COMPARISON OF ACCURACY OF HIV DIAGNOSIS BETWEEN RAPID HIV TEST KITS CONDUCTED IN NON-LABORATORY SETTINGS AND LABORATORY-BASED METHODS IN SOUTH AFRICA

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DECLARATION

I, Thato Nelly Chidarikire declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Where I used materials/thoughts from other sources, I have properly acknowledged through the conventional referencing.

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25th day of October, 2016

DEDICATION

I dedicate this work to my parents, my husband and my children, with all my love.

ABSTRACT

Introduction

South Africa has the largest absolute number of individuals living with human immuno-deficiency virus (HIV) in the world. The quality assurance (QA) of HIV rapid diagnostic tests (RDT) has not kept pace with the rate of expanded testing and utilisation of RDT. This has made it difficult to assess the accuracy of testing. In South Africa HIV counselling and testing (HCT) and the use of HIV RDT is the point of entry to HIV prevention, management, care, treatment and support.

HCT in public health facilities is delivered mainly through rapid testing by non-professional staff. Implementation of QA processes is crucial for accurate diagnosis of HIV. However, accuracy of HCT using rapid test kits in non-laboratory settings in South Africa will remain a challenge unless there is evidence that non-laboratory rapid HIV testing results are as reliable as the laboratory-based enzyme immunoassays.

This study aimed to determine the accuracy of HIV RDT in the context of an intervention. The objectives of the study were: i. To assess the sensitivity and specificity of rapid test kits in two provinces; ii. To assess the sensitivity and specificity of rapid test kits between the two provinces and New Start non-governmental organisation (NGO) which implemented a more comprehensive

quality management system (QMS); iii. To assess the accuracy of HIV RDT in the two provinces; iv. To assess the accuracy of HIV RDT between the two provinces and New Start sites.

The hypothesis was ‘the accuracy of HIV diagnosis using HIV RDT kits in non-laboratory settings in which an intervention has been introduced (internal quality control), also known as IQC, will not be different compared to settings that do not utilize IQC’.

Methods

In South Africa, the current laboratory-based gold standard for diagnosis of HIV infection in adults in the public sector as recommended by the National Health Laboratory Services (NHLS) Virology expert committee is a serial 2-test algorithm. Thus, a reactive enzyme immunoassay (EIA) test result must be confirmed by a second confirmatory EIA that must be different in terms of antigens and technology. The Expert Committee recommendation is that positive results should be confirmed by a separate sample 14 days later. In the case of HIV rapid testing the national HIV counselling and testing (HCT) policy, 2010, similarly recommends a serial 2-test algorithm for diagnosis where a reactive screening test is confirmed by a different confirmatory test. If the confirmatory test is reactive the diagnosis is positive. If test 1 is non-reactive then the diagnosis is negative. In case of discrepant results an enzyme-linked immunosorbent assay (ELISA) test was recommended as a tiebreaker. A new HIV testing services (HTS) policy was

approved in South Africa in 2016 and it further recommended that the first time discrepant results are found, the counsellor must repeat the algorithm and if on repeat, the results are still discrepant, then reflex testing is recommended where the blood (whole blood) of a client is taken to the laboratory for ELISA (NDOH, 2016).This algorithm has replaced the use of Western Blot is South Africa. The rationale for the change was based on the sensitivity and specificity of 3rd and 4th generation ELISAs, workload, costs and expertise.

With the introduction of the 3rd and latterly 4th generation EIA tests the above algorithm is in use in South Africa and has replaced the use of the Western blot as a confirmatory test. The rationale for the change is based on earlier detection of HIV infection, workload, costs and expertise. Further developments for a diagnostic algorithm include the use of a fourth generation test and if reactive to use a HIV-1 and HIV-2 discriminatory test and HIV viral load.

This study was cross-sectional and compared the performance of HIV RDT in selected sites in Limpopo province that had introduced an intervention viz., an internal quality control (IQC) as part of quality management system (QMS) implementation, and compared to Mpumalanga province that had not introduced the IQC and performed limited QMS activities. The sample size calculated for the study was N = 717. IQC is an independent internal quality control that is used to check that an analytical phase or test precision is optimal. The introduction of routine QMS in Limpopo was through implementation of IQC supported by

appropriate training and certification of implementers. IQC was implemented routinely as part of the provincial QA initiatives with the aim of supporting the implementation of HIV RDT in non- laboratory settings. There are other QA measures that may be implemented to support HIV RDT programmes including external quality assessment (EQA) such as proficiency testing (PT) which is a tool used to assess the testing process independently. EQA implementation was however not part of the Limpopo (LP) QMS implementation. Six high volume testing sites comprising of 3 hospitals and 3 clinics were selected per province. This was to avoid the risk of not meeting the required number of participants due to refusals, lack of results and challenges with reporting.

In order to mitigate risk, the study was oversampled, where a total of 457 participants from the LP sites were enrolled in the study and results were analysed and compared to those of 361 participants from the Mpumalanga (MP) sites resulting in a total sample size of 818. The analyses included demographics, performance of RT as measured by the number of discordant results, reliability and validity of rapid tests RT as measured by the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) results. The data between Limpopo and Mpumalanga were further analysed together with the data from selected sites from a non-governmental organisation (NGO) called New Start and the performance, reliability and validity of the HIV test results were compared. The main role of New Start was to offer HCT in support of the government priorities and it implemented several different QMS measures for HIV rapid testing,

namely, IQC, EQA, PT and re-testing, training for implementers, development and implementation of standard operating procedures (SOPs), and ensuring that all commodities were stored under appropriate conditions including temperature monitoring.

In order to determine the validity and reliability of HIV RDT against the gold standard ELISA in Limpopo, Mpumalanga and New Start sites, the rate of discordance, the sensitivity, specificity, PPV and NPV were determined. Logistic regression models were constructed to assess the association between the interventions in the provinces. Crude and adjusted odds ratios were used as a measure of association between exposure and outcome and a 95% precision of estimate was used to ascertain statistical significance. Exposure factors with $p<0.05$ were considered statistically significant.

Results

A total of 947 attendees for HCT services in selected sites in Mpumalanga and Limpopo provinces between August and April 2012, were screened and of these, 818 were enrolled into the study according to the study inclusion criteria. There was no significant difference ($p=0.05$) between the number of participants enrolled in Limpopo (457) as compared to Mpumalanga (361) though Limpopo enrolled more participants than Mpumalanga. All available data from New Start sites for the period 2008 was analysed. The gender, rate of discordance and HIV positivity rate

were significantly different between the two provinces ($p<0.05$). The study showed that the laboratory-based HIV prevalence rate in each setting was 22.9% in Limpopo, 26% in Mpumalanga and 11% in New Start sites. The prevalence rates reported by Shisana, 2014, were 21.8% for Mpumalanga and 13.9% for Limpopo.

The rate of discordant HIV test results between the 2 provinces and New Start sites was also measured where discordant results were defined as those that were different between HIV rapid test and the ELISA test. The rate of discordant HIV test results was 5.9% (27) in Limpopo, 11.0% (40) in Mpumalanga p= 0.010 and 1.4% (68) in New Start sites. False negative results accounted for all the discordant results.

Logistic regression models were used to estimate the Odds Ratio (OR) and the 95% confidence interval of the association between implementation of QA programme and the HIV test accuracy or the HIV discordance rate. Facilities without a QA intervention programme had an approximately 2-fold increased odds of HIV test discordance compared to facilities with a QA programme in place (crude OR 1.86, 95% CI: 1.10 – 3.12 and adjusted OR 1.90, 95% CI:1.08 - 3.30). This association was statistically significant. The sex and age of the participants was not associated with discordance rate.

The sensitivities of the HIV RDT in Limpopo, Mpumalanga and New Start sites were 86% (CI: 83.9-89.4), 72% (CI: 64.2-79.0) and 98% (CI: 97.6-98.4)

respectively. In this study, specificity ranged within 99% (CI: 98.9-99.9) in all sites (Provinces and New Start sites). The PPV in Limpopo, Mpumalanga and New Start sites were 98% (CI: 93.2-99.6), 97% (CI: 91.0-99.2) and 93% (CI: 92.3-93.7) respectively, The NPV results in Limpopo were 93% (90.5-95.2), Mpumalanga at 86% (CI:81.3-90.7). For New Start sites, the NPV was 99.6% (CI: 99.4-99.8). The sensitivities and specificities of the sites were used at a national prevalence rate of 18.8% to determine the national PPV and NPV and these were found to be 100% (CI: 100-100) and 91.3% (CI: 89.04-92.96) respectively.

Discussion

In all three settings the World health Organisation (WHO) recommended sensitivity (>99%) and specificity (>98%) were not met. There was a gradient of sensitivities and specificities that was associated with the extent of QA implementation. Thus, New Start sites with a more extensive set of QA activities had the highest sensitivity; LP with introduction of IQC, had an intermediate sensitivity and MP the lowest. Despite the introduction of an intervention LP was not able to meet the required level of QA implementation compared to New Start. Increased discordance was associated with the extent of implementation of QA as shown by the results of the logistic regression model (crude and adjusted). In this study there was a decline in sensitivity that resulted in some false negative results. To a lesser extent, some false positive results were also identified in New Start sites. In the case of LP and MP the potential contributory factors to false negative results

would include the extent of QA implementation and training. Further evidence of the relative poor implementation would include the M&E assessments and in the course of the study there lost results, poorly taken and missing specimens that led to data being excluded.

Conclusion

On the basis of these results, it is concluded that implementation of quality assurance measures is critical to ensure correct diagnosis of rapid HIV testing. Furthermore, implementation of a combination of aspects of QA is urgently required including training of all implementing staff on quality assurance of rapid HIV testing, monitoring and evaluation to assess kit performance through IQC and PT, as well as implementation of the current South African HIV testing Services (HTS) Policy. All PT methods should be explored for implementation and training and certification of implementers must be ensured.

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ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ARV	Antiretrovirals
CDC	Centres for Disease Control
CICT	Client-Initiated Counselling and Testing
DBS	Dried blood spots
DHIS	District Health Information System
DHT	Diagnostic HIV test
DOH	Department of Health
DTS	Dried Tube specimen
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immune-sorbent Assay
EMTCT	Elimination of mother to child transmission of HIV
EQA	External quality assessment
FDA	Food and Drug Association
GMP	Good manufacturing processes
HAART	Highly active antiretroviral therapy
HCT	HIV Counselling and Testing
HIV	Human Immunodeficiency Virus
HOD	Head of Department
HTA	High transmission area
IVD	In vitro diagnosis

IQC	Internal Quality Control
LP	Limpopo province
MMC	Medical male circumcision
MDG	Millennium Development goal
MP	Mpumalanga province
MTCT	Mother to Child Transmission
NDOH	National Department of Health
NGOs	Non-Governmental Organisations
NICD	National Institute for Communicable Diseases
NHLS	National Health Laboratory Services
NPV	Negative Predictive Value
NSP	National Strategic Plan for HIV, STIs and TB (2012-2016)
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PEPFAR	Presidents emergency programme for AIDS relief
PICT	Provider-Initiated Counselling and Testing
PMTCT	Prevention of Mother to Child Transmission of HIV
POC	Point of care
PPV	Positive Predictive Value
PT	Proficiency testing
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System

RDT	Rapid diagnostic test
RT	Rapid test
SANAC	South African National AIDS Council
SOPs	Standard Operating Procedures
STI	Sexually Transmitted Infection
SDG	Sustainable development goal
TasP	Treatment as prevention
TB	Tuberculosis
TOT	Training of trainers
UNAIDS	Joint United Nations HIV & AIDS Programme
VCT	Voluntary Counselling and Testing
WHO	World Health Organization

1 CHAPTER 1: INTRODUCTION

1.1 Summary

In this chapter, a general background of the global burden of HIV and acquired immune deficiency syndrome (AIDS) is provided. The chapter further explores the burden of HIV and AIDS in sub-Saharan Africa then finally in South Africa. The current rapid HIV diagnostic procedures and their challenges are explored. Justification of the study and critical review of the documented data on HIV rapid diagnostic tests (RDTs), QA measures including EQA methods, internal quality control (IQC) methods and retesting methods in non-laboratory settings are reviewed. The search strategy for literature review is articulated and the chapter ends with a description of the aims and objectives of the study.

1.2 Search strategy for literature review

In terms of literature review, the following search strategies were used: On line search strategy and search engines, dates of publications, and search words strategy.

1.2.1 On-line search

A comprehensive search of biomedical databases was carried out to find all relevant manuscripts published in English. The search aimed at identifying relevant peer-reviewed studies that would provide adequate information on the study topic. Different sources of literature were utilised. These included peer

reviewed published literature from PUB med, published policies and guidelines, literature published on the internet and some grey literature that was relevant, well cited and published though not necessarily peer reviewed, and published reports.

1.2.2 Search engines, dates of publications, and search words used

Relevant search words were used to develop a data base, for example: 'quality assurance of HIV rapid test kits', ‘HIV rapid diagnostic tests’; 'HIV epidemic', 'quality control of rapid test kits', 'sensitivity and specificity of rapid HIV test kits', 'negative predictive value', positive predictive value', 'HIV prevalence' and 'discordant HIV results'. A manual review was also conducted, for example of conference abstracts that were relevant to the study topic.

1.3 HIV and AIDS Burden

1.3.1 Global

HIV is still one of the world's most detrimental health and development challenges. An estimated 35.3 million people globally were living with HIV in 2012 (UNAIDS 2013). In 2014, this number increased to 36.9 million (UNAIDS, 2015; UNAIDS 2015b), mainly due to people receiving the life-saving antiretroviral therapy, more infections and general population growth. It was further reported that there were 2.3 million new HIV infections globally, showing a 33% decline in the number of new infections from 3.4 million in 2001 (UNAIDS 2013). At the same time the number of AIDS deaths declined with 1.6 million AIDS deaths in 2012, down from

2.3 million in 2005 (UNAIDS, 2013). A further decrease in deaths was reported in 2014 where 1.2 million people died (UNAIDS, 2015, UNAIDS, 2015b).

1.3.2 Sub-Saharan Africa

Sub-Saharan Africa, the most seriously hit region in the world by HIV, was reported to have 70% of people living with HIV in 2013, though it is only has about 13% of the world's population (UNAIDS, 2015; Population Reference Bureau. 2014; UNAIDS Gap report 2014). Most children (88%) with HIV live in Sub Sahara (UNAIDS, 2015b). In the same year, there were an estimated 1.5 million new HIV infections and 1.1 million AIDS-related deaths (UNAIDS Gap report 2014).

The region's epidemic is mostly generalized, meaning their national prevalence is greater than 1% (UNAIDS 2015). In 9 countries, 10% or more of adults are estimated to be HIV-positive. HIV prevalence in Western and Eastern Africa is reported to be low to moderate ranging from 0.5% in Senegal to 6% in Kenya (UNAIDS 2015). Swaziland has the highest prevalence rate in the world (27.7%). Recent data however shows that in many countries in the region, the national HIV prevalence and/or incidence is stabilizing or even declining (UNAIDS 2015).

1.3.3 South Africa

The latest Human Sciences Research Council (HSRC) household-based survey 2012, estimated that 6.4 million people were living with HIV in 2012 (Shisana O, 2014) with a prevalence of 18.8% in adults as compared to 17% in 2008 (Shisana O, 2009). This makes South Africa the country with the largest number of persons living with HIV world-wide. The high prevalence is a result of new HIV infections and reduced mortality among individuals who are on ARV treatment (Bor J, 2013; Shisana O, 2014). In adults aged 15-49 years, the four HSRC surveys estimated HIV prevalence at 15.6% (2002), 16.2% (2005) and 16.9% 2008 and 18.8% in 2012. The incidence for young people aged 15-24 was 1.49% with females at a higher 2.54% as compared to the male counterparts who had an incidence of 0.55%.

Data derived from population-based sero-surveys and sentinel surveillance of pregnant women suggest that the South African HIV epidemic has reached a plateau. The 2011 National Antenatal Sentinel HIV and Syphilis Prevalence Survey in South Africa reported an HIV prevalence of 29.5% in pregnant women (Health, 2012). In 2013, the ANC prevalence was estimated at 29.7 showing that the epidemic curve had plateaued and there has been no statistical difference in the national prevalence estimate in the past 10 years (Health 2013).The interpretation of prevalence is likely to become complex as the epidemic matures and prevention efforts try to mitigate it at the same time (Shisana O 2014).

1.4 The Response to HIV Epidemic

1.4.1 Global

In 2000, all nations agreed to global HIV targets with the goal of halting and reversing the spread of HIV by 2015, as part of the UN Millennium Development Goals (MDGs).The World Bank also launched its Multi-Country AIDS Program (MAP). The international community agreed upon new Sustainable Development Goals (SDGs), which include a target to end the AIDS epidemic by 2030 (United Nations 2015). The SDG related to Health is the SDG 3: Ensuring healthy life and promote well being for all at all ages (WHO 2014).

1.4.2 South African Response

In response to the HIV epidemic, the South African government reviewed the National Strategic plan (NSP) 2007-2011 (Health 2007) on HIV and sexually transmitted infections (STIs) and tuberculosis (TB) (Health, 2007) and developed the NSP (2012-16) (Health, 2012). The main objectives of the NSP (2012-2016) are to reduce the rate of new HIV infections by 50% by 2016 and to place 80% of patients who qualify for treatment on treatment by 2016 and retain them on treatment.

The NSP has four strategic objectives aimed at comprehensively responding to the HIV epidemic as follows: Addressing the structural, social, economic and behavioural factors that drive the HIV and TB epidemics; preventing sexual and vertical transmission of HIV and STIs, and TB infections and disease, using a

combination of prevention approaches; providing universal access to affordable and good quality diagnosis, treatment and care; and ensuring that rights are not violated when interventions are implemented, and that discrimination on the basis of HIV and TB is reduced and ultimately eliminated.

HCT expansion will play a crucial role towards achieving the NSP goals. Implementation of HCT in South Africa is guided by the national HCT Policy Guidelines that were revised and approved in 2010 (Health, 2010). The national policy guidelines support provision of HCT for everyone who is 12 years of age and above and further states that individuals require HCT for different reasons and/or circumstances including the following: individuals and couples who want to know their HIV status; pregnant women participating in the prevention of mother-to-child transmission (PMTCT) programme; clinical diagnosis as part of basic patient care; research and other screening purposes; domestic violence and sexual assault; prior to providing post-exposure prophylaxis (PEP) after a needle stick injury, sexual assault and rape (acts of sexual penetration), per court order of the accused in sexual offence cases - Criminal Law (Sexual Offences & Related Matters Amendment Act No. 32 of 2007).

On World AIDS Day 2014, UNAIDS set targets for 2020 aimed at ending the epidemic by 2030. The targets included achieving '90% of people living with HIV knowing their HIV status; 90% of people who know their HIV-positive status on treatment; and 90% of people on treatment with suppressed viral loads' (UNAIDS,

2014). WHO also revised the treatment guidelines and recommended the test and treat strategy for all those who test HIV positive (WHO, 2015). In order to align to the international standards, UNAIDS 909090 strategy and WHO guidelines as well as expand HIV testing, South Africa has embarked in the revision of the 2010 policy guidelines to incorporate quality testing. The use of rapid HIV testing plays a crucial role with expansion of testing to enrol patients into care. While it is critical to expand HIV testing to meet these new goals, it is also very crucial that quality assurance implementation is improved.

South Africa has made great strides in rolling out treatment as reflected by the number of patients in care from just under 1 million in 2010 to over 2 million in 2012 (Shisana O, 2014). Despite these efforts, there is still a large proportion of individuals who have not been tested.

