MAKERERE UNIVERSITY
COLLEGE OF HEALTH SCIENCES
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DEPARTMENT OF IMMUNOLOGY AND MOLECULAR BIOLOGY

Evaluation of the Diagnostic Validity of the HIV VISITECT CD4 Point-of-Care Rapid Diagnostic Test Using PIMA Analyzer as the Gold Standard in Uganda.

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A RESEARCH REPORT SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN IMMUNOLOGY AND CLINICAL MICROBIOLOGY OF MAKERERE UNIVERSITY

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DECLARATION

The work presented in this proposal is the result of my original research work. Where I have used the works of other persons, due and acknowledgments are clearly stated. No portion of this work has been submitted in support of an application for a degree or qualification to any other university or institute of higher learning. I present it without any reservations for external examination.

Name: Oliver Nakamaanya

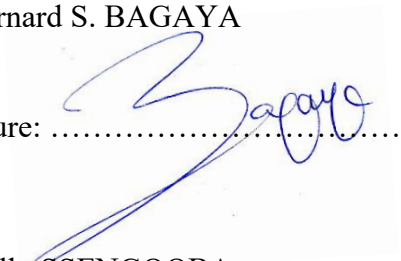
Signature:  Date: November 13,2023

APPROVAL

This proposal has been developed under my guidance and supervision.

Dr. Bernard S. BAGAYA

Signature: ……. Date: November 13,2023



Dr. Willy SSENGOOBA

Signature: Date: November 13, 2023

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ACRONYMS

AHD:	Advanced HIV Disease
AIDS:	Acquired Immunodeficiency Syndrome
CD4:	Cluster of differentiation 4
LFA:	Lateral Flow Assay
HIV:	Human Immunodeficiency Virus
IDI:	Infectious Disease Institute
POC:	Point-of-Care
WHO:	World Health Organization

OPERATIONAL DEFINITIONS

Advanced HIV disease: HIV disease characterized by CD4 count of <200 cells/mL, World Health Organization clinical stages 3 or 4 or any child <5 years of age. [1]

Negative Predictive Value (NPV): Is the probability that subjects with a negative test (CD4> 200 cells/mL) truly do not have the disease (AHD). [2]

Point-of-care testing: Medical testing at or near the site of the client/patient care so as to bring the testing services conveniently and immediately to the client/patient to increase the likelihood that the patient, physician, and the care team will receive the results quicker, and allow for immediate clinical management decisions to be made.[3]

Positive Predictive Value (PPV): Is the probability that subjects with a positive test (CD4< 200 cells/mL) truly have the disease (AHD). [2]

Sensitivity: The ability of the test to detect the health problem (AHD; CD4 <200 cells/mL) that it is intended to detect.[4]

Specificity: The ability of the test to detect a negative result (CD4>200) in people who do not have the disease of interest (ADH). [5]

ABSTRACT

Background: HIV is prevalent in Sub-Saharan Africa with Uganda inclusive. The use of a cluster of differentiation-4 (CD4) markers is widely recommended to support and monitor infection progression. The aim of this study was to compare the diagnostic validity of VISITECT CD4 LFA with instrument-based POC tests (Pima Analyzer) and to explore the factors influencing this performance in the Ugandan setting. **Methodology:** A prospective laboratory-based comparative study was conducted at Kisenyi Health Center IV, Kawaala Health Center IV and Kiswa Health Center IV to evaluate the diagnostic validity of the VISITECT CD4 LFA. **Results:** A total of three hundred fifty (351) subjects were enrolled into this study. Majority were females constituting 238/351 (68%) and 112 /351(32%) were males. The median age was 31 years (IQR 25 - 38). The Mean CD4 count using PIMA was 482 cells (±283.9 cells/µl). The sensitivity of VISITECT CD4 LFA was 86.25 % and Specificity 90.41%. The positive and negative predictive values were 72.63% and 95.70% respectively. All the samples collected and analyzed were venous blood. No factor considered was found to significantly affect the diagnostic validity of VISITECT LFA test. **Conclusion:** Based on diagnostic performance characteristics, VISITECT CD4 LFA is a reliable tool for guiding timely Advanced HIV Disease (AHD) screening among individuals with HIV/AIDS especially in resource limited setting. **Recommendations:** Focus should be on refining the test's accuracy and reducing the observed discrepancies in sensitivity and specificity, ultimately contributing to more precise and timely AHD screening for individuals living with HIV/AIDS.

Key words: CD4, VISITECT, PIMA, HIV

CHAPTER ONE
INTRODUCTION

1.1 Background

Human Immunodeficiency Virus (HIV) remains a highly prevalent disease worldwide, with developing countries inclusive of Uganda impacted most [6]. The epidemiological burden of HIV in sub-Saharan Africa remains unacceptably high. In 2021, 38.4 million (31.1 to 43.9 million) people worldwide were estimated to be living with (HIV), with Sub-Saharan Africa (SSA) accounting for 67% of them. Furthermore, in 2021, SSA was responsible for 670 000 of the 1.5 million new infections and 280 000 of the 650 000 AIDS-related deaths worldwide [7]. The Uganda population-based HIV Impact Assessment [8] found an HIV prevalence of 5.8% among people aged 15 and above. The reported prevalence varied by age category, geographical region, and population risk.

HIV infection progresses to cause severe immunosuppression and, eventually, death. The use of a cluster of differentiation-4 (CD4) markers is widely recommended to support and monitor infection progression [9]. The CD4 cells (also known as CD4+ T cells) are an immunological cell subpopulation of lymphocytes, one of the white blood cells that mount an immune response [10]. Specifically, the CD4 cells serve as an indicator of immune function in HIV infection and remain pivotal to global and national guidelines as part of the battery of tests performed before starting ART [11,12]. Presently, management of HIV starts with conducting baseline investigations such as CD4 cell count and viral load, among others [13,14]. Whereas the World Health Organization (WHO) recommends using viral load to monitor HIV treatment, patients with advanced HIV disease require a CD4 cell count to identify those (CD4 cell counts less than 200 cells/mm3) who require screening for opportunistic infections (including mycobacteria and cryptococcoses) [15–17]. Moreover, CD4 cell counts are important for detecting immunological treatment failure in patients who have already begun ART [14]. Indeed, the Ugandan HIV/AIDS treatment guidelines integrate CD4 testing into HIV care and monitoring [18].

In the past decade in Uganda, laboratory measurement of CD4 T cell counts has relied on Point-of-Care (POC)- or near-POC-based analyzers such as Abbott Pima (Abbott, Chicago, IL, USA) and BD FACS Presto (BD Biosciences, San Jose, CA, USA)[19,20]. The choice between the two analyzers is influenced by differences in diagnostic performance, clinical usability, supply

availability, and the associated operating costs. The Pima Analyzer has been reported to be more user-friendly than others, with more readily available supplies on the local market [21]. Furthermore, as CD4 cell counts fall below the clinical threshold of 350 cells/mL, the accuracy of the Pima analyzer improves [22]. Despite their vast clinical utility, point-of-care CD4 determination approaches are limited by prohibitive repair and operational costs, the need for constant power or batteries, extensive quality control procedures, and highly trained laboratory personnel [16,23]. These requirements have hindered CD4 testing in a variety of resource-limited settings, particularly among rural populations [24]. Also, POC testing are limited by inaccuracies, which are frequently associated with negative clinical decisions and poor patient outcomes, as well as tester capacity and inappropriate environmental storage conditions [25]. Also, analytical incapacities and proficiency gaps have resulted in high errors as more people seek HIV diagnosis [26]. More, inadequate storage conditions and near-expiry testing kits have also been linked to diagnostic errors [26,27]. These limitations portend the diagnostic desirability and global concerted efforts toward HIV care and monitoring. Owing to such limitations, and to meet the targets for decentralization of HIV/AIDS care services recommended by Vision 2030[17], more user-friendly, affordable, and non-instrument-based rapid diagnostic kits are required.

