The diagnostic accuracy of the HIV 1/2/subtype O Tri-line HIV rapid test in comparison to ELISA

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Dentistry.

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Declaration

I Shumani Charlotte Manenzhe declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Dentistry (MDent) in Oral Medicine and Periodontology at the University of Witwatersrand, Johannesburg. Neither the whole work nor any part of it has been submitted for any degree or examination in this or any other University.

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Signature of candidate

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Abstract

Background: Accurate HIV diagnosis is critical and can be life-saving. A Rapid Test (RT) is considered key to HIV prevention and management. Some studies have found RT to be comparable with ELISA whilst others have reported on lower sensitivity.

Aim and study design: The aim of this retrospective comparative descriptive study was to evaluate the sensitivity and specificity of the Tri-line HIV rapid test device in comparison to ELISA on patient records from Wits Oral Health Centre (WOHC) between 2014 and 2016

Method: The study population comprised records of patients older than18 months who had Tri-Line HIV RT and blood drawn for ELISA on the same day. Descriptive analysis of the data was carried out.

Results: The sensitivity of Tri-line was 80% (CI: 59-93%) and specificity was 100% (CI: 83-100%). The PPV was 100% (CI: 83-100%) and NPV was 80% (CI: 65-90%). ROC area of 0.9 at 95% CI was determined.

Conclusion: Due to a low sample size in this study a definitive conclusion could not be drawn. However on the basis of the results obtained, although the tri-line RT showed lower sensitivity it was shown to be a clinically useful test.

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My sister Vhuli I am so thankful for all the days you took my children so I can work and study.

Dedication

I dedicate this work to my husband Tami, my children (Murangi and Vhutali), my parents, my siblings and rest of my family and friends who supported, prayed with me and offered help when needed.

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Abbreviations and acronyms

AB/Ab:	Antibody
AG/Ag:	Antigen
AIDS:	Acquired immune deficiency syndrome
ART:	Antiretroviral therapy
ARV:	Antiretroviral
CI:	Confidence interval
CICT:	Client-initiated counselling and testing
CMJAH:	Charlotte Maxeke Johannesburg Academic Hospital
DNA:	Deoxyribonucleic acid
EBV:	Epstein-Barr Virus
ELISA:	Enzyme-linked immunosorbent assay
HAART:	Highly active antiretroviral therapy
HCT:	HIV counselling and testing
HIV:	Human immunodeficiency virus
HPV:	Human papilloma virus
HSV:	Herpes simplex virus
HTS:	HIV testing services
IgG:	Immunoglobulin G
IgM:	Immunoglobulin M
KSHV:	Kaposi's sarcoma-associated herpesvirus
MSM:	Men who have Sex with Men
NGO:	Non-governmental organisations
NPV:	Negative Predictive Value

PCR:	Polymerase chain reaction
PICT:	Provider-initiated counselling and testing
POCT:	Point-of-care HIV testing
PPV:	Positive Predictive Value
QA:	Quality assurance
RNA:	Ribonucleic acid
ROC:	Receiver Operating Characteristic
RT:	Rapid HIV test
RTs:	Rapid HIV tests
UNAIDS:	Joint United Nations Programme on HIV/AIDS
VCT:	Voluntary counselling and testing
VZV:	Varicella zoster virus
WHO:	World Health Organization
WOHC:	Wits Oral Health Centre

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1. CHAPTER 1

1.1 Introduction and Literature review

The human immunodeficiency virus (HIV) infection is a global problem, a burden to health care system costing governments substantial amounts of money annually. There are two known primary HIV strains: HIV-1 and HIV-2; with HIV-1 found throughout the world whilst HIV-2 predominates in West Africa (Agbelusi *et al*., 2013). However given the high rate of intercontinental and cross-country migration the two strains can be expected anywhere in the world. The HIV-1 and HIV-2 strains have several subtypes. The HIV-1 strain is reported to have four groups: the "major" group M, the "outlier" group O, groups N and P. In group M there are at least nine genetically distinct subtypes (or clades) of HIV-1. These are subtypes A, B, C, D, F, G, H, J and K (Lihana *et al*., 2009, Agbelusi *et al*., 2013). See Figure 1.1 below.

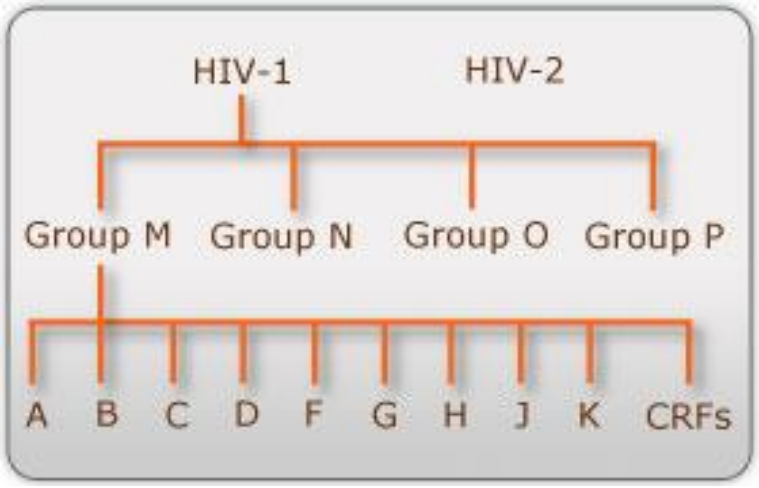

Figure 1.1 HIV strains, and their sub-classification into different groups and subtypes (Agbelusi *et al*., 2013)

The knowledge of the different strains of HIV is critical for all stakeholders involved in the diagnosis and management of HIV including associated diseases. The knowledge of the strains has improved diagnostic tests where currently most HIV tests incorporate both HIV 1 & 2 and others depending on regions, the different subtypes. In a highly globalized society it is imperative that the country of origin of the patient being tested is considered, because the patient may present with an HIV strain not prevalent or seen in that particular country.

It was estimated that 37 million people were living with HIV globally in 2016 (UNAIDS, 2016). South Africa, in particular has a generalized and maturing HIV epidemic, with the highest number of people infected with HIV world-wide estimated at 6.4 million (National HIV Testing Policy and Guidelines, 2016). There are also a substantial number of individuals who are unaware of their HIV positive status. Hence, in South Africa HIV infection represents the primary burden of disease amongst young and old people. The high prevalence in South Africa is mainly as a result of high rates of new infections on a daily basis; and the scale-up of antiretroviral treatment (ART)/ highly active antiretroviral therapy (HAART) which has increased life expectancy among individuals living with HIV (Shisana *et al*., 2014, Bor *et al*., 2013).

Intervention programs have been introduced, with education and screening at the forefront of these interventions to mitigate this catastrophe. The education and screening is aimed at educating the public about HIV and identifying individuals who have been infected so as to prevent re-infection or development of new infections. The key intention is to prevent the transmission and spread of the virus by those found to be infected once they know their status. The persistence of the HIV pandemic is reported to be in part the result of the inability to comprehensively test all at-risk individuals and failure to identify early infections (Louie *et al*., 2008).

A comprehensive approach central to HIV intervention has been introduced in the form of HIV testing services (HTS) at healthcare facilities. This is aimed at reducing the impact of the HIV epidemic from the government and societal point of view. It is comprised of a full range of services provided which include: the actual HIV counselling and testing (HCT); linkage of patients to appropriate HIV prevention, treatment, other clinical and support services; and coordination with laboratory services to support quality assurance and the delivery of correct results (National HIV Testing Policy and Guidelines, 2016) . Such interventions require close collaboration at all health service delivery system levels in NGOs, the public, and private sectors for the efficiency of point-of-care HIV testing (POCT). HCT is applied in the form of client initiated counselling and testing (CICT) previously VCT (voluntary counselling and testing) which is initiated by the patient, and provider initiated counselling and testing (PICT), which is initiated and recommended by the healthcare provider. The aim of PICT is early identification of patients for whom there is a high suspicion index of HIV infection due to presenting clinical signs and symptoms, high-risk sexual behaviour, or high HIV prevalence (National HIV Testing Policy and Guidelines, 2015, 2016).

Several methods used to test for HIV using different techniques have evolved over time in an effort to address the HIV/AIDS epidemic; these are shown in Table 1.1. The evolution of rapid tests (RTs) is focused on technologies with capabilities to detect early HIV infection based on the ability to detect various HIV markers as shown in Table 1.1 and Figure 1.2. In South Africa the recommended HIV testing involves the use of HIV RTs for children older than 18 months and adults utilising a specified algorithm (to be discussed below); whilst HIV polymerase chain reaction (PCR) is recommended for children younger than 18 months (National HIV Testing Policy and Guidelines, 2016).

Table 1.1 Different types of HIV tests

Type of test	**HIV markers detectable**		
	RNA/ DNA *	**Antigens**	**Antibodies**
PCR/viral load	X		
p24 only test (Ag)		X	
4th generation antigen (P24)/antibody (Ag/Ab) tests combo: ELISA or newer RT combos		X	X
3rd generation RT finger prick and oral swab test (Ab)			X
* Viral genetic material (qualitative and quantity)			

Guide to HIV testing: http://www.i-base.info/

Rapid testing has been integrated into the prevention and treatment programs, providing access for HIV testing to numerous people. Rapid HIV testing employed as a screening and diagnostic test is considered one of the key interventions in the national response to HIV and AIDS in South Africa (National HIV Testing Policy and Guidelines, 2015). RT programmes have significantly increased the number of people tested for HIV globally with approximately 60% of people reported to be aware of their HIV status in 2015 (UNAIDS, 2016).

Figure 1.2 shows the different RTs that can be used and compares the different generation's window period and the sequence of appearance of laboratory markers for HIV detection. The 1st and 2nd generation RTs are no longer in use. The 3rd generation RT detects Immunoglobulin G (IgG) or Immunoglobulin M (IgM) HIV antibodies whilst the 4th generation RT detects p24 antigen and IgM/IgG HIV antibodies. The 4th generation is shown to have a shorter window period when compared to the commonly used 3rd generation RT. This has been attributed to the additional detection of p24 antigen.