1.5 HIV Prevention

The South African government has embarked on the implementation of a combination of prevention programmes to interrupt HIV transmission. These interventions are segregated into three, namely, biomedical, socio-behavioural and structural factors. The main biomedical interventions implemented are HCT, condom distribution, MMC, STI management, youth HIV prevention, treatment, PMTCT, and high transmission areas (HTA) management. This process involves testing a large number of people using non-laboratory staff (Mashauri F, 2007). It is essential to ensure that QA procedures are followed from specimen collection,

testing and reporting of results in order to guarantee highly reliable and good quality HIV test results (Mashauri F, 2007). The programmatic interventions therefore required the implementation of high quality HCT as entry point. There is a need for simple HCT procedures especially in situations where HIV diagnosis is warranted, such as the PMTCT programmes (Cartoux M, 1998; Dabis F, 2002).

An example of the need for HCT is PMTCT. Availability of ARV drugs to HIV-infected pregnant women is crucial to prevent HIV transmission to their infants, extend prevention benefits to their HIV negative partners as well as to improve their own health. South Africa was one of the 22 priority countries participating in the Global Plan for elimination of new HIV infections among children by 2015. In order to achieve this goal, rapid HIV testing must be supported by scaling up QA measures.

One of the goals of the NSP 2012-2016 is to reduce transmission of HIV from mother to child to less than 2% at six weeks after birth and less than 5% at 18 months of age by 2016 (NSP, 2012-16). This included strengthening the management, leadership and co-ordination of the PMTCT programme and ensuring its integration with maternal and child health programmes.

1.5.1 The Testing Gap

With increasing availability of counselling and testing in many public health facilities in South Africa, uptake of counselling and testing is also increased. The

proportion of people who have ever had an HIV test and are aware of their status in South Africa has increased from 21% in 2002 to 50% in 2008 (Shisana O, 2002;Pettifor A, 2004). Knowledge of HIV status in South Africa between the age groups, 15 years and older increased from 49% in 2008 to 66% in 2012 (Shisana O, 2014).

In order to achieve the ambitious goals of the NSP, there is a need for an unprecedented effort to support health care facilities with delivery of reliable, high quality HIV diagnosis. The expansion of HIV counselling and testing (HCT) has implications for human resources (Taegtmeyer M, 2011), as it directly translates to expansion of not only services but also counsellors and testers. All the newly recruited personnel need to be trained on counselling, testing and quality assurance for testing to avoid compromising the quality of diagnosis while scaling up. In order to support the expanding implementation of quality testing, a need for task-shifting (a process where medical tasks are delegated to less specialised health care workers) has been identified and supported in response to the HIV epidemic (Samb B, 2007; Callaghan M, 2010).

HCT in public health facilities is delivered mainly through rapid testing by non-professional staff. The South African Minister of Health approved the provision of rapid HIV testing by non-professional staff in 2010 (Regulation gazette no. 9285 Volume 539, 2010). This legislation repealed the Human tissue act that allowed only professionals to conduct rapid HIV testing. The gazette specifies that all non-

professionals can provide HIV rapid testing provided they have been trained on the process. This supports the concept of task sharing between the professional nurses and the lay counsellors. Task sharing/shifting is an advantage in that it reduces the challenge of human resources for implementation of health care including HCT in public health facilities (Bedelu M, 2007;Chang LW, 2008; Sanjana P, 2009; Zachariah R, 2009; Callaghan, 2010). On the other hand, task shifting can also influence social dynamics in facilities (WHO, 2008; Tantchou and Gruénais, 2009). Other challenges for HCT include infrastructure where the public health facilities do not have enough rooms allocated for HCT (Tobi P, 2009) as well as requirement for training and supervision of lower cadres (Munga M, 2012).

HCT is South Africa is also delivered through outreach activities including campaigns and in community testing and home based settings. These modalities are supported by the national HCT policy of 2010. In May 2010, South Africa launched the biggest HCT campaign in the world and by June 2012, more than 13 million South Africans had tested for HIV (Mbengashe T, 2012). With the increasing demand for HIV testing as well as the fact that performing the test is not limited to health care professionals only, but includes lay counsellors and community care givers, the accuracy of HIV diagnosis using rapid test kits is crucial.

1.5.2 Statement of the Problem

With the scale up of rapid tests (RT), and in response to the goals of the NSP, it is critical that QA is implemented and sustained to support accuracy of diagnosis in non laboratory services. Given the burden of infections in South Africa there were efforts to expand testing. It was recognised that QA of rapid testing was a key component to the implementation of testing. The South Africa QA programme is guided and supported by the national reference laboratory, the National institute for Communicable Diseases (NICD). The NICD has coordinated the implementation of QA by working with the provinces. The first phase was the adaptation of training material followed by an awareness training at a high level of provincial HIV, AIDS, STIs and TB (HAST) staff. The provinces were expected to provide rollout plans for QMS. With the change in legislation the QMS programme re-focussed on training a different cadre of staff and the training curriculum was revised. In addition, it was observed that the training cascade was not effectively reaching the facility level. A revised strategy was introduced to focus on the facility level training. The strategy included the use of an Internal Quality Control (IQC) programme. This approach was initially applied in Limpopo Province that had demonstrated QMS implementation based on site assessments.

1.6 Addressing the Testing Gap

1.6.1 HIV Rapid Diagnostic Tests (RDTs)

HIV rapid diagnostic tests (RDTs) are single-use HIV assays that yield results in less than 30 minutes and do not have to be performed in the laboratory. Results of

RDTs are available quickly to enable individuals to learn their test result during the same site visit (Branson M, 2007). Early diagnosis provides an opportunity to access effective treatment earlier, with significant benefits for long-term survival and quality of life (Branson 2007). RDTs are also essential when immediate results are necessary to make decisions about treatment, for example, in pregnancy for PMTCT and in occupational exposure for PEP (Bulterys, 2004; Panlilio, 2005). In high-volume, high-prevalence settings, such as emergency departments, rapid tests can make testing more feasible and generate results quickly enough to influence clinical management (Lyss, 2007).

While knowledge of sero-status through antibody testing is the current entry point for HIV prevention and treatment programmes (WHO, 2009), the currently available conventional laboratory based enzyme immunoassays (EIAs) require instrumentation (incubators, mechanical washing and optical reading devices), and expertise, are expensive and do not provide same day results (Yao K, 2010). The introduction of rapid HIV tests has moved HIV diagnosis outside the traditional laboratory and integrated it into services such as point-of-care for ART, prevention of mother-to-child transmission (PMTCT) and HCT. Many studies have shown that with the scaling up of HCT world-wide, the introduction of rapid HIV assays is more appropriate than the traditional laboratory-based diagnosis as HIV rapid assays are more convenient for use in non-laboratory settings (Constantine N T 2005, WHO 2005, Plate DK 2007, Moodley D 2008, Yao K 2010).

The use and availability of rapid HIV tests has made field diagnosis of HIV inexpensive as well as technically feasible in resource-limited settings (Wright R J 2004). Rapid test processes are easy to perform, hence allow persons with minimal training and instruction to perform them (Ekwueme, D 2003). Results can be interpreted or read visually within a short space of time (Spielberg F, 1996, Giles R, 1999). Associations between incorrect HIV results given to patients and poor/no QA procedures at non-laboratory settings and their consequences have been shown in several studies (Gray J J, 1995; Gray RH, 2007; Azalao O, 2008, Bhattacharya R, 2008, Kagulire SC, 2011; Shanks L 2013, Shanks 2015)and the reasons this study seeks to establish the effect of a QMS programme on the accuracy of HIV rapid testing in HCT sites.

The advantages of rapid testing include the fact it allows for an increased testing coverage and that it can be performed by non- laboratory personnel without the need of a formal laboratory training (Yao K, 2010; WHO, 2010; Peeling and Mabey, 2010).Rapid tests have been found to be cost-effective and to have increased the proportions of individuals receiving their HIV results (Global Fund, 2009, Kates, 2009).

Due to expanding need to provide access to affordable HIV monitoring services in general, a great deal of effort focused on the development of point-of-care technologies (POCT) (Setty MK, 2014). POCT diagnostics are in-vitro diagnostics (IVD) that do not involve the use of laboratory staff and facilities to provide the results (Setty MK, 2014); for example, RDTs. POCT enabled testing in many

settings without access to formal laboratory services that are located primarily in low- and middle-income settings and have dominated this diagnostics space. These technologies have revolutionized HIV diagnosis in developing countries (Schito ML, 2010). They are affordable, sensitive, specific, robust, easy-to-use and equipment free (Peeling and Mabey, 2010). The different test samples can be in the form of blood, urine, saliva, and/or other bodily fluids and (semi-) solids. These tests accept a sample with little or no preparation and provide a result, within a short period of time (Meagher RJ, 2008; Peeling and Mabey, 2010).

Both RDTs and other POCTs have been at the core of many screening strategies, including voluntary testing and counselling, provider-initiated testing and counselling, home-based clinics, or community outreach–based testing and counselling. With the swift and widespread implementation of POCT globally, many barriers and impediments have been reported, which have prevented an ideal implementation (Reid SD, 2013; Johnson C, 2014).

1.6.2 The Scale Up of RDTs

The success of the public health approach to management of HIV lies in the large numbers of patients that have been tested and placed on treatment. There is no debate as to the success of the policy of using rapid HIV tests for HIV diagnosis to reach large numbers of people within a short time as shown by the high number of people in care (over 2.5 million in 2013) according to the NDOH annual report, (2014). The use of rapid HIV antibody tests in non-laboratory settings facilitates

access to care for patients. The South African HCT policy guidelines recommend that HIV rapid testing be prioritised in all non-laboratory settings and in communities to reach the populations that are at high risk of HIV acquisitions and/or transmission (Health, 2010). The national policy guidelines recommend the serial testing algorithm with the ELISA being utilised as the tie-breaker in discordant results (Health, 2010).

1.6.3 Challenges of RDTs

Despite the current successes, the use of rapid testing approach has many challenges if the protocols that govern its use are not rigorously followed. It is important that rapid test assays that are utilised in non-laboratory settings are of the same quality level when compared to that in laboratories. A combination of low levels of QA and inadequate staff training results in limitations to the effectiveness of non-laboratory-based rapid diagnostic testing programmes (Martin R 2005,Chang D 2006, Perkins MD 2006).

These challenges include monitoring the performance of the rapid test kits in terms of specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) as these may lead to unacceptably high rates of false positive and false negative results (Gray RH, 2007;Azala O, 2008;Kagulire SC, 2011).False-positive results with HIV RDTs have been widely reported and attributed to a variety of causes including serological cross reactivity, test storage and transport conditions, poor quality control, lack of training and supervision of staff, limitations of the assay itself, sub-optimal national testing algorithms, workload, SOPs not

being followed, contaminating proteins in specimen and possible manufacturing defects (Gray RH, 2007; Shanks L, 2013, Shanks L, 2015; WHO, 2015; Klarkowsky, 2015). They usually lead to discordant test results, which delay diagnosis and, if the frequency of discordant results is high, undermine confidence in testing. However, if the tests used in the diagnostic algorithm are susceptible to the same cause for false positivity, then this may lead to a false-positive HIV diagnosis with potentially devastating consequences. False positive results, usually lead to people being placed on treatment unnecessarily, subjecting them to stress and wasting resources (Shanks L, 2013).

In the case of false negative results, opportunities are missed, resulting in delayed treatment and continued infections (Shanks L, 2013). The implications of unreliable testing are deleterious and tragic. Reports indicate personal emotional distress, severe physical trauma from partners, abandonment and suicides, and some pregnant women have even been advised on and exposed to interventions to reduce mother-to-child transmission (Bhattacharya R, 2008;Shanks L, 2013). Appropriate monitoring for accurate HIV diagnosis will prevent misdiagnosis. Other reasons for misdiagnosing clients are associated with administrative errors, storage and transport conditions, poor quality assurance processes, lack of training and supervision of staff (Shanks L, 2013) .

1.6.4 HIV Treatment Cascade

Antiretroviral therapy is considered successful when it suppresses the viral load of a person living with HIV to undetectable levels. Research shows that people who have an undetectable viral load, they need access to a continuum of services such as HIV testing and load in their blood are less likely to pass HIV to others (Cohen MS, 2011) and more likely to live a long and healthy life (Nakagawa F, 2012). For one to achieve an undetectable diagnosis, linkage to appropriate medical care (and other health services), support while in care, access to antiretroviral treatment if and when they are ready, and support while on treatment. This sequence of steps is commonly referred to as the HIV treatment cascade or the HIV care cascade. Unfortunately, the cascade isn't seamless and some people "leak" out and are lost at each step, due to barriers to getting tested, staying in care, and starting or adhering to antiretroviral treatment. These barriers include poor access to services, stigma and discrimination, poverty, food insecurity and homelessness, and mental health and addiction issues (Hull MW, 2012).

As a result of these leaks at different points in the continuum, only a small proportion of people living with HIV are engaged in all the steps needed to achieve an undetectable viral load. For example, in South Africa, according to the national district health information system (DHIS) 2014, it was estimated that about 38% of people living on ART have an undetectable viral load, while 26% of patients not yet eligible for ART are enrolled in the pre ART register (figure 1.1).

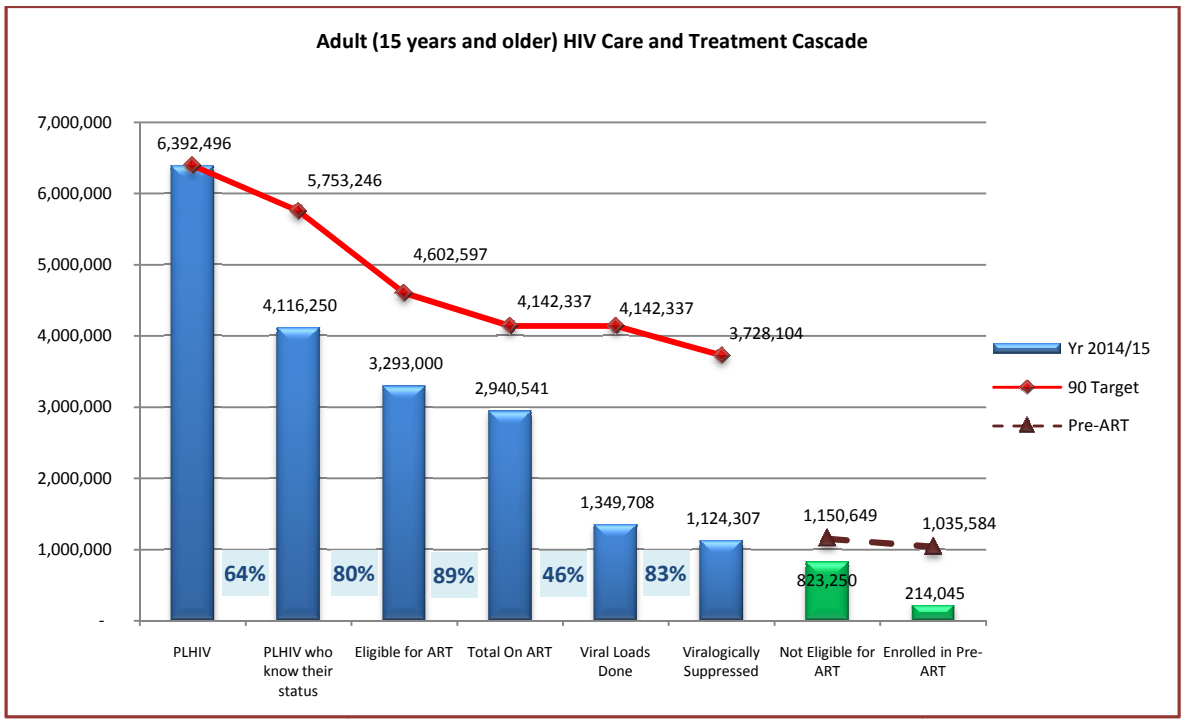

Figure 1.1. HIV treatment cascade for adults in South Africa including ANC, 2014. (Source: Adapted from the Centers for Disease Control and Prevention – Morbidity and Mortality Weekly Report, December 2nd, 2011.)

1.7 Different Strategies for HCT

The traditional HCT strategy is client-initiated counselling and testing (CICT) (Alwayo-Edyegu, 1999). CICT as a strategy has become relatively standardised as HCT programmes grow in size, number and maturity. Evolving epidemic dynamics and increased funding for HIV control have led to expanded service provision and targeting of population groups not reached by existing strategies (Menzies N A, 2009).

New HCT strategies have been developed, that target different population groups and utilise different service delivery methods (HEAIDS, 2008). These strategies include provider-initiated counselling and testing (PICT), which is offered to all patients accessing the health services at the health facilities, and mobile HCT offered in communities and homes (WHO 2012). Currently, in South Africa, in addition to CICT, PICT is offered in all public health facilities in order to expand access to the HCT services. Furthermore, mobile HCT in communities is also implemented through district government mobile clinics and non-governmental organisations. The PICT strategy in public health facilities was adopted and approved in South Africa in 2010 and incorporated into the national HCT policy guidelines (Health, 2010).

Since increasing the scale of HCT can raise knowledge of HIV status, HCT services should also be extended to previously underserved groups such as rural communities. Identifying HIV infected patients for treatment can be achieved by

expanding services to population groups in high prevalence areas. However, knowledge regarding how HCT may reduce HIV transmission is still debatable. Though two randomised control trials (Kamb M L 1998) have reported strong preventative effects, other studies (Matovu J K, 2005; Corbet E L, 2007; Sherr L, 2007) have found little or no impact on risk behaviour or HIV incidence, particularly in HIV negative clients. Several meta-analyses studies (Higgins D L, 1991; Wolitski RJ, 1997; Weinhardt LS, 1999; Denison J A, 2008) have reported preventative effects to be strongest among HIV positive clients and discordant couples. A similar conclusion was reached by an additional meta-analysis conducted in developing countries by Denison J A, (2008). In the Democratic Republic of Congo, Zambia and Rwanda, cohort studies have been conducted following discordant couples (Kamenga M, 1991; Allen S, 1992; Allen S, 2003). These studies have consistently found strong beneficial effects of HCT on condom use and HIV incidence (Kamenga M, 1991; Allen S, 1992; Allen S, 2003).

HIV testing is also essential for identification of HIV in pregnant women for prevention of mother-to-child-transmission of HIV (PMTCT), for protection of blood samples and to monitor disease trends in populations and clinical diagnostic purposes (Wright R 2004). Furthermore, HCT allows for proper monitoring of the HIV prevalence trends and the evaluation of the effectiveness of HIV prevention programmes (Wright R, 2004;WHO, 2005). Expanding the coverage of HCT can reduce HIV-associated stigma and discrimination as well as denial, hence mobilise communities to respond to the HIV epidemic (WHO, 2003). The use of rapid antibody tests has been endorsed by the WHO and have been adopted into

national guidelines in many countries in sub Saharan Africa including South Africa (WHO, 2004, WHO, 2015).

1.8 Accuracy of HIV Diagnosis in Non-Laboratory Settings

The monitoring of the quality of HIV diagnostic tests can have a significant impact on the accuracy of testing being provided to individuals. On the other hand, the lack thereof can compromise clinical management in that treatment may be wasted on patients that do not need it while patients who need treatment are delayed due to misdiagnosis (Peeling RW 2010).

To ensure consistency, the rapid HIV assays that are utilised in non-laboratory settings must be of the same level of quality as that expected of laboratory testing whether performed by professionals or non professionals (Chang D 2006).A Quality Management System (QMS) can be implemented to varying degrees, but the basic principles still apply to any service providing HIV testing results. All sites conducting HIV testing should implement a QMS that incorporates the 12 elements shown below in figure 1.2.