The Omega VISITEC CD4 advanced disease (VISITEC CD4) (Omega Diagnostics Scotland, UK) is one such invention. It is a lateral flow immunoassay (LFA)-based CD4 test that provides a semiquantitative estimation of CD4 cell count at a threshold of 200 cells/mL [17]. As a rapid diagnostic test (RDT), the test is simpler to perform, less laborious, and equipment-free. [11] Available diagnostic performance data indicated a specificity of 95.0% and sensitivity of 81.9% [17]. In our setting, no studies have been conducted to compare the diagnostic performance of the VISITEC CD4 assay to instrument-based POC methods such as PIMA. Moreover, the factors influencing this performance in our context remain unexplored. Therefore, this study aims to compare the diagnostic validity of LFA with instrument-based POC tests (Pima Analyzer) and to explore the factors influencing this performance in the Ugandan setting.

1.2 Problem statement

As more people with HIV enroll in care, the number of patients who require CD4 cell monitoring grows exponentially. As a result, developing countries like Uganda, which is the most affected by HIV, ought to increase their spending on CD4 cell count tests. Challenges with the current

point-of-care CD4 cell count platforms (primarily the Pima analyzer and BD FACs Presto, among others [14,18]) will amplify the budget and, more importantly, impede the dissemination of the much-needed CD4 cell count test to lower-level facilities. Current CD4 count platforms face challenges such as high reagent costs, averagely complex clinical usability, particularly in low-income health facilities, and frequent equipment breakdowns, necessitating routine repairs and scheduled maintenance [11]. Due to these challenges, Uganda's Ministry of Health HIV management program has not attained full dissemination of the PIMA and BD FACS Presto to all HIV/AIDS treatment and care centers in the country.

The VISITECT CD4 LFA is a newer non-instrument POC-based RDT for CD4 estimation used to detect patients with advanced HIV (CD4 counts of less than 200 cells/mL). The assay can be used to inform HIV-related clinical care in lower-level health facilities that do not have the capacity to support PIMA and FACSPresto platforms, providing an alternative to the existing instrument-based POC test. Additionally, the test is user-friendly, non-electricity dependent, has a turnaround time of 45 minutes, uses microsampling, low cost (6.00 Canadian dollars per test) [28]; which attributes makes it suitable for low resource settings. To support its diagnostic utility in our setting, validation data are urgently required. While the VISITECT test kits are now available on the market and address the majority of the limitations of the PIMA and BD FACs presto platforms, adequate performance data to support programmatic dissemination and its utility remains scarce.

Although studies from other settings have shown good diagnostic performance [19,20], no study has evaluated its performance in Uganda.

1.3 Research questions

What is the diagnostic performance and associated factors of the VISITECT CD4 LFA rapid diagnostic kit compared to the PIMA analyzer CD4 POC instrument in Uganda?

What are the factors affecting the VISITECT CD4 LFA rapid diagnostic kit diagnostic performance in Uganda?

1.4 Study objectives

1.4.1 General objective

To compare the diagnostic validity of the VISITECT CD4 LFA rapid diagnostic kit with the PIMA machine and determine factors affecting this validity among patients living with advanced HIV in Uganda

1.4.2 Specific objectives

i. To determine the advanced HIV/AIDS diagnostic validity of the VISITECT CD4 LFA rapid diagnostic kit using the Pima analyzer as the gold standard.
ii. To determine the factors that affect the diagnostic validity of the VISITECT CD4 LFA rapid diagnostic kit.

1.5 Justification

This research study would be among the first to provide evidence through a prospective study on the performance of this novel VISITECT CD4 LFA rapid diagnostic kit in HIV-positive patients in programmatic conditions. The obtained data will be part of the evidence to support or refute the rational deployment of LFA rapid diagnostic tests to facilitate and scale up CD4 testing and clinical decision-making for HIV care in areas where instrument-based techniques are challenging to deploy. This will help detect people with advanced HIV disease who need to receive the AHD care package, which will significantly improve clinical outcomes and prevent progression to very severe AHD. With this non-instrument-based CD4 test, decentralization of HIV/AIDS care will be more feasible. This will inform policymakers on the possibility of integrating the novel VISITECT CD4 LFA rapid diagnostic kit in a Ugandan setting.

As this will be among the first studies to report on the diagnostic performance of the VISITECT CD4 LFA rapid diagnostic kit, its findings will guide the innovations similar to the VISITECT CD4 LFA rapid diagnostic kit, and inform the desirability of such POC to suit their field applicability.

CHAPTER TWO
LITERATURE REVIEW

2.1 Epidemiology of HIV

Global statistics report that in 2021, 38.4 million (31.1 to 43.9 million) people worldwide were estimated to be infected with HIV. Of these, an estimated 25.7 million people live in Africa, and the global HIV epidemic has had the greatest impact on Sub-Saharan Africa accounting for 67% of all HIV infections in the world. Furthermore, in 2021, SSA was responsible for 670 000 of the 1.5 million new infections and 280 000 of the 650 000 AIDS-related deaths worldwide[7]. The Uganda population-based HIV Impact Assessment [29] found an HIV prevalence of 5.8% among people aged 15 and above. Moreover, the prevalence was higher (reported at 7.2%) among women than men (reported at 4.3%). Furthermore, HIV prevalence varied geographically in Uganda, ranging from 2.1% in the Northeast Karamoja region to 8.1% in the Central Buganda region[29].

2.2 Epidemiology of Advanced HIV Disease

According to World Health Organization, AHD is defined as either CD4 cell count less than 200 cells/mL, stage 3 or 4 of WHO clinical staging, or any child less than 5 years of age with HIV[1]. AHD poses significant public health challenges as it is associated with very high viral loads (viremia), fast progression of HIV/AIDS, and hence a higher risk of mortality. It is highly associated with opportunistic infections such as tuberculosis (TB), Cryptococcal meningitis, and other opportunistic infections as well as nutritional deficiencies [18].

It is hence important to screen all people living with HIV for advanced HIV disease for proper treatment which involves rapid ART initiation, TB preventive treatment, and treatment of other opportunistic infections as well as preventing advancement to very advanced HIV disease (CD4 count <100 cells/mL)[16]. Whereas clinical staging can be used to diagnose AHD, CD4 count testing remains the most recommended approach for this diagnosis[18]

2.2.1 Burden of Advanced HIV Disease

Among adults living with HIV, AHD is defined as having a CD4 cell count less than 200 cells/mL or stage 3 or 4 of WHO clinical staging, and it is associated with very high morbidity and mortality even with ART initiation[1]. The burden of ADH among adults globally varies

greatly in different countries. Among newly HIV-diagnosed patients in high-income countries such as China, the prevalence has been documented to be as high as 40%[30]. In Vietnam, a study conducted at twenty-two public HIV clinics among newly diagnosed ART-naïve patients between 2015 and 2017 indicated that 38.6% had CD4 ≤100 cells/μL[31]. By 2018, the prevalence of AHD among PEPFAR-supported countries was found to be between 11-22% of all newly diagnosed adult people living with HIV[15]. A meta-analysis conducted among sub-Saharan countries indicated that most people with HIV present with AHD (average CD4 count of 152 cells/mL at ART initiation[32]. A study conducted in three sub-Saharan African countries (Malawi, Kenya, and South Africa) among newly diagnosed HIV patients showed that the prevalence of AHD ranged from 7.8% to 11.8%, and this was more common among males and those who were not on antiretroviral therapy [33]. Another study in South Africa showed that the prevalence of AHD decreased from 46.8% in 2005 to 32.9% in 2016, with 16.8% having very advanced disease[34]. Other African countries, such as Senegal, reported an even higher prevalence of AHD of 71% among people with HIV newly initiated on antiretroviral therapy [35].

