, J., Chen, Z., Lu, Y., Wu, J., . . . He, F. (2024). Differences in socio-demographics status, risk behaviours, healthcare uptake and HIV/sexually transmitted infections (STIs) between brothel-based and street-based female sex workers in Yunnan, China. *International Journal of STD & AIDS, 35*(8), 584 - 592. doi:10.1177/09564624241239480

Cao, W., Thorpe, P. G., O'Callaghan, K., & Kersh, E. N. (2023). Advantages and limitations of current diagnostic laboratory approaches in syphilis and congenital syphilis. *Expert Review of Anti-infective Therapy, 21*(12), 1339-1354. doi:10.1080/14787210.2023.2280214

CDC. (2021). *Syphilis - STI treatment guidelines*. Centers for Disease Control and Prevention. From https://www.cdc.gov/std/treatment-guidelines/syphilis.htm

Chaudhry, S., Akinlusi, I., Shi, T., & Cervantes, J. (2023). Secondary syphilis: pathophysiology, clinical manifestations, and diagnostic testing. *Venereology, 2*(2), 65-75. doi:10.3390/venereology2020006

Chen, J., Huang, J., Liu, Z., & Xie, Y. (2022). Treponema pallidum outer membrane proteins: current status and prospects. *Pathog Dis., 80*(1), ftac023. doi:10.1093/femspd/ftac023

Ciepłucha, H. D., Bożejko, M., Honisz, J., Kruczek, F., & Sladkowska, K. (2025). Syphilis--the great imitator. Potential diagnostic problems: A literature review. *HIV & AIDS Review, 24*(1), 1-7. doi:10.5114/hivar/188797

Dery, S., Guure, C., Afagbedzi, S., Ankomah, A., Ampofo, W., Atuahene, K., . . . Torpey, K. (2024). Biobehavioral survey using time location sampling among female sex workers living in Ghana in 2020. *Front Public Health, 12*, 1137799. doi:10.3389/fpubh.2024.1137799

Dessie, G., Mulugeta, H., Wagnew, F., Zegeye, A., Kiross, D., Negesse, A., . . . Burrowes, S. (2021). Immunological Treatment Failure Among Adult Patients Receiving Highly Active Antiretroviral Therapy in East Africa: A Systematic Review and Meta-Analysis. *Current Therapeutic Research, 94*, 100621. doi:10.1016/j.curtheres.2020.100621

Do, D., Rodriguez, P. J., Gratzl, S., Cartwright, B. M., Baker, C., & Stucky, N. L. (2025). Trends in Incidence of Syphilis Among US Adults from January 2017 to October 2024. *Am J Prev Med., S0749-3797*(25), 00106-0. doi:10.1016/j.amepre.2025.03.008

Eriksen, J., Albert, J., Axelsson, M., Berglund, T., Brännström, J., Gaines, H., . . . Tegnell, A. (2021). Contagiousness in treated HIV-1 infection. *Infectious Diseases, 53*(1), 1-8. doi:10.1080/23744235.2020.1831696

Fan, L., Yu, A., Zhang, D., Wang, Z., & Ma, P. (2021). Consequences of HIV/syphilis co-infection on HIV viral load and immune response to antiretroviral therapy. *Infection and Drug Resistance, 14*, 2851-2862. doi:10.2147/IDR.S320648

Ferreira, S., Carvalho, J. K., Nascimento, A. K., Ferreira, A. G., Neto, M. S., & Bezerra, J. M. (2023). Clinical-epidemiological characteristics of pregnant women with HIV/syphilis coinfection: An integrative review. *Revista de Epidemiologia e Controle de Infecção, 13*(4), 232-239.