The 12 elements adopted from the WHO HTS guidelines, 2015, comprise of the following: i. Organisation: all testing sites have a quality policy that specifies the crucial aspects of the testing. ii. Personnel: Any testing service must ensure that all personnel are adequately trained, certified and supported to implement the service. iii. Equipment: All essential equipment must be available and fully

functional. iv Purchasing and inventory: All sites must ensure that adequate supplies of test kits and other items required for the testing process are available at all times. v. Quality control: Testing sites must ensure that testing procedures are performed correctly and that the assay works as expected. vi. Information management: All sites must ensure that transcription errors are minimal. vii. Documents and records: All documents and records for all aspects of testing and its quality management system. viii. Occurrence management: All non conformances must be addressed and corrected. ix. Assessment: Both internal and external assessment must be undertaken to assure quality of testing. x. Process improvement: All areas that require improvement must plan and undertake improvements and evaluate their effect. xi. Client service: Customer and client satisfaction must be ensured. xii. Facilities and safety: It is key that testing sites are well designed and maintained. The testing site should be clean, and comfortable with adequate lighting and free of any potential hazards (WHO 2015).

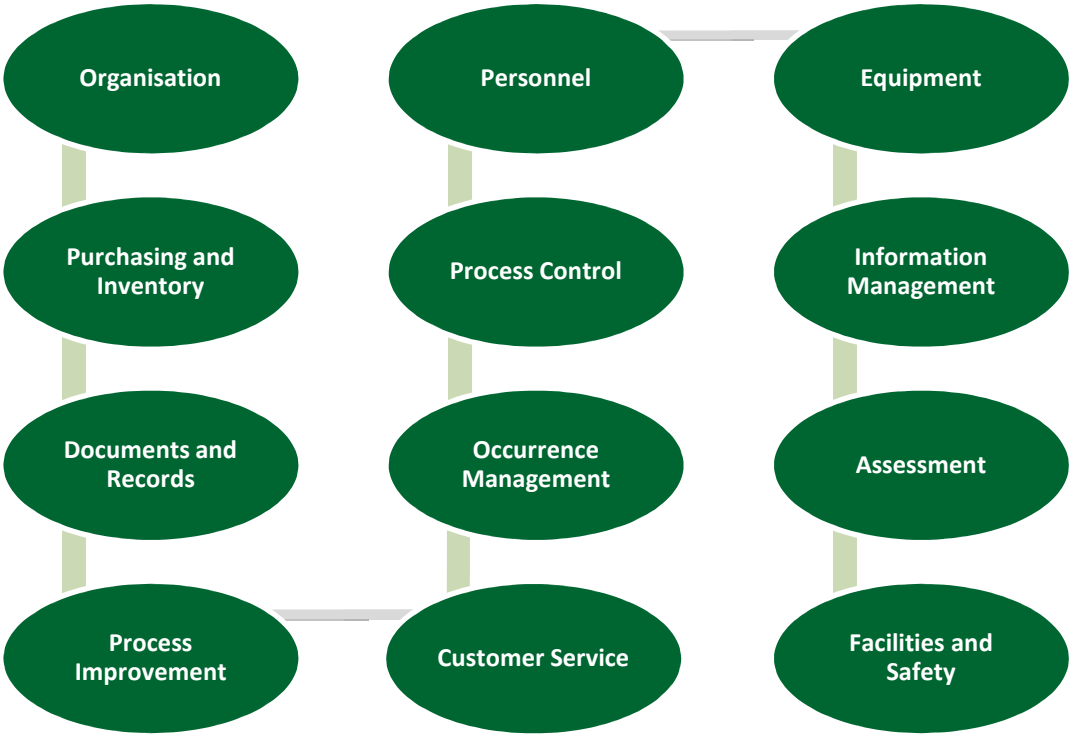

Figure 1.2. Twelve Elements of a Quality Management System

Monitoring of QMS is important in ensuring quality of testing. It is imperative that quality assurance is not seen as a once off activity that is undertaken by one person only. Instead, it should be seen as an integral part of the continuing roles and responsibilities of each and every implementer (WHO, 2015). Accuracy is crucial for effective testing programmes for diagnosis, surveillance or clinical monitoring as these inform prevention and treatment strategies as well as the management of individuals (Rouet, 2004; WHO, 2015). The implementation of the fundamentals of high quality management and monitoring outcomes through quality assurance programmes will reduce variability and increase the accuracy of

interpretation, therefore providing confidence in the testing process (Dax EM, 1999; Chalker V J, 2005; Martin R, 2005; Chang D, 2006). Several factors can affect the accuracy of testing (Dax EM, 1999; Learmonth, KM 2008; Moodley, 2008). While Spielberg F, (1989) demonstrated that the interpretation of assays for HIV detection read subjectively varied by 1.2-8.3% between readers, Learmonth KM, (2008) reported that both experienced and inexperienced readers in rapid testing interpreted the results with relative accuracy though they recommended that training and implementation of QA play a crucial role in accurate interpretation of rapid test results.

This study focuses on an intervention for the process control namely the Internal Quality Control (IQC). There are two types of process control namely the in-built kit control and the independent internal quality control (IQC) that is used to check that an analytical phase or test precision is optimal (Gust, A 2001; Kettlehut M M, 2003).The IQC are usually prepared independent of the kit manufacturer to avoid bias and is a well-characterised serological specimen. IQC confirms the quality of the test kit device before testing commences, on receipt of new batches/lots and in cases of extreme temperatures and prolonged storage.

EQA, also referred to as proficiency testing (PT) is the tool used to assess the testing process independently. It is important to note that EQA can be used to identify training opportunities and not only to monitor technical performances (Chang D, 2006). Adequate initial and on-going training needs may be identified

by using carefully constructed EQA schemes. These are an important measure to ensure both proficiency and accuracy (Prevention, 2005;Prevention, 2007). The QA of rapid test kits must include on-site performance monitoring and the retesting of samples as well as EQA (Martin R, 2005). EQA is a vital component of QA and can be implemented in three approaches as follows: participation in external PT programs, supervisory site visits by external experts and retesting a subset of specimens in another competent laboratory.

PT programs are normally effective as an EQA tool in recognizing poor performing sites (Parekh BS, 2010). Traditional PT programs and quality control reagents use serum/plasma specimens requiring stringent conditions for storage and transportation. To overcome the cold-chain transportation and PT panel delivery challenges, dried tube specimens (DTS) based PT are used as an alternative (Parekh BS, 2010).

Briefly, small volumes (20µl/tube) of specimen are allowed to dry in tubes overnight. Once dried, DTS are stable at room temperature for at least a month and can be rehydrated at the testing site for proficiency testing purpose. The DTS is a dry pellet and, similar DBS specimens, has several advantages. It is stable, safer and less bio-hazardous than liquid specimens (Parekh BS, 2010; Benzaken AS, 2014). In addition, the specimens are stable at temperatures expected in many countries, especially during storage and transport and hence can be transported at room temperature without the need for maintaining an expensive

cold chain. Once received at the testing facility, the specimens can be stored at room temperature for few days without negatively affecting the integrity of the specimens. This general approach may also be extended in preparation of QC materials. Several countries are in the expansion phase of implementing DTS-based PT programmes (Parekh BS, 2010).

The lack of appropriate and on-going training of rapid HIV test implementers represents severe limitations to achieving quality diagnostic testing as it can lead to misdiagnosis (Delaney K P, 2002; Ziyambi Z, 2002; Granade T, 2004; Kanal K, 2005). During the testing process mistakes could happen. These may include conducting testing outside of recommended temperatures, collecting specimens incorrectly, documentation errors, improper performance of testing, and incorrect interpretation of results (Greenwald J L, 2006). Adherence to QA procedures and ongoing test performance monitoring should reduce the number of testing mistakes that occur and increase the accuracy of results as reported by several studies (Delaney K P, 2002; Granade T, 2004; Wesolowski LG, 2006; Laura G Wesolowski, 2009; Wesolowsky L G, 2009).

The ability of untrained rapid testing staff to conduct rapid testing has been documented (Granade T, 2004), hence it is recommended that all personnel implementing rapid testing be trained and supervised to improve accuracy of results. Trained laboratory personnel may also have challenges in performing, interpreting and communicating the results of rapid HIV tests if the initial training

was not adequate or efficacious (Kanal K, 2005; Martin R, 2005; Prevention, 1998). Emphasis should be put on the importance of standardised on-going performance monitoring and training programmes which should be a requirement for all implementers (WHO, 2003). It has been reported that photographed rapid HIV test results may be useful as a cost effective training tool that could generate ongoing training interaction with the EQA processes (Learmonth KM, 2008).

A study was conducted by Learmonth KM, (2008) to determine the feasibility of using difficult to read, photographed rapid HIV test results to assess the proficiency of operator interpretation and, secondly, the potential for using the method as a training tool. The study reported that photographed results of rapid HIV assays could be interpreted with comparative accuracy and that the prior experience of operators resulted in more accurate interpretation of photographed results. The results of their study showed that laboratory personnel who participated in the study, both experienced and inexperienced with rapid HIV assays were able to interpret the photographed rapid HIV results with relative accuracy. The authors further reported that the amount of experience acquired on the job also played an important role in achieving a greater accuracy of interpretation. Furthermore, the authors proposed that this method could be used in providing a novel, cost-effective EQA for non-laboratory rapid HIV testing. Once established, programmes could be used for training and monitoring purposes, facilitating the more accurate interpretation of rapid HIV assays and thereby assisting HIV prevention efforts, especially in resource limited countries. Most

importantly, photographed test results will bring a practical and cost effective approach to QA for the much-needed scale up of non-laboratory HIV testing (Learmonth KM, 2008). In South Africa, however, this method will be a challenge to implement considering the quantities of testing in South Africa (more than 10 million tests per annum), where there is already lack of human resources (HR) to conduct the process, There will be a huge training need and training requirements and issues of timing for the process.

In addition to PT, other EQA methods include re-testing or re-checking, and on-site visits or evaluations as well as assessments including observations (Louis JF, 2013). It has been reported that untrained implementers with no laboratory background are able to perform and interpret the results of a rapid HIV test accurately provided the methods are simple enough to be performed by this category of implementers (Galli A, 2013). In some developing countries, such as Cambodia, counsellors are exposed to half-day training on implementation of rapid HIV testing. A study was conducted to evaluate the proficiency of trained non-laboratory health care personnel and laboratory technicians using the rapid test antibody technique. The accuracy of reports by counsellors was reported to be higher (100%) than that of laboratory technicians which supported the half day training that non-laboratory counsellors are usually exposed to before they can undertake HIV rapid testing in non-laboratory settings (Kanal K T, 2005).

The performance of rapid RDTs has improved greatly with new assays developed based on new technologies; as a consequence, most rapid test assays have

comparable performance with ELISA assays (Branson M, 2000; Ketema F, 2001; Ferreira CJ, 2005;Peeling RW, 2006; Everett DB, 2009). RDTs may either be immune-chromatographic (lateral flow) or immune-filtration (flow through) with assays to detect HIV antibodies to HIV-1/2 infection and/or HIV-1 p24 antigen in finger prick capillary blood, oral fluids, serum, plasma and venous whole blood specimens (Pai NP, 2007; Pai NP, 2012).

Serological RDTs for HIV-1/2 are categorised by generation characterised by antigen and/or conjugate used. The first three generations of HIV RDTs detect only antibodies to HIV-1/2 (WHO, 2012), thus individuals in the acute stage of infection, who may pose a substantial risk of HIV transmission to others may not be identified. The most recent generation of HIV RDTs (fourth generation) have been developed to detect HIV-1p 24 antigen, in addition to the HIV-1/2 antibodies tested for in previous generations of RDTs (Taegtmeyer M, 2011). Detection of the HIV-1 p24 antigen would allow earlier diagnosis of HIV when the infected individual is in the acute phase of infection and before development of antibodies to HIV-1/2. Fourth generation HIV RDTs are currently used in clinical practice in communities and clinical settings in both developed and developing countries (Taegtmeyer M, 2011). Fourth generation HIV RDTs are used as initial screening assays, followed by additional testing with two or three RDTs, depending on the underlying disease prevalence, to confirm the initial reactive test results.

A number of studies from different settings have been published showing the accuracy of the single available fourth generation HIV RDT to be suboptimal for the HIV-1p24 antigen detection component, with low sensitivity for identification of individuals in the acute phase and other individuals with low p24 antigen titres (Chetty V, 2012; Rosenberg NE, and 2012; Conway DP, 2014). Fourth generation HIV RDTs are more expensive than previous third and second generation RDTs and the proclaimed additional advantage of their detection of acute infection could be misplaced (Chetty V, 2012; Rosenberg NE, 2012; Conway DP, 2014). Furthermore, it has been reported in several studies that sensitivities of RTDs may differ in acute infections (Cohen MS, 2010; Cook D, 2010; Patel P, 2012).

1.9 HIV Testing Algorithms

Algorithms are defined as the sequence in which assays are performed to detect HIV antibodies in a body fluid (WHO, 2003). HIV testing strategies have clear objectives for diagnosis, surveillance and transfusion safety. The need for appropriate selection of testing platforms and protocol had also varied from setting to setting. Sensitivity, specificity and prevalence of HIV infection in general population play an important role in the choice of appropriate tests for different settings (WHO, 2015).

The decision to use parallel or serial testing algorithm is crucial and dependant on several factors including cost. In 2010, it was reported that if assays are properly selected, the positive predictive value (PPV) of 2 sequential reactive tests

(specificity ≥ 99%) is more than 90% even in low-prevalence populations (0.1%), and more than 99% in populations with a prevalence of 1% or higher (Parekh BS, 2010). The author further reported that if the tests have a specificity of 98%, the PPV is more than 90% in populations with a prevalence of 0.5% or higher , (Parekh BS, 2010).

One of the factors that may be used to determine the country's algorithm is the performance of the tests used. The tests are based on how closely a rapid HIV antibody test agrees with the gold standard viz., ELISA. In South Africa, as in many countries, the gold standard is the ELISA and the recommended algorithm is the serial testing algorithm. The advantage of using an algorithm with rapid tests as compared to ELISA and Western Blot for confirmation, is its simplicity and rapidity (Ferreira CJ, 2005). These characteristics can be useful when addressing high- risk populations in which only one opportunity for counselling and providing results is possible. The adoption of an appropriate strategy could expand the availability of HIV counselling and testing, which may increase the number of individuals reached for encouraging the adoption of risk-reducing behaviour or link to care.

Algorithms may consist of a screening test and a different confirmatory test to confirm initial positive results, or two tests in parallel with a third test as a tie-breaker for discordant results. These testing strategies are referred to as serial and parallel algorithms, respectively (Richard J, 2004). A serial testing algorithm is

one in which a single rapid test, if non-reactive, is not confirmed with a second test but recorded and reported as a negative result. If the first test is reactive however, it is confirmed with a second, different rapid test. Discordant results are resolved with the ELISA or a third RDT. The tie breaker approach is not supported by WHO, (WHO, 2011; WHO, 2015). Since HIV negative test results for the first or screening rapid test are not confirmed, the serial approach has the potential to miss some cases of HIV infection.

In many sub-Saharan countries, several rapid testing algorithms that are utilised are usually based on the availability of test kits and not the proven specifications of an algorithm. Several studies in sub-Saharan Africa have demonstrated that commercially available HIV rapid test kits yield favourable sensitivities and specificities at site level, (Nunn A J, 1993; Koblavi-Dème S, 2001; Urasa W, 2002; Van den Berk GEL, 2003; Foglia G, 2004; Menard D, 2005; Kroidl I, 2012). The WHO recommended sensitivity is 99% for first and second line assays (RDTs) and 100% EIA. While specificity is 98% for first line assays (RDTs and EIA) and 99% for second line assays (WHO, 2015). WHO does not recommend tie breakers for inconclusive results, but that clients be called back to re-test after 14 days as compared to the South African HCT guidelines (2010) that recommend ELISA as a tie breaker for inconclusive results and a sensitivity and specificity of 99% for first and second line assays (Health, 2010). Both quality and cost of rapid HIV diagnosis are critical in HIV rapid testing. It is, therefore, imperative to identify rapid testing algorithms that conserve finances by reducing the number of test kits

and support equipment used, while at the same time providing optimal performance (Wright R, 2004). With the expansion of HIV and AIDS treatment in Sub-Saharan Africa, knowledge of HIV status and choosing appropriate testing strategies remains critical.

Understanding the functioning of rapid antibody tests in different testing algorithms is crucial to inform local and national testing HIV testing policies. Many sub-Saharan countries have implemented the serial testing algorithm. A well-informed choice of a testing strategy can lead to better results and significant cost reduction. Correct identification of HIV positive patients is the gateway to most HIV and AIDS prevention and treatment programmes and will be vital to any systematic effort to eliminate the epidemic. In order to reduce the number of incorrect results, it is recommended that individual tests are used in combination algorithms that perform better than single tests alone and that testing is conducted in a manner that is consistent with the manufacturer's instructions (Wesolowsky L G, 2009).

The Food and Drug Administration (FDA) requires testing facilities that plan to perform rapid HIV testing to develop QA plans which include site specific testing procedures, plans for training (Mayhood MK, 2008), testing personnel and development of systems to identify and correct mistakes (Prevention, 2007). Adherence to QA procedures and ongoing test performance monitoring should reduce the number of testing mistakes and increase the accuracy of results (Wesolowsky L G, 2009). Despite the setting or the personnel who perform the

rapid HIV tests, the accuracy and reliability of diagnostics must be maintained for the success of HIV and AIDS programs.

To ensure reliability and minimize errors, quality assurance measures must be in place to address all aspects of testing as rapid testing expands into non-traditional settings where testing is conducted by people without a laboratory background or formal health care training (Yao K, 2010). These include, IQC implementation, compliance to the recommended algorithm and SOPs and correct interpretation of results. Increased need for HIV testing has created an urgent need to train large numbers of personnel to meet the demand.

There is a widely held assumption among healthcare workers that the rapid tests are as reliable and accurate in the field as in the laboratory, hence require little monitoring to ensure the quality of results. However, a review of eleven African countries with rapid testing programmes found that only seven had a system involving laboratory retesting, four conducted periodic on site observation, and two utilised proficiency testing panels (Plate, 2007).

Monitoring the quality of test kits after procurement and before distribution is critical, i.e. post marketing surveillance (PMS). Any deviation in end-point sensitivity and/or specificity may indicate suboptimal performance of the assay. The quality of the test kit may be affected adversely by suboptimal transport, storage temperature, humidity, or other environmental factors (Parekh BS, 2010).

Additional measures are needed to determine the quality of kits after procurement. It is recommended that stored kits be validated at regular intervals or randomly picked from field sites for post marketing surveillance. IQC can also serve this purpose at test site level. Furthermore, appropriate policies need to be in place for a course of action in case compromised test kits are detected.

1.10 Quality of Rapid Testing in the Field

It has been documented that in the field, i.e. at counselling and testing sites, the specificity and sensitivity of rapid HIV test kits decreases as compared to the laboratory setting (Mayhood MK, 2008;Black V, 2009). This may be due to several factors such as storage conditions of test kits, handling, transportation, temperature, lack of training, lack of basic QA application, record keeping, and evaluation of test kits, testing algorithm and on-going monitoring and evaluation (Plate DK, 2007;Moodley D, 2008) reported that the sensitivity of four different rapid HIV test kits was 92.5-97.3% when administered under field conditions (antenatal sites) and 100% when performed in a laboratory by technologists using ELISA testing in Kwa-Zulu Natal, South Africa. They further reported that weak positive results could be difficult to correctly interpret and might lead to false negative diagnosis in the field. Low sensitivity of rapid test kits was also reported by in a clinic setting in Cape Town. They attributed this to poor adherence to testing policies by staff as per their observation (Wolpaw BJ, 2010).