In Uganda, approximately 1.4 million people live with HIV with approximately 17,000 deaths [36], One-fifth of HIV patients starting ART have a CD4 count of less than 200 cells/mL, indicating advanced HIV disease. More, an estimated one-fifth of unsuppressed people living with HIV are returning to care with AHD[37]. The burden of AHD is estimated at 20%, and such patients present with severe life-threatening conditions as such face a high risk of death even after starting ART (WHO, 2017). This may reverse the longtime global concerted efforts to address HIV-related mortality in a high disease-burden setting. Moreover, the literature about advanced HIV disease remains very scant, which leaves a significant knowledge gap regarding the implementation of the AHD care package.

2.3. Diagnostic Validity of a Test

Healthcare involves conducting several tests as either part of the diagnostic or screening process for the different health conditions and the results vary since these tests have varying properties regarding validity which directly impacts their usefulness in practice [38]. Therefore, healthcare practitioners need to understand the diagnostic validity of the various tests so that they can interpret the results accordingly while making clinical decisions.

The validity of a test is defined as its ability to accurately measure what it is designed to measure and in clinical practice, this involves differentiating diseased from non-diseased individuals[39]. Test validity is determined by its sensitivity (ability to detect true positives) and specificity (ability to detect true negative cases). Normally, screening tests should have very high sensitivity rather than specificity to be able to capture all the possible cases for that particular health condition. Conversely, diagnostic tests should be highly specific rather than sensitive for the particular health condition to minimize false positives [40,41].

Studies determining the diagnostic validity of a test achieve so by comparing the test in question with the existing gold standard test which is usually the authoritative test that has been documented to determine the presence or absence of the disease being investigated [41]. Based on results from the two tests, sensitivity and specificity can be calculated as indicated in figure 1 below and expressed as percentages.

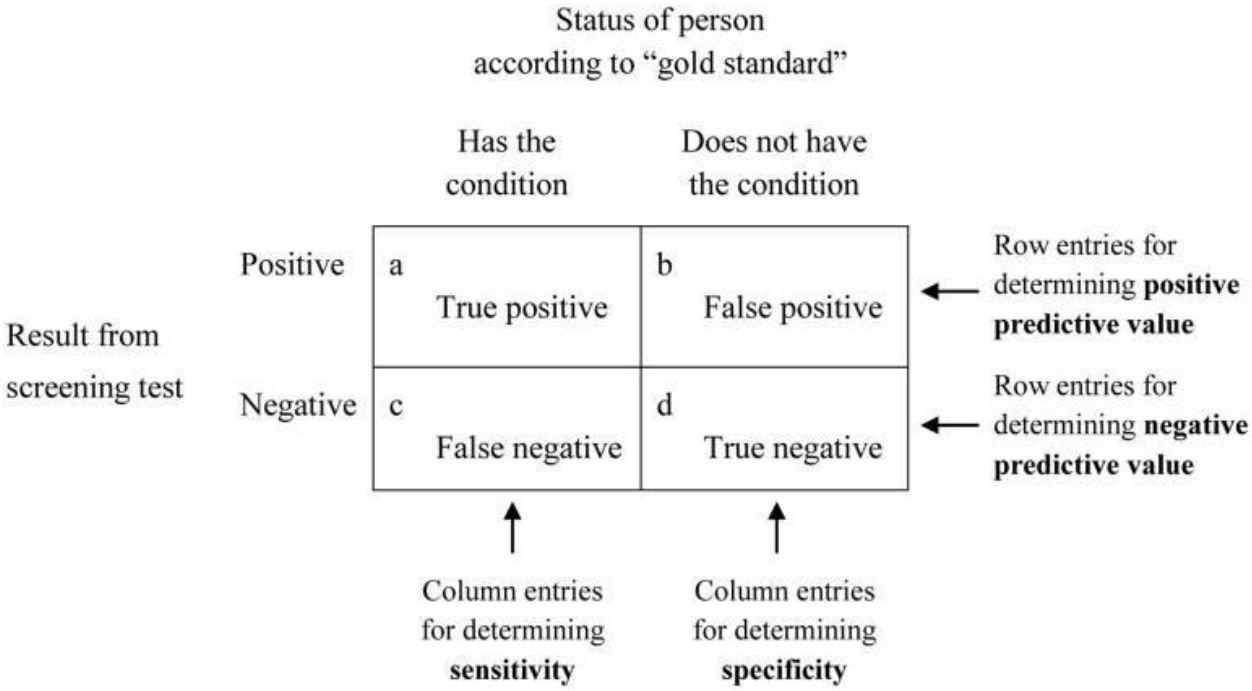

Figure 1 Derivation of sensitivity, specificity, and predictive values of a test.

2.4 Diagnostic performance and factors affecting diagnostic validity of the VISITECT CD4 LFA compared to the Pima analyzer.

Several research advances over generations have brought to light several investigations that can be used to monitor the progress of HIV treatment among people living with HIV/AIDS via viral load or CD4 cell count. These range from laboratory investigations using instruments like the BD FACSCalibur or the Beckman Coulter Cytomic FC 500 platform to near-POC BD presto and point-of-care tests like the Pima analyzer [42]. A retrospective cohort study conducted in Cape Town evaluating rapid point-of-care CD4 testing at mobile units discovered that a high proportion of patients who accepted point-of-care testing were linked to HIV care, indicating a possible benefit of POC CD4 testing in increasing HIV treatment uptake, which could potentially prevent AHD [43].

Thakar et al. (2012) found a significant positive correlation between the Pima analyzer and other laboratory instrument-based CD4 analyzers, such as FACSCount, FACSCalibur, and Cyflow SL3. The sensitivity and specificity of the Pima CD4 analyzer were found to be 96%-91% and 91%-96%, respectively, at a cut-off of CD4350 cell/μL, according to the same study [44].

In Uganda, a study conducted at seven Kampala Capital City Authority clinics on samples from 225 participants to determine the diagnostic performance of the Pima analyzer compared to the FACSCalibur analyzer, sensitivity was 100% and specificity was reported at 99%; at 350 cell/μL, sensitivity and specificity were 91.3% and 89.5%, respectively. At a CD4 count of 200 cells/μL, the sensitivity, specificity, and positive and negative predictive values were 91.9%, 97.3%, 87.2%, and 98.4%, respectively [22].

To date, there are limited studies that have evaluated the diagnostic performance of the VISTECT CD4 LFA. For example, a laboratory validation study for VISTECT CD4 LFA conducted in Botswana using venous blood from one thousand fifty-three participants indicated that the POC test had a sensitivity of 94.1% and specificity of 85.9% with an interrater agreement of 97.5% [45]. However, this study compared the VISTECT CD4 test with flow cytometry, unlike the proposed study.

Another study conducted in South Africa by Luchters et al that assessed the field performance and diagnostic accuracy of the VISTECT CD4 test compared to flow cytometry among pregnant

women with HIV showed a sensitivity and specificity of 81.7% and 82.6% on venous blood and 60.7% and 89.5% on finger prick blood, respectively[28]. However, this study used a CD4 count cut-off of 350 cells/μL, which may have led to the relatively lower sensitivity and specificity.

Unlike the above studies that compared the VISITECT CD4 LFA test with flow cytometry rather than other POC CD4 tests, a study by Ndlovu *et al* conducted among patients with HIV in three African countries compared the diagnostic performance of this test with flow cytometry and other POC CD4 tests. Findings from this study indicated that the VISITECT CD4 test has a sensitivity and specificity of 95.0% and 81.9%, respectively, using venous blood and 98.3% and 77.2%, respectively, using finger prick blood compared to flow cytometry at a cut-off of 200 cells/μL. Compared to the PIMA analyzer, VISITECT CD4 LFA had a sensitivity of 98.3% and a specificity of 77.2% using finger prick blood[17].

2.5. Factors that affect the diagnostic performance of the VISITECT CD4 LFA and Pima analyzer.

Despite the positive attributes of point-of-care testing approaches, and their suitability to meet the prevailing healthcare challenges, numerous factors have been studied and are considered key to their diagnostic performance.