Geremew, H., & Geremew, D. (2021). Sero-prevalence of syphilis and associated factors among pregnant women in Ethiopia: a systematic review and meta-analysis. *Systematic Reviews, 10*(1), 223. doi:10.1186/s13643-021-01786-3

Gilbert, L., Dear, N., Esber, A., Iroezindu, M., Bahemana, E., Kibuuka, H., . . . Ake, J. A. (2021). Prevalence and risk factors associated with HIV and syphilis co-infection in the African Cohort Study: A cross-sectional study . *BMC Infectious Diseases, 21*(1), 1123. doi:10.1186/s12879-021-06668-6

Gulersen, M., Lenchner, E., Eliner, Y., Grunebaum, A., Johnson, L., Chervenak, F. A., & Bornstein, E. (2023). Risk factors and adverse outcomes associated with syphilis infection during pregnancy. *Am J Obstet Gynecol MFM, 5*(6), 100957. doi:10.1016/j.ajogmf.2023.100957

Henninger, M. L., Bean, S. I., & Lin, J. S. (2022). Screening for Syphilis Infection in Nonpregnant Adults and Adolescents: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA, 328*(12), 1250-1252. doi:10.1001/jama.2022.8612

Kaminiów, K., Kiołbasa, M., & Pastuszczak, M. (2024). The significance of the cell-mediated host immune response in syphilis. *Microorganisms, 12*(12), 2580. doi:10.3390/microorganisms12122580

Kamori, D., Joachim, A., Mizinduko, M., Barabona, G., Mahiti, M., Kibwana, U., . . . Lyamuya, E. (2021). Seroprevalence of Human Herpesvirus Infections in Newly Diagnosed HIV-Infected Key Populations in Dar es Salaam, Tanzania. *Int J Microbiol, 2021*, 4608549. doi:10.1155/2021/4608549

Kassaw, B., Abera, N., Legesse, T., Workineh, A., & Ambaw, G. (2022). Sero-prevalence and associated factors of hepatitis B virus among pregnant women in Hawassa city public hospitals, Southern Ethiopia: Cross-sectional study design. *SAGE Open Medicine, 10*, 20503121221140778. doi:10.1177/20503121221140778

Khaw, C., Richardson, D., Matthews, G., & Read, T. (2018). Looking at the positives: proactive management of STIs in people with HIV. *AIDS Res Ther., 15*(1), 28. doi:10.1186/s12981-018-0216-9

Kularatne, R., Blondeel, K., Kasaro, M., Maseko, V., Bosomprah, S., Silva, R., . . . Peeling, R. W. (2024). Clinic-based evaluation of point-of-care dual HIV/syphilis rapid diagnostic tests at primary healthcare antenatal facilities in South Africa and Zambia. *BMC Infect Dis., 24*((Suppl 1)), 600. doi:10.1186/s12879-024-09463-1

Liu, M., Fan, Y., Chen, J., Yang, J., Gao, L., Wu, X., . . . Bao, F. (2022). Efficacy and safety of treatments for different stages of syphilis: A systematic review and network meta-analysis of randomized controlled trials and observational studies. *Microbiology Spectrum, 10*(6), e0297722. doi:10.1128/spectrum. 02977-22

Lyngdoh, C. J., Ramudamu, M., Agarwal, M., Verma, S., & Prasad, A. (2024). Evaluation of Serological Tests for the Diagnosis of Syphilis. *Cureus, 16*(5), e61007. doi:10.7759/cureus.61007

Mahmud, S., Mohsin, M., Muyeed, A., Islam, M. M., Hossain, S., & Islam, A. (2023). Prevalence of HIV and syphilis and their co-infection among men having sex with men in Asia: A systematic review and meta-analysis. *Heliyon*, *9*(3), e13947. doi:10.1016/j.heliyon.2023.e13947

Maseko, D. V., Valashiya, D., & Kularatne, R. S. (2022). Development and trial of a dried tube specimen (DTS) proficiency testing panel for dual HIV/syphilis rapid diagnostic tests. *Diagnostic Microbiology and Infectious Disease, 102*(3), 115607. doi:10.1016/j.diagmicrobio.2021.115607

NACC. (2024a). *Kenya HIV Prevention Revolution Roadmap: Count Down to 2030.* Nairobi: UNAIDS.

NACC. (2024b). *Kenya HIV County Profiles, HIV and AIDS Response in My County-My Responsibility.* Nairobi: UNAIDS.