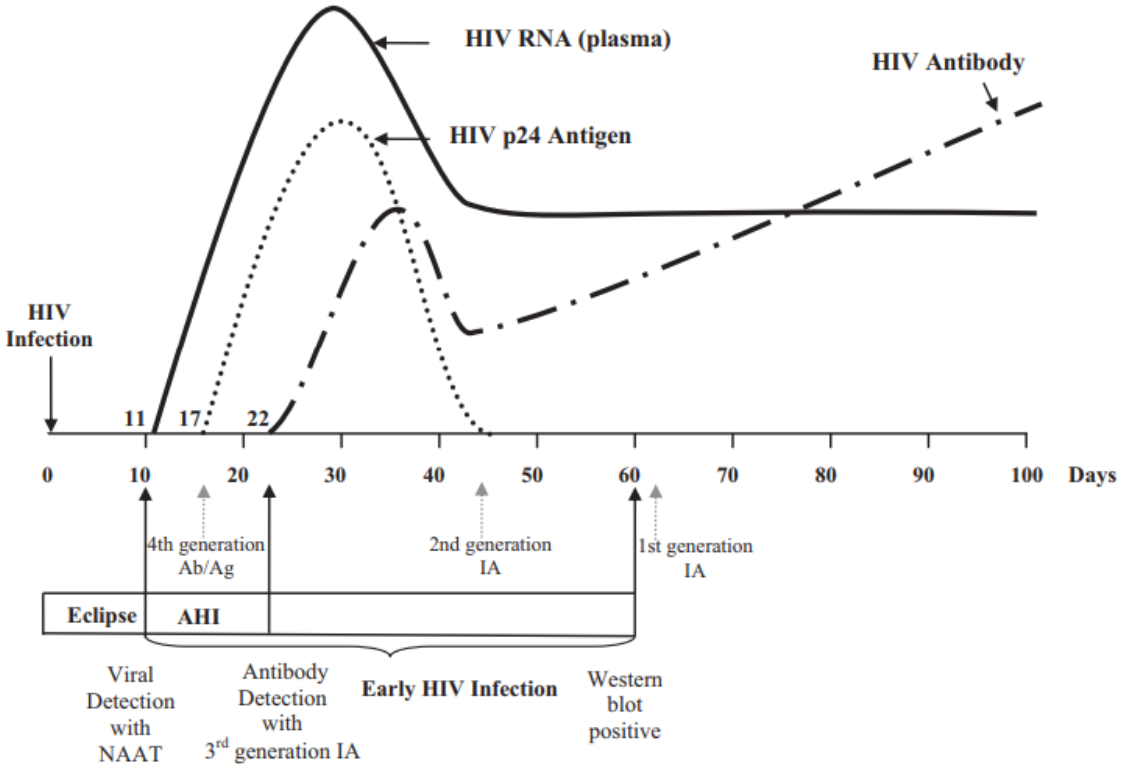

Figure 1.2 Sequence of appearance of laboratory markers for HIV-1 infection and window period for the different generations of RT (Patel *et al*., 2012, Branson *et al*., 2014).

The line graph shows the different laboratory markers for the different generation RT the intervals at which HIV infection can be detected. Eclipse period: the time after HIV acquisition when HIV RNA may be present in very small quantities but is undetectable. Nucleic acid amplification testing (NAAT) appears first at about 10 days Acute HIV infection (AHI): phase of early HIV infection when HIV RNA and p24 antigen (at about 15 days after infection) are detectable but HIV antibodies (detectable at about 22 days after infection) are not. Immunoassay (IA); Antibody (Ab); Antigen (Ag).

The 3rd generation RTs are widely used standard RTs and use immunochromatography for the detection of HIV antibodies in whole blood collected from a finger prick, and have been in use since the late 1980s at numerous testing sites globally in outreach, POCT), and nonclinical settings (Branson, 2000, Wolpaw *et al*., 2010, Patel *et al*., 2012, Adetunji *et al*., 2018). This test is a diagnostic tool of choice in resource limited areas due to low cost, relative ease of use, speed in obtaining results (≤ 30 minutes). Furthermore it minimizes the rate at which clients fail to return for test results (Wolpaw *et al*., 2010, Patel *et al*., 2012, Moodley *et al*., 2008). It is a convenient and non-invasive practical way to provide information about HIV status on an individual basis and in large groups of people with results

available on site during the same visit (Kassler *et al*., 1997, 1998). The test is performed in the presence of the patient thus incorrect labelling of the specimen is minimized (Moodley *et al*., 2008). Other HIV RTs make use of saliva samples that are also interpreted at the point of testing (Pilcher *et al*., 2010).

To promote access to POCT, HTS has been extended to non-health care facilities and integrated into community HIV prevention programmes (Bock *et al*., 2017). This promotes access to HIV screening and informs health authorities on possible intervention strategies in centres where individuals or groups can be accessed and where access to laboratory services is limited (Johnson *et al*., 2015); such as in outreach programmes where RTs are crucial for timely identification of individuals infected with HIV, and for instituting HIV prevention strategies including treatment (Louie *et al*., 2008). High levels of competencies have been shown amongst counsellors in outreach programmes at community level in studies done in South Africa and Malawi (Jackson *et al*., 2013, Molesworth *et al*., 2010, Bock *et al*., 2017).

HIV RTs allow a timeous identification of those infected with the virus in emergency rooms, doctor's consultation rooms, and clinics to facilitate appropriate treatment (Louie *et al*., 2008). This is especially in cases where establishing a diagnosis of HIV infection is critical for clinical decision-making and timely provision of appropriate therapy (National HIV Testing Policy and Guidelines, 2015) as an HIV diagnosis can be life-saving. This forms part of the PICT to aid in the management of patients and also support the upscaling of HIV testing as part of HIV prevention strategy.

The HIV testing algorithms were introduced to improve the accuracy of HIV testing; and involve the use of RTs that have been designed to achieve predictive values close to 100 %, either in sequence (serial testing) or parallel (parallel testing). In parallel testing two RTs are used simultaneously each test being a check on the other (Mbachu *et al*., 2015) and when there is discordant results, a third RT is used as a tiebreaker. This approach has been challenged and reported to result in high rates of misdiagnosis (Johnson *et al*., 2017a, b). Serial testing on the other hand involves the use of two RT kits with sensitivity $\geq$ 99% as per WHO (World Health Organization) recommendations (Mbachu *et al*., 2015) and is the recommended approach in South Africa. A serial 2-test algorithm for HIV diagnosis has been recommended for RTs that allows linkage of the individual to appropriate services. The 2 tests constitute an initial screening RT and a second confirmatory RT (National HIV Testing Policy and Guidelines, 2015).

In the recommended serial 2-test algorithm, the 2 antibody RTs are employed sequentially with serial laboratory based ELISA (enzyme-linked immunosorbent assay) tests where needed as follows (National HIV Testing Policy and Guidelines, 2016):

1. When the screening RT is reactive, a confirmatory RT is then performed to confirm the positive result of the screening test. If the confirmatory test is reactive then the diagnosis is positive for HIV.
2. When the screening test is non-reactive then the diagnosis is negative for HIV and result should be reported as such. However the possibility of recent exposure must be considered and when deemed necessary the period of re-testing should be determined. Retesting for window period is recommended after six weeks from the possible date of exposure and should be determined based on patient's perceived risk to help determine the frequency.
3. When there is discrepancy between the 2 tests then the tests are to be repeated immediately, if still discordant then whole blood is drawn for an ELISA test which is employed as a tiebreaker in determining the HIV status. The patient is then requested to return within seven days for the results which can take up to 2 weeks.
4. In the laboratory a serial testing algorithm using fourth generation ELISA is conducted. If the initial ELISA result is non-reactive, a negative result is reported, and if results are reactive, a positive result is reported. When the results of the two ELISA tests are discordant and not resolved by further re-testing the HIV testing should be repeated after six weeks or as determined based on assessed patient risk.

There are currently several HIV rapid diagnostic tests and all the WHO prequalified RTs have a sensitivity of ≥99% and specificity ≥98% and are reported to be accurate when used correctly in a validated national testing algorithm (WHO, 2004, Johnson *et al*., 2017a).

1.1.1 Challenges with HIV RT

The standard RTs only test antibodies against HIV, and individuals only test positive after seroconversion, which is the time between infection and the generation of detectable antibodies. Hence it is reported that although the sensitivities of rapid HIV tests for established HIV infection are high, the results are variable when it comes to the detection of HIV infection prior or during the early seroconversion period (Patel *et al*., 2012). A RT that

tests for antibodies in whole blood would read negative if the patient has not seroconverted or in those with agammaglobulinaemia or severe immunosuppression, who even though infected, may not have sufficient and detectable antibody titres. Patients often find it particularly difficult to understand that a negative result can be positive 3 to 6 weeks later. A study by Patel and colleagues highlights missed opportunities for HIV diagnosis and prevention. In their study the researchers found that RTs failed to detect early HIV infection in half of the cases studied. Sensitivities for early HIV infection ranged from 55–57% with the third generation and 76–88% with the fourth generation RTs (Patel *et al*., 2012). This is in agreement with Figure 1.2 that shows the time lines following exposure to HIV to identification of HIV infection or missed diagnosis depending on RT used.

Individuals with false negative results can transmit the infection to others, thus making the intervention programmes counterproductive. The communication of inconclusive HIV results and coping with the uncertainties that come with it, is difficult for both the health care provider and the patient (Johnson *et al*., 2017a). Those who test whilst on HAART may also test negative and if not followed up they may think they are cured and default on treatment; hence the importance of education and need for laboratory HIV testing. These and other factors are the primary drivers for a decreased sensitivity with RT (Bock *et al*., 2017).