Various studies have assessed the performance of different point-of-care tests at different CD4 cell cut-offs. In these studies, the sensitivity and specificity were reportedly higher at a CD4 cell cut-off below 100cells/ μL[22]

In Uganda, a study conducted at seven Kampala Capital City Authority clinics reported variations in the specificities and sensitivities of the Pima analyzer compared to the FACSCalibur analyzer. Accordingly, these increased accuracies with decreasing CD4 cell count, and the highest diagnostic performance was reported at a CD cell count of less than or equal to 100 cells/μL [22]. In the same study, the instrument diagnostic performance was reduced with increasing CD4 cell counts, and this finding highlighted the critical point of the use of such an analyzer. More, this study reported a variance in diagnostic performance with personnel competence and technical considerations. Specifically, the training staff to use it and the turnover time for processing results were factors that affected the test's performance [11, 20]. Relatedly, in Uganda, a previous study indicated that Pima showed an increased accuracy with decreasing

CD4 cell count, whereby at 100 cell/L, sensitivity was 100% and specificity was 99%; at 350 cell/μL, sensitivity and specificity were 91.3% and 89.5%, respectively. At a CD4 count of 200 cells/μL, the sensitivity, specificity, and positive and negative predictive values were 91.9%, 97.3%, 87.2%, and 98.4%, respectively [22]. Also, Thakar et al. (2012) reported a CD4 cell count correlation with the Pima analyzer and other laboratory instrument-based CD4 analyzers, such as FACSCount, FACSCalibur, and Cyflow SL3. In all these instruments, the diagnostic performance was higher at a lower CD4 cell count, specifically at a cut-off of CD4350 cell/μL [44].

Studies that have assessed the performance of VISITECT CD4 LFA have reported varied factors that affect its diagnostic performance. For example, a study by Ndlovu *et al* reported a sensitivity and specificity of 95.0% and 81.9%, respectively, using venous blood and 98.3% and 77.2%, respectively, using finger prick blood. More, compared to the PIMA analyzer, VISITECT CD4 LFA had a sensitivity of 98.3% and a specificity of 77.2% using finger prick blood [17]. Therefore, from this study, finger prick blood had higher sensitivity and lower specificity compared to venous blood. Also, from this study, other factors were reported to affect the diagnostic performance of VISITECT CD4 LFA including sample incubation period, time lag from processing to reading (re-reading stability), and some participant sociodemographic factors such as sex and age[17]. More, a study conducted in South Africa by Luchters et al reported varied diagnostic performance from varied samples collected using venipuncture and finger prick [28]. From this study, the site of blood sample collection affected the diagnostic performance with venous blood being more sensitive while finger prick blood was more specific.

2.6 Point-of-Care CD4 cell counting

Presently, there are at least three commercially available point-of-care (POC) or near-POC CD4 assays on the market. These include Abbott Pima (Abbott, Chicago, IL, USA), BD FACSPresto (BD Biosciences, San Jose, CA, USA), and CyFlow miniPOC (Sysmex Partec, Goertlitz, Germany). In addition, a recent invention of the VISITECT CD4 LFA which is a non-instrument-based POC rapid diagnostic kit has been rolled out. The VISITECT CD4 LFA is a semiquantitative immunochromatographic assay that can be manually performed to estimate the CD4 cell count at a cut-off of 200 cells/μL using either capillary or venous blood[46]. As the Abbott Pima and BD FACSPresto are routinely used in our setting, their testing framework has presented below;

2.6.1. CD4+ Cell count using Pima™ Analyzer

Pima Analyzer is a point-of-care test used to estimate CD4 cell count working by the volumetric principle and hence giving absolute cell counts [44]. Pima CD4 is an automated, portable bench-top fixed volume cytometer used for image-based immune hematology tests intended for the rapid in vitro quantitative measurement of absolute CD3+/CD4+ T cells (T -helper cells) in capillary or venous whole blood, and is intended to be used for the ongoing monitoring of absolute CD4 lymphocyte counts in patients with a documented diagnosis of immunodeficiency disease. The disposable Pima CD4 test cartridge is equipped with the means to take up approximately 25 µL of sample and contains dried reagents needed to perform the test. After insertion of the Pima CD4 test cartridge into the analyzer, the peristaltic movement first transports the sample into the incubation compartment where the sample interacts with specific antibodies labeled with two different fluorescent dyes emitting light at two different wavelengths (dye 1 and dye 2). One antibody is an anti-human CD3 monoclonal antibody conjugated to dye 1, and the second antibody is an anti-human CD4 monoclonal antibody conjugated to dye 2. After the incubation time, the stained sample is transferred into the detection channel of the cartridge. The Pima analyzer is equipped with miniaturized multi-color fluorescence imaging optics. Fluorescence signals are detected by an onboard camera and analyzed using proprietary software algorithms on board an embedded computer. T-helper cells carry both CD3 and CD4 surface antigens and therefore emit light at wavelengths specific for both antibody-dye conjugates. This allows the specific differentiation of T-helper cells from other blood cell types carrying only one of the two surface antigens. During the course of test processing, data is recorded, analyzed, and interpreted using software embedded in the analyzer. Upon completion of the test, the cartridge is removed from the analyzer and a test result is displayed. The test results are reportable from the analyzer within 20 minutes (https://www.globalpointofcare.abbott/en/product-details/pima-cd4-cartridge.html).

A previous study that assessed the diagnostic performance of the Pima CD4 cell counting approach reported it highly effective in identifying patients with CD4 less than or equal to 100 cells/µL (100% sensitivity, >99% specificity). Moreover, two-thirds of patients tested were accurately detected at a CD4>350 cells/µL, indicating Pima's accuracy in detecting CD cell counts [47].

2.6.2. CD4+ Cell count using BD FACSPresto Analyzer

The BD FACSPresto is based on fluorescence imaging and absorbance reading technology, with embedded software that reads patient samples from a single-use disposable cartridge. It can be operated by a rechargeable battery. The cartridges use dried-down fluorescently labeled antibodies. Integrated software provides automated analysis and calculates results for CD4 absolute counting, %CD4, and total haemoglobin concentration.

A previous study that assessed the diagnostic performance of the BD FACSPresto reported a good correlation was obtained between the BD FACSPresto, Pima, and Facscalibur. The mean difference with absolute CD4+ T-lymphocyte values obtained from the BD FACSPresto system correlated well with PIMA, FACSCount, and FACSCalibur method showed no significant differences and further analysis revealed a close agreement between all three instruments with no significant difference between the methods [48].

Both the point-of-care methods present desirable features including, shorter turnaround times, and ease of sampling as they can use capillary blood samples, and may use a battery. In addition, the two analyzers perform internal quality control using inbuilt cartridge beads and require minimal operational space. Despite these attributes, the analyzers are costly as they require considerable initial capital investment on instrumentation, usually more than USD 5,000 United States Dollars, as well as continuous service and maintenance for instrument repairs. It has also been previously reported that instrument-based POC CD4 technologies are prone to breakdowns and can generate a considerable amount of invalid test results, therefore, adding to the test cost [49].As such, there is a need for instrument-free and affordable POC CD4 technologies amenable to decentralization to expedite the implementation of the AHD package which has a mortality benefit.

2.6.3. CD4+ Cell count using VISITECT CD4 LFA

Developed by the Burnet Institute, the Omega VISITECT CD4 Advanced Disease Lateral Flow Assay (Visitect CD4 LFA) (Omega Diagnostics, Scotland, UK),

It is a disposable and instrument-free POC test that can provide actionable information for HIV/AIDS care in settings such as developing countries where laboratory CD4 testing is not

feasible for all patients with HIV[50]. Since it detects the CD4 cell count at a cut-off of 200 cells/μL, this test is useful for detecting patients with AHD.