Nam, S. W. (2025). Acute Macular Neuroretinopathy in a Patient with Syphilis: Case Report. *Journal of Retina*, *10*(1), 79-83. doi:10.21561/jor.2025.10.1.79

Negash, M., Wondmagegn, T., & Geremew, D. (2018). Comparison of RPR and ELISA with TPHA for the Diagnosis of Syphilis: Implication for Updating Syphilis Point-of-Care Tests in Ethiopia. *J Immunol Res.*, *2018*, 2978419. doi:10.1155/2018/2978419

Njau, A. F., Robert, M., Rwebembera, A., Kisendi, R., Maro, C., Dennis, G., . . . Msangi, M. (2025). Prevalence and associated factors for HIV, HBV and syphilis coinfections among pregnant women attending antenatal care in Tanzania. *PLoS One, 20*(8), e0329068. doi:10.1371/journal.pone.0329068

Nyeri County. (2021). *Nyeri County HIV & AIDS Strategic Plan. 2015/2016 - 2018/2019.* Nyeri. Retrieved March, 2026 from https://nsdcc.go.ke/wp-content/uploads/2021/08/nyeri.pdf

Orbe-Orihuela, Y. C., Sánchez-Alemán, M. Á., Hernández-Pliego, A., Medina-García, C. V., & Vergara-Ortega, D. N. (2022). Syphilis as re-emerging disease, antibiotic resistance, and vulnerable population: Global systematic review and meta-analysis. *Pathogens*, *11*(12), 1546. doi:10.3390/pathogens11121546

Parczewski, M., Gökengin, D., Sullivan, A., Amo, J., Cairns, G., Bivol, S., . . . Rockstroh, J. K. (2025). Control of HIV across the WHO European region: progress and remaining challenges. *Lancet Reg Health Eur., 52*, 101243. doi:10.1016/j.lanepe.2025.101243

Pham, M. D., Ong, J. J., Anderson, D. A., Drummer, H. E., & Stoové, M. (2022). Point-of-Care Diagnostics for Diagnosis of Active Syphilis Infection: Needs, Challenges and the Way Forward. *Int J Environ Res Public Health*, *19*(3), 8172. doi:10.3390/ijerph19138172

Purwoko, M. I., Devi, M., Nugroho, S. A., Fitriani, F., Pamudji, R., & Candra, N. C. (2021). Laboratory examination of syphilis. *Bioscientia Medicina: Journal of Biomedicine and Translational Research, 5*(8), 726-745. doi:10.32539/BSM.V5I3.339

Queiroz, J. H., Correa, M. E., Ferreira, T. D., Marques, M. F., Barbosa, M. D., Marchioro, S. B., & Simionatto, S. (2022). Detection of Treponema pallidum in whole blood samples of patients with syphilis by the polymerase chain reaction. *Revista do Instituto de Medicina Tropical de São Paulo, 64*, e75. doi:10.1590/S1678-9946202264075

Ramchandani, M. S., Cannon, C. A., & Marra, C. M. (2023). Syphilis: A Modern Resurgence. *Infect Dis Clin North Am., 37*(2), 195-222. doi:10.1016/j.idc.2023.02.006

Rathore, P., Mishra, V., Bansal, N., Sharma, A., Saini, A., & Verma, I. (2024). Unraveling the Enigma of Syphilis: A Comprehensive Review. *Journal of Young Pharmacists, 16*(3), 385-390. doi:10.5530/jyp.2024.16.50

Rosset, F., Celoria, V., Delmonte, S., Mastorino, L., Sciamarrelli, N., Boskovic, S., . . . Quaglino, P. (2025). The Epidemiology of Syphilis Worldwide in the Last Decade. *Journal of Clinical Medicine, 14*(15), 5308. doi:10.3390/jcm 14155308

Satyaputra, F., Hendry, S., Braddick, M., Sivabalan, P., & Norton, R. (2021). The Laboratory Diagnosis of Syphilis. *J Clin Microbiol., 59*(10), e0010021. doi:10.1128/JCM.00100-21