Rapid tests in infants pose challenges as infants may receive antibodies from an infected mother and test positive as a result, even when not infected. Moreover, infants, due to their underdeveloped immune systems, may not produce antibodies in response to the HIV infection and subsequently have negative test results (WHO, 2005). Hence in this group of patients the PCR test is most suitable, especially for those under 18 months old (National HIV Testing Policy and Guidelines, 2016).

Studies have shown that on site RTs do not yield the same accuracy as tests performed in laboratories (Moodley *et al*., 2008, Wolpaw *et al*., 2010, Bock *et al*., 2017). Although RTs increase HIV status awareness in large communities, they often fail to detect early HIV infection with resultant dire consequences and missed opportunities for HIV prevention (Patel *et al*., 2012). It has also been suggested that RT kits may underperform and fail to detect a substantial number of infected individuals (Wolpaw *et al*., 2010, Patel *et al*., 2012). This also works against the national strategic plan to reduce HIV infections by 50% (National HIV Testing Policy and Guidelines, 2015) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) goal to identify 90% of individuals infected with HIV by 2020. The

ability to detect HIV infection in recently infected individuals is of paramount importance because the highest rate of HIV transmission per coital act has been found to occur in the early stage of infection (Wawer *et al*., 2005). The early infections contribute significantly to high prevalence of HIV infection (Shisana *et al*., 2014, Patel *et al*., 2012).

Although high levels of competencies have been shown for RTs, even amongst lay counsellors, inadequate quality assurance (QA) and user error contributes to poor performance of RTs leading to misdiagnosis (Bock *et al*., 2017). This was shown in a study that reported higher sensitivity and specificity when RTs were performed by skilled laboratory technicians in comparison to lay counsellors and nurses (Moodley *et al*., 2008). Human error and suboptimal testing strategies are reported to have a significant impact on HIV misdiagnosis (Johnson et al., 2015, Johnson *et al*., 2017b, Bock *et al*., 2017). Figure 1.3 depicts several points in the HIV testing continuum where misdiagnosis and errors can occur. As depicted on Figure 1.3, HIV misdiagnoses and testing errors cannot be pinned down to a single cause or underlying factor as the diagnostic errors can occur across multiple steps within the HIV testing continuum (Johnson *et al*., 2017a).

One of the challenges is the subjectivity in the interpretation of positive bands on RTs especially the weak positive bands (Gray *et al*., 2007) that can be interpreted as false negative or positive. In their study, Wolpaw *et a*l., 2010 found the most significant contributor to inaccurate results to be errors in the administration of tests and not following the recommended testing algorithm. Johnson et al. confirmed that the use of suboptimal testing algorithms was the cause for misdiagnosis especially in false positive results. In the same review the researchers found that retesting of patients on HAART was the most common cause of false negative results (Johnson *et al*., 2017b).

The reported reduced sensitivity and specificity devalue the long term cost effectiveness of RTs. Furthermore Moodley and colleagues emphasised user dependent reliability of RTs, particularly in the interpretation of weak positive band on test devices (Gray *et al*., 2007, Shanks *et al*., 2013, Klarkowski *et al*., 2014). This finding is corroborated by Mwisongo and colleagues who observed that the quality of RT is highly compromised by poor adherence to manufacturer's guidelines (Mwisongo *et al*., 2016).

Although current commercialised RTs meet the required international performance specifications; there are manufacturer variations in sensitivity and specificity which have a significant impact on the sensitivity (ability of the kit to correctly detect specimens

containing HIV antibodies) and specificity (ability of the kit to correctly detect specimens that do not contain HIV antibodies) (Moodley *et al*., 2008, Mwisongo *et al*., 2016).

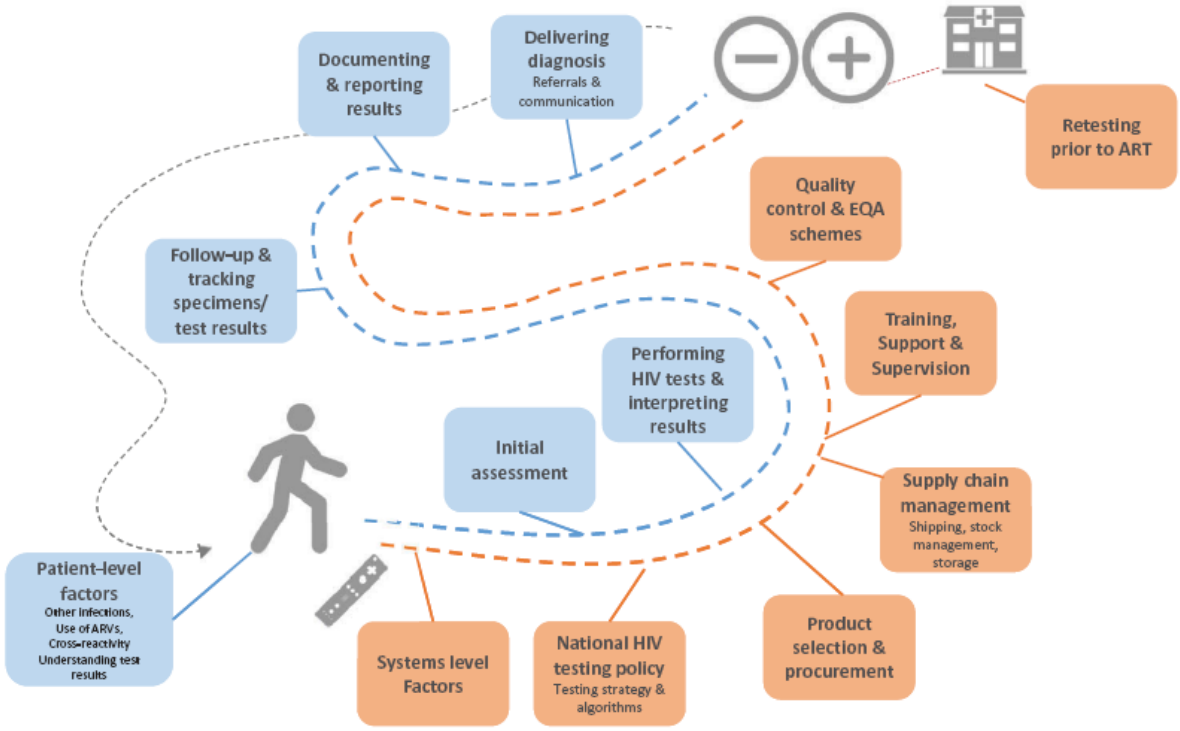

Figure 1.3: HIV testing continuum and the patient, provider, facility and system-level settings where diagnostic errors and HIV misdiagnosis can occur. From Johnson *et al*., 2017b

HIV misdiagnosis with RTs is believed to be under-reported. The contributing factors include programme reputation and publication bias (Johnson *et al*., 2017a). This underreporting limits discussions and investigation that are required to determine possible causes of misdiagnosis so that efforts can be made to address them systematically. This is important as misdiagnosis has deleterious individual and public health implications.

1.1.2 Studies on RTs

A study in five African cities including Durban in South Africa assessed three HIV rapid antibody tests (the OraQuick, Determine, and Unigold) found the RTs to have high sensitivity and specificity, similar to or slightly below the values indicated in the package insert for each test kit. Whilst all test kits achieved above 98% sensitivity and specificity none demonstrated 100% sensitivity and 100% specificity (Piwowar-Manning *et al*., 2010). In another study from Uganda evaluating three RTs namely, Determine HIV-1/2/O, Stat-Pak Ultra-Fast, and Uni-Gold Recombinant HIV-1/2 test against ELISA reported low sensitivity and specificity.

Of 295 positive results, 129 were found to be false positive whilst 4 of 1222 negative results were false negative. Of the 129 false positive results, 123 were obtained from Determine and Uni-Gold tests (Gray *et al*., 2007). In addition the researchers found 37 samples with weak positive bands to be negative on ELISA and Western blot. Overall 94% of weak positive bands were not confirmed as positive by ELISA (Gray *et al*., 2007).

A study conducted in a clinic in Cape Town reported on a period during which rapid HIV testing sensitivity was estimated at 68.7% with more than 1,100 HIV positive individuals having received negative results (Wolpaw *et al*., 2010). In this study after the change of one RT to another, the sensitivity was increased to up to 95%. Moodley and colleagues however, in their study found that HIV rapid test results were comparable with ELISA results especially when performed by laboratory technicians with sensitivity and specificity at 100%. They reported sensitivity and specificity of RTs up to 97% and 98% by lay counsellors and nurses respectively (Moodley *et al*., 2008). The four RTs included in the study were: First Response HIV Card 1-2-0; Pareekshka HIV Triline; Abbott Determine HIV 1\2; and Sensa. In another study in India RTs fared poorly when compared to ELISA (Mehra *et al*., 2014).

In view of the limited ability of antibody-only-RTs to detect acute HIV infection, a 4th generation RT capable of detecting HIV antibodies and the p24 antigen has been introduced (Chetty *et al*., 2012, Beelaert and Fransen, 2010). Combination of the 2 technologies in a RT, facilitates the detection of early infections prior to seroconversion, particularly during the acute phase of infection when individuals are highly infective and asymptomatic (Chetty *et al*., 2012, Beelaert and Fransen, 2010). This early detection of acute HIV infection is important for clinical diagnosis and the prevention of viral transmission. This combo test should be considered an alternative diagnostic and screening tool for antigen detection in high-risk population groups in resource constrained settings.