Although limited studies have evaluated the diagnostic performance of the VISITECT CD4 LFA test, one study by Ndlovu *et al* reported a sensitivity and specificity of 95.0% and 81.9%, respectively, using venous blood and 98.3% and 77.2%, respectively, using finger prick blood compared to flow cytometry at a cut off of 200 cells/μL. Compared to the PIMA analyzer, VISITECT CD4 LFA had a sensitivity of 98.3% and a specificity of 77.2% using finger prick blood[17]. From this study, the VISITECT CD4 LFA test is a promising equipment-free technology and may suit most limited resource settings as well as field applications. It is simpler to perform and may offer more attributes including non-electricity dependence as well as micro sampling (Burnet Institute, 2019). With its fieldable advantage, the need for validation studies is timely, and may upscale HIV care, particularly AHD at risk of opportunistic infections and treatment monitoring[50].

CHAPTER 3. METHODS

3.1 Study design

This was a laboratory-based comparative study to evaluate the diagnostic validity of the VISITECT CD4 lateral flow immunoassay (LFA) with that of the PIMA analyzer, which is an instrument-based point-of-care machine and the factors that affect the sensitivity and specificity of the LFA.

3.2 Setting

The study was conducted at three district health centers; Kisenyi Health Center IV, Kawaala Health Center IV and Kiswa Health Center IV. Clinical information and blood samples were obtained from patients undergoing routine CD4 monitoring at the centers and analyzed at the respective laboratories on-site. The sites offer general medical, surgical, and laboratory diagnostic services mainly to people in Kisenyi, Kawaala and Kiswa and the neighborhoods. They also offer general HIV diagnostic and treatment services.

Kisenyi, Kawaala and Kiswa Health Center IVs are located in Kisenyi Central division, Kampala Lubaga division and Bugolobi Nakawa division respectively and they are both under the jurisdiction of Kampala Capital City Authority (KCCA) and the HIV clinics there are run by Infectious Disease Institute (IDI). Makerere University houses the Infectious Disease Institute (IDI) main offices.

3.3 Study population

3.3.1 Target population

Objective 1 of this study used left-over clinical samples collected from HIV patients for purposes of monitoring their CD4 counts at Kawaala HCIV, Kiswa HCIV and Kisenyi HCIV. This was because these facilities utilize PIMA CD4 test platforms in their routine care.

Objective 2 of study described selected factors that affect the validity of the VISITECT CD4 LFA. Factors to consider included storage conditions of the VISITECT CD4 LFA kits, interrater agreement on result interpretation, age, gender and opportunistic infections.

3.3.2 Inclusion and Exclusion

Inclusion criteria

1. Samples for CD4 analysis for advanced HIV disease screening, ART initiation, ART monitoring or follow-up CD4 count
2. Samples with adequate volume to complete the study assays.
3. Samples that are less than 24 hours old from the time of collection
4. All health workers involved in CD4 analysis at the selected study sites

Exclusion criteria

1. Samples exceeding 24 hours from the time of collection.
2. Samples with little volume and not able to complete the test
3. Health workers not involved in CD4 analysis

3.3.3 Sample size determination

The sample size was determined based on proportions. The sample size estimation for anticipated sensitivity and specificity was determined by Buderer's formula for incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity[51].

Sample size (n) based on sensitivity $=\frac{Z^2 \times S_N \times (1-S_N)}{L^2 x\ Prevalence}$

Sample size (n) based on specificity $=\frac{Z^2 \times S_P \times (1-S_P)}{L^2 x\ (1-Prevalence)}$

where n = required sample size,

Z^2 = Critical value of Z-distribution =1.96

S_N = anticipated sensitivity of VISITECT CD4 LFA =98.3%, and S_P = anticipated specificity of VISITECT CD4 LFA = 77.2%, reported among patients living with HIV in three selected African countries [17].

L = margin of error =5%

Prevalence = prevalence of advanced HIV (CD4 Cell count <200 cells/mm3) determined using PIMA from various health facilities across the country = 14.5% in 2016 [21]

After inserting the figures in the formula above;

Sample size for sensitivity, $n_{sensitivity} = 177$

Sample size for specificity, $n_{specificity} = 316$

Specificity requires a large sample size; hence, it is what was considered.

By adding 10% to cater to the sample non-viability rate (32), the required sample size was 348.

The respective proportions of samples at the different study sites will be determined according to the ratios of participants at the respective sites.

3.4 Sampling and recruitment procedures

Consecutive sampling was used. All samples fulfilling the inclusion criteria were consecutively included into the study until the required sample size was reached.

3.5 Data collection and Laboratory methods

Data collection was conducted in two phases from 1st October 2023 to 30th December 2023: 1) From the analysis of left-over clinical samples that are collected for CD4 analysis. These samples were run concurrently on the PIMA and the VISITECT CD4 LFA 2) Using self-administered questionnaires that were given to health workers involved in CD4 analysis at the selected facilities.

3.6 Principle of VISITECT CD4 LFA

VISITECT CD4 LFA is a disposable Point of care test (POCT) that offers an estimation of the CD4 protein on the surface of CD4+ T cells, providing semi-quantitative results at a threshold of 200cells/mm^3.

The VISITECT CD4 Advanced Disease Rapid Test is an immunochromatographic assay that estimates full-length CD4 protein associated with CD4+ T cells in human whole blood and is directly correlated with CD4+ T cell levels. A capture monoclonal antibody (MAb) specific for the cytoplasmic domain of CD4 is applied as a line on the nitrocellulose membrane. Whole blood

is added directly to the VISITECT CD4 Advanced Disease Rapid Test where red blood cells and monocytes are retained in the blood collection pad and following the addition of buffer, other white blood cells (including CD4+ T cells) migrate to a reaction area where cell lysis occurs, resulting in the release of full-length CD4 for capture in the test strip. Colloidal gold-labeled MAb conjugate against CD4 binds the captured CD4 and forms a test line. These complexes are visualized as a pink/purple line. A reference line (200 lines) is included to allow the estimation of CD4 levels by comparison to a set cut-off (equivalent to the signal level generated by samples containing 200 CD4+ T cells/μL)[50].

3.7 Sample Description

The study used left-over clinical samples that were collected for purposes of CD4 monitoring. The samples have to be collected in tubes containing the Ethylenediaminetetraacetic acid (EDTA) anticoagulant and with a minimum volume of 2mls. These samples were anonymized with unique identification numbers and run in the respective laboratories on both the VISITECT LFA and the PIMA simultaneously.

For CD4 LFA, the collected sample were then incubated for 3 minutes to remove red blood cells, add 1 drop of buffer, and then incubate for 17 minutes to release CD4 cells, which were captured by the test strip. Finally, 3 drops of buffer were added to form the monoclonal-antibody conjugate, which was used for the reference and control lines on the test kit (see figure 2). The results were interpreted after 20 minutes according to the standard operating procedure by visual inspection of the intensity of the formed test line in relation to the reference line. If the test line was darker than the reference line, this shows a CD4 count of > 200 cells/mm3, while a similar or fainter test line compared to the reference line shows CD4<200 cells/mm3. Visual inspection of each test was performed by two independent blinded technicians or service providers, and any disagreements were resolved by involving the principal investigator.