Shukla, M., Pereira, L., & Pillay, A. (2022). Treponema. In I. Filippis (Ed.), *Molecular Typing in Bacterial Infections* (Vol. I, pp. 191-213). Springer Cham. doi:10.1007/978-3-030-74018-4

Simões, L. A., Mendes, J. C., Silveira, M. R., Costa, A. M., Lula, M. D., & Ceccato, M. D. (2022). Factors associated with HIV/syphilis co-infection initiating of antiretroviral therapy. *Revista de Saúde Pública, 56*, 59. doi:10.11606/s1518-8787.2022056003904

Ssenyonjo, J., Mistler, C., Adler, T., Shrestha, R., Kyambadde, P., & Copenhaver, M. (2024). Examining HIV Knowledge and Sexually Risky Behaviors among Female Sex Workers in Kampala, Uganda. *Int J Environ Res Public Health., 21*(2), 163. doi:10.3390/ijerph21020163

Tang, Y., Zhou, Y., He, B., Cao, T., Zhou, X., Ning, L., . . . Liu, S. (2023). Investigation of the immune escape mechanism of Treponema pallidum. *Infection, 51*(2), 305-321. doi:10.1007/s15010-022-01939-z

Tsega, N. T., Abebe, B., Ebabu, T., Asmare, T., Kassa, M., Haile, T. T., . . . Wondie, K. Y. (2022). Sexually transmitted infections and associated factors during pregnancy in Gondar city, Northwest Ethiopia, 2021: A multicenter study. *Clinical Epidemiology and Global Health, 16*, 101096. doi:10.1016/j.cegh.2022.101096

UK Health Security Agency. (2025). *Sexually transmitted infections and screening for chlamydia in England: 2024 report.* London: UK Government. From https://www.gov.uk/government/statistics/sexually-transmitted-infections-stis-annual-data-tables/sexually-transmitted-infections-and-screening-for-chlamydia-in-england-2024-report

Vargas, S., Calvo, G., Qquellon, J., Vasquez, F., Blondeel, K., Ballard, R., & Toskin, I. (2022). Point-of-care testing for sexually transmitted infections in low-resource settings. *Clin Microbiol Infect., 28*(7), 946-951. doi:10.1016/j.cmi.2021.05.052

Wall, K. M., Workowski, K., Young, M., & Stafford, I. A. (2025). Point-of-care testing to combat congenital syphilis—the time is now. *JAMA, 333*(13), 1115-1116. doi:10.1001/jama.2025.0171

WHO. (2023). *The diagnostics landscape for sexually transmitted infections.* Geneva: World Health Organization.

WHO. (2025, September 10). *Sexually transmitted infections (STIs).* From World Health Organization:Retrieved from https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis)

Wong, N. S., Powers, K. A., Tucker, J. D., Lee, S. S., Goh, B. T., Zhao, P., . . . Mitchell, K. M. (2021). Modelling the impact of a sex work crackdown on syphilis transmission among female sex workers and their clients in South China. *Sexually Transmitted Infections, 97*(1), 45-50. doi:10.1136/sextrans-2020-054497

Yang, L., Fu, Y., Li, S., Liu, C., & Liu, D. (2022). Analysis of Treponema pallidum DNA and CXCL13 in Cerebrospinal Fluid in HIV-Negative Syphilis Patients. *Infect Drug Resist., 15*, 7791-7798. doi:10.2147/IDR.S394581

Yuindartanto, A., Hidayati, A. N., Indramaya, D. M., Listiawan, M. Y., Ervianti, E., & Damayanti, D. (2022). Risk factors of syphilis and HIV/AIDS coinfection. *Berkala Ilmu Kesehatan Kulit dan Kelamin, 34*(2), 114-119. doi:10.20473/bikk.V34.2.2022.114-119

Zeng, X., Ouyang, Y., Wang, H., Liu, L., Chen, J., Zhu, C., . . . Liu, P. (2025). Risk factors of serofast state in patients undergoing syphilis: a meta-analysis of 17 cohort studies. *Front Immunol., 16*, 1689904. doi:10.3389/fimmu.2025.1689904

