THE ACCURACY, SENSITIVITY AND SPECIFICITY OF RAPID POINT-OF-CARE TESTING FOR CD4+ T CELL COUNT ENUMERATION AND TB DIAGNOSIS

MANDISA SKHOSANA

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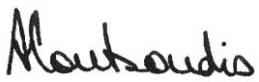
INVESTIGATOR:
MANDISA SKHOSANA
STUDENT NUMBER: 212557377
Department of Paediatrics and Child Health
Nelson R. Mandela School of Medicine
University of KwaZulu-Natal

RESEARCH SUPERVISOR

Prof Anna Coutsoudis
Department of Paediatrics and Child Health
Nelson R. Mandela School of Medicine
University of KwaZulu-Natal

As the candidate's supervisor, **I agree** to the submission of this thesis.



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DEDICATION

To the countless Scientists and Health workers who have dedicated their lives to fighting the HIV/AIDS epidemic.

To my mother, Mrs Nokuzola Gasa, who has been my angel on earth. Thank you for being my inspiration, my role model, my friend, my rock and the best mother anyone could ever ask for.

To my children, Tshiamo and Nelisa. Thank you, my babies, for understanding even when mommy could not be there with you all the time while working on this thesis.

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ABREVIATIONS

AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AO	Auramine O
ART	Antiretroviral Treatment
ARV's	Antiretrovirals
AZT	Azidothyimidine
BA	Bland Altman
BMI	Body Mass Index
BREC	Biomedical Research Ethics Committee
CI	Confidence Interval
CD	Cluster of Differentiation
CDC	Centre for Disease Control
cDNA	Complementary DNA
CV	Co-efficient of Variation
DNA	Deoxyribonucleic acid
DoH	Department of Health
FBC	Full Blood Count
FDA	Food and Drug Administration
FIDSSA	Federation of Infectious Diseases Societies of Southern Africa
FN	False Negatives
FP	False Positives

GFR	Glomerular Filtration Rate
Gp	Glycoprotein
GRID	Gay-Related Immune Disease
Hb	Haemoglobin
HCT	HIV Counselling and Testing
HHS	Health and Human Services
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSRC	Human Science Research Council
HSV	Herpes Simplex Virus
HTLV-1	Human T-Lymphotropic virus – 1
HTLV-2	Human T-Lymphotropic virus – 2
IM	Immunotrol
IQR	Interquartile Range
IPT	Isoniazid Preventative Therapy
IUATLD	International Union Against Tuberculosis and Lung Disease
KZN	KwaZulu-Natal
LAV	Lymphadenopathy-associated virus
LJ	Lowenstein-Jensen
LOA	Limits Of Agreement
LPVr	Lopinovir
LTNP's	Long Term Non Progressors
LTR	Long Terminal Repeat
MDR	Multi-Drug Resistant
MGIT	Middlebrook Mycobacteria Growth Indicator

mRNA	Messenger RNA
µL	Microliter
MTB	Mycobacterium Tuberculosis
NASBA	Nucleic Acid Sequence Based Amplification
NAT's	Nucleic acid based tests
NHLS	National Health Laboratory Services
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NPV	Negative Predictive Value
NSI	Non-syncitia-inducing
NSP	National Strategic Plan
NVP	Nevirapine
PANTA	Polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin
PCR	Polymerase chain reaction
PHC	Primary Healthcare Clinic
PLG	Pan Leucogating
PLG/CD4	Enumeration of CD4 using SA-NHLS Beckman Coulter flow cytometry using panleucogating.
PMTCT	Prevention of mother to child transmission
POC	Point of Care
PPD	Protein purified derivative
PPV	Positive Predictive Value
PTB	Pulmonary TB
PTLC	Predictors of pre-treatment loss to care
QC	Quality Control
RNA	Ribonucleic acid

SA	South Africa
SANAC	South African National AIDS Council
SD	Standard Deviation
SI	Syncitia-inducing
SIM	Similarity
SIV	Simian Immunodeficiency Virus
STI's	Sexually Transmitted Infections
TA's	Transcription-based amplification system
TB	*M. Tuberculosis*
TB LAMP	TB Loop Mediated Isothermal Amplification
TDF	Tenofovir Diproxyl Fumerate
VL	Viral Load
WHO	World Health Organization
XDR	Extensively Drug Resistant
ZN	Ziehl Neelsen

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ABSTRACT

Objectives:

The PIMA CD4+ T cell count analyser has been favourably evaluated for use in point-of-care (POC) situations in Mozambique and Zimbabwe, has also been recommended by the World Health Organisation (WHO), however, there is limited information on its use in Primary Healthcare (PHC) settings in KwaZulu Natal (KZN) South Africa. The main aim of this study therefore assessed the accuracy, sensitivity and specificity of the Alere PIMA Point of Care (POC) analyser CD4+ T cell count enumeration compared to the South African National Health Laboratory Services (SA-NHLS) methodology, which uses Beckman Coulter with Panleucogating (PLG/CD4). The potential role of using the PIMA CD4 analyser as a predictor of antiretroviral therapy (ART) eligibility was also assessed.

Material and Methods:

The study took place at Lancers Road clinic, a busy primary health clinic (PHC) facility under the eThekwini Health Unit.

An extra two millilitres of venous blood was drawn from the same blood draw as for the routine CD4 NHLS test (Beckman Coulter) into another EDTA tube for the comparison of the enumeration of CD4+ T cells using the PIMA analyser during January – July 2013.

Results:

A total of 268 patients were recruited for the PIMA analyser comparison with NHLS PLG/CD4 while a sub-set of 100 blood samples were also analysed on the FACS calibur.

In the 100 samples the PIMA analyser results correlated better with the FACS calibur results (mean bias of 7.52, Bland Altman limits of agreement -111 to 126 and correlation of 0.970) than with the NHLS PLG/CD4 results (mean bias of -12.78, Bland Altman limits of agreement -226.041 to 200.481 and correlation of 0.90).

In the 268 samples the overall mean difference between the PIMA analyser – NHLS PLG/CD4 was 17.5 cells/µl (95% CI 6.2 to 28.8). The percentage similarity (SIM) between the two (Mean ± SD) was 106 ± 15.5; indicative of acceptable agreement between the two tests. When

categorised by the following CD4+ T cell counts of: ≤350 cells/µl; 351-500 cells/µl; ≤500 cells/µl and > 500 cells/µl , the mean difference of PIMA analysers – NHLS PLG/CD4 was 33 cells/µl (95% CI 23 to 42); 22 cells/µl (95% CI -3.5 to 47); 30 cells/µl (95%CI 21 to 39); and cells/µl (95% CI -78 to 6.1) respectively.