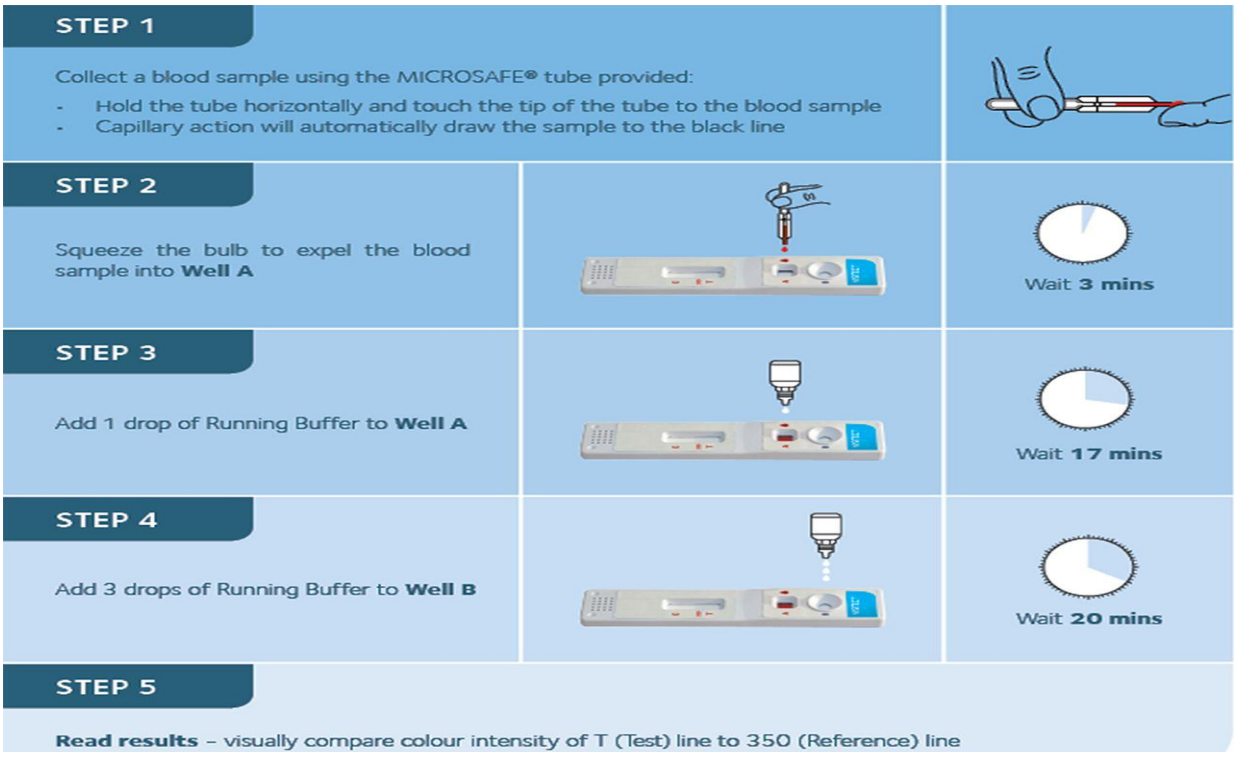

Figure 2: Procedure and timing of steps in conducting VISITECT CD4 LFA

For PIMA, the collected sample were processed as per the laboratory and standard operating procedure in the PIMA CD4 device manufacturer's manual [52,53]but in summary; To initiate a test run and open the cartridge slot door, the operator pressed✓. The cartridge slot door opens and the Pima Analyzer prompts the operator to insert a cartridge and the door was closed by pressing ✕returning the operator to the «Run test» window. The cartridge was inserted into the Pima Analyzer in the direction indicated by the arrow on the cartridge, upon inserting the Pima test cartridge, a sensor in the Pima Analyzer recognized that a cartridge has been inserted and automatically starts the analysis process by fully drawing the cartridge into the Pima Analyzer and closing the cartridge slot door. The display screen changed briefly to «Reading cartridge» at this moment. Once the Pima Analyzer has accepted the cartridge, it prompted the operator to enter both the Operator and Sample ID. During data entry, the test analysis continued in the background. After the analysis was successfully completed, the «Analysis done. Remove cartridge» window appeared and prompted the operator to remove the cartridge from the Pima

Analyzer. Once the cartridge was removed, the Pima Analyzer automatically displayed the first of four result windows, showing the Sample ID and test result and Test Report was printed at this time.

The results gave absolute CD4 cell counts from the collected sample. All these procedures and interpretations were conducted by a well-trained technician or any other healthcare service provider at the health facility.

After conducting the above procedure, the technicians or any other healthcare provider completed a self-administered questionnaire assessing opinions regarding the usability of both the CD4LFA and PIMA. Prior to completing the questionnaire, it was developed and tested in two facilities and the feedback from the healthcare workers were used to fine-tune it for final dissemination.

The results from all procedures were collected and entered into Microsoft Excel sheet. The questionnaire also captured additional information about other independent variables, such as participant demographics and those related to sample collection.

All collected data were password protected and hard copies kept under lock and key and only accessible by or with permission from the principal investigator. The forms were checked for completeness or incomplete data for which respective laboratory records or persons were contacted to obtain the missing information if any.

3.8 Data analysis

The data was cleaned, coded, and then imported into STATA version 17 for analysis. Data quality assessment was performed monthly. Listings of missing data and data inconsistencies were checked regularly and corrected to obtain the most complete and cleanest final database for data analysis.

Data about the sociodemographic characteristics of participants were expressed as the mean or median with corresponding standard deviations or interquartile ranges for continuous variables, whereas frequencies and percentages (proportions) were reported for categorical variables. The measurement of the CD4 count for the Pima analyzer was evaluated at the recommended cut-off of 200 cells/mL (AHD) against VISITECT CD4 LFA.

The sensitivity, specificity, and positive and negative predictive values of VISITECT CD4 LFA were calculated and expressed as percentages relative to the Pima analyzer with their corresponding 95% confidence intervals.

Additionally, factors that could affect the validity of the VISITECT LFA results were assessed. These include interrater agreement on result interpretation by two independent technicians that were assessed using the Cohen's kappa index according to the formula:

$$\kappa = \frac{p_o - p_e}{1 - p_e} = 1 - \frac{1 - p_o}{1 - p_e},$$

Where: P_o = the relative observed agreement among raters, and P_e = the hypothetical probability of chance agreement. Only interrater agreement of "near perfect" (0.81 – 0.99) to "perfect" (1) were considered. Other factors to be described were: Storage conditions of the VISITECT CD4 LFA kits, age, gender and opportunistic infections.

3.9 Ethical considerations

The study was reviewed and approved by the School of Biomedical Science (SBS) and the Research Ethics Committee (Makerere University College of Health Sciences REC). With the above approvals, administrative approval was sought from Kampala Capital City Authority (KCCA) prior to conducting research at the facilities.

Participation in the study was entirely voluntary for both the patients and the health workers. Participants were informed that they may withdraw at any time and that they were to receive the same tests and treatment irrespective of whether they participated or not. Participants who were not included in the study or who had refused to be part of it or who had decided to withdraw received standard health care without any negative consequences related to this decision.

Participants' study information was not released outside of the study without the written permission of the participant, except as necessary for monitoring by Ethics Review Committee. This shared data was de-identified to protect participant confidentiality. Confidentiality was always maintained at all times. All study-related information were stored securely by the lead investigator. All laboratory specimens, reports, data collection instruments, process logs, and administrative forms were identified by a coded number to maintain participant confidentiality.

Study records were restricted to the study team and the database was password-protected for security of access. Confidentiality of the data provided to us was maintained by assigning unique identifiers to samples.

3.10 Dissemination plan

At the end of the study, a report was written highlighting the important findings, and copies was made available at all study sites for reference by the clinicians and patients. Policymakers will also receive policy briefs extracted from the findings for consideration during the formulation of policies related to the management of AHD. A dissertation was written and submitted to the Department of Immunology and Molecular Biology and the University library. Finally, the manuscript will be written and submitted for peer review and publication in a peer-reviewed scientific journal.

CHAPTER FOUR

RESULTS

4.1 Sociodemographic and clinical characteristics of study subjects

A total of three hundred fifty one (351) subjects were enrolled into this study. Of the 351 study subjects, majority were females, constituting 238 (68%) and 112 (32%) were males. The median age in the study participants was 31 years (IQR 25 - 38). All the 351 subjects had valid results on both PIMA and VISITECT and were include in the final analyses. The Mean CD4 count using PIMA was 482 cells (±283.9 cells/µl). The prevalence of Advanced HIV disease (AHD) subjects at a CD4 count threshold of ≤200 cells/µl of blood was 22.79% (Table 1).