APPENDICES

Appendix I: Informed Consent Explanation and Consent Form

Title of study: Comparative Analysis of VDRL, RPR, and TPHA Using PCR as the Gold Standard for Testing *Treponema Pallidum* in HIV Patients Visiting Nyeri County Referral Hospital

Introduction: My name is James Wachira Wambugu. I am a master's student in Medical Laboratory Sciences (Immunology) at Jomo Kenyatta University of Agriculture and Technology. You are invited to participate in a study about detecting syphilis among HIV patients. You are eligible because you are receiving ARV treatment at the Comprehensive Care Clinic (CCC) at Nyeri County Referral Hospital (NCRH). Please read this form carefully before deciding to take part. If you cannot read, you can ask the researcher or a hospital staff member to read it to you.

Description: Syphilis is a sexually transmitted infection (STI) caused by the pathogenic spirochete *Treponema pallidum* subsp. The infection is currently increasing, especially among HIV-positive patients. Syphilis management requires accurate and timely diagnosis. Various tests are used to diagnose syphilis. In this study, we aim to evaluate how each of these tests performs in detecting syphilis. The results of this study will help us and the hospital provide urgent treatment options and preventive measures for syphilis.

Procedures: If you agree to participate in this research and sign this consent form, you will have a brief face-to-face interview using a questionnaire. About 4 ml (equivalent to 4/5 of a teaspoon) of venous blood will be drawn from you. The entire process, including the interview and blood collection, should take only 20-30 minutes.

Risks and benefits: The chance of harm (physical or psychological) from participating in the study is minimal or nonexistent, as the risk will not be higher than that of routine medical practice. Only a small discomfort from drawing blood is expected, which will last just a few seconds. There is no monetary benefit for participating in this study.

The potential benefit includes determining if you are infected with syphilis and advising your doctor for prompt and appropriate treatment.

Data/Information Storage: During the study, data will be extracted from your medical records. We will confidentially store your data (including information from interviews, medical records, and biological samples) throughout the study and will destroy it afterward.

Test results: Anyone who tests positive for syphilis will be advised to seek prompt treatment from their healthcare providers.

Time involvement: This study will take less than 30 minutes during your usual visit to the clinic.

Subject's rights: If you have read this form and decided to participate in this project, please understand that your participation is voluntary, and you can withdraw your consent or stop participating at any time without penalty.

You have the right to refuse to answer specific questions. Your privacy will be protected in all published and written data from the study.

My questions have been answered. My decision whether or not to participate in the study is voluntary. If I choose to join, I can withdraw at any time. By signing this form, I do not give up any rights I have as a research participant.

_____	_____	_____
Participant Name	**Signature**	**Date**
_____	_____	_____
Principal investigator	**Signature**	**Date**

Appendix II: Questionnaire

Thank you for agreeing to take this survey. The survey is being done by James Wachira astudent at Jomo Kenyatta University as part of his Master's Project. All of the answers you provide in this survey will be kept confidential. The survey data will be reported in a summary version only and will not identify any individual person.

This survey will take you less than 20 minutes to complete.

Demographic Information

1. Date of visit (day/month/year) ___/___/___
2. Date of birth (day/month/year) ___/___/___
3. What is your marital status?

- ☐ Single (never married)
- ☐ Married
- ☐ Divorced/Separated
- ☐ Widowed
- ☐ Cohabiting
- ☐ Other (specify) _____

4. Gender? (_____)

Medical History

5. When did you test for HIV? (_____)
6. Have you suffered any STI? (_____)
7. For male respondents. Are you circumcised?