Under the current South African guidelines of ≤350 cells/µl CD4+ T cells, the sensitivity of the PIMA analyser was 83.5% and specificity 92%. At this threshold of ≤ 350 cells/µl there were 35 (13%) misclassifications, of which 27 were false negatives. This implies that 27 patients would have been falsely deemed ineligible for ART according to the PIMA analyser. The mean difference between the PIMA analyser and NHLS PLG/CD4 in this group of 27 patients was 112 cells/µl. The positive predictive value was high at 95% such that 95% of the patients eligible for treatment according to PIMA analysers would have also been deemed eligible for treatment on the NHLS PLG/CD4 test. Using future South African treatment guidelines threshold of CD4+ T cell counts ≤500 cells/µl , a high sensitivity of 94% was observed at the sacrifice of lower specificity of 78%.

According to the NHLS PLG/ CD4 test result, 164/268 (61%) of patients were eligible for ART (CD4+ T cell count ≤350 cells/µl) compared to 145/268 (54%) with the PIMA analyser POC CD4+ T cell test. Of those eligible for ART according to the ART register at Lancers Road PHC, 110/164 (only 67%) of these patients were initiated on ART. Of those who did not return for their results 35/268 (13%), twenty of 35 (57%) were eligible for ART according to the NHLS PLG/CD4 laboratory CD4 test result, all of whom were not initiated on ART.

Conclusion:

The overall agreement between the PIMA analyser POC and NHLS PLG/ CD4+ T cell count enumeration in adult HIV positive individuals was acceptable with clinically insignificant mean bias. Together with high positive predictive value, and sensitivity and acceptable specificity the PIMA analyser POC lends itself to an excellent facilitator of improved healthcare.

Key Words:

HIV, CD4+ T cell count, point of care testing, PIMA, ART eligibility.

CHAPTER 1 - BACKGROUND

1.1. HIV DISEASE

The disease is caused by the Human Immunodeficiency Virus (HIV) which belongs to the *Retroviridae* family of the genus *Lentivirus*. Lentiviruses infect many species and typically cause long- length illnesses with long incubation periods (Levy 1993). HIV causes AIDS which is a condition that affects humans whereby there is progressive failure of the immune system as well as some cancers. (Weiss et al., 1993)

HIV is mainly transmitted through sexual contact where there is transference of blood, semen, pre-ejaculate and/or vaginal fluid. Vertical transmission of HIV can also occur in utero, perinatally and post-natally through breast milk.

1.1.1 Epidemiology

According to the World Health Organization (WHO) statistics, the global prevalence of the Human Immunodeficiency Virus (HIV) was estimated at 35 million adults between the ages of 15 and 49 living with HIV in 2013 (WHO 2013a). In the same year, the total number of children under the age of 15 living with HIV was at a staggering 3.2 million with a recorded 1.5 million number of deaths due to HIV and Acquired Immunodeficiency Syndrome (AIDS) related causes. Africa alone accounts for over 67% of global HIV infections. It is estimated that 93% of all children infected with HIV are in Africa. In 2012, 1.6 million deaths due to HIV-related illnesses were recorded globally and 1.2 million of those deaths occurred in Africa (WHO 2012). In South Africa, there were about 6.3 million people who were living with HIV in the year 2013. An estimated 2.9 million of these adults were women 15 years of age and above and another 460 000 of these were children under the age of 15 years (UNAIDS 2013). The National Antenatal Sentinel HIV and Syphilis Survey estimates that 29.5% of pregnant women aged 15-49 were living with HIV in 2011 (SANAC 2012). This survey was based on a sample size of 36 000 women attending 1445 antenatal clinics across all provinces in South Africa. KwaZulu Natal recorded the highest HIV prevalence in the country in 2011, with 39.1% of pregnant women testing HIV positive in 2006. This figure has since decreased to approximately 37% in 2011 among women attending antenatal clinics in SA (Avert 2011).

The Human Science Research Council (HSRC) 2012 household survey conducted in 2012 and published in the Mail and Guardian in 2013 (Mail & Guardian 2013) showed that in all provinces, the HIV prevalence has increased in the age group 15-49, with KwaZulu Natal having the highest proportion of infections at 27% and Mpumalanga at 26%. The HSRC concludes that antiretroviral treatment has increased the life-span of HIV infected people, thus increasing the proportion of people living with HIV.

1.1.2 Replication of HIV

The following steps occur during HIV replication:

a. Fusion of HIV to the CD4 cell surface and entry into the cell

Entry begins with the interaction of the envelope complex (gp 160 spike) to both the CD4 T cell receptor and the chemokine receptor (either CXCR5 or CCR5) on the surface of the host cell. HIV attaches to the the CD4 binding domains of gp 120 and the CD4 T cell receptor. After the CD4+ T cell and gp120 receptors are bound, that complex on the viral envelope exposes the chemokine binding domains of gp 120 due to a structural change and allows them to bind to the chemokine receptor of the host cell (NIAID 2009).

b. Viral enzymes enter the host cell and replication and transcription occurs

After HIV has attached to the target cell, various enzymes including reverse transcriptase, ribonuclease, integrase and protease are released into the cell. The viral single-stranded ribonucleic acid (RNA) genome is transcribed into double stranded deoxyribonucleic acid (DNA), which is then integrated into a host chromosome (NIAID 2009).
This reverse transcription process is highly susceptible to errors and results in mutated HIV strains which may elude the immune system or cause drug resistance (NIAID 2009).

c. Integration, generation of viral proteins, immature and mature HIV virus forms.

The integration of the viral DNA into the host genome is facilitated by an enzyme called *integrase*. In the latent stages of HIV infection, this viral DNA lays dormant (NIAID 2009).

The integrated DNA pro-virus is transcribed into messenger-RNA (m-RNA) which is translated into regulatory proteins Tat and Rev. Tat encourages the production of new viruses and Rev accumulates and binds to viral m-RNA and allows the unspliced RNA to leave the nucleus. The full length is actually the viral genome; it binds to the Gag protein and is parcelled into new virus particles (NIAID 2009).

The final step of the HIV replication cycle is the assembly of new HIV-1 virions. This begins with the Env polyprotein (gp160) which is cleaved, resulting in the 2 glycoproteins gp41 and gp120 (NIAID 2009).

The Gag polyproteins (p55) and Gag-Pol (p60), along with the HIV RNA, fuse with the inner surface of the plasma membrane of the host cell as the developing new virions starts to bud out of the host cell (NIAID 2009).

The cleavage of the budded virion is mediated by the viral enzyme protease. (NIAID 2009).

1.1.3 Pathophysiology of HIV

During acute infection, the level of HIV may rise to several million virions per milliliter of blood. (Piatak et al. 1993). This is accompanied by a persistent destruction of T cells leading to the decline of CD4+ T cells (Sousa, et al. 2002).

The probability of HIV transmission occurring after a single exposure is about 1 in 200 to 1 in 1000. HIV remains trapped in the genital mucous lining and on the surface of the epithelium and doesn't penetrate the exterior epithelial cell layers (Wilson et al., 2012c).