The 4th generation RT in one study was reported to detect early HIV infection in 76% of studied specimens which was twice that of the 3rd generation RT (Patel *et al*., 2012), similar to the findings by Beelaert and Fransen, 2010. In the latter study, the 4th generation RT was able to detect 82% acute HIV infection in specimens due to the sole presence of the HIV Ag bar in the combo test and showed a higher sensitivity when compared to the antibody only test. Although a significant number of early infections were detected, five of the seven negative results were weakly reactive and the remaining two were positive when tested by Vironostika ELISA test and with the Vironostika ELISA test. The Determine^{TM} RT thus

showed lower sensitivity (82%) for acute infections than the 92% sensitivity attained with the Vironostika ELISA test (Beelaert and Fransen, 2010).

A Kwazulu-Natal study on pregnant women evaluated a 4th generation antigen/antibody RT against a 3rd generation antibody RT and found that although the 4th generation RT was not able to detect acute HIV infection, it showed superior sensitivity in antibody detection when compared to the widely used 3rd generation RTs (Chetty *et al*., 2012).

From the studies above, the antigen and antibody RTs are shown to be less sensitive than the ELISA and detect HIV antigens when the viral load is high (Beelaert and Fransen, 2010, Eshleman *et al*., 2018). The variability in RT sensitivity favours the use of ELISA as a mainstay intervention particularly in high risk individuals.

1.1.3 The use of ELISA

The use of immunoassay technology, the gold standard for HIV diagnosis, has improved substantially over time, with most tests replaced by 4th generation HIV-1 and -2 antigen plus antibody combination immunoassay (Miller, 2015). Although false negative and positive results are possible the 4th generation immunoassay tests are reported to be more sensitive and specific as they can be reactive even prior to seroconversion. The interval between infection and a reactive result is reported to be 14-21 days (Miller, 2015). The increased sensitivity of the 4th generation immunoassay was also shown by Patel and colleagues who found RT sensitivity for early HIV infection to range from 22-33% compared to 76–88% for the 4th generation immunoassay (Patel *et al*., 2012). However Moodley and colleagues found that 98% -100 % of HIV rapid test results were comparable with ELISA (Moodley *et al*., 2008).

Beelaert and Fransen, 2010 found in their study that the 4th generation Determine^{TM} HIV-1/2 Ag/Ab combo RT exhibited a slightly lower sensitivity for the detection of viral Ag when compared to the Vironostika HIV Uni-Form II Ag/Ab ELISA test. The lower sensitivity of the 4th generation RT in comparison to the 4th generation ELISA was also reported by Patel and colleagues (Patel *et al*., 2012). Thus the antigen and antibody HIV RTs were reported to be less sensitive than the ELISA and detect HIV antigen when the viral load is high (Beelaert and Fransen, 2010, Patel *et al*., 2012, Eshleman *et al*., 2018). From the above studies ELISA has shown higher sensitivity than 3rd and 4th generation RTs, hence it is considered the gold standard when it comes to HIV testing.

Although ELISA is the gold standard, its widespread use is hampered by several factors including complexity in the collection and processing of venous blood, the transportation and appropriate storage of the specimens, particularly in resource-poor settings, where access to electricity or refrigeration may be inadequate or absent (Pilcher *et al*., 2010). In addition, laboratory testing may also have errors with sample mislabelling leading to patients receiving false results. Hence RTs, have been widely adopted as the standard of care in most testing sites and identification of those infected remains the cornerstone of a global HIV infection prevention strategy (Pilcher *et al*., 2010).

1.2 Summary

HIV RTs are important health promotion tools and their usage increases awareness of HIV status. They have also paved the way for use in non-health facilities and emergency units other than recognized clinics in areas with increased HIV infections (Pilcher *et al*., 2010).

Due to the challenges faced when testing infants it is recommended that, results be interpreted with caution given an increased rate of false negative and false positive results in this population (WHO, 2005). The specificity and sensitivity of RTs are unreliable in infants and are largely dependent on timing of the test in relation to the period during which the infection was acquired.

The prevention of new infections and/ or re-infections in a country like South Africa where multiple new infections occur daily necessitates more accurate and reliable tests to be administered as part of the HTS prevention and treatment programme. Although, some studies have reported the performance of RTs and laboratory-based enzyme-linked immunosorbent assay (ELISA) to be comparable (Moodley *et al*., 2008, Mehra *et al*., 2014), concerns regarding sensitivity and specificity of the RTs (Gray *et al*., 2007, Wolpaw *et al*., 2010) warrant significant consideration given that the sensitivity and specificity of RTs may be affected by user training and competency, testing environments, the testing algorithm used, test kit handling and storage as well as performance of a specific test kit (Bock *et al*., 2017).

Previous studies have demonstrated the limitations of the 4th generation RT when compared to ELISA (Chetty *et al*., 2012, Beelaert and Fransen, 2010, Eshleman *et al*., 2018). With the reduced sensitivity and specificity, the long term cost effectiveness of the 4th generation RTs has to be thoroughly interrogated.

Although the use of ELISA bears great advantage over RTs, the need for adequate laboratory infrastructure makes it inappropriate for use at on-site testing facilities considering the current infrastructure and resource constrains.

1.3 The rationale for the study

This study was based on an observation made in the Oral Medicine clinic at the WOHC where a number of patients who presented with Group 1 lesions as described in the EC-Clearinghouse Classification (Table 1.2) and were testing negative with RTs and positive with ELISA when requested. Following this a request was made for patients presenting with such lesions to have both tests performed. Although meant for European and United States populations, this classification is widely used in other parts of the world including Africa. Consensus on the classification of the oral manifestations of HIV infection and their diagnostic criteria based on presumptive and definitive criteria was reached in 1992. The presumptive criteria alludes to the clinical appearance of the lesion (high suspicion index for suspected lesion/condition) whilst the definitive criteria refers to diagnosis following special investigation (ECC/WHO, 1993).

The EC-Clearinghouse Classification groups lesions into Group 1, 2 and 3; based on the frequency of their occurrence amongst HIV positive patients (see Table 1.2). Group 1 lesions represent those lesions commonly seen with HIV infection. Hence, in clinical practice, where a person's HIV status is unknown and presents with such lesions, there should be a high suspicion index for HIV infection (Maeve *et al*., 2005, Shangase *et al*., 2004) and such patients should be tested. The first line test in the clinic is the RT. For patients presenting with Group 1 lesions the two testing techniques are recommended even if the RT results are negative. Some of the lesions in group 2 and 3 such as HPV infections, TB, salivary gland enlargement, herpes zoster, etc. are common in our setting and when present are highly suggestive of HIV.

Oral lesions may be the first clinical signs suggestive of HIV infection and may thus be used for early clinical diagnosis and management of such patients(Greenspan *et al*., 1992, Agbelusi *et al*., 2013). Expectedly, the oral cavity is a reservoir for most micro-organisms that cause oral lesions associated with HIV. These include viruses (KSHV, EBV, and HPV etc.), *Candida albicans,* and several bacterial species; and co-infection with HIV may lead to their reactivation or potentiation of their virulence.

Increased awareness of lesions strongly associated with HIV in the public and amongst other health care providers outside dentistry is necessary. This can assists key stakeholders in education, prevention programmes and integration of oral health when developing interventions to curb the epidemic.

Table 1.2. Classification of oral lesions associated with HIV

Group 1: Lesions strongly associated with HIV infection	**Group 2: Lesions less commonly associated with HIV Infection**
Candidiasis: *Pseudomembraneous candidiasis* *Erythematous candidiasis* *Angular cheilitis* Hairy leukoplakia Kaposi's sarcoma Non-Hodgkin's lymphoma Periodontal diseases *Linear gingival erythema (LGE)* *Necrotizing periodontal diseases*	Bacterial infections: *Mycobacterium tuberculosis* Melanotic hyperpigmentation Necrotizing (ulcerative) stomatitis Salivary gland disease *Dry mouth due to decreased salivary flow* *Unilateral/bilateral swelling of salivary glands* Thrombocytopenia purpura Non-specific ulcerations Viral infections: *Herpes simplex virus (HSV)* *Varicella-zoster virus (VZV)* *Herpes Zoster* *Human papillomavirus (HPV)* Condyloma acuminatum Focal epithelial hyperplasia Verruca vulgaris
Group 3: Lesions seen in HIV infection **Bacterial infections:** *Actinomyces israelii* *Escherichia coli* *Klebsiella pneumonia* *Cat-scratch disease* *Epitheliod (bacillary) angiomatosis* Drug reactions (ulcerative, erythema multiforme, lichenoid, toxic epidermolysis) Fungal infection other than candidiasis Cryptococcus neoformans *Geotrichum candidum* *Histoplasma capsulatum* *Mucoraceae (mucomycosis/zygomycosis)* *Aspergilus flavus* Neurological disturbances: *Facial palsy* *Trigeminal neuralgia*	

Adapted from ECC/WHO, 1993. EC-Clearinghouse and WHO Collaborating Centre on Oral Manifestations of HIV infection

The routine testing protocol applied at the WOHC (Wits Oral Health Centre) is as follows: Patients are informed and counselled before and after testing regardless of the results. The RT is performed after obtaining the patient's informed consent. For positive or discordant results venous blood is drawn for ELISA. The ELISA used during the period of the study was the 4th generation Siemens Advia Centour assay that detects HIV p24 Antigen and Antibodies to HIV 1, Including Group O (HIV-1 + "O") and/or HIV-2. If the RT result is negative, it is accepted as such and no further testing is done. The RT test used at WOHC as first line is the ABON™ HIV 1/2/O Tri-Line HIV RT device (ABON Biopharm (Hangzhou)

Co. Ltd, China) referred to in the text as Tri-line. The HIV 1/2/O Tri-line HIV rapid test device used is a rapid chromatographic immunoassay for the qualitative detection of antibodies to HIV-1, including subtype O, and HIV-2 in venous and capillary whole blood, serum and plasma specimens. The product can be used as an aid in the diagnosis of HIV infection. It was accepted for the WHO list of prequalified HIV diagnostics and was listed on 25 August 2014 (WHO PQDx Public Report, 2017). The use at the WOHC appears to have been before this acceptance into the WHO list of prequalified HIV diagnostics. This RT is reported to have 99.9% relative sensitivity and 99.8% relative specificity as per manufacturer information leaflet.