Table 1 Demographic and CD4 count characteristics of the study participants

	Overall population n =351	PIMA ≤200 CD4 cells/µl n= 80	PIMA >200 CD4 cells/µl n=271
Gender			
Female n (%)	238(68%)	47(19.75%)	191(80.25%)
Male n (%)	112 (32%)	33(29.46%)	79(70.54%)
Age (years), median (IQR)	31 (25-38)	31(27-38)	31(24-38)
Mean (SD) CD4+ PIMA	482 (±283.9)	107 (±63.1)	531 (±248)

4.2 Performance Characteristics of the PIMA

Ninety-five (95) subjects were classified as positive i.e., having Advanced HIV Disease by the VISITECT and two hundred fifty-six (256) as negative i.e., did not have AHD by the VISITECT. Using results from the PIMA as the gold standard, there were 11 false negative and 26 false positive AHD results. At the currently recommended AHD cutoff (≤ 200 CD4 cells/µl) using PIMA as a gold standard in this study, the sensitivity of VISITECT CD4 LFA was 86.25 % and Specificity 90.41%. The positive and negative predictive values were 72.63 and 95.70

respectively (Table .2).

Table 2 VISITECT Performance characteristics at the ≤200 CD4 cells/µl threshold.

		PIMA		
		≤200cells/µL (AHD +)	**>200cells/µL (AHD -)**	
VISITECT	**≤200cells/µL (AHD +)**	**69**	**26**	**95**
	>200cells/µL (AHD -)	**11**	**245**	**256**
Total		**80**	**271**	**351**
Sensitivity %, (95% CI)			**86.25 (76.73 – 92.93)**	
Specificity %, (95% CI)			**90.41 (86.26 – 93.64)**	
PPV %, (95% CI)			**72.63 (64.57-79.44)**	
NPV (95% CI)			**95.70 (92.78-97.48)**	
LHR+ (95% CI)			**8.99 (6.17-13.09)**	
LHR-, (95% CI)			**0.15 (0.09-0.26)**	
Accuracy			**89.46 (85.76- 92.47)**	
Prevalence			**22.79%**	

4.3 Reasons for clinical request of a CD4 Count test

Overall, Majority 204/351 (58.12%) of the participants were tested for CD4 Count as a requirement for ART initiation, followed by clinical suspicion of AHD 122/351 (34.76%). Among those being tested to initiate ART, the VISITEC CD4 LFA test found that 142/204 (69.61%) had CD4 count >200 cells/µl, indicative of AHD while PIMA machine detected 149/204 (73.03%) of those tested for initiation of ART (Figure 4). Of those tested for initiation

of ART, PIMA and VISITEC LFA detected 55/204 (26.96%) and 62/204 (30.39%) respectively as having CD4+ Count of ≤200 cell/µl (Figure 5).

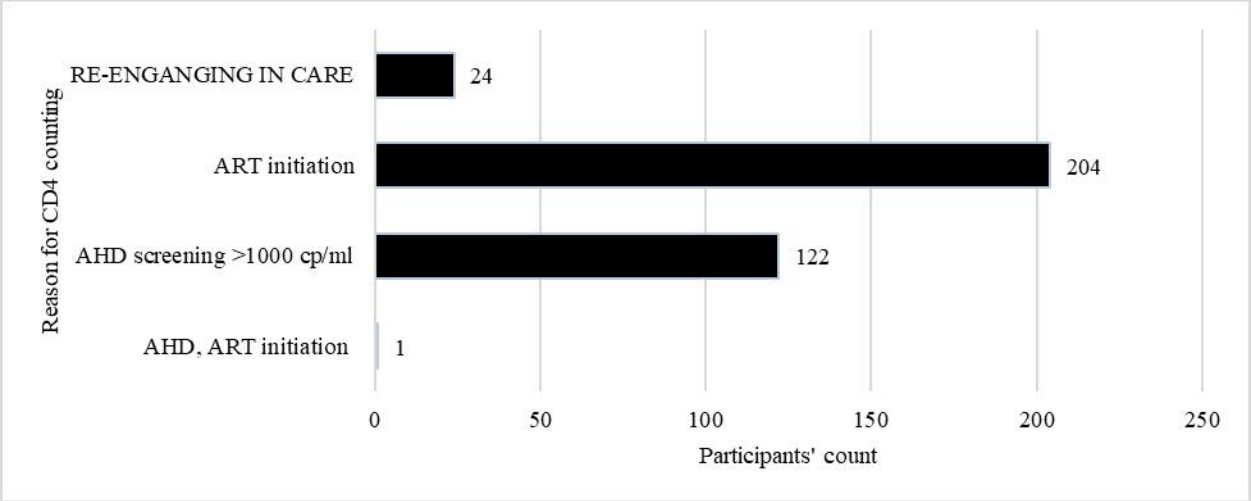

Figure 3 Number of participants based on reason for CD4+ Cell counting

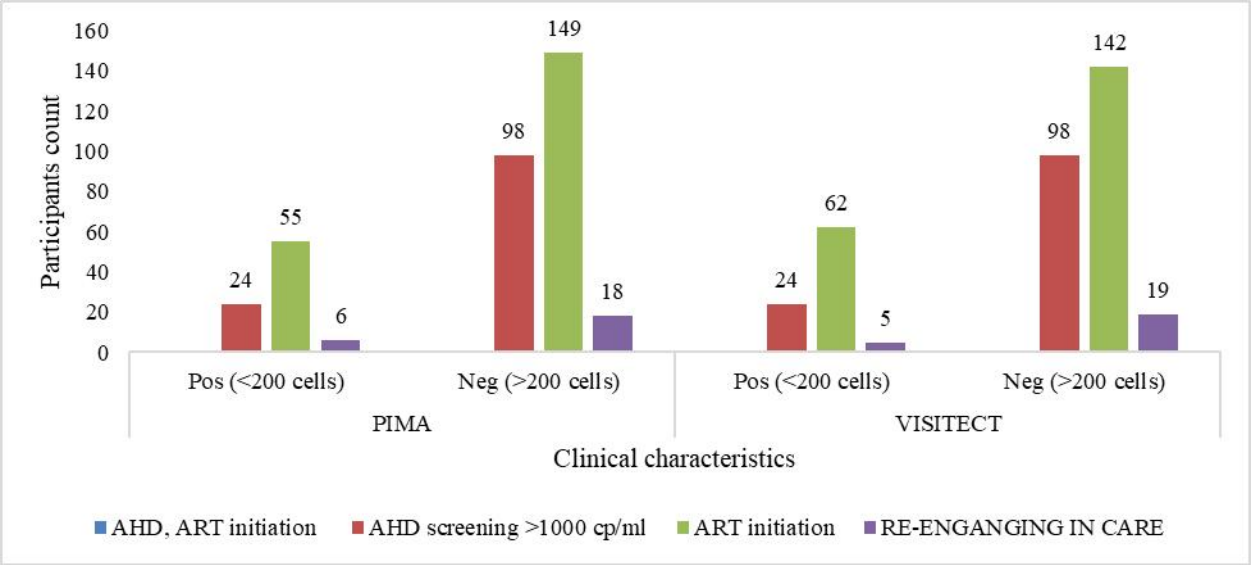

Figure 4 Number of participants based on CD4 Cell counts and reasons for Testing

4.4 Factors Affecting the diagnostic validity of the VISITECT CD4 LFA rapid diagnostic kit

All the 3 laboratories used in the study had environmental condition monitoring (temperature and humidity) and they are all within acceptable ranges. The Laboratories were participating in EQA with pass rate of EQA varying from 8-9 of 10. The staff had the required professional qualifications and had in-house training on VISITECT used by the PI and the overall interrater agreement was 95.7% (Cohen's kappa 0.957). All the samples collected were venous blood. No factor considered was found to significantly affect the diagnostic validity of VISITECT LFA test