The acute vireamia is associated with stimulation of CD8+ T cells and is also associated with the production of antibodies. The CD8+ T cell response has been shown to be essential in bringing virus levels down which increase soon after infection and decrease as the CD4+ T cells recover (Pantaleo et al., 1997). Better disease outcome and slower progression to AIDS has been associates with a strong CD8+ Tcell response. Prospective studies on cohorts of HIV infected individuals have shown that rates of HIV disease progression may vary among individuals (Pantaleo et al., 1997). The mechanism of CD4+ T cell depletion differs in the acute and chronic phases (Hel et al., 2006).

The role of CD4+ T cells is to help other lymphocytes, especially B cells and CD8+ T cells, recruit monocytes, basophils, neutrophils, and eosinophils as well as minimize responses to T regulatory cells (Zhu et al., 2010). Changes in CD4+ T cells is mediated by positive and negative feedback regulatory mechanisms (Phetsouphanh et al., 2015).
T follicular helper cells and regulatory T cells are key players in driving B cell activation and lymphocyte activation respectively, within HIV-1 infected lymphoid tissue.

The loss of CD4+ T cells can be due to the direct infection of this subset (Haase et al., 1999), and chronic immune activation (Brenchley et al., 2012) leading to morbidity and mortality if untreated. Effective CD4+ T cell responses are vital in the development of cellular and humoral immunity against viral infections. Specific p24 cytotoxic CD4+ T cells (Norris et al., 2004; Zaunders et al., 2004) are important in HIV-1 infection particularly in long term non-progressors (LTNPs) and Elite Controllers for the control of viral replication, unlike in progressive disease where non-proliferative CD4+ T cells are present (Zaunders et al., 2013).

1.1.4 Biological factors affecting transmission

Transmission of HIV through sexual contact occurs after non-keratinized genital surfaces come into contact with infected genital secretions or blood. Transmission is influenced by the following factors (Wilson et al., 2012a.):

a. Viral load - the higher the viral load, the higher the risk of transmission
b. Acute HIV infection – there is a high viral load during the acute phase of infection and this leads to an increased risk of infection
c. Stage of HIV infection – a low CD4 count is associated with increased infection rates
d. Untreated sexually transmitted infections (STI's) – the risk is increased with both ulcerative and non-ulcerative infections in either partner
e. Herpes Symplex Type 2 (HSV2) infection – recurrences of HSV2 is associated with high viral loads in genital secretions
f. Bacterial Vaginosis in young women – this is associated with high transmission as the overall vaginal flora is unbalanced
g. Cervical ectopy in young women

h. Circumcision – halves the risk of transmission to HIV-uninfected men but the risk may increase if sexual intercourse occurs before the circumcision wound is healed
i. Rare inherited mutations of chemokine receptors (e.g homozygous CCR5 delta 32 deletion mutation) – individuals with this mutation have CD4+ T cells which lack the main receptor required for the attachment of HIV onto the CD4+ T cell surface

1.1.5 Stages of HIV-1 Infection

There are 3 main stages of HIV infection: acute infection, clinical latency and AIDS.

Acute HIV infection

The initial period following infection with HIV is called acute, primary HIV or acute retroviral syndrome (Kahn et al., 1998). Many individuals develop influenza-like symptoms 2-4 weeks following exposure while some experience no symptoms (Kelly et al., 2009).

Clinical latency

The acute stage of infection is followed by a stage called clinical latency, asymptomatic HIV or chronic HIV (The US Department of Health and Human Services (HHS), 2012). Without treatment, this second stage can last from anything from 3 to 20 years. There are no symptoms at this stage but CD4+ T cells slowly are depleted so that near the end of the stage many individuals experience weight loss, fever, gastro-intestinal problems and muscle pains (Kelly et al., 2009).

AIDS

Once CD4+ T cell count drops to under 200 cells/µl or opportunistic infections become serious the patient then progresses to the clinical AIDS stage.

1.1.6 HIV Disease Management

SOUTH AFRICAN GUIDELINES

In response to the HIV/AIDS epidemic, the South African Department of Health has compiled a detailed 2012-2016 National Strategic Plan (NSP) on HIV, sexually transmitted infections (STIs) and TB (SANAC 2011). The plan is driven by a 20 year vision for the country which is:

a. Zero new infections due to vertical transmission
b. Zero preventable deaths associated with HIV and TB
c. Zero discrimination associated with HIV and TB

In line with this 20 year vision, the NSP has the following goals:

a. Reducing new HIV infections by at least 50% using combination prevention approaches
b. Initiating at least 80% of eligible patients on ART, with 70% alive and on treatment 5 years after initiation
c. Reducing the number of new TB infections, as well as the number of TB deaths by 50%
d. Ensuring an enabling and accessible legal framework that protects and promotes human rights in order to support implementation of the NSP
e. Reducing self-reported stigma and discrimination related to HIV and TB by 50%

The KwaZulu-Natal (KZN) Department of Health, in consultation with the South African National AIDS Council (SANAC) Treatment Technical Task Team, have compiled a set of guidelines for the management of HIV/AIDS (KZN Dept of Health 2013). The guidelines aim to achieve certain goals such as:

a. To achieve the best health outcomes in the most cost-efficient manner
b. Implement nurse-initiated treatment
c. Decentralize service delivery to primary healthcare clinic (PHC) facilities
d. Integrate services for HIV, TB, maternal and child health (including prevention of mother to child transmission), sexual and reproductive health, and wellness
e. Diagnose HIV earlier
f. Prevent HIV disease progression
g. Avert AIDS-related deaths
h. Retain patients on lifelong therapy
i. Prevent new infections among children, adolescents, and adults
j. Mitigate the impact of HIV/AIDS (KZN Dept of Heath 2013)

The objectives of the program include strengthening the public and private health sectors' capacity to deliver integrated health services, to ensure timely initiation of antiretroviral drugs (ARVs) for treatment and prevention and to minimize drug toxicities (KZN Dept of Heath 2013).

Viral load testing can provide an early indication of treatment failure in patients receiving ART. The 2013 WHO guidelines for ART treatment recommend using viral load testing for detecting virological failure and/or confirming treatment failure among people with evidence of clinical and/or immunological failure. This kind of monitoring is now recognized as the gold standard for monitoring the response to ART in high income settings. Several clinical and epidemiological studies show that the risk of HIV transmission is very low when the viral load is lower than 1000 copies/ml. The Guidelines Development Group also recommended lowering the viral load threshold for treatment failure from 5000 copies/ml to 1000 copies/ml (WHO, 2013b). As part of HIV disease management, current guidelines require viral load testing at 6 and 12 months after commencement of ART and then every 12 months.

1.1.6.1 HIV testing

In South Africa, HIV testing must be conducted ethically within a supportive environment by a trained professional. Verbal consent should be obtained before testing. Disclosure of the results and the implications thereof should comply with the promotion of human rights (KZN Dept of Health 2013).