Four RT kits were evaluated in Cameroon and it was found that the test sensitivities varied from 94.1% to 100% and the specificities varied from 88% to 98.8%. The PPVs varied between 85.3 and 100% in a population with 11% HIV prevalence with the exclusion of discordant results as shown in table 1.1a. With the inclusion of discordant results the PPVs varied between 59.1 and 92.9% with a prevalence of 11% as shown in table 1.1b (Aghokeng FA, 2004).

Table 1.1a Results of evaluation of 4 rapid test kits in Cameroon without indeterminate results

Test kit	Sensitivity (%)	Specificity (%)	PPV: 11% prevalence	NPV: 11% prevalence	PPV: 7% prevalence	NPV: 7% prevalence
Genie II	98.9	100	100	99.3	100	99.3
Immuno Comb II	99.3	99.5	98.1	99.6	97.5	99.6
Determine HIV 1+2+0	100	98.3	94.4	100	91.5	100
Camstix-HIV1+2	100	98.3	94.4	100	91.5	100

Table 1.1b Results of evaluation of 4 rapid test kits in Cameroon with indeterminate results

Test kit	Sensitivity (%)	Specificity (%)	PPV: 11% prevalence	NPV: 11% prevalence	PPV: 7% prevalence	NPV: 7% prevalence
Genie II	98.9	98.2	92.9	99.3	88.9	99.4
Immuno Comb II	99.3	97.4	84.6	99.5	84.6	99.6
Determine HIV 1+2+0	100	90.6	72.4	100	62.5	100
Camstix-HIV1+2	100	88.3	67.9	100	57.4	100

Although evaluations conducted in resource-constrained countries have demonstrated that rapid HIV tests perform well in research settings, their performance may differ when used on a wide-scale due to lack of trained staff, poor laboratory infrastructure and weak quality assurance programmes (Kline RL, 1994; Andersson S, 1997; Stetler HC, 1997).

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The performance of two rapid HIV test kits was tested and validated under field conditions in Northern Tanzania and it was reported that the sensitivities, specificities, PPV and NPV of the test kits were reduced from 100% to a range of 98-99% (Mayhood MK, 2008). In addition, the diagnostic performance of two rapid tests for HIV-1/2 in plasma and in whole blood were evaluated, where lower sensitivities and specificities of the RDTs were reported in the field (Kroidl I 2012). These results support earlier studies (Gray J J, 1995; Ramalingam S, 2002;Van den Berk GEL, 2003; Ferreira CJ, 2005; Menard D, 2005; Plate DK, 2007).

In order to address the QA issues, efforts have been invested by international organizations such as CDC, WHO, PEPFAR and at a National level. Examples include CDC/WHO network meetings and stemming from such meetings is the development of a QA curriculum that addresses HIV rapid testing specifically (WHO, 2004). This responds to the need for reliable diagnostic assays capable of ensuring the correct identification of infected individuals and the safety in blood transfusion (Aghokeng FA, 2004). The use of rapid point-of-care tests, introduction of services to prevent mother-to-child transmission, and increasing provision of

antiretroviral drugs were key events that facilitated the expansion of HIV counselling and testing services (Marum E, 2012). Innovations in service delivery included providing HIV testing in both clinical and community sites, including mobile and home testing (Marum, 2012). Promotional campaigns were conducted in many countries and evolutions in policies and guidance facilitated expansion and uptake (Marum E, 2012).

The HIV diagnostic market has grown greatly producing a number of different rapid HIV test kits in the market. Not all test kits are manufactured under good manufacturing processes (GMP) standards. Some kits may be produced by the same manufacturers and sold under different names by several different companies. This means that occasionally the same test kit or its components are manufactured in multiple facilities; some of which may lack appropriate inspection or approval by regulatory agencies (Parekh BS, 2010). Several steps should be followed to assess the quality of test kits at various levels of the evaluation process (Figure 1.3).

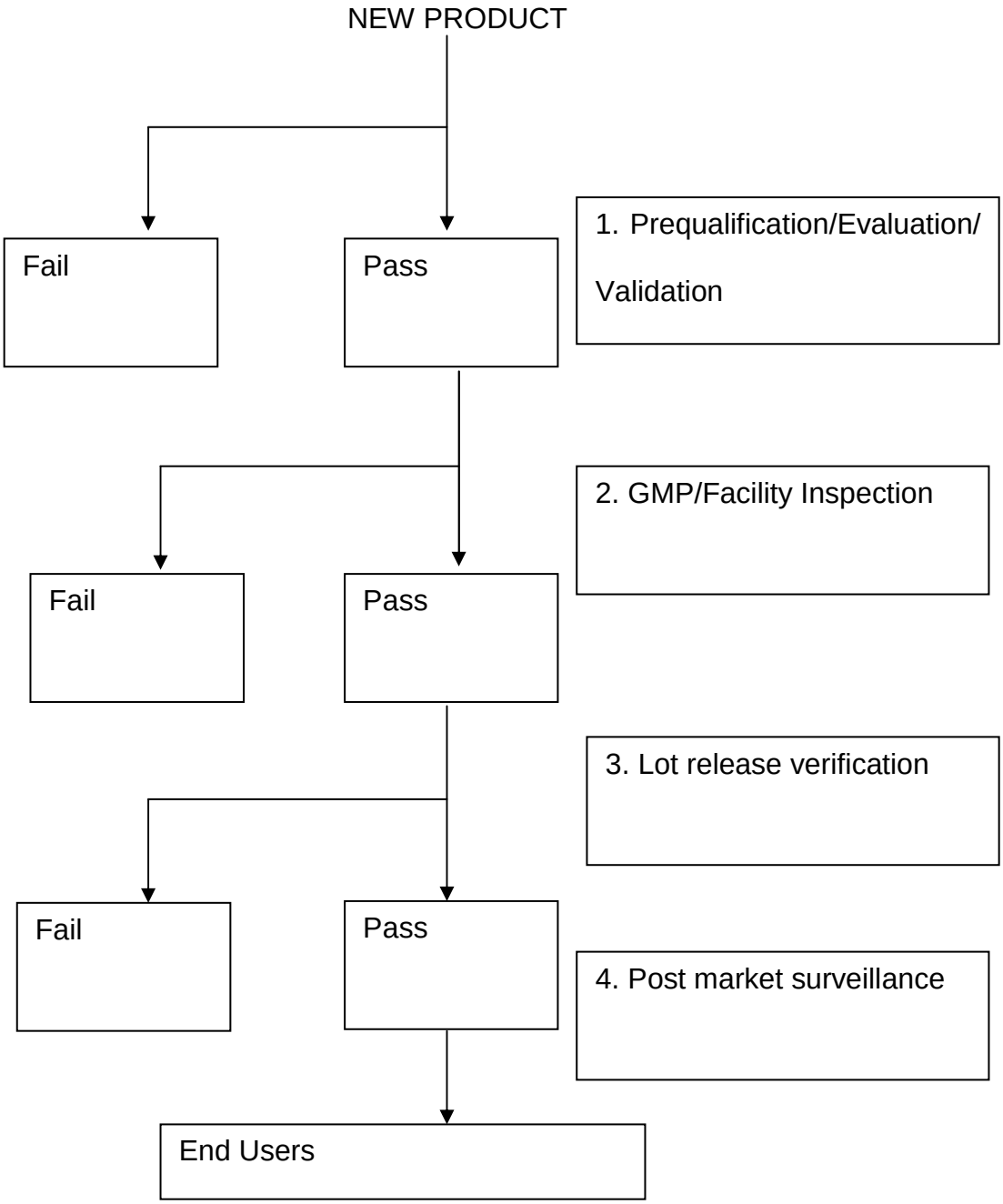

Figure 1.3 Assessment of the quality of HIV rapid test kits: WHO, GMP: Good manufacturing processes (Parekh BS, 2010).

Furthermore, to ensure that rapid tests perform as expected, WHO developed guidelines for country-based evaluation and implementation of rapid HIV testing (WHO, 2004). These guidelines recommend a three-phase approach (figure 1.3): In phase 1, candidate rapid HIV test kits are evaluated for sensitivity and specificity in a reference laboratory. Tests that demonstrate acceptable performance are then selected for phase 2, which is, the field evaluation. This assesses the performance of rapid test kits singly or in combination testing algorithms at point of care (POC) sites under the conditions of intended use.

Depending on the evaluation results, a rapid HIV testing algorithm is developed using 2 or 3 rapid HIV tests that fulfil the selection and performance requirements (Chaillet P, 2009). Phase 3 recommends the implementation of the suitable algorithm with a system of continuous quality assurance that optimally includes training, supervision and competency assessment of personnel who perform the tests, site visits to observe testing and EQA based on re-testing a proportion of specimens by reference laboratories (figure 1.4).

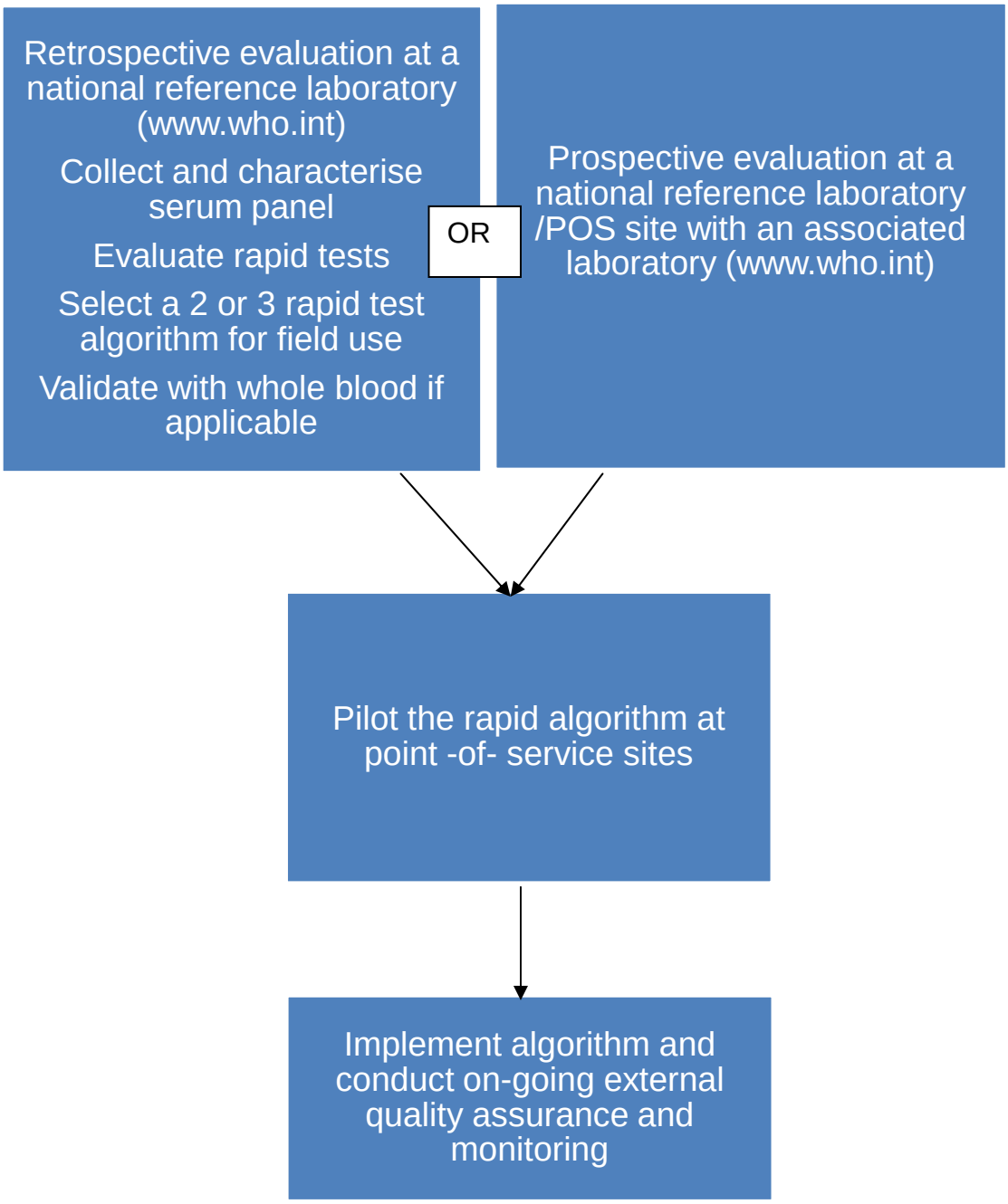

Figure 1.4. In-country evaluation of rapid HIV tests Adapted from Guidelines for appropriate Evaluations of HIV testing technologies (WHO, 2003) (Plate DK, 2007).

Following the implementation of the pre-qualification programme, the United States Agency for International Development (USAID), in collaboration with U.S. CDC, instituted a vigorous test kits validation program to support and enable different implementing countries to have access to HIV rapid test kits that are not yet FDA approved but meet the performance criteria. This evaluation is performed using a well-characterized global panel of serum/plasma specimens obtained from several countries with diverse HIV-1 subtypes and HIV-2. Validation also includes a comparison of 3 kit lots to assess lot-to-lot consistency. Test kits are rated for different characteristics including provision of all testing components and devices used, recommended storage temperature, shelf life, training needs and ease of use and interpretation.

The expectation from WHO was that each country would develop appropriate plans that would result in an implementation of QA at testing sites as well as monitor the effectiveness of the implementation. The guideline was developed to review aspects such as consensus on the training curriculum, followed by phases such as direction setting, alignment, readiness, implementation and monitoring and evaluation. South Africa developed the national HCT policy guideline and the national QA guidelines in 2010 to guide the implementation of HCT (Health, 2010).

1.11 QA Strategies

Several tools and models may be used to monitor and improve the quality of testing. These include algorithm choice, procurement and storage, training and

refresher training, reporting, supervision, observed practice, use of a logbook for data collection (Appendix 1) and implementation of an EQA programme as well as an internal quality control (IQC) programme. The use of a standardised logbook to monitor and improve the quality of testing on-site is important. Incomplete information, lacking test kit names, lot numbers and expiration dates makes it difficult to trouble-shoot problems (Parekh BS, 2010).

The retesting strategy involves the collection of every 10^{th} specimen as serum or dried blood spot (DBS) and sent to a reference laboratory for retesting to determine the extent of field site accuracy. While such strategies are important to improve the accuracy of rapid testing, some limitations in resource- limited settings may include the following: cost and the lack of trained personnel for implementation (Learmonth KM, 2008).

1.12 QMS Implementation Strategies in South Africa

Ideally, to implement a high quality strategy for QMS the following must be taken into consideration: direction setting to define goals, alignment to all relevant stakeholders, system readiness, implementation to scale and monitoring and evaluation (Yao K, 2010). South Africa has a national QA guideline that was developed in 2009 to guide provinces regarding implementation of quality assurance. The NDoH is currently implementing the following interventions: post-marketing surveillance, both active (testing new lots) and passive (IQC and PT), by NICD (national reference laboratory) for all test kits on tender, standardised

training curriculum for QMS, training of trainers programme by the NICD throughout the country for implementation of QMS and development of standard operating procedures. The trainers are expected to disseminate the training to all end users in their facilities.

Consultations have been conducted with provincial programme managers to make them aware of the expectations for QA implementation. The IQC programme piloted in Limpopo province is currently being rolled out to other provinces. This programme involves using known HIV positive samples and HIV negative samples to validate the test kits before use at the facilities. NICD provides both HIV negative and HIV positive controls for validating test kit device performance during the pilot study. All staff members who routinely offer HIV testing were trained through the TOT model prior to commencement of the pilot. The staff was monitored regularly and offered technical support. Based on the results, the intention is to expand the roll out the programme.

In South Africa, a testing strategy using Dried Blood Spot (DBS) was implemented by a national HCT implementing partner, namely, New Start. This approach is, however, only statistically valid for sites with a high volume through-put, i.e. 5,000 specimens or more per time period. It is not recommended to retest specimens for low volume sample through-put, as a much higher proportion (40% to 90%) of specimens will have to be retested in order to be able to detect significant error rates of 1-5% (WHO, 2004). Furthermore, the re-testing programme has certain

constraints including requirement of staff, time and lack of timely results to affect the outcomes. While South Africa supports the different QA strategies that New Start implements, re-testing may not be practical in public health settings due to the quantities tested on a monthly basis, lack of technical staff to implement and logistics. The country has opted to implement PT in addition to IQC and focus on training, mentoring and supervision of implementers. Countries such as Rwanda, Cameroon and Tanzania have implemented EQA (Granade T, 2004;Mashauri F, 2007;Chaillet P, 2009).

1.13 New Start: NGO QA Strategies

New Start is an NGO that is funded by PEPFAR to complement the government HCT programme (www.newstart.co.za). It has implemented HCT in non medical sites in all 9 provinces since 2004. New Start is one of the biggest HCT implementing partners in the country and it has sites whose only role is to provide HCT services in the community. New start sites are called non-medical sites because they do not provide any primary health care to participants. New Start was selected for the study as it implemented several quality assurance measures at all sites for HIV testing therefore presented a good opportunity for comparison. These included, QA procedures covering the equivalent 12 elements applied to laboratory-based QA, IQC, EQA viz., proficiency testing panels (PT) and the re-testing process. New Start sites employed a quality manager who was a laboratory technologist and training was provided on competency for HIV testing and quality assurance to all employees involved in the implementation of HIV rapid testing.

Furthermore, New Start sites also had monitoring and evaluation managers for HIV rapid test quality management, who monitored the progress of the programme and the implementers (counsellors and testers). New Start utilised the National Institute for Communicable Diseases (NICD) for quality assurance of rapid HIV testing, which included HIV ELISA testing as a confirmatory method for rapid HIV testing (retesting), IQC and PT panels (plasma). For retesting, 10% of specimens (DBS) were sent to NICD for validation.

There is paucity of documented assessment of the implementation of QA at HIV rapid testing sites in South Africa, i.e. there is no systematic programme to monitor the implementation of the necessary trainings at facility level, as well as the implementation of quality assurance measures and guidelines in the country (SEAD, 2010; Moodley, 2008). The SEAD study aimed at analysis of VCT implementation in South African public health facilities. The study was an evaluation that identified potential problems observed during HCT service such as availability of test kits on tender, swabs, stop watches, wearing gloves, counselling, availability of registers and any form of QA, but did not quantify accuracy of testing, nor look into sensitivities, specificities, PPVs or NPVs.

South Africa is currently implementing the following QA aspects:

Test product validation: All rapid HIV test kits that are utilised in public health facilities in South Africa are subjected to a pre-qualification validation by the National reference laboratory, NICD. After selection and prior to implementation,

all test kits on the tender are subjected to a post-marketing surveillance process to validate the test kits against specific requirements set out such as sensitivity, specificity and stability (Parekh BS 2010).

Training: A country-specific rapid testing quality management system (QMS) curriculum was adopted in 2006. Provincial training rollout plans were developed in 2007, followed by training of project management and master trainers since 2008 by the National reference laboratory (NICD). Training is ongoing as required. New Start staff has undergone this training.

Training strategy rollout plan: The rollout strategy has been developed. The strategy is followed by QMS training of key personnel (ToTs) including master trainers, programme managers, mentors and regional training centres (RTCs) by NICD. It is expected that training will be cascaded to district and facility level by each province. Developed provincial plans are followed by continuous monitoring of effectiveness of training.

QA guidelines: In South Africa, QA guidelines were developed in 2009 (Health, 2009). The QA guidelines were circulated in provinces through provincial coordinators. Provincial coordinators have been trained on implementation of the guidelines and are expected to cascade the training throughout the provinces. Provincial coordinators have been trained on the implementation.

IQC: The IQC pilot project was implemented by the Department of health and NICD in Capricorn district in Limpopo in 2010. The rollout of IQC in the Limpopo province has been completed.