HIV tests were administered by nurses trained on HTS in the Maxillo-Facial and Oral surgery ward. The patients tested were referred mainly from the Oral Medicine and Maxillo-Facial and Oral surgery clinics.

The Tri-line RT is used for screening. The confirmatory tests used were the First Response HIV 1-2-0 Card Test was used in 2014-2015 and Advanced quality Rapid HIV Test in 2016. The parallel testing approach is also used, this however is not practiced routinely it would seem the algorithm used depends on the nurse performing HTS at a particular time. With the parallel testing algorithm the two rapid tests mentioned above are used simultaneously, and if discordant the tests are repeated 2 to 3 times and if still discordant then blood is drawn for ELISA.

In the data extracted from the patients records between 2014 and 2016 both RTs were concordant in all patients.

1.3 Research Questions

What is the sensitivity, specificity and discriminatory power of the Tri-line RT in comparison with ELISA?

1.4 Aim of the study

The aim of this study was to evaluate the sensitivity and specificity of the HIV 1/2/O Tri-line HIV rapid test device in comparison with ELISA as the gold standard in patient records from the WOHC at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH).

1.5. Objectives

The objectives of the study were:

- To collate records of all patients at the WOHC who had been tested for HIV at the same visit using the HIV 1/2/O Tri-line HIV rapid test device and ELISA.
- To report on negative and positive results for both the RT and ELISA performed at the same visit.
- To report on the sensitivity, specificity, PPV (positive predictive value), NPV (negative predictive value), positive and negative likelihood ratios of the HIV RT for detection and exclusion of HIV infection in comparison to the gold standard laboratory test ELISA.
- To determine the clinical usefulness of HIV 1/2/O Tri-line HIV rapid test device.

CHAPTER 2: Methodology

2.1 Study design

This is a retrospective comparative descriptive pilot study conducted on the records of patients seen and tested for HIV between 2014 and 2016 at the WOHC. Patients included in the study were tested for HIV with the ABON™ HIV 1/2/O Tri-Line HIV RT device [ABON Biopharm (Hangzhou) Co. Ltd, China] and the 4th generation ELISA: Siemens Advia Centour assay that detects HIV p24 Antigen and Antibodies to HIV 1, including Group O (HIV-1 + "O") and/or HIV-2.

2.2 Data collection

Data was collected from 111 medical records of patients who were tested for HIV at WOHC from 2014 to 2016. Approval to access the WOHC HIV test register was obtained from the relevant authority, the letter is attached as Appendix C.

The medical records were retrieved from the WOHC database and the following data were extracted and recorded in a structured data collection sheet: year of testing; age; gender; ELISA test results (positive / negative), if positive, CD4-T cell count where available; and Tri-line HIV rapid test result (positive / negative). The data collection sheet is attached as Appendix D. The patient data evaluation was retrospective and anonymous; and informed consent was not required. Each eligible patient record was assigned a study number and data extracted from the patient's HIV register were entered into a pre-designed excel spread sheet.

2.2.1 Sample size calculation

For the estimation of sensitivity and specificity at levels of 98% and 95%, respectively, with 2% precision, at the 95% confidence level, with a prevalence of HIV of 80% in the study group, the sample size calculated for the study was N = 2281. Sample size requirements were based on the key research question, namely the determination of the sensitivity and specificity of the Rapid Test. The sample size calculations were carried out in G*Power (Buchner, 2007).

2.2.1 Inclusion criteria

Medical records of patients 18 months and older, who had been counselled and from whom informed consent to have the HIV test done, had been obtained. Medical records from 2014-to 2016 indicating a Tri-line test administered on the same day venous blood was drawn for ELISA were included in the study.

2.2.2 Exclusion criteria

- Medical records where consent was not obtained.
- Medical records where only one of the tests was performed.
- Medical records where tests were conducted using the HIV 1/2/O Tri-line HIV rapid test and ELISA at different visits.
- Medical records of patients younger than 18 months.

2.3 Statistical methods

Descriptive analysis of the data was carried out as follows: Categorical variables were summarised by frequency and percentage tabulation, and illustrated by bar charts. Continuous variables were summarised by the mean, standard deviation, (or median and interquartile range), and their distribution illustrated by histograms.

Estimates of sensitivity, specificity, PPV (positive predictive value), NPV (negative predictive value), positive and negative likelihood ratios were determined, together with their 95% confidence intervals. These quantities were calculated as shown in Table 2.1

Table 2.1. Calculating estimates of sensitivity, specificity, PPV, NPV, positive and negative likelihood ratios

		True status (ELISA)	
		Condition Positive	Condition Negative
Test outcome (Rapid test)	Test Positive	True positive (TP)	False positive (FP)
	Test Negative	False negative (FN)	True negative (TN)

Sensitivity = TP / Condition Positive

Positive Predictive Value = TP / Test Positive

Positive Likelihood Ratio = Sensitivity / (1-Specificity)

Specificity = TN / Condition Negative

Negative Predictive Value =TN / Test Negative

Negative Likelihood Ratio = (1-Sensitivity) / Specificity

Data analysis was performed using a statistic program (STATA). Quantitative data was summarized in tables and figures and described using medians and ranges; counts with percentages (%); the Fischer's exact test; and the Mann-Whitney U test. A p-value of <0.05 was considered statistically significant.

A Receiver Operating Characteristic (ROC) curve was drawn to determine the discriminatory power of the Tri-line Rapid Test kit compared to ELISA (gold standard). The area under a ROC curve specifies the discriminatory power of the diagnostic test. A theoretically perfect diagnostic test records an area of 1.0 which signifies a sensitivity of 100% and specificity of 100% (Fan et al., 2006, Hajian-Tilaki, 2013). A ROC area of 0.0 signifies a specificity of 0% and sensitivity of 0%, indicating a theoretically imperfect test. Statistically, a diagnostic test of ROC area equal to or less than 0.75 is not clinically useful while a ROC area of greater than 0.75 is clinically useful. A test with a ROC area of more 0.97 and above is deemed very useful (Fan et al., 2006).

"Discriminatory power" is the extent a test score recorded by an instrument varies with regards to differences in traits with the aim of distinguishing subjects or participants with high traits from those with low traits (Ferrando, 2012). In this study, it is the ability of the Tri-line RT to distinguish between individuals with HIV positive results from those with HIV negative results. Discriminatory power predicts the clinical usefulness of a diagnostic tool.

2.4 Ethical Approval

Ethical clearance for this study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa, under the protocol number M170572 (Appendix A).

2.5 Protocol approval

The protocol was assessed and approved by the assigned assessors and the dedicated committee (Appendix B).

CHAPTER 3: Results

3.1 Study population demographics

A total of 111 medical records were reviewed, out of which 45 met the inclusion criteria. The available sample size of 45 patients corresponds to a precision of 14%, rather than 2%. Hence it is understood that this is a pilot study.

Sixty six records excluded from the study comprised records where only one of the tests was performed and those where the Tri-line test and ELISA were performed independently at different visits. In 2 medical records the ELISA and RT were conducted 3-4 days apart. One patient had tested positive with both tests whilst the other tested negative with both tests. It can be argued that these 2 records could have been added to the study group. The inclusion criteria were adhered to; to prevent result bias, maintain objectivity and control of the study. Furthermore, the statistician further advised that their inclusion would not have a significant impact on the results; therefore, the 2 records were excluded from the study.

Two medical records with negative result with the Tri-line test were also excluded: one patient tested positive 2 months later with ELISA whilst the other patient had an indeterminate result. The second patient, whose results were indeterminate, was diagnosed with deep fungal infection. The patient who tested positive 2 months later with ELISA was diagnosed with Kaposi's sarcoma.

In six medical records where both tests were conducted, the Tri-line results were not recorded in 3 whilst in the remaining 3 ELISA was performed but results were not available. The remaining records comprised 15 needle stick injuries where ELISA was not done as per protocol at the WOHC, and the in remaining 41 medical records ELISA was not done. Of the excluded records 6 had an HIV positive RT result whilst 54 had HIV negative RT results. The RT results were not recorded in 6 other excluded medical records.

The majority of the study population was female (61%) as shown in Table 3.1. The average age was 35 years with a range from 3 to 61 years (Table 3.2). Most cases were recorded in 2015, with only one case in 2014 (Table 3.4 and Figure 3.2).

Table 3.1: Gender of participants

Variable	Variable	n	%
Gender	F	27	61
	M	17	39
	Not specified	1	

Table 3.1 shows that the majority (61%) of the participants included in this study are female.

Age was recorded in 42 patients, with 3 patients whose age was not recorded. Thus in terms of age there was 7% missing data. The mean age of the patients was 35 years with a SD =14 years and a range of 10-70 years as shown in Table 3.2 above.
From Table 3.3, 26 out of the 42 participants with identified age were female in gender.