HIV test results are reported as:

HIV NEGATIVE

a. Report as negative to patient
b. Encourage patient to repeat the test in 3 months to exclude the possibility of the window period
c. Discuss the appropriate prevention package with the patient

(KZN Dept of Health 2013)

HIV POSITIVE

At intial HIV diagnosis a blood sample is taken for a CD4+ T cell count. According to the South African Treatment Guidelines 2013, if a person is:

HIV POSITIVE > 350 CD4+ T cells/µl, then

a. Transfer to a wellness program for regular follow-up and repeat CD4 testing 6-monthly
b. Advise on how to avoid HIV transmission to sexual partners and children
c. Initiate INH prophylaxis if asymptomatic for TB
d. Provide counseling on nutrition and contraceptive and do annual pap smear

(KZN Dept of Health 2013)

HIV POSITIVE ≤ 350 CD4+ T cells/µl - Eligible to start ART

a. CD4 count <350 cells/µl irrespective of WHO clinical stage

   OR

b. Irrespective of CD4+ T cell count
   i. All types of TB (in patients with TB/HIV drug resistant or sensitive TB, including extra pulmonary TB)
   ii. HIV positive women who are pregnant or breast feeding

   OR

   iii. Patients with Cryptococcus meningitis or TB meningitis (defer ART for 4-6 weeks)
c. WHO stage 3 or 4 irrespective of CD4+ T cell count

(KZN Dept of Health 2013)

REQUIRE FAST TRACK (i.e. ART initiation within 7 days of being eligible)

d. HIV positive women who are pregnant or breast feeding

   OR

e. Patients with low CD4 ≤200 cells/µl

   OR

f. Patients with Stage 4, irrespective of CD4+ T cell count

   OR

g. Patients with TB/HIV co morbidity with CD4+ T cell count ≤ 50 cells/µl

(KZN Dept of Health 2013)

1.1.7 Standardized National Monitoring for Adults and Adolescents with HIV

1.1.7.1 At initial diagnosis of HIV:

a. Confirm HIV result with rapid antibody test to ensure that national testing algorithm has been followed
b. Check CD4+ T cell count if HIV positive and WHO clinical staging to assess eligibility for ART and eligibility for fast-tracking
c. Screen for pregnancy or ask if planning to conceive to identify women who need ART for life or ARV prophylaxis for prevention of mother-to-child transmission (PMTCT)
d. Screen for TB symptoms using the WHO questionnaire to identify TB/HIV co-infected
e. Check haemoglobin (Hb) or a full blood count (FBC) if requires zidovudine (AZT) to check for anaemia and neutropaenia
f. Check creatinine levels if requires Tenofovir Diproxil Fumerate (TDF) to detect renal insufficiency
g. For patients initiated on Nevirapine (NVP) based regime check Alanine Transaminase (ALT) levels to exclude liver disease

(KZN Dept of Health 2013)

1.1.7.2 Laboratory Monitoring for Patients on ART

a. CD4+ T cell count at 1 year on ART to monitor immune response to ART
b. Viral Load (VL) at month 6, 1 year on ART and then every 12 months to identify treatment failures and problems with adherence
c. Check ALT only if on NVP and develops symptoms of NVP toxicity
d. FBC at month 3 and 6 if on AZT to identify AZT toxicity
e. Creatinine at month 3, 6 and 12 and then annually if on TDF
f. Fasting cholesterol and triglycerides at month 3 if on Lopinavir/Ritonavir LPV/r to identify LPV/r toxicity

(KZN Dept of Health 2013)

1.1.7.3 At routine follow-up visits for those not yet eligible for ART

a. Repeat CD4+ T cell count at 6 months to see if they have become eligible for ART
b. WHO clinical staging at every visit to see if they have become eligible for ART
c. Screen for TB symptoms to identify TB suspects
d. Offer Isoniazid Preventative Therapy (IPT) if no TB symptoms to identify TB/HIV co-infection to prevent TB activation
e. Offer prevention for HIV positives to prevent HIV transmission and re-infection to prevent sexually transmitted infections (STIs)

1.1.7.4 Indications for urgent up-referral prior to initiation or when on therapy

a. Glomerular Filtration Rate (GFR) less than 60 ml/min
b. Hb less than 8 g/dl
c. Body Mass Index (BMI) less than 18.5 kg/m2

(KZN Dept of Health 2013)

1.1.8 Staging Immune Status Through the Use of CD4+ T Cells

The CD4+ T cell: White blood cells or "leukocytes' are responsible for defending the body against infectious diseases. There are 5 different types of white blood cells, namely, Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils. All leucocytes are produced and derived from a haematopoietic stem cell in the bone marrow (Ross M. 2010). The main distinguishing feature of leucocytes is the presence of cytoplasmic granules, hence they are characterized as granulocytes and agranulocytes.

CD4+ T helper cells are white blood cells that are important in the body's defense against invading pathogens; they are the co-ordinates of the immune response. CD4+ T helper cells are the primary targets of HIV.

A prospective cohort study conducted by Van Asten et al. (2004) concluded that immune status prior to HIV infection affects disease progression after sero-conversion. This was demonstrated

in a study where 51 HIV negative intravenous drug users were recruited and followed up after sero-conversion.

1.1.8.1 Enumeration of CD4+ T lymphocytes in HIV/AIDS

According to the WHO, 9.7 million people living in low and middle income countries had access to ART as of December 2012. The goal of WHO was to scale up HIV treatment and have 15 million people on ART by the end of 2015. There is a need to consider inexpensive treatment as well as affordable laboratory testing in order for this goal to be achieved (WHO, 2014a).

In HIV infected patients, the CD4+ T cell count in peripheral blood is used for :

a. Assessing the extent of immune deterioration and speed of development of AIDS
b. Making a decision to initiate ART, taking into account the patient's clinical picture
c. Deciding on the initiation of prophylactic treatment
d. Monitoring the efficacy of ART

In adults, the absolute CD4+ T cell count is measured, whereas in infants and young children, the percentage of CD4+ T cells among total lymphocytes is more accurate (WHO, 2007).

1.1.8.2 Techniques used to Count CD4+ T Cell

Techniques that are currently used are methods which comprise of large automated flow cytometers that enumerate a variety of cells and dedicated systems that only do CD4+ T cell testing such as the FACSCount.

Automated methods

Flow cytometry is the gold standard technology for CD4+ T cell enumeration because of its accuracy, precision and reproducibility. However, this technology is relatively complex and costly. The operators need to be well trained in the technical and biological aspects of the methodology. Cost-effective running of flow cytometry requires regular maintenance. This makes the use of flow cytometry limited in resource-limited settings to centralized laboratories. The introduction of simpler and portable instruments which require little operator training has been highlighted by the WHO.