1.14 Study Aims and Objectives

1.14.1 Aim

To determine the accuracy of HIV rapid testing in two provinces in South Africa- one in the presence and one in the absence of a Quality Assurance indicator (IQC).

1.14.2 Objectives

- To assess the sensitivity and specificity of rapid test kits in two provinces
- To assess the sensitivity and specificity of rapid test kits between the two provinces and New Start NGO which implemented a more comprehensive QMS
- To assess the accuracy of HIV rapid testing in the two provinces
- To assess the accuracy of HIV rapid testing between the two provinces and New Start sites.

1.15 Hypothesis

The accuracy of HIV diagnosis using HIV rapid test kits in non-laboratory settings in which an intervention has been introduced (IQC) will not be different compared to settings that do not utilize IQC.

2 CHAPTER 2: METHODOLOGY

2.1 Introduction

This chapter describes the study background, design, setting, population, implementation and data management. Eligibility criteria for recruitment into the study are described in detail. The prospective and retrospective datasets used for the analysis are also described, including the measures taken to ensure credible data quality. The chapter ends with a review of the methods used for data processing and ethical considerations.

2.1.1 Limpopo Pilot Project

Between June 2009 and September 2010, the Department of Health and the NICD initiated an IQC pilot project in Limpopo in support of the rapid HIV testing programme. The pilot project was followed by full implementation of the use of IQC as part of a quality assurance initiative for HIV rapid testing. The pilot study was to determine the feasibility of the intervention and with the aim of full implementation after the pilot. The effectiveness of IQC on the quality of HIV rapid testing was not known. In this pilot project, IQC was implemented routinely in 23 selected sites as part of QA measures in the Capricorn district.

In South Africa, the current gold standard for diagnostic approach within the public sector as recommended by the National Health Laboratory Services (NHLS) Virology Expert Committee is a serial 2-test algorithm. The recommendation is that

in the case of a reactive test result the second confirmatory result should be different in terms of antigens and technology. The Expert Committee recommendation is that positive results should be confirmed with a second independent specimen in 14 days. In the case of HIV rapid testing the national policy similarly recommends a serial 2-test algorithm for diagnosis where a reactive screening test is confirmed by a different confirmatory test. If the confirmatory test is reactive the diagnosis is positive. If test 1 is non-reactive then the diagnosis is negative. In case of discrepant results an ELISA test is recommended as a tie-breaker (HIV Counselling and Testing (HCT) Policy Guidelines 2010). The above algorithm has replaced the use of the Western blot as a confirmatory test in South Africa. The rationale for the change was based on the sensitivity and specificity of 3rd and 4th generation ELISAs, workload, costs and expertise.

2.2 Training

In both Provinces all lay counsellors were routinely trained on the 10-day HIV counselling and testing and 2 day HIV rapid test competency training. A counsellor was deemed competent if there was successful demonstration of a rapid test performance and results interpretation during training.

In Limpopo, in addition to the 10-day HCT and the rapid test competency training, the counsellor received the following training curriculum developed by the NICD and approved by the NDOH: training of trainers (ToTs) on QMS (3 days), HRT (2

days refresher), IQC (2 days) and PT (1 day). All nurses and counsellors who provided rapid HIV testing in facilities were trained in the three areas. SOPs were developed and all staff were oriented on the use of SOPs. Job aides were also developed for use by implementers. All ToTs were conducted by NICD and NDOH. All participants received competency certificates for each training. SFH had developed for its New Start franchises a detailed training programme as part of its QMS including RDT, IQC, and EQA, data management. Counsellors also had to go through competency training prior to start of testing.

2.3 Implementation of HCT

2.3.1 QC

The IQC specimens were characterised by serology testing including two ELISAs, RTD and WB testing in the case of positive specimens and provided as HIV negative and HIV positive serum samples. SOPs for storage and use of IQC samples were provided with the samples and training on SOPs was conducted. Each counsellor used these QC samples first to confirm validity of the test kits prior to testing of patients. The IQC specimens were used at the beginning of each week and on receipt of new batches. The results were recorded in a logbook. The counsellors were closely monitored by the sister-in-charge to ensure compliance. In cases of IQC failure, root cause analysis was conducted and recorded in the log-books.

2.3.2 RDT in HCT services

Rapid HIV testing was performed by trained lay counsellors in both provinces and in New Start sites. New Start further used ELISA testing per a diagnostic algorithm. All counsellors implemented HCT following the serial testing algorithm as per South African HCT Policy (Health 2010) (Figure 2.1) where in case of discrepant results ELISA was used as a tiebreaker.

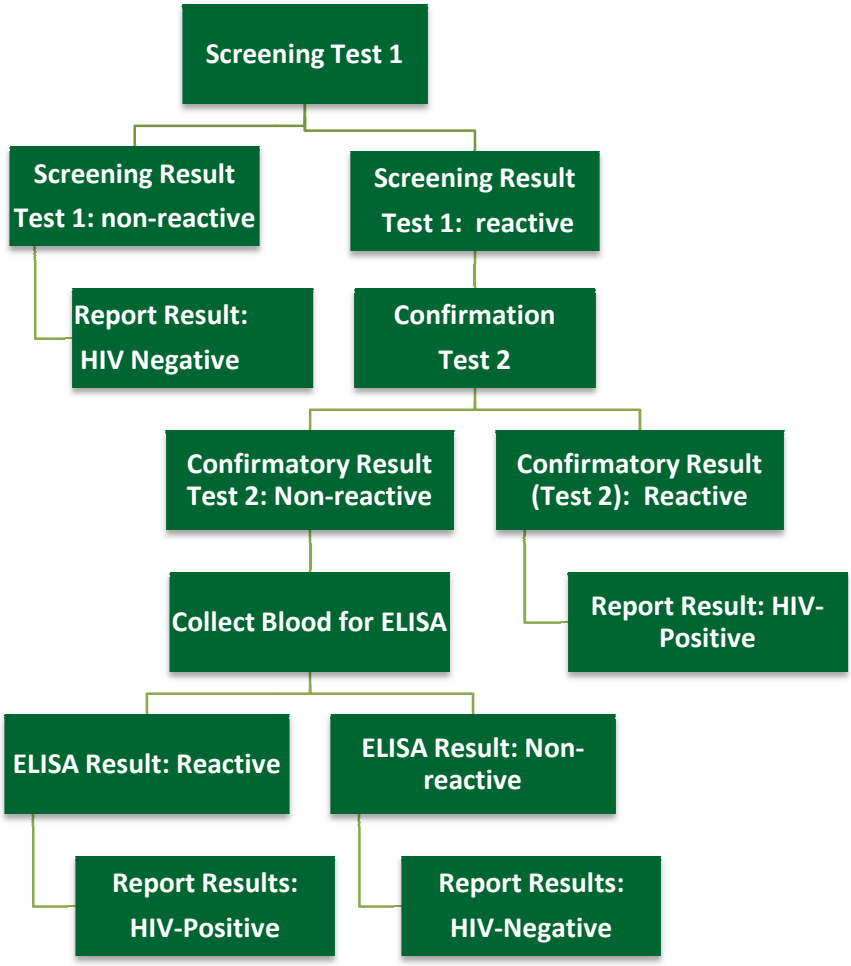

Figure 2.1 National HIV rapid Testing algorithm Source: HCT Policy Guidelines: 2010, National Department of Health.

HCT was offered in counselling rooms and delivered in the form of client-initiated counselling and testing (CICT) also known as voluntary counselling and testing (VCT). In this study, HCT was offered only by trained lay counsellors. There were 3 counsellors per site on the study in Mpumalanga while Limpopo had 5 counsellors per site implementing HCT. Attendees who presented for HCT at all the selected study facilities were introduced to the facility general health information session which included basic information on HIV and AIDS. The study was introduced to the attendees during this briefing session.

The information session was followed by individual pre-test counselling sessions, where participants were given detailed study information to enable them make informed decisions regarding participation. Recruitment was made based on the study inclusion criteria as described. Informed consent forms were administered to participants who agreed to participate in the study. Thereafter, rapid HIV testing was performed on all study participants following the national algorithm (figure 2.1). The national HIV rapid testing algorithm was a serial testing process, where screening was conducted using ABON™ HIV 1/2/O Tri-line HIV Rapid Test Device (Trinity Biotech Manufacturing Ltd, China). All non reactive results were recorded as HIV negative. All reactive screening results were confirmed with First Response® HIV 1-2.0 rapid test kit (Premier Medical Corporation, Ltd, India). If the confirmatory result was reactive then patients were reported as HIV positive. All discordant results were sent for ELISA as a tie breaker. The screening and

confirmation test kits were allocated through the national tender to both provinces where the study was conducted.

All participants were informed of their rapid HIV test results, received post-test counselling and were referred appropriately for further management, based on their HIV test results as per standard national guidelines. All participants who tested HIV negative on rapid test were requested to come back for re -testing in 6 weeks in cases of possible recent exposure per policy guidelines. All participants who tested HIV positive were managed appropriately according to the national guidelines (Health 2010). All ELISA testing results were sent back to the facility to be recorded. This provided an opportunity to call back patients in case of discrepant results.

2.4 ELISA Assays

All participants provided whole blood for ELISA testing irrespective of their rapid HIV test results. The ELISA testing algorithm used was a serial testing algorithm in both Limpopo and in Mpumalanga NHLS laboratories (figure 2.2). The screening was conducted with Cobas E411, Roche, Germany, while confirmatory was conducted with the Advia Centaur ® HIV 1/O/2, Enhanced (EHIV) Siemens, Germany. Both ELISA assays were 4th generation tests. All non reactive results with the screening tests were reported as HIV negative. All reactive results were confirmed and if confirmatory is reactive, the participant was reported as HIV positive. If the confirmatory results were non reactive, the patient were recorded as discordant.

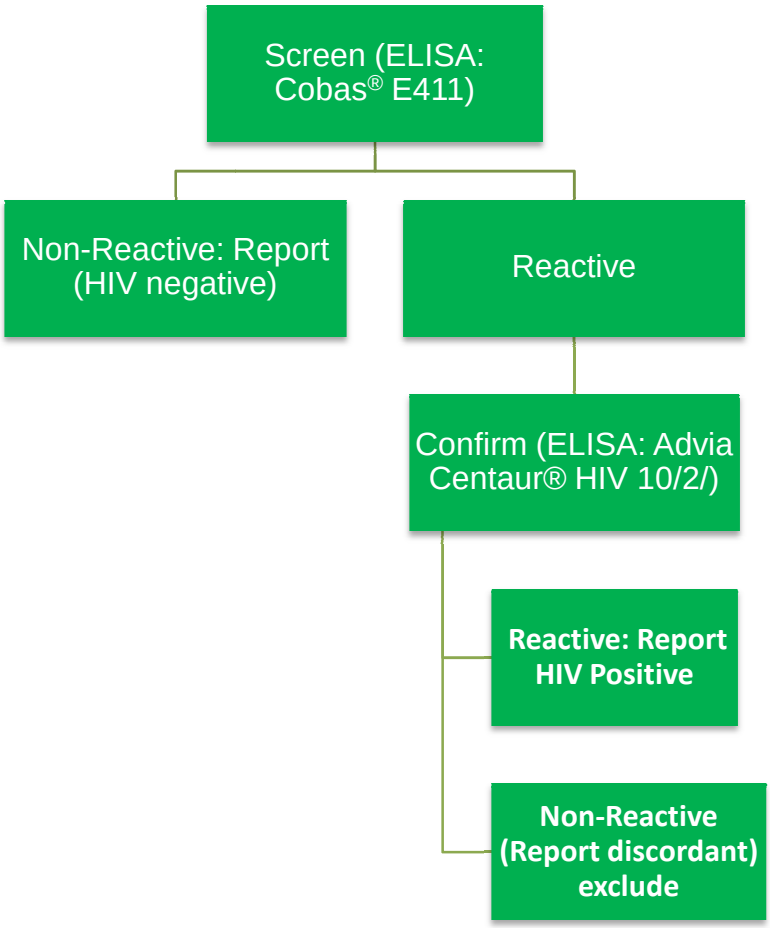

Figure 2.2 NHLS HIV ELISA diagnostic testing algorithm

2.4.1 Basic Principles of the Assays

i. Advia Centaur ® HIV 1/O/2, Enhanced (EHIV) Siemens, Germany.

The Advia Centaur ® HIV 1/O/2 is an antigen sandwich immunoassay. It utilises a sandwich assay that includes a biotinylated monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides, and monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides.

ii. Cobas® E411 Roche, Germany

The Cobas® E411 utilises a sandwich assay that includes a biotinylated monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides, and monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides.

2.5 Specimen Identification

All rapid HIV test results were bar-coded and recorded in the facility HCT register. All blood samples sent to the laboratory had a bar-coded sticker on the specimen bottle as well as the laboratory request form in order to track and trace the laboratory results back to the rapid HIV test results. All the bar-coded stickers for the study were in triplicate: one sticker was placed in the HCT register, one on the specimen bottle and the last one on the laboratory request form. All results were filed securely at the facility.

2.6 Retesting: DBS Retesting EQA for Retrospective Data

Participants tested at New Start facilities were also tested through CICT/VCT by lay counsellors. DBS re-testing was an EQA tool used by New Start sites. For every tenth rapid test performed a dried blood spot was collected at the New Start testing facilities and sent to the NICD for HIV ELISA testing. All reactive ELISAs were confirmed with a second and different ELISA (Screen: Genscreen HIV ½ v2, Bio Rad; Confirmatory, Vironostika HIV Uniform II Plus). Both ELISA tests were third generation tests.

2.7 Study Design

Rationale: The study set out to determine the comparison between two tests (exposure and outcome) at a given time so a cross sectional design was most suitable to respond to this study objectives even though it may have certain limitations. While there are other designs such as a cohort design that could have allowed for a comparison of results with and without exposure to QA interventions, this study is looking at direct comparison of results at a given time and does not provide for follow-up testing. A cohort design would therefore, not have been suitable. Furthermore, this study was also nested within the operational interventions of the HCT programme in the selected sites which would have made a cohort design more challenging. Limitations of different alternative designs such as a cluster randomized control trial (RCT) and a stepped wedge RCT, are noted and discussed in the limitations section.

The study undertaken was of a cross sectional design in nature to evaluate the accuracy of testing in Limpopo Province and compared the results to Mpumalanga Province that had not instituted any quality assurance initiatives. The study data collection was conducted between August 2012 and April 2013. In addition, retrospective data from Society of Family Health franchises called New Start were sourced from the NICD laboratory for analysis in order to compare the accuracy of testing in non-medical sites implementing QA activities to that of the two provinces.

2.7.1 IQC Pilot in Limpopo

The known control samples were tested before commencement of rapid HIV testing at all times to identify any possible quality challenges and confirm the accuracy of the test kits. The IQC data were recorded in logbooks and validated by the facility manager (appendix 1). Standard operating procedures (SOPs) for HIV rapid testing and for IQC implementation were developed and all the testing staff were trained on these documents. All IQC samples were stored appropriately in a freezer and temperature was monitored and recorded on temperature charts. The implementation of IQC in these facilities was closely monitored. Re-training was conducted where needed. Baseline data were collected, followed by 2 monitoring visits. The implementation was on-going for 2 years before this study was initiated.

2.8 Study Setting and Population

The decision to choose two provinces was due to the fact that the study aimed to compare the accuracy and extent of misclassification of HIV rapid testing in two provinces to determine effectiveness of the (IQC) intervention in one compared to other where the intervention was not applied. The study also tried to avoid bias in the one province (Limpopo) as the intervention was not entirely blinded within the province. Mpumalanga on the other hand, did not have the intervention at all in all the facilities. New Start was used as a reference to compare with the provinces as it had fully developed and implemented mature QA systems for a longer duration with a better degree of accuracy and therefore a good basis for comparison for the study. New Start also utilised the same algorithm as the provinces and provides VCT which is conducted by counsellors.

2.8.1 Limpopo

From the 23 sites that were selected to implement IQC pilot project in Limpopo between June 2009 and September, 2010, convenience sampling was applied and six sites were selected to participate in the study. The sites were identified by the provincial health authorities and selected based on the HCT head count of ≥ 100 per month. The 6 sites identified comprised of 3 clinics and 3 hospitals namely, Nobody clinic, Seshego clinic, Sebayeng clinic, Seshego Hospital, Zebediela Hospital and Lebowakgomo Hospital.

2.8.2 Mpumalanga

There were 6 sites conveniently selected to participate in the study comprising 3 clinics and 3 hospitals. These sites were selected to participate in the study by the provincial health authorities based on their monthly HCT head count of ≥100. The 6 sites included Ka-Bokweni clinic, Elukwatini clinic, Beatty clinic, Themba Hospital, Embhuleni Hospital, and Witbank Hospital. All the sites in Mpumalanga did not participate in the IQC programme implementation.

2.8.3 NGO Implementing Sites

New Start was an NGO funded by PEPFAR to complement the government HCT programme. New Start had HCT sites in selected districts in the 9 provinces and was one of the biggest HCT implementing partners in the country with sites whose only role is to provide HCT services in the community. New Start sites were called non-medical sites because they did not provide any primary health care services to participants; they i.e. only supported the government with the provision of HCT.

New Start was identified for the study as it implemented several HIV testing quality assurance measures in all sites. These included IQC and EQA namely, proficiency testing panels (PT) and DBS retesting of 10% of previously tested data. New Start sites employed a quality manager who was a laboratory technologist and training was provided on quality assurance and competency testing of all employees. New Start utilised the National Institute for Communicable Diseases (NICD) for quality assurance of rapid HIV testing, which included HIV ELISA testing as a confirmatory method for discordant rapid HIV test results, IQC and EQA. DBS re-

tested data for all New Start sites collected in 2008 were included in the study for analysis.

2.9 Sampling Strategy

2.9.1 Sample Size Calculation for Prospective Data

For the prospective data, the sample size was calculated using Open Epi programme (Open Source Epidemiological Statistics for Public health) Version 2.3.1. The following were the assumptions for the sample size calculation:

National HIV Prevalence was estimated at 18.8% (Shisana O 2014). Confidence limit: 99.9%; $P<0.05$. The sample size calculated was N = 717. At the time of the study design, there was no data to use that was based on assessing accuracy of testing or misclassification, hence national prevalence was used for the calculation as a proxy measure.

There was some objectivity in the calculation of the sample size and the study was focussed on achieving the required sample size. The sample size for the study was calculated to be 717. Divided equally across the study sites, this gives an average of 60 participants per site. However, the study was oversampled to take into account poor specimens, loss of specimens and administrative challenges.

2.10 Sample Size Calculation for Retrospective Data

Retrospective data, consisting of results of re-testing of 4825 samples (10% of total samples) collected in 2008 from all New Start sites were utilised for analyses.

While New Start was available in all 9 provinces, the available data for analyses was from 7 provinces and data from Mpumalanga was not available. The data was compared to ELISA to determine the degree of discordance.The rationale for analysing all available New Start data was to determine the accuracy of testing in systems that have matured QMS systems.