Table 3.2 Age distribution

Age (years)							
N	**Mean**	**SD**	**Median**	**Interquartile range**		**Minimum**	**Maximum**
42	35.1	13.9	33	26	46	10	70

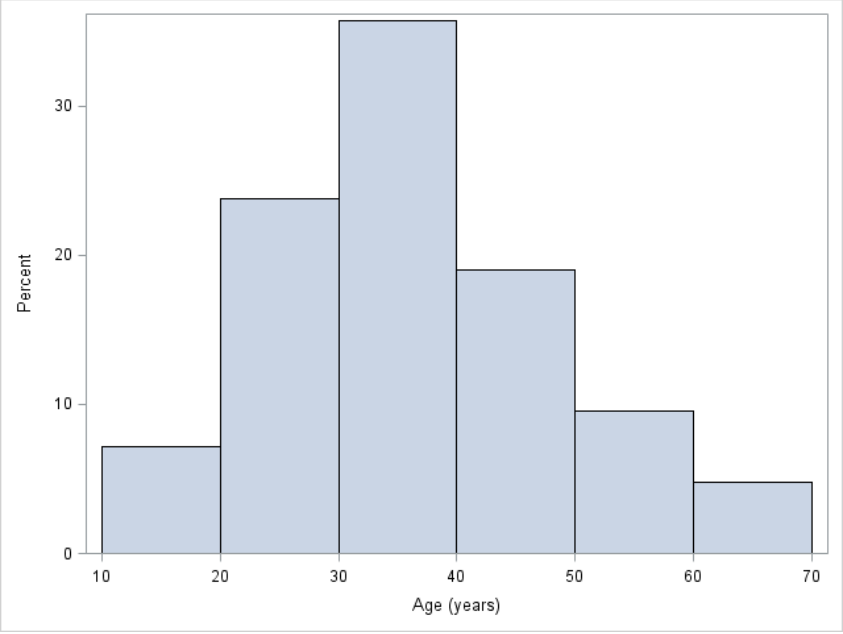

Figure 3.1 The age distribution

Table 3.3: Age and gender distribution of participants

Age Category (3 missing)	Gender (1not specified)		Total
	Male	Female	
0-20 years	1	2	3
21-40 years	10	17	27
Above 40 years	5	7	12
Total	**16**	**26**	**42**

The majority (60%) of the participants included in this study were screened in the year 2015 whilst 17 (38%) and 1 (2%) were screened in the year 2016 and 2014 respectively (Table 3.4).

Table 3.4: Year of HIV Screening

Variable	Variable	n	%
Year	2014	1	2
	2015	27	60
	2016	17	38

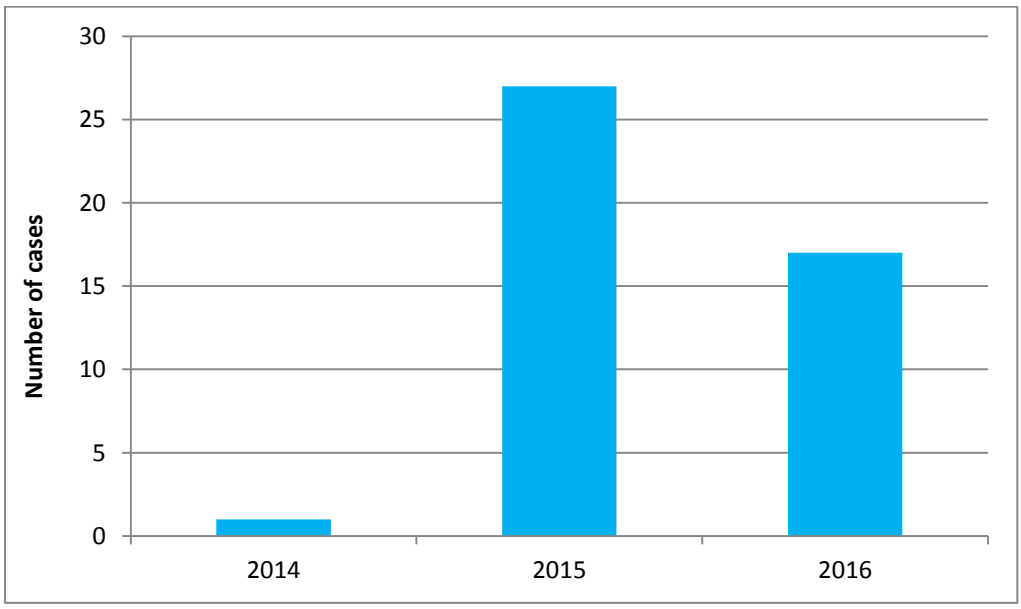

Figure 3.2 Number of cases per year

3.2 Findings from ELISA and RT

The results of the HIV screening test conducted using the two screening tools (ELISA and Tri-line) were different. Of the 45 medical records that met the inclusion criteria ELISA test results show 20 participants (44%) were negative whereas Tri-line found 25 participants (56%) negative (Table 3.5). Twenty five of these records had a positive outcome with 25/45 (56%) testing HIV positive with ELISA and 20/45 (44%) with Tri-line RT.

Table 3.5: HIV Test Results from ELISA and Rapid Test

Variable	**Variable**	**n**	**%**
ELISA test	NEGATIVE	20	44
	POSITIVE	25	56
Rapid Test	NEGATIVE	25	56
	POSITIVE	20	44

Table 3.6 shows that the majority (28) of the participants included in the study were between the ages of 21 and 40. The ELISA test result found 8 participants within this category to be negative and 19 positive. The RT on the other hand found 10 of them negative and 17 positive. Twelve of the participants were above 40 years. ELISA found six participants in this category to be negative and 6 positive whereas rapid test detected 9 of them as negative and 2 of them as positive. Three of the participants were between the ages of 0 and 20. The ages of three participants were missing from the data available to the researcher. ELISA and RT results of the 0-20 year category and the missing category were the same, i.e. all being negative.

Table 3.6: Age and HIV test result distribution

Age Category	**ELISA Test**		**Tri-line Rapid Test**		**Total**
	Positive	**Negative**	**Positive**	**Negative**	
0-20 years	0	3	0	3	3
21-40 years	19	8	17	10	27
Above 40 years	6	6	3	9	12
Missing	0	3	3	0	3
Total	**25**	**20**	**25**	**20**	**45**

Seven of the 45 participants included in this study had oral lesions strongly associated with HIV/AIDS recorded; five had candidiasis, one Kaposi's sarcoma and one plasmablastic lymphoma. ELISA test detected all the participants with lesions as HIV positive. The RT on the other identified four of the participants with candidiasis as HIV positive and those with Kaposi's sarcoma and plasmablastic lymphoma as HIV negative (Table 3.7).

The CD4-T cell count was also recorded, however with more than 30% of the data missing in the ELISA-positive cases; the data could not be analysed. Attention can however be drawn to the one patient with a false HIV negative Tri-line result and a CD4-T cell count of 36 cell/µL. A patient from Sudan had a CD4-T cell count of 264 and tested negative with both Tri-line and ELISA

Table 3.7: HIV associated oral lesions

Lesions	**ELISA Test**		**Tri-line Rapid Test**		**Total**
	Positive	**Negative**	**Positive**	**Negative**	
Candidiasis	5	0	4	1	5
Kaposi's sarcoma	1	0	0	1	1
Plasmablastic lymphoma	1	0	0	1	1
High grade Malignant Haematolymphoid neoplasia	1		1		1
Ulcer NOS	1		1		1
Squamous papilloma		1		1	1
Total	9	1	6	4	10

Twenty five (56%) of the 45 cases screened in this study period tested positive for HIV with ELISA whilst the remaining 20 (44%) tested positive with Tri-line RT. Of the 25 patients who tested positive with ELISA, 20 also tested positive with Tri-line with five patients testing false negative for HIV with the Tri-line test (Table 3.8). No patients were found to be positive with the Tri-line test and negative with ELISA and thus there were no false positive

results. In the false negative group four were male and one was female. Of the 5 false negative results four were recorded in 2015, and one in 2014 (the only case in 2014 that met the inclusion criteria), and none were recorded in 2016.

Table 3.8: Cross-tabulation of the Tri-line RT and ELISA results

RT	ELISA		
	POSITIVE	**NEGATIVE**	**Total**
POSITIVE	20	0	20
NEGATIVE	5	20	25
Total	25	20	45

Given the low sample size the estimates of sensitivity and specificity were subject to wide confidence intervals as depicted in Table 3.9. To determine the validity and reliability of the Tri-line RT against the gold standard ELISA the rate of sensitivity, specificity, PPV, NPV, positive and negative likelihood ratios were determined. The sensitivity of Tri-line was 80% (CI: 59-93%) which indicates that 80% of patients were identified as HIV positive, and specificity was 100% (CI: 83-100%) indicating that all patients who were negative were identified as such. This is shown in Table 3.4. The PPV was 100% (CI: 83-100%) and NPV was 80% (CI: 65-90%). Specificity and PPV are high (albeit with wide confidence intervals), but sensitivity and NPV are lower than anticipated (Table 3.9).

The positive likelihood ratio is the ratio between the probability of a positive test result given the presence of the disease and the probability of a positive test result given the absence of the disease, i.e. Sensitivity / (1-Specificity). This was calculated as infinity since Specificity is 100%. The negative likelihood ratio is the ratio between the probability of a negative test result given the presence of the disease and the probability of a negative test result given the absence of the disease, i.e. (1-Sensitivity) / Specificity (Table 3.9).

Table 3.9 Diagnostic indicators

Indicator	Estimate	95% CI
Sensitivity	80%	59-93%
Specificity	100%	83-100%
PPV	100%	83-100%
NPV	80%	65-90%
Positive Likelihood Ratio	infinity	-
Negative Likelihood Ratio	0.20	0.09-0.44

Sensitivity, specificity, PPV, NPV, positive and negative likelihood ratios of the Rapid Test compared to the gold standard ELISA test.

3.3 Discriminatory power of the Tri-line rapid test with reference to ELISA

The results of the non-parametric ROC curve indicated the ROC area of 0.9 at 95% confidence interval (Figure 3.1 and Table 3.10). Table 3.10 outlines the details of the values represented in the ROC curve in Figure 3.3. They are shown as the area under the curve specifying the discriminatory power of the diagnostic test (Tri-line RT).