Flow cytometry offers several technical options to obtain absolute CD4+ T cell counts (Hubl et al., 1996):

a. **Double platform assays**: The flow cytometer is used to deliver the percentage of CD4+ T cells and this is combined with parameters from the haematological analyser to obtain the absolute CD4+ T cell count.
b. **Single platform assays**: The flow cytometer is used on its own to obtain the percentage of CD4+ T cells and/or absolute CD4+ T cell counts.

Dedicated cytometers

These are designed to exclusively perform absolute CD4+ and CD8+ T cell counts without a haematology analyser. These are simpler to operate and cheaper than automated flow cytometers but are more rigid in terms of assay and reagent choices. The running costs can also be high yet the sample throughput can be lower than in automated methods. The FACSCount is the only dedicated cytometer that has been validated and widely used (Wade et al,. 2014).

Currently, CD4+ T cell testing is done using flow cytometry, which is considered the gold standard for CD4 testing. In many parts of the world flow cytometry such as the BD FACS Count or BD FACS Calibur, PARTEC Cyflow or GUAVA platforms are used (Mwau et al., 2013; Manasa et al., 2007). The long turn-around times of these platforms delay clinical decision making and put an extra burden on patients who have to make numerous visits to the health facility prior to being initiated on ART, resulting in patients lost to follow-up (Myer et al., 2013).

PIMA POC CD4+ T cell testing

The PIMA analyser is a promising device in resource-limited settings, as it uses cartridges which have a long shelf-life and are stable in a wide range of climatic conditions (Mwau et al., 2013).

The PIMA analyser is a compact, automated machine weighing just 2.5kg. The Alere PIMA Analyser is powered either by a rechargeable battery, or can be run from mains A/C power. It offers a one stop, same day result for absolute CD4+ T cell count in less than 20 minutes (Alere

HIV 2012). Consisting of the Alere PIMA Analyser and Alere PIMA CD4 Cartridge, the Alere PIMA CD4 test enables CD4+ T cell analysis from a fingerstick or venous whole-blood sample, providing an effective and affordable tool in the management of HIV patients whilst at the clinic facility (Alere HIV 2012).

1.1.8.3 Review of studies evaluating the performance of the PIMA

Until recently, there have been very few studies which have evaluated the performance of the PIMA analyser in CD4+ T cell enumeration. A study done in Kenya in nine health facilities that offer CD4+ T cell testing, evaluate a point of care (POC) testing using the PIMA analyser. These sites provided a range of socio-economic and climatic conditions, from rural, peri-urban and urban settings and temperatures ranging from 18ºC to 38ºC and humidity levels ranging from 25% to 75% (Mwau et al., 2013). A total of 1549 patients were recruited from the nine sites with at least 49.7% of these patients on ART. Venous and capillary blood specimens were collected from each patient and the venous blood was used for CD4+ T cell testing using flow cytometry. The capillary blood was used for CD4+ T cell testing using the Alere PIMA analyser.

The coefficient of determination/correlation (CV) was 0.885 (Mwau et al., 2013) when comparing CD4+ T cell counts between the BD FACS Count and the BD FACS Calibur from 312 patients. There was a mean bias of 76.5cells/µl suggesting that these 2 platforms are not interchangeable (Mwau et al., 2013). The sensitivity of the PIMA analyser was 89.6% and the specificity 86.7% in those who were 5 years and above (n=396) when compared to the BD FACS Calibur. When the threshold was lowered to 200 cells/µl, the sensitivity of the PIMA analyser was 86.7% and the specificity was increased to 94.12% (Mwau et al., 2013). Furthermore the PIMA analyser had a sensitivity of 79.4% and a specificity of 83.4% in those aged 5 years and above (n=822) when compared to the BD FACS count. In this comparison, the sensitivity and specificity were 83,0% and 98.2% respectively when the threshold was lowered to 200 cells/µl (Mwau et al., 2013).

These authors concluded that POC testing may not provide the same accuracy, sensitivity and specificity as the "gold standard". The measurement of the precision of the PIMA analyser gave a coefficient of determination of 10.3%, which was found to be undesirable and also much lower than that of the FACS Count and the FACS Calibur. However, it was acknowledged that

POC testing may still provide CD4+ T cell count results to patients who may otherwise not have them at all. (Mwau et al., 2013). An encouraging finding in this study was that there was minimal bias using either capillary blood or whole blood samples on the PIMA analyser, which makes the PIMA analyser ideal for use in a variety of settings. These authors concluded that by virtue of its nature, the PIMA analyser can also still expand access to CD4+ T cell count testing because it can be used in rural settings where there is no access to electricity. Clinic personnel can conduct CD4+ T cell count testing with minimal technical training (Mwau et al., 2013).

The results of the Kenyan study were vastly different from those conducted in India, to assess the utility of the PIMA analyser to enumerate CD4+ T cells. This research was done in 21 ART clinics across India between June and August 2011. Blood samples were collected from 1790 patients and were tested using the PIMA analyser and the gold standard methods i.e. FACS Calibur, FACS Count and Cyflow SL3. Additionally 175 paired venous and capillary samples were collected for testing by both the PIMA analyser and the FACS Calibur (Thakar et al., 2012). Inter-machine comparisons and repeatability of the CD4+ T cell counts were assessed using venous blood samples prior to distributing all machines to the 21 ART centres. The coefficient of variation was less than 10% for both fresh blood samples and for stabilized blood samples (Thakar et al., 2012). The accuracy of the PIMA analyser at different CD4+ T cell levels was determined by comparing the median values obtained for different CD4 ranges by both the PIMA analyser and the reference analysers. A CV of less than 10% was considered to be acceptable for this analysis (Thakar et al., 2012). The data showed admirable correlation between the CD4+ T cell count results obtained by PIMA analyser and the reference gold standard analysers; with correlation coefficients of 0.983, 0.988 and 0.977 compared to the FACSCount, FACS Calibur and Cyflow SL3 respectively. The p value was less than 0.001 in all comparisons. The differences in the median CD4+ T cell counts counts obtained by the PIMA analyser and by flow cytometry were less that 0.05 and therefore not significant (Thakar et al., 2012).

The sensitivity and specificity was measured to determine the clinical significance of the variations in the measured values in patients requiring ART. This was found to be 91% but also showed that most of the mismatched samples were between 320 and 380 CD4+ T cell counts. Comparison of the finger-prick with the venous sample were analysed using the same methods.

The study found that proper training of operators was necessary in order for finger-prick samples to be tested efficiently on the PIMA analyser as samples of unsatisfactory volume gave doubtful results (Thakar et al., 2012). The conclusions in this study in terms of the suitability of the PIMA analyser for resource-limited settings and health centres with limited electricity supply were similar. This study did not indicate whether the analyses were performed in a wide range of climatic conditions or not, hence it is not clear if the performance of the PIMA analyser could be affected by climatic conditions.