- ✓ Yes
- ✓ No

If yes, Traditional or medical procedure (_____)

8. Are you pregnant?

- ✓ Yes
- ✓ no

9. Do you suffer from any other disease?
   - ✓ Yes
   - ✓ No

If yes which one? (_____)

10. What is your Current ART regimen (_____)?
11. Any change of ART regimen?
    - ✓ Yes
    - ✓ No

If yes, what is the reason for change? (_____)

12. What is your most recent viral load results? (_____)
13. What is your most recent cd4 count results? (_____)
14. Have you ever been tested for syphilis?
    - ✓ Yes
    - ✓ No

If yes, was it positive or negative? (_____)

15. Do you have any chancre?
    - ✓ Yes
    - ✓ No
16. Have you ever been treated for STI?
    - ✓ Yes
    - ✓ No

If yes, which ones, and what were the results? (_____)

17. What is your history of blood transfusion? (_____)
18. Do you have any history of organ transplant? (_____)

Appendix III: Maelezo ya Kibali na Fomu ya Ithini

Kichwa cha masomo: Muhtasari wa Ulinganishaji wa VDRL, RPR na TPHA kutumia PCR kama kielekezo katika kupima Treponema Pallidum kati ya wagonjwa walio na virusi vya ukimwi wanaohudhuria Kituo cha Utunzaji Kikamilifu (CCC) katika Hospitali ya Rufaa ya Kaunti ya Nyeri (NCRH Utendaji wa)

Utangulizi: Jina langu ni James Wachira Wambugu. Mimi ni mwanafunzi wa Sayansi ya Maabara ya Matibabu (chanjo) kutoka Chuo Kikuu cha Kilimo na Teknolojia cha Jomo Kenyatta. Unaalikwa kushiriki katika utafiti juu ya kugundua syphilis kati ya wagonjwa wa ukimwi. Wewe ni mshiriki anayeweza kufanikiwa kwa sababu unapokea matibabu ya ARV katika Kliniki ya Huduma ya Utunzaji kamili (CCC) katika Hospitali ya Rufaa ya Kaunti ya Nyeri (NCRH). Kwaheri soma fomu hii kabla ya kukubali kuwa katika utafiti huu. Ikiwa huwezi kusoma, unaweza kumuuliza mtafiti au mshiriki wa wafanyikazi wa hospitali kukusomea.

Maelezo: Syphilis maambukizo ya zinaa (STI) yanayosababishwa na pathogenic spirochaete Treponema pallidum subsp. Ugonjwa huo kwa sasa unaongezeka haswa miongoni mwa wagonjwa wenye ukimwi. Tiba ya Syphilis inahitaji utambuzi sahihi na kwa wakati unaofaa. Kuna vipimo tofauti ambavyo hutumiwa kwa utambuzi wa syphilis. Katika utafiti huu tunataka kutathmini jinsi kila moja ya maonyesho haya ya mtihani kugundua syphilis. Matokeo ya utafiti huu yatatusaidia na hospitali kutoa njia za haraka za kutibu kaswende na pia kuizuia

Taratibu: Ikiwa unakubali kushiriki katika utafiti huu, na kusaini fomu hii ya idhini, utapitia mahojiano mafupi ya uso kwa uso kwa kutumia dodoso. Karibu 4ml damu ya venous itatolewa kutoka kwako. Mahojiano na ukusanyaji wa damu inapaswa kuchukua dakika 20-30 tu za wakati wako.

Hatari na faida: Uwezo wa kudhuru unaotokana na ushiriki katika utafiti utakuwa mdogo au haupo kwani hatari haitakuwa kubwa kuliko ile inayotokana na mazoezi ya kawaida ya matibabu. Usumbufu mdogo tu unaohusishwa na kuchota damu utatokea ambao utadumu kwa sekunde chache. Hakuna faida ya kifedha kwa ushiriki wako

katika utafiti huu. Faida ambayo inaweza kutarajiwa kusababishwa na utafiti huu ni pamoja na kutathmini ikiwa umeambukizwa na syphilis na ushauri wa daktari wako kwa matibabu ya haraka na sahihi.

Uhifadhi wa data / Habari: Wakati wa utafiti, data zitatolewa kutoka kwa rekodi zako za matibabu. Tutahifadhi siri yako ya siri (kutoka kwa mahojiano, rekodi za matibabu na vifaa vya kibaolojia) wakati wa masomo haya na kuharibiwa baadaye.

Kuhusika kwa wakati: Utafiti huu utadumu chini ya dakika 30 wakati wa ziara yako ya kawaida kliniki.