Table 3.10: Details of ROC Curve in Figure 3.3

Detailed report of sensitivity and specificity

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-
(>= 1)	100.00%	0.00%	55.56%	1.0000	
(>= 2)	80.00%	100.00%	88.89%		0.2000
(> 2)	0.00%	100.00%	44.44%		1.0000

Obs	ROC Area	Std. Err.	Binomial Exact [95% Conf.	Interval]
45	0.9000	0.0408	0.78779	0.97525

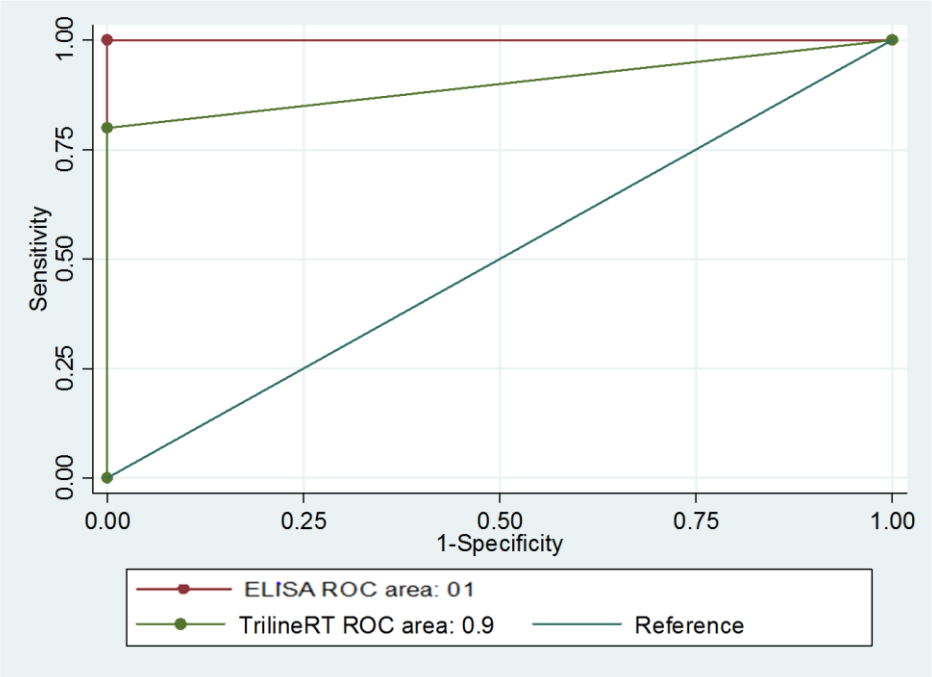

Figure 3.3: Receiver Operating Characteristic (ROC) Curve for Rapid Test compared to ELISA (Gold Standard)

Summary of results

The sensitivity of Tri-line was 80% (95% CI: 59-93%), and specificity was 100% (95% CI: 83-100%). The calculated PPV was 100% and the NPV was 80%. The discordant results were false negative, and none were false positive. The results of the non-parametric ROC curve demonstrated an ROC area of 0.9 at 95% confidence interval.

CHAPTER 4: Discussion

4.1 Discussion

In this study, of the 45 records with valid ELISA results, none had HIV positive results with the Tri-line test that turned out negative with ELISA. Hence the specificity and PPV in this study were found to be high, whilst sensitivity and NPV were found to be lower than anticipated, and this finding is congruent to other studies (Wolpaw, 2010, Patel *et al*., 2012, Bock *et al*., 2017). The estimates of sensitivity and specificity for this study were subject to wide confidence intervals as a result of the low sample size, which is expected in a pilot study.

Although in this study high specificity of 100 % (95%CI 83-100%) was observed, with no false positive results with the Tri-line test, was observed, false positive results have been reported in other studies (Gray *et al*., 2007, Shanks et al., 2013, Klarkowski *et al*., 2014). Consequently, the retesting of patients already on ART is advocated to ensure that all patients have been accurately diagnosed. The high specificity in the study rules in HIV infection with a high degree of confidence for those testing positive with the Tri-line.

False positive results are common in infants born to HIV mothers. Although there were three medical records where age was not reported, these were included based on reports from the Maxillo-Facial and oral surgery ward, in that there were no patients seen and tested for HIV who were < 18 months old. If individuals under 18 months were present they would have been excluded on the basis that PCR instead of RT would have been conducted.

In the current study, the sensitivity was lower than the WHO recommended sensitivity of ≥99%. The Tri-line test did not meet the sensitivity reported by the manufacturer of 100% (95% CI 99.2 % - 100%). This result implies an increased probability of missing HIV positive patients. This raises serious concerns and further studies should be conducted to determine the possible reasons for the underperformance of the Tri-line test. These uncertainties may result in the validity of the tests being questioned by patients and thus compromise efforts made to reduce the spread of the virus. The sensitivity and NPV were found to be 80% (95% CI: 59-93% and 65-90% respectively), inferring that patients who tested negative may in fact be HIV infected. This poses a serious impediment on the impact of intervention programmes as patients who falsely test negative may subsequently and unknowingly transmit the infection to others.

False negative results translate to missed opportunities to implement appropriate management with subsequent delays in the initiation of ART and continued spread of HIV. RTs that test for antibodies only can give false negative results when patients have not seroconverted, have agamaglobulinaemia, severe immunosuppression or are on HAART. This leaves health care workers in the frontline with an immense challenge of having to explain to individuals being tested that a negative result may not necessarily exclude the possibility of positive result at a later stage.

The exact cause for the five false negative results in this study could not be determined. In other studies, the main contributing factor attributed to discordant false negative results was acute HIV infection (Adetunji *et al*., 2018). It is possible, based on the data collected, that the five false negative results could be linked to acute HIV infection, improper administration of Tri-line RT or severe immunosuppression in the patients tested. The latter is believed to be the most probable contributing factor in a patient who tested negative with the Tri-line test and positive with ELISA despite a CD4-T cell count of 36 cells/µL. Tests conducted during the early stages as opposed to later stages of HIV infection, have been shown to have lower sensitivity and may therefore produce false negative results. The aforementioned factors are feasible particularly because most patients were specifically referred for an HIV test by health care workers when HIV infection was suspected. Furthermore, the use of different testing algorithms in one setting as is the case at WOHC may be confusing and result in the manufacturer's guidelines not being followed as prescribed. The recommended WHO testing algorithm that was also employed has its flaws. The algorithm can lead to misdiagnosis of HIV infection when the screening RT and the confirmatory RT gives a false positive or negative result.

Therefore individuals presenting with group 1 lesions as described in the EC Clearing House classification, should as a standard protocol have testing inclusive of both the RT and ELISA test where RT is the first line test. This can be life-saving as was the case in the current study for two patients diagnosed with oral malignancies (plasmablastic lymphoma and Kaposi's Sarcoma) strongly associated with HIV whose results were negative with the Tri-line test but positive with ELISA. The wide spread use of RTs in high-risk, high-incidence populations is reported to be of limited benefit considering the possibility of missed infections (Wawer *et al*., 2005, Patel *et al*., 2012). The value of RTs should be continuously challenged so that better RTs with increased sensitivity and specificity are developed. Although the value of RTs may be questionable as stated above, the tests provide immediate results and optimize

intervention programmes. The challenge is with patients who test negative and opt not to subject themselves to additional tests even when they are at risk of being infected. As this current study demonstrated, the patients did not subject themselves to the ELISA test for various reasons and were accordingly excluded.

This challenges the widespread use of RT in patients where there is a high index of suspicion for HIV infection given its failure to detect HIV infection in the early stages with resultant missed opportunities to interrupt onward HIV transmission and timeous management (Patel *et al*., 2012). The use of RTs in high risk populations should therefore be re-considered or other more sensitive tests explored for early identification of HIV infection, since individuals with early HIV infection are more likely to transmit HIV given the higher viraemia early in the disease process (Wawer *et al*., 2005).

Similar to findings in the literature, the majority of HIV positive participants in this study were female (UNAIDS, 2009, Mbachu *et al*., 2015). The highest rates of HIV infection were between the ages of 20 and 41. This finding, having accounted for all other factors, aids in the profiling of patients most likely to be infected with HIV in our setting. Patients in this age group presenting with lesions associated with HIV, should have both the RT and ELISA test conducted as a precautionary measure.

In most cases, it is not always possible to determine when an individual got exposed, so choosing the most appropriate test may be a challenge. Therefore considering that a negative RT result does not preclude the possibility of infection with HIV; tests with a high sensitivity and specificity, independent of serostatus or viral titre, would be ideal diagnostic tools to eliminate inaccurate HIV diagnoses which could have dire consequences for the patient and society (Granade *et al*., 2004). This is important as the achievement of prevention and treatment goals of HIV infection relies on individuals knowing their status, making HTS the gateway to a continuum of care (National HIV Testing Policy and Guidelines, 2015). Given the limitations of standard widely used RTs one is of the view that if the status quo remains, eradication of HIV infection will not be realized any time soon, one false negative result is one too many, given the prevalent high risk lifestyles.

In a setting where laboratories are out of range, patients can be enrolled on HAART unnecessarily. At WOHC, patients testing HIV positive with RT are sent for ELISA to confirm the RT results; and in the Oral Medicine clinic given the high rate of negative results (anecdotal), patients presenting with lesions associated with HIV are also sent for ELISA

despite the RT results. There were some instances where patients were sent for CD4-T cell count only, which is of no use in the diagnosing HIV as a low CD4-T cell count can be seen in other conditions other than HIV. Requesting CD4-T cell count only or even viral load does not help exclude HIV infection. In this study one of the cases was that of a patient who presented with severe oral mucosal erosive lesions with a CD4-T cell count of 264 cells/µL and tested HIV negative with both the Tri-line and ELISA. It is thus important for clinicians to know what test to request for purposes of either diagnosing or excluding HIV infection.

An understanding of factors contributing to HIV misdiagnosis in specific contexts is critical as it can aid health care providers and policy makers to come up with approaches that will address and prevent HIV misdiagnosis allowing the scale-up of HIV RT programmes (Johnson *et al*., 2017a). It is important that in the absence of newer advanced HIV testing algorithms, the current recommended testing algorithm is followed and that the appropriate WHO prequalified RTs are employed in HIV diagnostics.