Another study looking at the performance of the PIMA analyser using capillary blood was evaluated in a multisite study where 300 patients were recruited, including HIV-infected patients as well as HIV negative patients for controls. Capillary blood samples were collected for CD4+ T cell count testing using the PIMA analyser and venous and capillary blood samples were collected for CD4+ T cell testing using both the PIMA analyser and FACS Count (Diaw et al., 2011).

Similar CD4+ T cell counts were obtained by the PIMA analyser and FACS Count using either HIV positive or HIV negative blood. However, venous blood performed better with a concordance co-efficient of 0,97 and a Pearson's coefficient of 0,98 compared with capillary blood which had a concordance coefficient and Pearson's coefficient of 0,88 and 0,89 respectively (Diaw et al., 2011).

In terms of the sensitivity and specificity for clinical decision making to start ART at 200 cells/µl there was not much difference between the venous and capillary blood samples. The sensitivity of the PIMA analyser was 90% and 91% with a specificity of 98% and 96% for venous and capillary blood respectively. When the CD4+ T cell count threshold was increased to 350 cells/µl, the sensitivity of the PIMA analyser was 98% for venous blood and 91% for capillary blood, suggesting that the higher the CD4+ T cell count the lower the sensitivity of the PIMA when using capillary blood. The specificity was 79% and 80% with venous and capillary blood respectively. Furthermore, with the capillary samples there were 14% aborted analyses due to errors (Diaw et al., 2011).

The findings of this study by Diaw et al., 2011 were confirmed by the findings of the study conducted by Mwau et al., 2013. In both the studies the performance of the PIMA analyser was found to be substandard as compared to flow cytometry when the type of sample used is

capillary blood compared to a finger-prick. Of note, Mwau et al., 2013 did not assess the performance of the PIMA analyser using venous blood samples. In Mwau et al's study venous blood samples were tested only with flow cytometry.

The limitations of capillary testing with the PIMA analyser were also highlighted following a an evaluation of the analyser in South Africa. The aim of the study was to assess and report the performance of the PIMA analyser in adults, using venous blood in a controlled laboratory environment compared to using capillary blood sampling in a primary health care (PHC) clinic. Capillary sampling was standardised across all phases using the same lancet, herein referred to as "Lancet 1". In phase 2B a different lancet, "Lancet 2" was used to assess whether the type of lancet had contributed to the poor performance observed in the previous phases. (Glencross et al. 2012).

The study was divided into 3 phases:

Phase 1 was done to assess the baseline accuracy and sensitivity of the 4 PIMA analysers in a controlled laboratory environment. A venous sample volume of 20µl was pipetted into the PIMA cartridges. The reproducibility of the machines was evaluated using n=77 matched venous and capillary samples. Low and normal Immunotrol (IM) controls were tested every day for nine consecutive days. There was very good instrument precision with % CV's of < 2.5% (Glencross et al., 2012).

Phase 2 was conducted in an antenatal clinic using 2 of the PIMA instruments validated in Phase 1. Testing was done using capillary sampling as per manufacturer's instructions. One operator was trained to perform the tests in order to eliminate operator-related variations in the results. A larger negative bias was observed. Loss of precision in relation to that seen when venous blood was tested by the PIMA and a wider % CV was seen. The CV's for venous vs venous blood; capillary vs venous blood and venous vs capillary blood were 6.7%, 23.3% and 26.5% respectively (Glencross et al., 2012).

Phase 3A comprised of field tests performed in two semi-rural, less resourced clinics in Limpopo. In this phase, finger pricks were performed and tests on the PIMA analyser conducted by multiple unspecified operators. Additional venous samples were drawn for CD4 testing using flow cytometry. Although poorer sampling testing performances were noted at

these sites, bead precision for low and high controls was noted with a CV of 2.88% (Glencross et al., 2012).

Of the 111 samples that were collected in phase 3A, 8 were clotted and could not be tested, 7 had invalid PIMA analyser results. In the final analysis of the 96 matching samples, significant differences to predicate reporting and very wide limits of agreement, particularly in the very clinically significant ≤350 cells/µl range, were found. Further analysis of the results in this group revealed that 10 out of 32 patients would not have been initiated to ART if tested by the PIMA analyser. Furthermore 23,5% of patients with CD4 counts between 350 cells/µl - 500 cells/µl would have missed the opportunity for pre-ART intervention, as they had higher CD4 counts by the PIMA analyser (Glencross et al., 2012).

Phase 3B was conducted in an inner city clinic in Johannesburg. Two nursing assistants conducted the tests. Additional venous samples were drawn for testing using flow cytometry. The operators were given additional manufacturer-driven training to assess if that would improve the poor performance of the PIMA analyser observed in the previous phase. The results in this phase were in sharp contrast with those obtained in phase 3A. Less bias and tighter limits of agreement variations were observed, irrespective of whether lancet 1 or lancet 2 was used. The additional training produced better results but venous blood still yielded better CD4+ T cell count results than capillary sampled blood (Glencross et al., 2012).

Capillary sampling for use with the PIMA analyser has been proven to produce less than desirable results. (Mwau et al., 2013; Thakar et al., 2012; Diaw et al., 2011 and Glencross et al., 2012). However Glencross et al. commented that the challenges with capillary sampling are widespread and not just unique to the PIMA analyser. In their study Glencross et al. have successfully eliminated other possible causes of poor performance by the PIMA analyser, such as the type of lancet. They further argue that the type of sample per se should not affect the performance of the PIMA analyser as venous blood is not expected to be different from capillary blood with regard to haematological values. Therefore having excluded most other possible causes of poor precision, they have concluded that the quality of the sample is most likely the reason for the poor performance of the PIMA analyser, along with the lack of proper quality control and lack of attention to testing protocol in PHC (Glencross et al., 2012).

Glencross et al. highlights the general lack of understanding of the differences between a "finger-prick" and capillary sampling in PHC's; that the sampling that is required for the PIMA analyser is different from that required for other PHC tests such as the rapid HIV test. The authors emphasize the importance of re-enforcing the differences between these techniques as well as strict adherence to the instructions of the manufacturer when using the PIMA analyser in order to ensure accuracy and precision of the instrument. Quality control of the capillary bleed is suggested as being crucial in order to overcome the challenges related to POC testing. Using venous blood instead of capillary sampling is further presented as a viable option in this study (Glencross et al.,2012).