2.11 Inclusion Criteria

2.11.1 Participants

- Both male and female attendees, 18-64 years of age
- Must be at the clinic for HIV counselling and testing
- Must be willing to sign an informed consent form for the study
- Must be willing to provide blood for ELISA testing irrespective of the RDT results

2.12 Data

- The results for rapid HIV test kits and those for ELISA must be available

2.13 Sites

2.13.1 Limpopo

- IQC implementation
- Head count of ≥100 patients per month for HCT

2.13.2 Mpumalanga

- No IQC implementation
- Head count of ≥100 patients per month for HCT

2.13.3 New Start

- IQC and retesting methods implemented

2.14 Exclusion Criteria for Study Participants

2.14.1 Participants

- Attendees under 18 years of age and over 64 years of age
- Attendees who were not willing to provide whole blood samples for ELISA testing

2.15 Data

2.16 Data Cleaning and Quality Checks

For prospective data, all participants' HIV test results were bar-coded and recorded in the facility HCT register by the counsellor. Data were then extracted from the HCT register and entered into a pre-designed Excel spreadsheet. Variables extracted included age, gender and rapid test results. Retrospective data from selected New Start facilities were accessed from the NICD HIV results database. Data were tabulated to check for consistency and completeness. Missing variables were corrected by confirming with source documents (e.g. clinic HCT register). All errors and inconsistencies that were identified in this process were rectified.

2.17 Statistical Analysis

The Sensitivity (true positive rate), Specificity (true negative rate), Positive predictive value (PPV) and Negative predictive value (NPV) were determined for

the two provinces and New Start sites (Table 3.8). Calculations were conducted as per table 2.1.

Table 2.1 Calculations for sensitivity, specificity, NPV and PPV

	HIV Positive	HIV Negative	
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	

- Sensitivity = A ÷ (A+C)
- Specificity = D ÷ (B+D)
- Positive Predictive Value = A ÷ (A+B)
- Negative Predictive Value = D ÷ (C+D)
- A = true positives; when the test under evaluation yield a positive result and the "gold standard" (the reference test method used to compare all other test results) yielded a positive result as well.
- C = false negatives; when the test under evaluation yielded a negative result, while the “gold standard” or true value was positive.
- A+C = all people who are truly infected with HIV
- B = false positives; when the test under evaluation yields a positive result, while the “gold standard” or true value was negative

- D = true negatives; when the test under evaluation yields a negative result and the “gold standard” or true value yielded a negative result
- B+D = all people who are truly un-infected with HIV Calculating Sensitivity, Specificity, PPV and NPV.

To achieve objectives 3 and 4: To assess the accuracy of testing between the two provinces and New Start sites:

Logistic regression models were constructed to estimate the Odds Ratios (OR) crude and adjusted) and these were used as a measure of association between exposure and outcome. Statistical significance was ascertained at 95% CI and a p value <0.05 was considered evidence against the null hypothesis. The sensitivity, specificity, PPV and NPV for the RDT and their 95% CI were calculated. All data analyses were conducted in STATA release 12 (Stata Corp., College Station, Texas, US).

2.17.1 Descriptive statistics

Data were analysed and presented as frequency distribution of characteristics. Frequency distribution was described for the following variables: test results (positive, negative and discordant). Age was analysed as a continuous variable and presented as frequency polygons.

2.17.2 Bivariate Analysis

Bivariate analysis was done to identify association between factors using Pearson's Chi square test for categorical variables. A p value of <0.05 was considered significant.

2.17.3 Multivariate Analysis

Logistic regression models were constructed to assess the association between the interventions in the provinces. Odds ratios were used as a measure of association between exposure and outcome and a 95% precision of estimate was used to ascertain statistical significance. Exposure factors with p<0.05 were considered statistically significant.

2.18 Validity and Reliability of the HIV test kits

Sensitivity, specificity, PPV and NPV were used to determine the reliability and validity of the rapid test kits (Table 3.8). The sensitivity and specificity values for Limpopo, Mpumalanga and New Start sites were further used with the national prevalence of 18.8% (Shisana O 2014) to determine the PPV and NPV at national level based on the following formulas from Baye's theorem (Okeh and Ugwu, 2008):

$$PPV = \frac{sensitivity \times prevalence}{sensitivity \times prevalence + (1 - specificity) \times (1 - prevalence)}$$

And

$$NPV = \frac{specificity \times (1 - prevalence)}{(1 - sensitivity) \times prevalence + specificity \times (1 - prevalence)}$$

National Prevalence estimated at 18.8% (Shisana O 2014).

2.19 Ethics approval

Letters of support and permission to conduct the study were received from the provincial Heads of Department (HOD) for both provinces (Appendix 5 and 6). Meetings were convened with both provinces to introduce the study in the two provinces at district and facility levels.

For analyses, only records from facility registers and laboratory forms were used. These were coded and kept in a locked cupboard at all times for the duration of the study.

Informed consent for participation was obtained after a verbal explanation of the study to each participant during pre-test counselling sessions (Appendix 3 and 4). Participants were offered a copy of the signed informed consent form. It was explained to the participants that the purpose of the study was to compare the results of HIV rapid testing in clinical and hospital facilities to those of laboratory based ELISA method in order to improve HIV diagnosis methods in the facilities. All HIV negative participants were requested to re visit the facility after at least 6

weeks after testing to close the window period. All ELISA testing results were sent back to the facility to be recorded. This provided an opportunity to call back patients in case of discrepant results and in case they do not come back to the facility for re testing. Ethical approval was obtained from the Human research (Medical) Ethics committee of the University of the Witwatersrand. Permission from relevant provinces and facilities was obtained prior to implementation of the study (clearance certificate number: M111141) (Appendix 2).

3 CHAPTER 3: RESULTS

3.1 Introduction

This chapter provides results that respond to the objectives of the study. The overall study characteristics of the prospective data are given as well as the 2009/10 piloted RDT IQC programme in Limpopo that was evaluated to determine whether it improves accuracy of RDT. In this chapter, the evaluation of accuracy of RDT kits versus ELISA in the two provinces, Limpopo and Mpumalanga is also presented. The results are compared to New Start sites.

3.2 Pilot study for IQC Implementation in Limpopo

Prior to the implementation of this study, an IQC pilot was conducted in LP as a PEPFAR supported intervention for rapid HIV testing programme in selected sites. On completion of the pilot, the study was conducted based on the results of the pilot.

For IQC implementation, known HIV negative and HIV positive control blood samples sourced from NICD were utilised daily for the first three months and then the process was changed to weekly testing for the rest of the study. The frequency of IQC was first daily to ensure that the implementers are familiar with the process and once that was established, in three months, the frequency was changed to weekly and every time a new batch was opened.

There were no discrepant results between expected and recorded results during the pilot. The frequency of IQC was varied to ensure correct implementation in the initial period as this was a new technique and very close monitoring was need. After three months the frequency of use of the IQC was changed to once weekly as it was found that the counsellors had adapted sufficiently well to the routine use and recording of IQC results. IQC were also used on receipt of a new batch of RT for the remaining period of the study as part of regular QA processes for rapid HIV testing.

The Limpopo IQC pilot study evaluated the feasibility of implementing IQC in HCT sites. The objectives of the pilot study were to establish: simplicity - ease of operation & adaptability, acceptability - staff willingness to institute IQC, logistics - storage and distribution and quality data management. Monitoring visits were conducted with an assessment tool to determine compliance to IQC implementation. Compliance was measured at three levels: full compliance which was measured as 80% compliance to standard requirements, partial compliance, measured as 50% of compliance to standard requirements and non-compliance, which was measured as less than 50% compliance to standard requirements. Figure 3.1below shows the number of IQC tests performed against the targets.

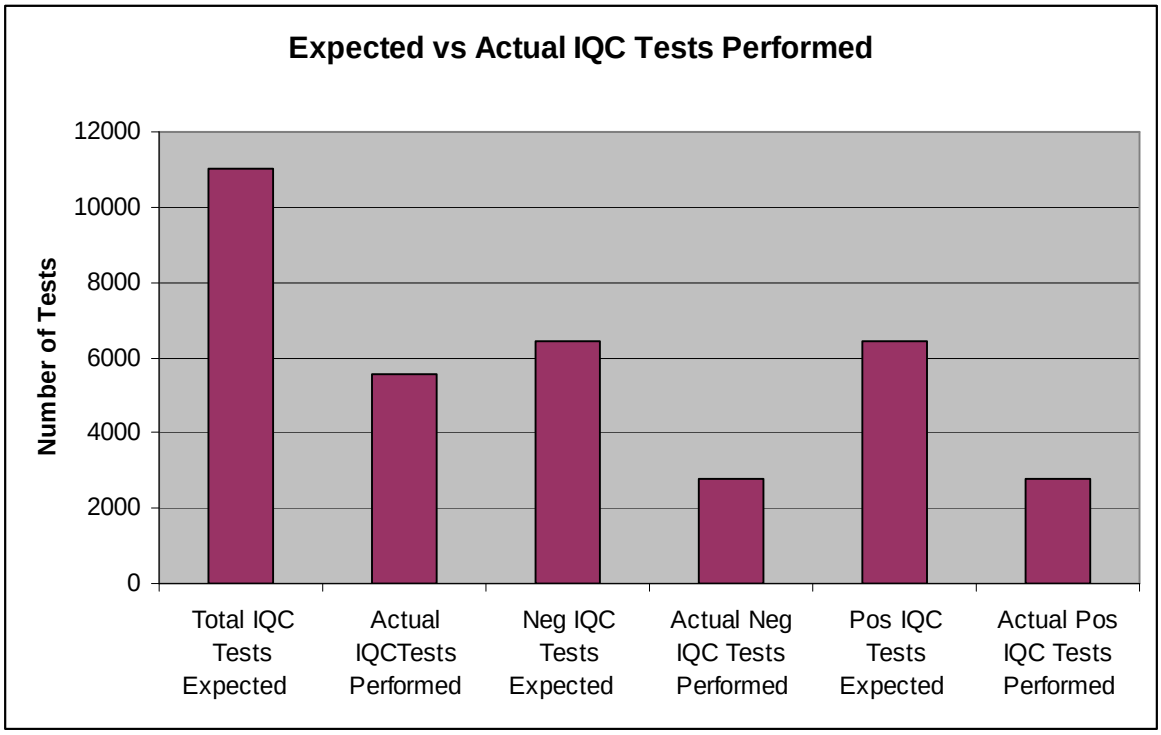

Figure 3.1. IQC implementation during the pilot project: Expected vs actual IQC performed

Some implementers were not implementing IQC as expected and this was also rectified by additional training, support visits and mentoring. Standard requirements were defined as training of site personnel, staff participation at the site, SOPs and QA guidelines availability and use at the sites, control samples storage/integrity maintenance, data completeness, testing of both test kits in the algorithm, usage of both negative and positive controls, adherence to established frequency of IQC and continuity. Baseline data were collected, followed by 2 monitoring visits. The results of the monitoring visits are shown in figure 3.2 below:

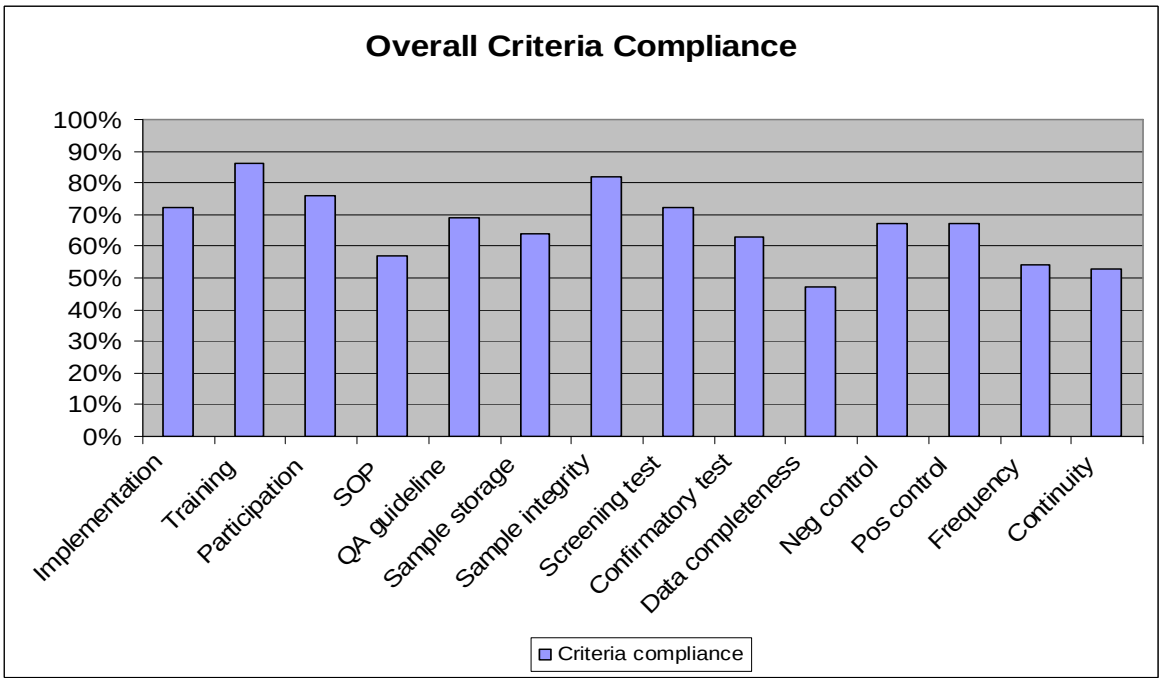

Figure 3.2. IQC pilot Project: Criteria for compliance

Only training and sample integrity were above 80%which was deemed full compliance. Data management and completeness was lower than 50% and deemed non-compliant. The other elements were partially compliant e.g. there were differences in the number of IQCs performed for the screening test than for the confirmatory test. More training and mentoring were conducted to ensure full compliance on all elements before implementation and the results were full compliance.

3.3 Recruitment of study participants

3.3.1 Enrolment Criteria

A total of 947 participants who attended HCT in the selected facilities in Mpumalanga and Limpopo between August and April 2012, were screened to participate in the study. Out of the 947 participants who attended HCT in the facilities, 51 were excluded due to the fact they were outside of the age inclusion criteria. Of the 896 participants enrolled, 78 were excluded as HIV test results were incomplete; they were missing either the rapid test results or the ELISA results. The results of 818 participants remaining in the study were analysed. (See figure 3.3 below). Out of the 78 participants excluded due to unavailability of HIV results, 54 were from Mpumalanga while 26 were from Limpopo.

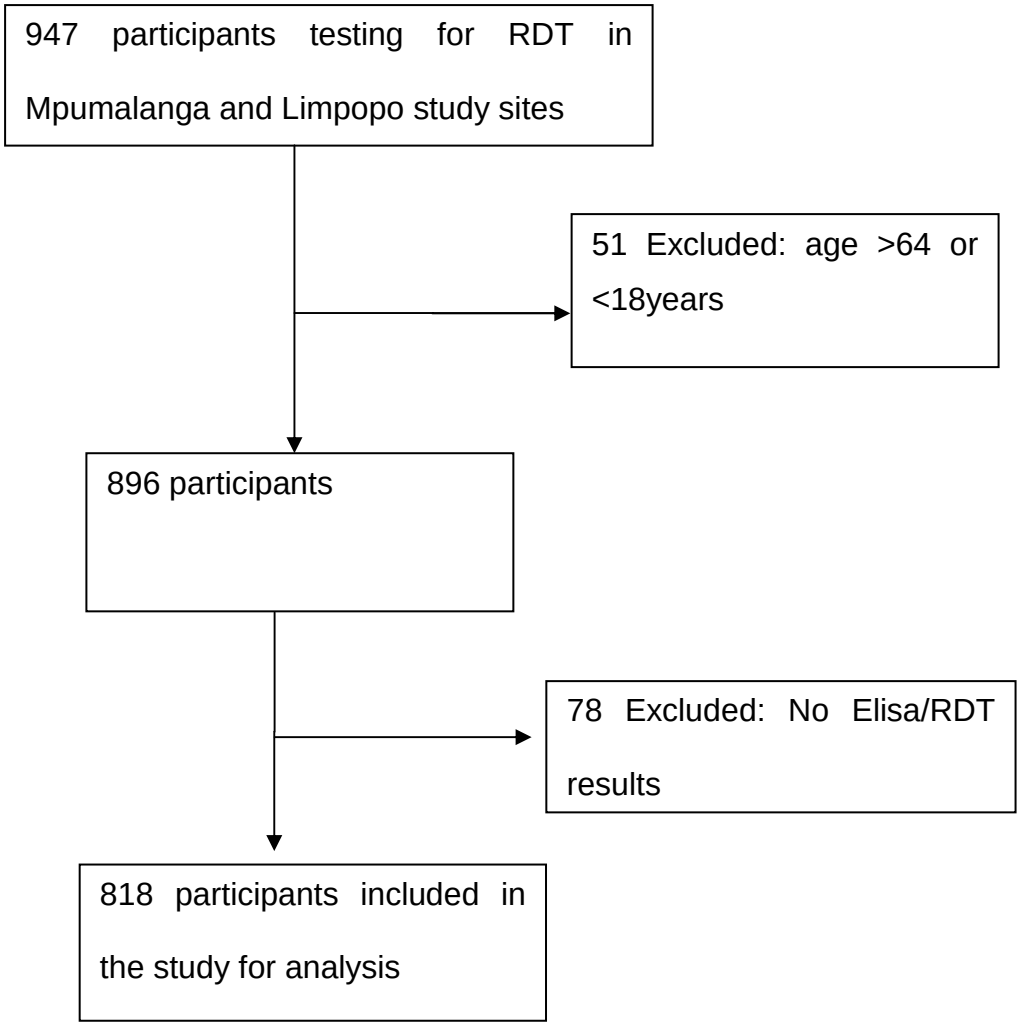

Figure 3.3 Inclusion and exclusion criteria for study participants

Exclusion criteria were as follows: 54 participants were excluded due to falling outside age inclusion while 78 participants were excluded due to the fact that they did not have both the HIV test results. The provincial distribution for exclusion was as follows:

Table 3.1. Exclusion criteria for all participants

Province	Age exclusion	No RDT/ELISA results
Limpopo	19	26 (9 RDT, 17ELISA)
Mpumalanga	32	52 (15 RDT, 37 ELISA)

3.4 Demographic data for Limpopo and Mpumalanga

Out of a total of 818 participants, 457 (55.9%) participants were from Limpopo and 361 (44.1%) from Mpumalanga sites (table 3.2). The data were significantly different, p = 0.021, (CI: 0.02-0.022). Of the 457 participants from Limpopo province, 102 (22.3%) were males and of the 361 participants from Mpumalanga province, 131 (42%) were males, p= 0.026. The mean age was 32.7 years (S.D ±9.8) in Limpopo and 35.9 years (SD ±10.2) in Mpumalanga. The majority of the participants in both Limpopo (n=154, 33.4%) and Mpumalanga (n=122, 39.1%) were in the 25-35 years age group.

In terms of age categories, the data from the two provinces were not significantly different, p=0.095. Gender and HIV status of participants, however, were significantly different, p=0.026 and 0.031 respectively between the 2 provinces. The rate of discordant HIV test results were significantly different in the provinces, p = 0.010. The HIV positivity rate between the two provinces was significantly different at 22.9% versus 26.0 % in Limpopo and Mpumalanga respectively (p value 0.012). In Limpopo the age of 26 (5.6 %) of participants was not known and

in Mpumalanga 49 (12.7%) were of unknown age. Missing data on age were restricted only on age analysis and not on other variables such as RDT and ELISA test if results for the participants were available.

Table3.2 Characteristics of study participants

Variable	Limpopo: N (%)	Mpumalanga: N (%)	p-value
Gender			
Male	102 (22.3)	131 (42)	0.026
Female	355 (77.7)	181 (58)	
Age Category			
(years)18-25			
26-35	144 (31.5)	81 (26.0)	0.095
36-45	154 (33.7)	122 (39.1)	
46-64	83 (18.2)	62 (19.9)	
Unknown	50 (11.0)	47 (15.0)	
	26 (5.6)	49 (13.6)	
HIV Status			
True HIV Positive	105 (23.0)	94 (26.1)	0.031
True HIV Negative	325 (71.1)	227 (62.8)	
Discordant	27 (5.9)	40 (11.1)	0.010
HIV positivity rate	105 (22.9)	94 (26.0)	0.012
Totals	457 (55.9)	361 (44.1)	0.021

The clinics in Limpopo enrolled most of the participants as compared to hospitals, with Nobody clinic having enrolled 127 (27.8%) and Seshego and Zebediela hospitals having enrolled 33 (7.2%) each (table 3.3). Clinics enrolled more HCT clients than hospitals due to the fact that clinics were the first point of entry for health care including HCT. The patients diagnosed for HIV in hospitals were likely to be chronically ill with poorer outcomes.