In view of the WHO recommendations that ART should be administered immediately after a positive HIV diagnosis regardless of the CD4-T cell count; preventing and addressing misdiagnosis is of paramount importance (Johnson *et al*., 2017a, Shanks *et al*., 2013). The "test and treat" approach is like a double edged sword on the one hand it can be life-saving when patients are truly positive and on the other, it increases the risk of unwanted effects by initiating ART in patients who do not have HIV infection (Shanks *et al*., 2013).

The oral health team can contribute towards addressing issues of misdiagnosis and reaching the goal of diagnosing 90% of people with HIV, by identifying those patients presenting with lesions strongly associated with HIV. The efforts to accelerate HIV diagnosis and linking individuals to treatment should be complemented by efforts to improve the quality of HTS, strengthening the use of validated testing algorithms and strategies (Johnson *et al*., 2017b). The HTS at the WOHC does not use one testing algorithm and the parallel testing algorithm is not followed as prescribed, mainly due to unavailability of the third RT recommended as a tiebreaker. Whilst repeating the tests two or three times using the same method each time may be redundant; Mbachu and colleagues in 2015, found in their study that the serial testing algorithm had a higher sensitivity. Their study showed that using an established algorithm in HIV screening and diagnosis improves the accuracy of the RT with regards to sensitivity, specificity, positive predictive value and negative predictive value (Mbachu *et al*., 2015).

There is no doubt that in a country like South Africa, plagued with multiple new infections daily, the 4th generation RT should be standard at all testing sites. Such RTs when compared to the use of separate tests for HIV antibody or p24 antigen alone would be an important tool for the diagnosis of HIV (Beelaert and Fransen, 2010). Hence given the expanded use of RTs in health facilities and at community level, one is of the view that the widespread use of the 4^{th} generation RTs should be promoted. Although the 4^{th} generation RTs has distinct challenges, they address major deficiencies with an antibody only RT such as the Tri-line test. Their widespread use will stimulate further research into more advanced assays with high sensitivity and specificity that would be of great benefit in the fight against HIV infection.

Studies on HIV RTs can help improve HIV testing and advance the development of algorithms for specific settings such as in oral health care. The HTS at the WOHC was found to be mainly via PICT with no CICT recorded cases. This is significant when one considers that 65534 patients were seen at the WOHC from 2014-2016 and only 111 were tested for HIV.

This study has shown that although much improvement is deemed necessary with regards to use of RT, the results in this study indicating a ROC area under the curve of 0.9 at 95% CI, makes the Tri-line RT a clinically useful test.

In a country with a high mortality rate of HIV infected individuals with associated diseases, access to HIV testing and diagnosis is life-saving and essential in combating the HIV pandemic. Rapid HIV testing increases the effectiveness of testing and prevention programmes. The introduction of RTs in resource-limited areas has resolved many logistical issues including limited access to laboratories, delayed results turnaround time, the complexity and costs of ELISA technology (Moodley *et al*., 2008). Increased access to rapid HIV tests however is of limited value if internal and external quality control measures are not monitored regularly. False results and incorrect diagnoses could undermine the public confidence in HIV testing with a subsequent negative impact on all HIV prevention, treatment and support programmes (Moodley *et al*., 2008).

Study limitations

This was a retrospective review study which lends itself to all the limitations of these types of studies including a small sample size. The lack of complete data in the hospital records meant that a large number of medical records were excluded from the study. Of the 65534 patients seen at the WOHC from 2014 to 2016 only 111 according to obtained records were sent for HTS. The study focused on patients who had the HIV tests done at the WOHC, therefore patients who had the tests conducted elsewhere were excluded from the study. The primary research could not verify whether the manufacturer's guidelines were adhered to, by the healthcare workers who conducted the RTs or if the RT kits used were defective or not.

Chapter 5. Conclusion and recommendations

The sample size for this study was not enough to reach a concrete conclusion. However, on the basis of the results obtained within the limitations of the study, the Tri-line test showed lower sensitivity when compared to ELISA. The sensitivity observed was also lower than that recommended by WHO. The Tri-line test was however shown to be a useful clinical test as depicted on the ROC curve. The results of this study however should be interpreted in the context of its limitations which amongst others include a rather small sample size. The authors recommend that Tri-line RT is not used as the sole diagnostic test for HIV, especially where a negative result is registered with the RT.

Studies on HIV RTs can help improve HIV testing and advance the development of algorithms for specific settings such as in oral health care. In order to reach targeted population presenting at the WOHC a variety of HTS modalities should be encouraged so that HTS is not only through PICT but CICT as well. In this way the WOHC being a tertiary clinic can play a significant role in up-scaling HTS, which can translate to HIV prevention and timeous management.

The 4th generation RT can reliably detect HIV antibodies and antigens; therefore, it can be used for targeted HIV testing, enhance existing HIV testing programmes and provide timeous identification of HIV infection. The antibody RT can be used for screening and the 4th generation RT as a confirmatory test. The combo RT would be of great benefit in oral health centres where lesions strongly associated with HIV are seen, especially considering that this can present in the early stages of HIV infection prior seroconversion or during the late stages when antibodies titres are low, when antibody only RTs are most likely to give false negative results.

For high risk individuals presenting with lesions strongly associated with HIV, and questionable lifestyle risks, ELISA should be standard especially at health facilities where the services are available. The dental staff working in clinics should thus be trained in HTS so as to facilitate diagnosis of HIV infection for timeous intervention. Patients seen in various departments at the WOHC should be encouraged to have HIV tests done and those considered to be at high risk or with lesions strongly associated with HIV should have blood drawn for ELISA even when results are negative. When false negative results are suspected patients should be encouraged to have the RT test repeated to avoid missed HIV diagnoses,

which can compromise the patient's health and result in ongoing transmission of the virus (Bock *et al*., 2017).

A standard protocol for patients presenting with oral lesions associated with HIV that involves the use of RT and ELISA in all circumstances, regardless of the RT result should be developed and instituted at the WOHC. The protocol may be expanded and adopted as the standard protocol across oral health care centres. This will help ensure uniformity amongst clinicians and the HTS nurses; and compliance with acceptable protocols as advocated by the national department of health; which include standardization of the algorithm in order to avoid the tragic and harmful implications of unreliable testing strategies.

The current study highlighted the important role that the oral health care team can play in HIV diagnosis, prevention and management through identification of those who are infected and present with oral lesions associated with HIV. To help with development and implementation of proposed strategies more studies are needed, that specifically look at RT used in the WOHC and/ or nationally, and oral manifestations of HIV.

Good record keeping should be encouraged as it will aid future research in this area. Patients who refuse to be tested should be recorded and requested to sign a refusal of treatment form especially where it has an impact on their management going forward. The HTS nurses should be provided with lesions most commonly associated with HIV. The clinician referring patients for HTS should stipulate reasons for requesting HIV testing on the laboratory request form so that clarity may be sought from the referring clinician if a request for ELISA has not been specified.

Other health personnel outside oral health should be familiarized with the EC Clearing House lesions to facilitate adequate intervention strategies. There is a need for personnel trained on HTS to be stationed at the WOHC where the general patients present to cater for all patients coming to the clinic who may want to make use of CITC and for those requiring PICT. For relative ease of access there may be a need for government to establish laboratory services at major testing centres.

The challenges faced with RTs and difficulties with QA can be minimized by increasing training and oversight. It should be borne in mind that even with optimal QA measures the accuracy of on-site RT can never be perfect (Pilcher *et al*., 2010). To ensure the efficiency of RTs there should be an improvement in the reporting of misdiagnoses, so that efforts can be

made to prevent and address them. Regular quality control of RTs is also crucial and studies reflecting their accuracy may validate or invalidate the use of RTs. Public confidence may be enhanced by studies evaluating the accuracy of RTs used in their setting to address issues relating to the distrust of testing programmes.

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APPENDIX A:

Ethics Clearance Certificate

R14/49 Dr Shumani Charlotte Manenzhe

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170572

NAME: (Principal Investigator)	Dr Shumani Charlotte Manenzhe
DEPARTMENT:	Oral Medicine and Periodontology
PROJECT TITLE:	The Diagnostic Accuracy of the HIV 1/2subtype O Tri-Line HIV Rapid Test in Comparison to ELISA
DATE CONSIDERED:	26/05/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Sindisiwe Shangase and Dr Sizakele Ngwenya

APPROVED BY: Prof P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 29/05/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in May and will therefore be due in the month of May each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date 01/06/2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B

Protocol Approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

05 January 2018
Person No: 698672
PAG

Dr SC Manenzhe
PO Box 4624
The Reeds
0158
South Africa

Dear Dr Manenzhe

Master of Dentistry: Approval of Title

We have pleasure in advising that your proposal entitled *The diagnostic accuracy of the HIV 1/2/subtype O Tri-line HIV rapid test in comparison to ELISA* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX C

Approval from the School of Oral Health Science

7 York Road, Parktown, 2193 South Africa • Telegrams "Witsmed" • Telephone (011) 717-2000 • Fax (011) 484-2717

Department of Maxillofacial and Oral Surgery
Telephone 0117172130
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27 April 2017

Dear Dr. Manenzhe

As per your request for your study entitled "***the diagnostic accuracy of the HIV 1/2 subtype O Tri-line HIV rapid test in comparison to ELISA***," approval is hereby being granted for you to access records of patients who were tested for HIV in ward 384 for the period 2014 to 2016.

Although these patients have given consent for the HIV testing at Wits Oral Health Centre, their privacy and confidentiality however need to be respected at all times and at no stage should these patients be identified by their names in the ultimate collection and analysis of the data.

Yours sincerely

Dr. RE Rikhotso
Head of Department MaxilloFacial and Oral Surgery
University of Witwatersrand

APPENDIX D

Data Collection Sheet

Patient no.	Date of testing (year)	Age	Gender	ELISA results Positive/negative cases		Tri-line HIV RT results Positive/negative cases	