Table 3 Analysis of factors affecting diagnostic validity of VISITIECT LFA

Factor	**PIMA/VISITECT Agreement**		**Chi value (X^2)**	**P value**
	Yes, n(%)	**No, n(%)**		
Opportunistic infection			10.128	0.072
Candidiasis	6 (100.0)	0 (0.0)		
Cryptococcal infection	5 (100.0)	0 (0.0)		
Hypertension	0 (0.0)	1 (100.0)		
Tuberculosis	18 (90.0)	2 (10.0)		
Pneumonia	1 (100.0)	0 (0.0)		
None	285 (89.62)	33 (10.38)		
Smoking			0.9356	0.333
Yes	8 (100.0)	0 (0.0)		
No	307 (89.5)	36 (10.5)		
Alcohol intake			2.008	0.156
Yes	44 (95.65)	2 (4.35)		
No	271 (88.85)	34 (11.15)		
Interrater Agreement				
Cohen's Kappa	0.957			

CHAPTER FIVE
DISCUSSION

The results of this study provide valuable insights into the performance characteristics of the VISITECT LFA test in determining AHD based on CD4 cell count, using the PIMA test as the gold standard. The findings revealed a sensitivity of 86.25% and a specificity of 90.41% at the recommended AHD cutoff of ≤ 200 CD4 cells/µl. Additionally, the positive predictive value (PPV) was 72.63%, and the negative predictive value (NPV) stood at 95.70%. These values offer important information regarding the accuracy and reliability of the VISITECT LFA test in identifying individuals with AHD. To contrast with a related study done by Ndlovu *et al* compared to the VISITEC CD4 LFA to PIMA analyzer, VISITECT CD4 LFA had a sensitivity of 98.3% and a specificity of 77.2% using finger prick blood[17]. Therefore, the sensitivity and specificity of VISITEC CD4 LFA in this study was slightly lower compared to the previous study. This difference specificity could be attributed to various factors such as differences in the study populations, variations in sample collection or handling, differences in the diagnostic techniques used, or potential variations in the performance of the VISITEC CD4 LFA and/or PIMA across different settings or conditions.

The sensitivity of 86.25% indicates that the VISITECT test correctly identifies a significant proportion of individuals who are truly classified as having AHD based on the gold standard, PIMA. This suggests that VISITECT is can be important in detecting valuable percentage of those who require timely AHD care package, which is crucial for managing HIV/AIDS and preventing disease progression to very AHD. However, it is imperative to note that there were 11 false negatives, implying that some AHD individuals may be misclassified by VISITECT as non-AHD. This could potentially delay the initiation of the AHD care package which would be life-saving, emphasizing the importance of further refining the test to reduce false negatives.

On the other hand, the specificity of 90.41% is indicative of the VISITECT test's ability to accurately identify individuals who do not have AHD. This is vital in preventing unnecessary treatment and associated costs with reduced turnaround time (TAT) of CD4 test results. This can as well be important in the implementation of the universal test and treat strategy hence

improving management and treatment outcomes of HIV patients especially in resource constrained settings. However, the 26 false positives observed in this study suggest that a notable number of individuals may be wrongly identified as AHD by VISITECT. This can lead to unnecessary emotional distress and expenses of implementing AHD management package on the side of the HIV program. Hence, reinforcing the need to optimize the test's specificity.

The PPV of 72.63% indicates the probability that an individual identified as AHD by VISITECT LFA is truly eligible. The high NPV of 95.70% is reassuring, indicating that when VISITECT LFA identifies an individual as non-AHD, it is highly likely to be accurate, minimizing the risk of missing individuals in need of treatment. In resource-constrained settings, where access to advanced diagnostics may be limited, the VISITECT LFA test shows promise as a valuable tool for AHD assessment. The VISITECT CD4+ LFA test demonstrates favorable sensitivity, specificity, and negative predictive value at the recommended AHD CD4+ cell count cutoff. However, efforts should be directed towards improving its positive predictive value and reducing false negatives and false positives to enhance its clinical utility. As a point-of-care diagnostic tool, VISITECT LFA holds promise in expanding access to timely AHD diagnosis and optimizing healthcare resources in the fight against HIV/AIDS. Additionally, considering factors such as cost-effectiveness, accessibility, and ease of use will be vital in optimizing the practical utility of VISITECT CD4 LFA in resource limited healthcare settings.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The findings from this study emphasize the potential of VISITECT CD4 LFA as a reliable tool for guiding timely Advanced HIV Disease (AHD) screening among individuals with HIV/AIDS especially in resource limited setting. In comparison to PIMA, VISITECT LFA based on its test diagnostic performance characteristics (sensitivity, specificity, positive and negative predictive values) can improve management and treatment outcomes of HIV patients and minimize progression to Advanced HIV disease (AHD).

6.2 Recommendations

Future research should focus on refining the test's accuracy and reducing the observed discrepancies in sensitivity and specificity, ultimately contributing to more precise and timely AHD screening for individuals living with HIV/AIDS. The study's results should be considered in the context of patient outcomes and long-term treatment adherence as these factors play a crucial role in HIV/AIDS management. As a point-of-care diagnostic tool, VISITECT CD4 LFA holds promise in expanding access to timely AHD screening and optimizing healthcare resources in the fight against HIV/AIDS in resource limited settings.

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APPENDICES

3.9 Work plan

Task	2023							
	Feb	March	April	May	June	July	Aug	Sept
Proposal writing								
Submission to college								
IRB approval								
Administrative approvals								
Sample collection								
Data analysis								
Dissertation and manuscript writing								
submission								

4.0 Budget

ITEM	**QUANTITY**	**UNIT COST(USD)**	**TOTAL COST (USD)**
VISITECT AHD KITS	20	99.50	1990.00
PIMA CD4 CARTRIDGE KITS	8	1350.00	10800.00
TIMERS	6	13.52	81.12
CRYOVIALS	500	5	340.00
IRB	1	30.00	30.00
OTHERS		1000.00	1000.00
TOTAL			**14241.12**

4.1 Data collection tools

HEALTH CARE WORKER

DATE:…../…../…… CLINICIAN ID ……

STUDY SITE /HIV CLINIC ……. AGE ……. GENDER ……..

PROFESSION OF HCW……

HIGHEST LEVEL OF TRAINING

Certificate ….. Diploma…. Bachelor's degree…..

Have you had any formal training to use VISITECT CD4 test? YES….. NO…….

Trained by: 1) Organization:……

2) In-House:……..

Type of sample collected: Venous…… Capillary (finger prick)……

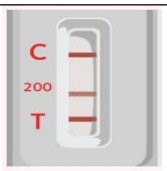
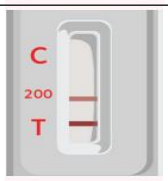
Time of collection……..

Time of processing ……

Participant's CD4 cell count: PIMA …….. VISITECT…….

Test result interpretation

	Test result example	Result Interpretation			Comment
1.		¬ Positive	¬ Negative	¬ Invalid	
2.		¬ Positive	¬ Negative	¬ Invalid	
3.		¬ Positive	¬ Negative	¬ Invalid	

4.		¬ Positive	¬ Negative	¬ Invalid	
5.		¬ Positive	¬ Negative	¬ Invalid	
Score / Number of correct				**……../ 5**	**…… %**

FACILITY

1- How many samples do you work on in a week?
2- Do you have a storage unit
3- Are its temperatures and Humidity monitored?
4- Does the lab participate in external quality assurance (EQA) for CD4 analysis?
5- What is the pass rate on the last three EQA cycles?

PARTICIPANT

Participant's ID ……..

Age……..…. Gender ……..

Occupation…….. Region…….

1- What is your reason for CD4 counting? (Tick the most appropriate)

1) Advanced HIV disease screening
2) ART initiation
3) Re-engaging in care

2-Do you have any opportunistic infection? (Circle what applies)

Tuberculosis (TB) Pneumonia Salmonella infection

Candidiasis Toxoplasmosis. None

3-Do you have any non-communicable diseases? (Circle what applies)

Diabetes Cancer Heart disease

Chronic lung disease Mental health condition None

4- Are you a smoker?

YES NO

5- Do you drink alcohol?

YES NO

If 'YES' how many drinks a day?