Table 3.3 Age, gender, HIV status and HIV status participants in Limpopo sites

Variable	Limpopo Province N (%)	Zebediela hospital N (%)	Lebowakgomo hospital N (%)	Seshego Hospital N (%)	Sebayeng Clinic N (%)	Seshego clinic N (%)	Nobody clinic N (%)
Gender							
Male	102 (22.3)	11 (33.3)	36 (46.2)	18 (54.6)	13 (11.6)	11 (14.9)	13 (10.2)
Female	355 (77.7)	22 (66.7)	42 (53.9)	15 (45.4)	99 (88.4)	63 (85.1)	114 (89.8)
Age (yrs)							
18-25	144 (31.5)	5 (15.2)	16 (20.5)	12 (36.4)	42 (37.5)	36 (48.7)	33 (32.4)
25-35	154 (33.7)	13 (39.4)	22 (28.2)	6 (18.2)	37 (33.0)	30 (40.5)	46 (45.1)
35-45	83 (18.2)	11 (33.3)	16 (20.5)	9 (27.3)	26 (23.2)	4 (5.4)	17 (16.7)
45-64	50 (11.0)	4 (12.1)	24 (30.8)	6 (18.2)	6 (5.4)	4 (5.4)	6 (5.9)
Unknown	26 (5.6)				1 (0.9)		25 (19.7)
HIV Status							
Positive	105 (22.9)	8 (24.2)	24 (30.8)	17 (51.5)	26 (23.2)	14 (18.9)	16 (12.6)
Negative	325 (71.1)	22 (66.7)	50 (64.1)	12 (36.4)	82 (73.2)	54 (73.0)	105 (82.7)
Discordant	27 (5.9)	3 (9.1)	4 (5.1)	4 (12.1)	4 (3.6)	6 (8.1)	6 (5.7)
HIV Positivity	105 (22.9)	8 (24.2)	24 (30.8)	17 (12)	26 (23)	14 (19)	16 (12.6)
Totals	457 (100)	33 (7.2)	78 (17.1)	33 (7.2)	112 (24.5)	74 (16.2)	127 (27.8)

Some of the clinics in Mpumalanga did not enrol more participants as compared to hospitals and this could be due to the excluded data for analysis where there were missing results (table 3.4).

Table 3.4 Age, gender, HIV status and HIV status participants in Mpumalanga sites

	Mpumalanga Province N (%)	Witbank hospital N (%)	Embhuleni hospital N (%)	Themba Hospital N (%)	Beatty Clinic N (%)	Elukwatini clinic N (%)	Kabokweni clinic N (%)
Gender							
Male	**131 (42.0)**	**33 (67.4)**	**27 (69.2)**	**23 (48.9)**	**22 (24.4)**	**-**	**26 (29.9)**
Female	**181 (58.0)**	**16 (32.7)**	**12 (30.8)**	**24 (51.1)**	**68 (75.6)**	**-**	**61 (70.1)**
Age (yrs)							
18-25	81 (26)	12 (24.0)	8 (20.5)	9 (19.2)	25 (27.8)	-	27 (31.1)
25-35	122 (39.1)	24 (49.0)	9 (23.1)	15 (31.9)	37 (41.1)	-	37 (42.5)
35-45	62 (19.9)	10 (20.4)	8 (20.5)	13 (27.7)	17 (18.9)	-	14 (16.1)
45-64	47 (15)	3 (6.1)	14 (35.9)	10 (21.3)	11 (12.2)	-	9 (10.3)
Unknown	-	-	-	-	-	49 (100)	-
HIV Status							
True Positive	94 (26)	15 (30.6)	9 (23.1)	10 (21.3)	25 (27.8)	12 (24.48)	23 (26)
True Negative	227 (62.8)	30 (61.2)	26 (66.6)	32 (68.1)	57 (63.3)	31 (63.26)	51 (58)
Discordant	40 (11.1)	4 (8.2)	4 (10.3)	5 (10.6)	8 (8.9)	6 (12.24)	13 (14.9)
HIV Positivity	94 (26.0)	15 (30.6)	9 (23,1)	10 (21.3)	25 (27.8)	12 (24.48)	23 (26.4)
Totals	361 (100)	49 (13.6)	39 (10.8)	47 (13.0)	90 (24.9)	49 (13.6)	87 (24.0)

3.5 HIV rapid testing in Limpopo and Mpumalanga sites

Data for HIV rapid and ELISA test results for Limpopo and Mpumalanga sites were compared to determine the difference between the rate of discordant HIV test results between the 2 provinces (Table 3.2). Discordant results were defined as those that were different between HIV rapid test and the ELISA test. The rate of discordant HIV test results in Mpumalanga was significantly higher than in Limpopo (Table 3.2). There was a range of discrepancy in the rate of discordant results within the facilities in both provinces though the numbers per site were relatively small (Tables 3.3 and 3.4).

3.6 Accuracy of HIV RDT

The sensitivity, specificity, PPV and NPV are presented in table 3.8.

Table 3.5: Two by two Profile of HIV status for participants in Limpopo sites

		ELISA RESULTS		
		Positive	**Negative**	
RDT RESULTS	**Positive**	**105**	**0**	
	Negative	**27**	**325**	
				457

The number of true HIV positive results in Limpopo was 132 while that of true HIV negative results was 325 (table 3.5).

Table 3.6: Two by two Profile of HIV status for participants in Mpumalanga sites

		ELISA RESULTS	
		Positive	**Negative**
RDT RESULTS	**Positive**	**94**	**0**
	Negative	**40**	**227**
			361

The number of true HIV positive results in Mpumalanga was 134 while the total number of true HIV negative results was 227 (table 3.6).

Table 3.7: Two by two Profile of HIV status for participants in New Start sites

ELISA RESULTS

		Positive	**Negative**	
RDT RESULTS	**Positive**	**537**	**14**	
	Negative	**54**	**4220**	
				4825

The number of true negatives in New Start was 4234 and the true positive results were 591 (table 3.7). New Start data for HIV positivity and discordant rate is shown in table 3.8 where 1.4% results were discordant and the positivity rate was 11%.

Table 3.8: Data from New Start sites

SITE	NEG	POS	TOTAL TESTED			
				Discordant	Concordant	% Discordant
CAPE TOWN	689	36	725	12	713	1.66
DURBAN KZN	474	93	567	12	555	2.12
GAUTENG JHB	662	92	754	1	753	0.13
FREESTATE BLOEM	645	76	721	2	719	0.28
MIDDLEDRIFT	280	16	296	1	295	0.34
EAST LONDON	442	54	496	12	484	2.42
PIETERMARITZBURG	192	40	232	3	229	1.29
MTHATHA	270	37	307	10	297	3.26
MAFIKENG	449	60	509	12	497	2.36
LIMPOPO MUSINA	171	47	218	3	215	1.38
GRAND TOTAL	4274	551	4825	68	4757	1.41

3.7 Validity and of HIV rapid test results

In order to determine the validity and reliability of RDT against the gold standard ELISA in Limpopo, Mpumalanga and New Start sites, the sensitivity and specificity, PPV and NPV were determined (table 3.9). The positivity rate (prevalence) in each setting was 22.9% in Limpopo, 26% in Mpumalanga and 11% in New Start sites. The sensitivities of the RDT in Limpopo, Mpumalanga and New Start sites were 86.5% (CI: 83.9-89.4), 72.0% (CI: 64.2-79.0) and 98.0% (CI: 97.6-98.4) respectively which indicates that 86%, 72% and 98% (CI:95%) of participants were identified as HIV positive. Specificity was 99.4% (98.9-99.9) both in LP and MP, and 98.5 5 (98.2-98.8) on New Start sites.

The PPV in Limpopo, Mpumalanga and New Start sites were 98% (CI: 93.2-99.6), 97% (CI: 91.0-99.2) and 93% (CI: 92.3-93.7) respectively and thus participants who tested HIV positive were likely to be HIV infected. The NPV results in Limpopo and Mpumalanga were 93% (CI: 90.5-95.2) and 86% were (CI: 81.3-90.7). For New Start sites, the NPV was 99.6% (CI: 99.4-99.8).

Table 3.9. Sensitivity, Specificity, PPV and NPV calculations

Sites	Limpopo (%)	Mpumalanga (%)	New Start sites (%)
Discordance rate	**5.9**	**11**	**1.4**
Sensitivity (95% CI)	**86.5 (83.9 – 89.4)**	**72.0 (64.2 – 79.0)**	**98.0 (97.6-98.4)**
Specificity (95% CI)	**99.4 (98.9 – 99.9)**	**99.4 (98.9 –99.9)**	**98.5 (98.2-98.8)**
PPV (95% CI)	**98.1 (93.2 – 99.6)**	**97.0 (91.0 – 99.2)**	**93.0 (92.3-93.7)**
NPV (95% CI)	**93.1 (90.5 – 95.2)**	**86.1 (81.3 –90.7)**	**99.6 (99.4–99.8)**
Total tested	**457**	**361**	**4825**

3.8 Association of QA with HIV discordance rate

Logistic regression models were used to estimate the Odds Ratio (OR) and the 95% confidence interval of the association between implementation of QA programme and the HIV test accuracy or the HIV discordance rate (table 3.10). Facilities without a QA intervention programme had an approximately 2-fold increased odds of HIV test discordance compared to facilities with a QA programme in place (crude OR 1.86, 95% CI: 1.10 – 3.12 and adjusted OR 1.90, 95% CI:1.08 - 3.30). This association was statistically significant. The sex and age of the participants was not associated with discordance rate. There were no more variables in the study to compare and determine the barriers and/or enablers for accuracy of testing. These would inform future studies as they would provide policies and programmes with relevant data to make informed decisions.

Table 3.10 Impact of QA on discordance rate

Factor	Crude Odds Ratio	95% CI	p-value	*Adjusted Odds Ratio	95% CI	p-value
QA intervention						
Yes	**1.00**	**reference**	**-**	**1.00**	**reference**	
No	**1.86**	**1.10 – 3.12**	**0.020**	**1.90**	**1.08 - 3.30**	**0.025**
Sex						
Female	1.00	reference		1.00	reference	
Male	1.17	0.67 – 2.04	0.578	1.12	0.63 - 2.00	0.693
Age	0.99	0.96 – 1.01	0.280	0.98	0.96 - 1.01	0.194

Table 3.11The PPV and NPV at national level (prevalence of 18.8% using Baye's Theorem)

Predictive value	National (%)
PPV	**100.0**
NPV	**91.3**

The national prevalence (Shisana O, 2014) was used to calculate the PPV and NPV using Baye's theorem (Okeh and Ugwu, 2008). The PPV was found to be 100% (CI: 100-100) and the NPV was found to be 91% (CI: 89.04-92.96). This means that the probability of having a positive result if there was infection was 100% and that of having a negative result if there was no infection was 91% (table 3.11).

3.9 Summary of key findings

Sensitivity was found to be lower in the two provinces as compared to the WHO recommendations of 99% for first and second line RDT assays. Even in Limpopo where IQC was implemented the sensitivity was 86%. In Mpumalanga sensitivity was even lower at 72%. This means a higher probability of missing HIV positive clients. The PPVs in all sites were high and the NPVs were high in Limpopo and

New Start sites while relatively low in Mpumalanga. The PPVs calculated at National level was 100% while NPV was 91%. While there was an obvious decline in the sensitivity in the provinces, the discordant results were false negatives and not false positives.

4 CHAPTER 4: DISCUSSION

4.1 Introduction

The overall goal of this study was to determine the accuracy of HIV rapid testing in two provinces in South Africa-one in the presence and one in the absence of a Quality Assurance indicator (IQC), with the view of improving the accuracy of testing in non-laboratory settings.

The main outcomes and the possible reasons for the results of this study are discussed in detail in this chapter. Firstly, the key findings are summarised, then discussed in detail. The significance of the research data that were collected as well as implications are presented. The chapter concludes with a consideration of the limitations of the study and highlights recommendations for future research. This study has advanced the field through an assessment of the state of implementation of a QA intervention and comparing it to a mature QA system. Furthermore, this study quantified the accuracy of testing and made recommendations based on the results.

4.2 Summary of key findings.

The key findings of this study were as follows:

In terms of recruitment, the study was over sampled to take into account administrative problems and loss of samples. The clinics enrolled more participants than hospitals and the HIV positivity rate was found to be higher in hospitals than in clinics. Though the study was oversampled, there were still 78 participants that were excluded because they lacked critical data for the study to analyse, either rapid HIV results or ELISA results.

The fact that 78 participants were excluded highlighted the overall problems of implementing QA measures in order to conduct rapid testing correctly. The loss of rapid test results is a critical part of data management that needs strengthening through training and mentoring. Participants were enrolled and recorded but their rapid HIV results were not reflected in the register. The other set of results that was missing was the ELISA results. The missing ELISA results were due in part to samples clotting, which translated to improper handling of the blood samples, as well as results not reaching the facility from the laboratory, which could be a result of lack of SOPs guiding the process. These problems highlight the overall requirement of strengthening and or implementing key quality control measures in public health facilities.

The degree of QA implementation was associated with specific QA indicators in the three settings. The rate of discordant results was significantly different between the two provinces and when compared with New Start sites. Mpumalanga having the highest rate of discordant results followed by Limpopo and New Start sites with lowest rate of discordance. The sensitivity for New Start sites was highest at 98% as compared to Limpopo at 86% and Mpumalanga at 72%. The reported sensitivities were not in line with the WHO-recommended sensitivities of >99% for first line assays RDTs while specificity is at >98% for first line assays RDTs (WHO 2015) in the case of the public health facilities. The PPVs were very high among all sites as well as the NPVs. Using the sensitivities and specificities of Limpopo, Mpumalanga and New Start sites, at a national prevalence of 18.8%, the national PPV was determined to be 100% while NPV was 91.3%.

4.3 Accuracy of RDT in comparison with the gold standard ELISA

Challenges to HIV rapid testing include test sensitivity, specificity and the subjective interpretation of weak positive results in field settings. The WHO guidelines (WHO, 2009) recommend that of the available testing technologies, EIAs and rapid tests are the most practical and cost-effective for diagnostic purposes, and provide results comparable to an EIA/Western blot algorithm. Studies have shown that the testing algorithms using EIAs and rapid tests are as reliable for confirmation as western blots (WHO, 2009). In addition, compared with Western blots, EIAs and rapid tests are less expensive, do not require as high a level of technical expertise to perform and interpret, and produce fewer

indeterminate results (UNAIDS, 1997; UNAIDS/WHO 1999). UNAIDS and WHO therefore, recommend alternative testing strategies using combinations of EIAs or rapid tests to confirm initial reactive test results where the tests should be highly sensitive and specific to provide reliable detection of antibodies in a specimen.

The current results show that the implementation of QA including the use of a process control in LP resulted in a higher sensitivity of the testing but did not reach the recommended sensitivity. It is possible to achieve as near to the recommended sensitivity as observed in New Start sites. New Start sites compared to LP had a wider range of QA tools implemented. The results show that with incremental implementation of QA tools accurate testing is feasible. The results also suggest that the consistent implementation and monitoring of QA can achieve the required objective. Where the sensitivities do not to reach the required target it would be useful to review current algorithms. The New Start sites followed more closely the recommended WHO guidelines in terms of the testing strategy. The decline in sensitivities in the public health sector poses a challenge of missing HIV positive individuals.

This study has contributed to the field in that it quantified the accuracy of testing and made recommendations informed by the results based on sensitivities and specificities of the tests unlike previous studies such as the SEAD (2010) study that focussed on a process evaluation to identify potential problems observed during the HCT service provision. The SEAD study did not quantify accuracy of

testing though their recommendations were implementation of QA to improve quality of service provision. The study recommendations were focussed on improving the actual service of HCT rapid testing and complying with the processes. The SEAD study focussed on operations and analysis of VCT implementation in selected South African public health facilities (for example, availability of stop watches, compliance to incubation times, availability of test kits on national tender, swabs and buffers, availability of SOPs, putting gloves on during testing, availability of registers, etc). It aimed at identifying potential problems observed during HCT service provision. It may be known already that misclassification occurs but this thesis focuses on an intervention for the process control namely the Internal Quality Control (IQC) and in general QMS. This is compared to sites that do not have the intervention and sites that have a mature QA/QMS intervention. South Africa does not have this data. At the design of this study there was no data available for the rate of misclassification in public health facilities in South Africa which is why the sample size had to be based on proxy national prevalence. This study has provided the data on rate of misclassification.

The decline in sensitivity in the field i.e. at counselling and testing sites has been reported in several studies and may be due to non compliance to storage and transportation of the test kits, lack of quality control and lack of training (Mayhood MK, 2008;Black V, 2009; Ramalingam S, 2002, Van den Berk GEL, 2003;Ferreira CJ, 2005;Plate DK, 2007; Wolpaw BJ , 2010). A decrease in sensitivity means that there is a higher probability that patients who are HIV positive may be missed. As

compared to the manufacturer's reported sensitivity of 100% per test kit and NICD laboratory evaluation, the New Start sensitivity remained high indicating a low probability of missing positive results. This will be a problem for South Africa based on the volumes that are tested annually though the re-testing process may partially mitigate the problem as evidenced by the extent of testing overall. The specificity, however, ranged between 99 to 100% between the 2 provinces and New Start sites, translating to the fact that it is highly likely that patients who tested HIV negative are not infected. Given the high PPVs in Limpopo, Mpumalanga and New Start sites, the likeli-hood of HIV diagnosis on a positive result is good. This will play a crucial role for the 909090 strategy. By contrast, the results are different between the two provinces in terms of NPV, 93%, 86% for Limpopo, Mpumalanga sites respectively. New Start sites had NPV of 99%.

Awareness of one’s HIV status through HIV testing is an important entry point to a comprehensive package of care for HIV and AIDS prevention and treatment (NDOH, 2010). Population-level HIV testing in South Africa is among the highest globally, with a large proportion of South Africans knowing their HIV status. Shisana O, (2014) reported that in their survey, 65% of participants (n=28 997) knew their HIV status (Shisana O, 2014). The authors further reported that in 2012, the total number of people who were HIV positive was estimated at 6.4 million with over 2.5 million on treatment (Shisana O, 2014). It thus remains necessary to maintain high levels of testing for individual awareness of HIV status, and ART initiation. In addition to acting as a treatment for HIV, ART can also be

considered as treatment as prevention TasP(Cohen MS 2010). TasP reduces HIV viral load, which, in turn, decreases the likelihood of onward transmission of HIV (Donnell D 2010).

4.4 Reliability and validity of rapid test kits

The overall effect of reduced test sensitivity and specificity at a national level was calculated. The sensitivities and specificities of Limpopo, Mpumalanga and New Start sites were used to determine the PPV and NPV nationally with a prevalence of 18.8% (Shisana O, 2014). The PPV was found to be 100% while the NPV was lower at 91.3 %. This means that nationally, a positive result means that patients who test HIV positive are likely to have HIV infection. The NPV was 91% which means that there is a possibility that patients who test HIV negative are HIV infected. New Start with a mature QMS system showed high sensitivities and low discordant rates meaning that QA plays an important role in improving the quality of testing including sensitivities. Limpopo followed and Mpumalanga had the lowest sensitivities and highest discordance rate.