While this was not part of their research study, Glencross et al. further claim that the use of the PIMA analyser at the POC may be less cost-effective, compared to using conventional flow cytometry (Larson et al., 2012a) provided a cost analysis using data from a South African program implemented during 2010 in Gauteng Province in which POC was piloted using the PIMA analyser. The run-of-the-mill cost per CD4+ T cell test performed by the mobile HIV Counselling and Testing (HCT) program using the PIMA analyser was $23.76 yet laboratory-based testing was estimated at $7–8 per test (Larson et al., 2012a). Although POC technology may lead to better clinical outcomes by providing rapid results and be cost-effective the focus should also include improving sequential steps in the continuum of HIV (Hyle et al, 2014). The economic and clinical impact of providing POC testing was evaluated using a simulated cohort of 2 million patients. This analysis made by Hyle el (2014) suggests that although performance characteristics of a new diagnostic test are a high priority, the impact of POC on health sytems and linkage to HIV care significantly outweighs the effects of the performance chracteristics of a diagnostic test within narrow ranges. The goal of immunological staging is to accelerate linkage to care for those who require it for better clinical outcomes even if some patients are misclassified (Hyle et al, 2014). The PIMA POC analyzer received a score of 4.0 when an objective and standardized scorecard was used to assess the operational specifications of POC diagnostic tests currently available. Characteristics of the devices that were assessed included, but not limited to portability, electrical requirements, result storage, quality control requirements and operator skills required (Lehe et al, 2012).

1.1.8.4 Review of studies showing the importance of providing timely CD4+ T cell results

The realization of the NSP goals requires a scale-up of services in the health sector such as early HIV diagnosis and linkage to care as well as access to treatment.

Loss to follow-up of diagnosed patients is a significant challenge which often results in patients presenting late to hospitals with low CD4+ T cell counts and subsequent high morbidity and mortality. Loss to follow-up of HIV positive patients following diagnosis can exceed 50% in some low-income settings (Jani et al., 2011).

Challenges with returning to the healthcare facility resulting in loss to follow-up include: distance from healthcare centers, male gender, referral by 3rd parties, psychosocial factors and undocumented death rates. In South Africa, CD4+ T cell count results become available within 1 week. This is due to the fact that CD4+ T cell testing takes place at an off-site laboratory (Faal et al., 2011).

This finding was concurred by two studies conducted in Durban which also cited a prior history of tuberculosis as an additional risk factor for loss to follow up. This study found that the more risk factors a patient has, the more likely the patient will be lost to follow-up. Patients with 1, 2 or 3 risk factors were found to be 1.88, 2.50 and 3.84 times respectively more likely to be lost to follow up , prior to treatment initiation when compared to patients with no risk factors (Losina et al., 2010).

Nearly half the patients infected with HIV failed to have CD4 staging following an HIV diagnosis in 2 prospective cohort studies in KwaZulu Natal, a province with the highest HIV prevalence in South Africa. The primary objective of the studies was to determine the predictors of pre-treatment loss to care (PTLC), which was defined as failure to undergo CD4 staging within 8 weeks of receiving an HIV diagnosis (Losina et al., 2010).

In a similar study also conducted in Durban, South Africa, from July to December 2006, about 45% of patients were found to have been lost to follow-up in the first year following ART initiation. ART eligibility was defined as a CD4+ T cell count of ≤ 200 cells/µl. Patients who did not initiate treatment within 3 months were contacted telephonically and correlates of loss to care were evaluated using logistic regression. The delay from CD4 count to ART training

and treatment initiation, even among those with the lowest CD4 counts, was found to be the main stage at which loss to follow-up was occurring (Basset et al., 2009).

Contrary to this, Rosen et al. 2011 conducted a systematic review to ascertain the exact stage at which patients testing HIV positive in PHC's are being lost. Retention in HIV care was categorized into 3 stages:

Stage 1: From HIV testing to receipt of CD4+ T cell count results or clinical staging
Stage 2: From staging to ART eligibility
Stage 3: From ART eligibility to ART initiation (Rosen et al., 2011)

Although, there has been a few studies that have followed up patients through all the stages, the average retention in Stage 1 was 59% (35%–88%); Stage 2, 46% (31%–95%); and Stage 3, 68% (14%–84%), showing that most patients were lost at stage 2 (Rosen et al., 2011). However, the retention rates at stage 3 varied greatly between studies, with some studies reporting retention rates as low as 14% and some as high as 84% (Rosen et al., 2011).
Another observational study conducted in Khayelitsha, South Africa (Patten et al, 2013), to assess the impact of providing POC testing on ART initiation found that when POC testing was introduced, 90% of patients were assessed for ART eligibility with 50% initiating ART as opposed to 67% and 40% assessed for and initiating ART respectively, prior to the introduction of POC testing. While these findings were not statistically significant, the authors argue that not providing patients with immediate CD4+ T cell count results leaves patients with uncertainty regarding their prognosis and without the knowledge of their need to be initiated on ART, they may prefer to ignore their HIV positive diagnosis. Awareness of the progression of one's HIV disease may lead to better uptake of ART before becoming severly immunocompromised.

These studies have highlighted the urgent need for shortening the time taken for HIV positive patients to be linked to care, however, due to the fact that there has not been many studies on retention in pre-ART care and factors determining pre-ART retention, it remains unclear which groups might benefit most from any directed supportive intervention (Rosen et al., 2011). The results by Rosen et al. were supported by a study conducted in Hlabisa which reported that

retention is poorest in younger individuals and those who are in early stages of HIV disease who are not yet eligible for ART (Lessells et al., 2011).

Gender has also been shown to play a role in linkage to HIV care and management. In 2009, a study conducted in Gugulethu, Cape Town, revealed that men generally enter ART programs later than women and with more advanced disease. This was attributed to the fact that in SA, ART in the public sector is offered through PHC's and that because PHC's mostly cater for the needs of women, men may be disadvantaged by this in resource-limited settings (Cornell et al., 2009).

There have been several studies which have gone a step further to assess the value of providing immediate CD4+ T cell count results by introducing POC CD4 testing to address these challenges.

A pilot study conducted in a mobile HCT clinic in Johannesburg from May to October 2010 documented the outcome of integrating POC testing using the PIMA analyser to improve linkage of HIV positive patients to care. 3 outcomes were analyzed: 1) Assignment to testing group, 2) Successful follow-up, and 3) Completion of referral visit for HIV care 8 weeks following diagnosis. In this study, out of 508 patients who tested HIV positive, 311 patients were offered the POC CD4+ T cell count test and 194 were not offered this, but offered the routine CD4+ T cell count test through the National Health Laboratory System (NHLS). Patients who were offered POC were found to be more likely to visit a referral clinic after testing, suggesting that providing rapid CD4+ T cell count results may improve linkages to care. (Larson et al., 2012b).

The value of providing immediate CD4+ T cell counts to patients testing HIV positive at PHC clinics has also been demonstrated in a study conducted by Faal et al. in 2011. In this study 344 HIV positive patients were recruited and randomized into 3 arms:

a. Immediate receipt of CD4 results n = 124
b. Standard collection of CD4 results with written information (leaflet) n= 108
c. Standard collection of CD4 results only (Faal et al., 2011) n = 112

The authors found that in the immediate provision of CD4 results arm, 43 patients were eligible for ART at the time of testing. Provision of immediate CD4+ T cell count results led to more

people reporting for ART initiation (65%). In this arm, 81 patients had CD4+ T cell counts > 215 cells/µl and of this 81, only 38% patients reported for pre-ART.