Haki za mada: Ikiwa umeisoma fomu hii na umeamua kushiriki mradi huu, tafadhali eleza ushiriki wako ni wa hiari na una haki ya kuondoa idhini yako au kuacha kushiriki wakati wowote bila adhabu.

Una haki ya kukataa kujibu maswali fulani. Usiri wako wa kibinafsi utadumishwa katika data yote iliyochapishwa na kuandikwa inayotokana na utafiti.

Maswali yangu yamejibiwa. Uamuzi wangu wa kushiriki au kutoshiriki katika utafiti ni hiari. Ikiwa nitaamua kujiunga na masomo naweza kujiondoa wakati wowote. Kwa kusaini fomu hii, mimi haitoi haki yoyote ambayo mimi kama mshiriki wa utafiti.

_____	_____	_____
jina la Mshiriki	Saini	Tarehe

_____	_____	_____
Mpelelezi mkuu	Saini	Tarehe

Appendix IV: Orodha ya Maswali ya Uchunguzi

Asante kwa kukubali kuchukua utafiti huu. Utafiti huo unafanywa na James Wachira msomi katika Chuo Kikuu cha Jomo Kenyatta kama sehemu ya Mradi wake. Majibu yote unayotoa katika utafiti huu yatakuwa ya siri. Hesabu ya uchunguzi itaripotiwa katika toleo la muhtasari tu na haitamgundua mtu yeyote. Utafiti huu utakuchukua chini ya dakika 20 kukamilisha.

Habari ya Idadi1. Tarehe ya kutembelea (siku / mwezi / mwaka) ___ / ___ / ___

2. Tarehe ya kuzaliwa (siku / mwezi / mwaka) ___ / ___ / ___

3. Je! Hali yako ya ndoa ni ipi? Sio ndoa (haijawahi kuoa)Kuolewa Kugawanywa / Kutengwa Mjane

4. Kufanya kazi Nyingine (taja) _____ Jinsia?
5. ()Historia ya Matibabu5. Je! Ulipimwa li HIV? (_____)

6. Je! Umepata magonjwa yoyote ya zinaa? (_____)

7. Kwa wahojiwa wa kiume. Je! Wewe umetahiriwa?Ndio/ Hapana

Ikiwa ndio, Utaratibu wa kitamaduni au matibabu (_____)

8. Je! Wewe ni mjamzito? Ndio/hapana

9. Je! Unaugua ugonjwa mwingine wowote?Ndio/Hapana

Ikiwa ndio moja? (_____)

10. Je! Ni aina gani ya mfumo wako wa sasa wa ART (_____)?

11. Mabadiliko yoyote ya regimen ya ART?Ndio/Hapana

Ikiwa ndio, ni nini sababu ya mabadiliko? (_____)

12. Je! Ni nini matokeo yako ya hivi karibuni ya upakiaji wa virusi? (_____)

13. Je! Matokeo yako ya hesabu ya hivi karibuni ya cd4 ni yapi? (_____)

14. Je! Umewahi kupimwa syphilis?Ndio/Hapana

Ikiwa ndio, ilikuwa nzuri au hasi? (_____)

15. Je! Una chancre yoyote?Ndio/hapana

16. Je! Umewahi kutibiwa magonjwa ya zinaa?Ndio/ Hapana

Ikiwa ndio, ni ipi, na matokeo yalikuwa nini? (_____)

17. Je! Ni historia yako gani ya kuongezewa damu? (_____)

18. Je! Una historia yoyote ya kupandikizwa kwa chombo? (_____)

Appendix V: Research Coordinators

Research Coordinators are Mary Achieng, Victoria Karoki and Benson Karanja

Their roles include:

- Screening subjects for eligibility using protocol specific inclusion and exclusion criteria, documenting each potential participant's eligibility or exclusion
- Informing participants about study objectives.
- Prepares study materials as requested by the Principal investigator
- Administering questionnaires.
- Adhering to ethical standards.
- Assist the Principal Investigator in sample collection, coding and analysis
- Ensuring that the necessary supplies and equipment for a study are in stock and in working order.
- Engaging with subjects and understanding their concerns.
- Communicate feedback of the study outcome to the participants