There were no reported IQC failures in the pilot programme in Limpopo and thus the test devices performed as expected. At commencement of the pilot programme, logbooks were distributed to all selected facilities to monitor compliance. This included monitoring the results of the HIV rapid tests conducted as recorded in the register. Based on the results of the reports of the monitoring visits, and the results of this study it was recommended that IQC be rolled out to

all health facilities in Limpopo and eventually throughout the country as it clearly improved the quality of diagnosis of HIV rapid testing in non-laboratory settings. The lower sensitivity nevertheless, points to the fact that despite the implementation of IQC it did not to address all problems likely to be associated with QA.

4.5 Discordant Results

Increased discordance was associated with the extent of implementation of QA as shown by the results of the logistic regression model (crude and adjusted). Facilities in Mpumalanga, which did not have a QA intervention programme had an approximately 2-fold increased odds of HIV test discordance compared to facilities in Limpopo with a QA programme in place (OR 1.86, 95% CI: 1.10 – 3.12). This association was statistically significant. The gender and age of the participants was not associated with discordance rate. Mpumalanga province which had the highest level of discordance had limited evidence of QA implementation as compared to Limpopo province where there was evidence of IQC implementation for a period of two years. New Start sites, where the discordance was lowest, had a mature system with the highest level of QA implementation including formal QA systems such as IQC, EQA and QA managers.

The rate of discordance differed within facilities in Limpopo which implies that even though all the facilities were implementing IQC, there may still be some challenges that are affecting the results of diagnosis. This means that the implementation of

QA measures need to be continually monitored and supported by training and mentoring to ensure good quality diagnosis

4.6 HIV Diagnosis

The overall characteristics for the sites were similar in both provinces. There were only certain differences that did not affect the results. The clinics enrolled most of the participants as compared to hospitals generally. This was due to the fact that clinics were the first point of entry for health care including HCT. Patients who presented at hospitals were originally referred from the clinics due to complications and complexity of the cases. The patients diagnosed for HIV at hospitals were likely to be chronically ill with poorer outcomes and this might explain why the HIV positivity rate was higher in hospitals as compared to clinics. The positivity rate was also higher in Mpumalanga as compared to Limpopo and New Start sites. This also agrees with published data by Shisana O (2014) regarding the positivity rates of the different provinces.

4.7 Misclassification

In this study there was a decline in sensitivity that resulted in false negative results. To a lesser extent, some false positive results were also identified in New Start sites. False negative results accounted for all the discordant results in LP and MP. Some of the reasons for false negative results include ongoing seroconversion, divergent HIV strain, inhibitory factors in specimen, insufficient specimen added, too much buffer, storage and transportation and possible manufacturing defects (WHO, 2015). While access to HIV diagnosis is life-saving;

the use of rapid diagnostic tests in combination may be vulnerable to wrongly diagnosing HIV infection when both assays give a false positive result (Shanks L, 2013, Cruccitti, 2011; Baveewo S, 2012). This has been documented and is reported to be caused by predominantly, serological cross reactivity (Kleinman et al., Gray RH, 2007; Anzala O, 2008). False positive diagnosis can result in patients being placed on treatment unnecessarily which translates to wasting of resources and devastation on the misdiagnosed patient. False positive results are found to be higher in low HIV prevalence contexts as even the rapid tests with high sensitivity can perform poorly. False positive diagnosis was also reported in resource limited settings (Shanks L, 2013). Other reasons for misclassification are linked to administrative errors, test storage and transport conditions, poor quality control, lack of training and supervision of staff, limitations of the assay itself, sub-optimal national testing algorithms, workload, SOPs not being followed, contaminating proteins in specimen and possible manufacturing defects (Shanks L, 2013; WHO, 2015).

4.8 South Africa's Future for QA of RDT

In order to strengthen its HIV RDT programmes, South Africa needs to review and strengthen the current HIV programme plans and standards with regards to incorporation of QA programmes. The HIV testing programme already has high engagement with the Ministry of Health, the National reference laboratory (NICD), the programme managers and non-governmental implementing partners as recommended by WHO (WHO, 2015). The national testing policy has been

revised and this provides guidance to implementation of RDTs including improved quality assurance programmes and algorithms to minimize discordance (NDOH, 2016). This policy needs to be implemented in all public health facilities, private sector and implementing partners. Implementation of the approved standard operating procedures and job aides is of critical importance.

Since the study shows that accuracy of RDTs is improved by the implementation of QA interventions accompanied by appropriate training and certification, and monitoring of the use of RDTs, plans need to be put in place to gradually phase in implementation at all sites. Introduction of IQC should be rolled out in all Public health facilities and other sites that implement RDTs. Furthermore, EQA methods should also be implemented to complement IQC. These should include PT methods where there is capacity within the laboratories for assessments of the panels, re-testing methods, especially in non-governmental organizations where the through-put is not very high and assessments through site visits including observation methods. Assessment methods may include the use of tools such as the stepwise process for improving the quality of HIV rapid testing (SPI-RT) checklist. The checklist provides the following:

- a solid foundation for ensuring the quality of testing in Public health facilities
- a guideline to evaluate a RT site against the requirement for quality improvement
- a guideline for the development of policies and procedures

It is crucial that South Africa implements these EQA methods to identify and prevent misdiagnosis. Implementation of an HIV rapid testing quality improvement initiative (RTQII) aims to ensure quality of testing and expand upon current in country HIV rapid testing quality improvement work (http://www.rtqi.com.)

RTQII comprises of the following 5 key action areas:

- Policy engagement
- Human Resources
- PT programmes
- Standardised register data collection tool
- Post market surveillance

Strengthening of data collection processes, data analysis, timely reporting of results to sites followed by corrective action is crucial for monitoring and evaluation of the programme. Strengthening supply chain processes is important to ensure availability of testing kits at all times.

Individuals on ART are not recommended for routine re testing. This is because the sensitivity of RDTs and laboratory tests can be reduced by exposure to ART because of viral suppression, making this method unreliable for confirming a positive diagnosis. Confirmation for patients on ART requires antigen tests such as the polymerase chain reaction (PCR).

4.9 Limitations

The limitations of the study were as follows:

1. The main limitation of the study was time differences in New Start data and the study prospective data for LP and MP as the study could not provide a direct comparison of results between all sites on a real time basis.

2. The study was not a randomised cluster assessment design within LP in order to avoid a problem of contamination as most facilities were either already implementing QA or in the process of initiating implementation, the study was not fully blinded in Limpopo. Other study designs were also explored such as an impact evaluation design, matched control design, cohort, a cluster randomised control design, and a stepped wedge RCT study. A cross sectional design was best suited for this study.

3. There is also potential bias in comparing LP and MP when determining whether the intervention of IQC was effective in improving the accuracy of testing. The assumptions are that facilities in both provinces are staffed by the same categories of staff, hierarchy of reporting was similar, and processes were the same. The only differences being the implementation of QA and IQC specifically.

4. Potential bias in convenient sampling in both Provinces but based on practical considerations such as accessible sites and proximity to laboratory services

5. Potential bias in reading and interpreting results as the study design does not provide for inter-reader variability, for example, as diagnosis was determined by one end-user at a given time as they received the results. This is standard practice in routine testing

6. Potential bias using two different NHLS labs. Assumption was that the performance of both labs was not different.

7. Use of 4th generation testing (ELISA) as a final diagnosis compared to RTD. Given the maturity of the HIV epidemic, it is likely that acute infections are a small proportion of the HIV infected population and would not explain extent of loss of sensitivity.

8. Further analysis by OD signal value of ELISA and Western blot banding pattern could have been useful to understand the causes of RT false negatives other than the operator.

5 CHAPTER 5 CONCLUSION

The conclusion is based on the results and discussion of the study objectives. The implementation of QA measures plays a crucial role in the accurate diagnosis of HIV rapid testing. The accuracy of HIV diagnosis is improved by implementation of QA. This is seen in the higher sensitivities of New Start and Limpopo sites. Based on the results of the study, it is concluded that even though the sensitivity of New Start and Mpumalanga rapid testing did not meet the WHO criteria for sensitivities in the field, implementation of QA plays a critical role on the improvement of accuracy of rapid testing as also seen in New Start where sensitivity was very high. South Africa must implement several aspects of QA, including PT methods of EQA, training, mentoring and supervision, this increases the accuracy of testing and the sensitivities of the tests. Increasing sensitivities will also play a crucial role in the event of a possible move towards self-testing and the move towards the 909090 strategy. Should the sensitivities not reach the recommended levels in the presence of a QMS implementation, the algorithms must be re- evaluated. Limitations of the study must be taken into consideration in order to guide more studies regarding quality assurance of rapid diagnostic test kits.

5.1 Recommendations

- Implementation of quality management systems through a quality assurance quality improvement activity such as the PEPFAR-supported Quality Improvement Initiative: Stepwise Process for Improving HIV rapid testing that has a series of steps including monitoring of implementation (http://www.pepfar.gov/documents/organization/217761.pdf)
- Implementation of the new South African HTS Policy
- Improved use of site data such as IQC and PT as part of the passive post-marketing surveillance to detect any technical problems with the device and limit false negative and positive results.
- Review of test algorithm if it is found that despite improvements in QA implementation performance is still less than recommended sensitivity/specificity.
- Adequate on-going training, refresher training and supportive supervision of HIV testing providers, with requirement for certification should be in place.
- Implementation of the WHO guidelines including
    - Repeat HIV rapid testing in case of discordant results
    - Replace the use of the laboratory-based tiebreaker ELISA testing with referral for laboratory-based diagnostic testing per NHLS guidelines
- Explore the use of electronic readers to improve consistency of reading.

Explore future technologies such as m-health and cell phone technology for ensuring quality of testing.

6 CHAPTER 6: APPENDICES

APPENDIX 1: LOG BOOK FOR DATA COLLECTION

NATIONAL HIV RAPID TESTING QUALITY ASSURANCE LOGBOOK

Name of Counsellor :				Facility:						Province:
Number	Client/patient code	Age (years)	Gender	Date tested	1st Test kit name Lot No Exp Date	2nd Test kit name Lot No Exp Date	Result given to client	Observed	Registered Nurse Signature	Specimen for HIV Elisa (Tie Breaker)
1			M F		R NR INV	R NR INV	P N IND	Y N		

Page Total	⇩	⇩	⇩	
Total Reactive/Positive				
Total Non Reactive/negative				
Total invalid/indeterminate				

R= Reactive, NR=Non-Reactive and INV=Invalid P=Positive, N=Negative, and IND= Indeterminate

APPENDIX 2: ETHICS CLEARANCE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Thato Chidarikire

CLEARANCE CERTIFICATE	M111141
PROJECT	Comparisons of Accuracy of HIV Diagnosis between Rapid HIV Test Kits Conducted in Non-Laboratory Settings and Laboratory-Based ELISA Methods in South Africa
INVESTIGATORS	Ms Thato Chidarikire.
DEPARTMENT	School of Pathology
DATE CONSIDERED	28/10/2011
M1111410DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 28/10/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Professor Adrian Puren

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX 3: STUDY INFORMATION SHEET

INFORMATION DOCUMENT (will be translated in the local languages should the need arise)

Study title: Comparison of accuracy of HIV diagnosis between rapid HIV test kits conducted in non-laboratory settings and laboratory-based ELISA methods in South Africa

Greeting and Introduction:

Good day, my name is Thato Chidarikire. I am a PhD student from the University of the Witwatersrand. We are doing research on the comparison of accuracy of diagnosis between rapid HIV test kits conducted in non-laboratory settings(such as this clinic where only a finger prick is rapidly analysed) and laboratory–based ELISA methods. Research is a process that one undertakes in order to answer a particular question. In this study, we want to find out if the accuracy of HIV diagnosis between the rapid HIV test kits and the laboratory-based ELISA method is the same or of acceptable standards so that doctors don't have to refer HIV test results to the laboratory for confirmation when a patient has come to the facility and can rely fully on HIV rapid test kits results. This will shorten the time it takes to wait for laboratory results especially for patients who need to be fast tracked in to the treatment programme. This study is being conducted in selected public health facilities in Limpopo and Mpumalanga provinces.

Invitation to participate: We would like to request for your participation in the study as this would greatly assist us in terms of finding an answer to this very important question.

What is involved in the study – This study will be conducted in selected public health facilities in Limpopo and Mpumalanga provinces. After pre test counselling and signing of the informed consent form, the study participants will be offered a rapid HIV test which will be conducted following the national HCT guidelines where you will be screened for HIV with a rapid HIV test kit and if your results are HIV negative you will be reported as HIV negative. If your results are HIV positive, you will be confirmed with another HIV rapid test kit. If your results are HIV positive you will be reported as HIV positive and you will be referred for further management.

If you agree to participate the following procedures will carried out. A drop of blood will be drawn from you using a finger prick method. Blood will be tested for HIV using the rapid HIV test kits. Following the rapid test, irrespective of your HIV status, whole blood (about 5ml on 1 teaspoonful from your arm) will be collected from you for an ELISA test at the NICD laboratory closest to the clinic that you attend. You will only be in the study for the, although the total duration of the study in the facilities will be six months. The procedure for HIV counselling and testing and drawing of venous blood may take approximately 30 minutes. All participants will be given their HIV test results for both the rapid method and the ELISA method. A total of 1200 (600 per province) participants will be involved in the

study. If you need further counselling and support this will be provided to you at the facilities through the current services.

Risks

There will be no risk for you if you participate in the study. Blood will be collected by a finger prick (1-2 drops) and by venesection (5ml). This may cause brief minor physical discomfort to your finger and at the site of the vein.

Benefits: There are no direct benefits for participating in the study but taking an HIV test is very beneficial as it will let you know your HIV status so that you may make informed decisions based on your HIV status including early access to treatment if needed. You will receive all information on the study while you are involved in it and after the results are available.

Participation is voluntary: Your participation in this study is completely voluntary. If you refuse to participate in the study, there will be no penalties or loss of any benefits that you may be otherwise entitled to. You will still receive your standard medical care. You may also discontinue your participation in this study at any point without any penalties or loss to benefits that you were otherwise entitled to receive.

Reimbursements: There will be no reimbursement for participation in this study.

Confidentiality: Efforts will be made to keep all personal information confidential. Codes will be used on the data collection forms and these will be linked to patient

names in a separate record sheet kept under lock and key and only accessible to the researcher and supervisor. Data will be analysed as grouped data and presented as such. Even if results are published, confidentiality will still be maintained.

For further information related to the study, you may contact me at the following contacts: Ms Thato Chidarikire, Tel: +27 12 395 9153 or Chidat@health.gov.za/chidarikiret@gmail.com

For reporting of complaints or problems, you may contact Ms AnisaKeshav, University of the Witwatersrand Human Ethics Committee (HREC) on the following contacts: Ms AnisaKeshav, Tel: +27 11 717 1234 or anisa.keshav@wits.ac.za

Thank You

Yours Sincerely,

Thato Chidarikire

University of the Witwatersrand, South Africa

APPENDIX 4: STUDY INFORMED CONSENT FORM

INFORMED CONSENT FORM

Study title: Comparison of accuracy of HIV diagnosis between rapid HIV test kits conducted in non-laboratory settings and laboratory-based ELISA methods in South Africa

I understand that I have been asked to participate in the abovementioned study. I have heard the aims and of the Research Study that is proposed and I was given an opportunity to ask question in order to understand the study fully.

I understand that taking part in this research study is completely voluntary, that is, of my own choice and I know that I may withdraw from the study at any point without being penalized.

I understand that the researchers will make every effort to keep personal information confidential.

1. I hereby agree to participate in this research study as per the information letter

   …… ……

   Name of participant Place

   …… ……

Signature of participant	Date
……	……
Name of witness	Place
……	.....
Signature of witness	Date
……	.....
Name of Health Care Worker	Signature and Date

APPENDIX5: APPROVAL FROM LIMPOPO PROVINCE

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH

Enquiries: Selamolela Donald

Ref:4/2/2

Chidarikire TH

University of the Witwatersrand

Johannesburg

0001

Greetings,

Re: Permission to conduct the study titled: Comparison of accuracy of HIV diagnosis between rapid HIV test kits conducted in non-laboratory settings and laboratory-based Elisa methods in South Africa.

1. The above matter refers.
2. Permission to conduct the above mentioned study is hereby granted.
3. Kindly be informed that:-
   - Further arrangement should be made with the targeted institutions,
   - In the course of your study there should be no action that disrupts the services.
   - After completion of the study, a copy should be submitted to the Department to serve as a resource.
   - The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.

Your cooperation will be highly appreciated.

Mathebela

Head of Department

2011/12/29

Date

18 College Street, Polokwane, 0700, Private Bag x9302, POLOKWANE, 0700
Tel: (015) 293 6000, Fax: (015) 293 6211/20 Website: http/www.limpopo.gov.za

APPENDIX 6: APPROVAL FROM MPUMALANGA PROVINCE

MPUMALANGA PROVINCIAL GOVERNMENT

Building No.3
No. 7 Government Boulevard
Riverside Park Extension 2
Nelspruit
1200
Republic of South Africa

Private Bag X 1128,
Nelspruit, 120(
Tel: 013 766 329(
int: +27 13 766 329(
Fax: 013 766 346(
int: +27 13 766 346(

Department of Health
Office of the HOD

Litiko Letemphilo | Umnyango WezaMaphilo | Departement van Gesondheid

Enquiries: Mr M.T. Matlou
Chief Director: Integrated Health Planning
Tel: 013-766 3293

MPUMALANGA PROVINCE
HEAD OF DEPARTMENT
PRIVATE BAG X 11285 NELSPRUIT 1200
2011 -01- 23
RECEIVED
DEPARTMENT OF HEALTH & SOCIAL SERVICES

Ms T.N. Chidarikire
P.O. Box 43
Carlswald
Kyalami
JOHANNESBURG
1685

Tel: 012 395 9153 (082 977 2410)
Email: chidarikiret@gmail.com / chidat@health.gov.za

Dear Ms Chidarikire,

RE: COMPARISON OF ACCURACY OF HIV DIAGNOSIS BETWEEN RAPID HIV TEST KITS CONDUCTED IN NON-LABORATORY SETTINGS AND LABORATORY-BASED ELISA METHODS IN SOUTH AFRICA

1. The Provincial Research and Ethics Committee hereby grants approval for your research project which is being conducted as part of your PhD degree at the University of the Witwatersrand, to compare the accuracy of HIV diagnosis between HIV rapid test kits in non-laboratory settings and the laboratory-based ELISA method with the view to improving the accuracy of testing in non-laboratory settings.

2. It is noted that the study will be conducted at the following six public health care facilities in Mpumalanga:
   - KaBokweni Community Health Centre (CHC) and Themba Hospital in Ehlanzeni District

We care Do you? Siyanakekela

MPUMALANGA A Pioneering Spirit

- Eerstehoek/Elukwatini Clinic and Embhuleni Hospital in Gert Sibande District, and
- Beatty Street Clinic and Witbank Hospital in Nkangala District

These facilities have a relatively high patient uptake for HCT services in general headcount and represent the demographic- and social diversity of the province in terms of location (peri-urban, rural and urban) and general population.

3. Ethical approval was obtained from the Human Research (Medical) Ethics Committee of the University of the Witwatersrand.

4. The onus lies with the researcher to seek approval from the mentioned public health facilities prior to implementation of the study.

5. It should be noted however, that the department will be expecting a report on the findings, once the research project has been completed.

Yours faithfully,

ACTING HEAD OF DEPARTMENT
MR M.R. MNISI
DATE: 02/07/2012

cc: Chief Director: Primary Health Care: Ms I Makwetla
Director: HIV and AIDS : Dr J Ngomane
HCT Provincial Co-ordinator: M Madalane

7 CHAPTER 7: REFERENCES

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