In the leaflet arm, 35 patients were eligible for ART at the time of testing and of these patients only 37% initiated ART. A total of 72 patients had CD4+ T cell counts > 215 cells/µl and of these patients, 33% reported for the pre-ART program.
In the standard collection arm, 36 patients were eligible for ART at the time of testing but of the 36 patients, only 25% reported for ART initiation. A total of 67 patients had CD4 counts > 215cells/µl and of these 67 patients, only 42% reported for pre-ART (Faal et al., 2011). Although the evidence for the impact of POC CD4+ T cell count testing on linkage to HIV care and treatment after HIV testing is limited, it is nevertherless positive (Larson et al., 2013).

1.2. TUBERCULOSIS (TB)

1.2.1 Epidemiology

TB is a major health problem. The current global picture of TB indicates that there is continued progress and that the incidence of TB has been falling in all six WHO regions but the rate of decline is not fast enough (WHO Global tuberculosis report, 2013). An estimated 8.6 million people developed TB in 2012 globally and about 1.1 million were HIV positive. Of the people diagnosed with TB in 2012, 1.3 million died from the disease. About 75% of these cases were in the African region. Globally, in 2012, there were 450 000 recorded cases of MDR-TB resulting in about 170 000 deaths (WHO, Global tuberculosis report, 2013).

The number of TB infections among children under the age of 15 was estimated at 530 000 cases globally, with 74 000 TB deaths among HIV negative children (WHO, Global tuberculosis report, 2013).

In 2012, about 410 000 women died from TB and 160 000 of those women were HIV positive. TB remains among the top 3 killers of women globally. The majority of TB cases were found in South-East Asia (29%), Africa (27%) and Western Pacific (19%). India and China also accounted for 26% and 12% of the cases, respectively (WHO, Global tuberculosis report, 2013).

The African continent had approximately one quarter of the world's TB cases in 2012. The cases and death rates relative to population in the African region were among the highest in the world, with 255 incident cases per 100 000.

In South Africa, out of a country population of 52 million, there were 296 996 new TB notifications in 2012, and 52 586 cases of re-treatment bringing the total to 349 582 TB case notifications. Of these cases, 37 310 cases were tested for Multi-Drug Resistant (MDR) TB, 15 419 were confirmed as MDR-TB but only 6 494 patients were put on treatment for MDR-TB. The national budget for the TB program was USD 475 million. The program is mostly funded domestically, with only 3% of the funding obtained internationally (WHO, Global TB report, Country profiles, 2013).

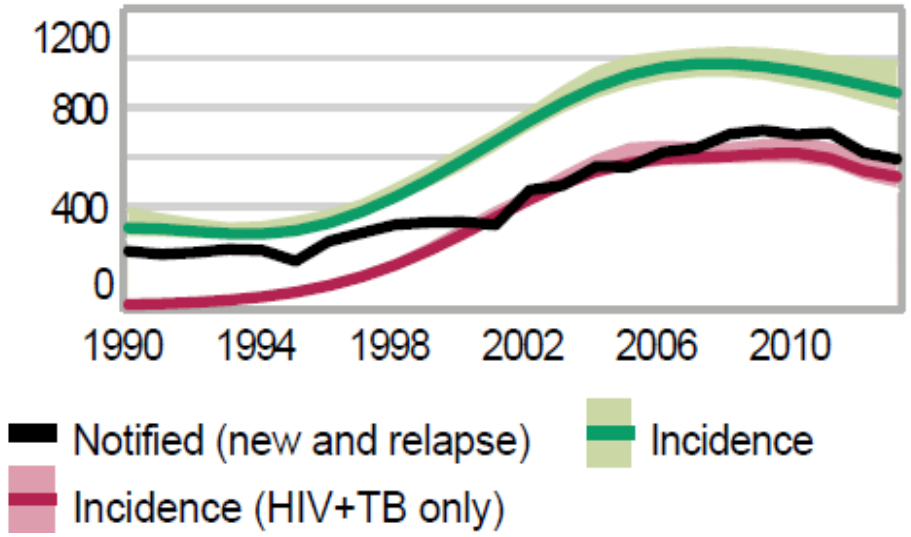

Figure 1: Rate of TB infections per 100 000 population per year

Credit: World Health Organisation country profiles, 2013

The province of KwaZulu Natal (KZN) is the epicenter of the HIV and TB co-epidemics in South Africa, with a TB notification rate of 1094 cases per 100 000 population. A study was conducted by Wallengren et al., 2011, following the 2005 outbreak of extremely drug-resistant TB (XDR-TB) in KZN, South Africa. Laboratory records from 2 laboratories in the province were analyzed. All samples from 2001 to 2007 from patients with MDR-TB were tested for susceptibility to 2nd line anti-TB drugs to determine the number of MDR-TB and XDR-TB cases in each health district. The study found that in 2007, a total of 2799 cases of MDR-TB

were identified in KZN and MDR-TB accounted for 2.3% of cases in the province (Wallengren et al., 2011).

In addition, the study found that MDR and XDR-TB cases spiked in 2007. A 10-fold increase was seen between 2001 and 2007, from 216 to 2799 cases of MDR-TB and from 6 to 270 cases of XDR-TB. The district most affected were the Umkhanyakude and Umzinyathi districts, with rates of 57 and 48 cases per 100 000 population. This reported MDR-TB incidence rate is among the highest in the world (Wallengren et al., 2011).

1.2.2 TB and HIV Co-infection

There are over 1 million people co-infected with HIV and TB worldwide. The burden of the twin epidemics is the highest in Sub-Saharan Africa (WHO 2014b). The emergence of HIV in the 1980's changed the epidemiological profile of TB globally (Lemos et al., 2008).
In 2010, there were 8.8 million new cases of TB of which 12-14% were also infected with HIV. Africa accounts for 82% of TB cases among HIV infected people. In 2010, 5 countries with the highest number of TB cases were India, China, South Africa, Indonesia and Pakistan (WHO 2014b).

The weak health systems and policies in these countries have been cited as the main obstacles in achieving access to TB care. In South Africa, the collision of the two epidemics has been linked with the pre-1994 history of South Africa. The political, economic and land restriction policies gave rise to migrant labourers, overcrowding, and the resultant unsanitary hostels and slums, causing the spread of diseases particularly amongst black people in South Africa, e.g TB from mining. This has led to South Africa, a middle income country, having worse health outcomes than some of Africa's low-income countries (Coovadia et al., 2009).

This analysis is concurred by Karim S. et al. 2009, adding that lack of political will and poor implementation of policies and programmes post-1994 have led to HIV and TB being one of the greatest challenges that South Africa faces.

The hypothesis that migrant labourers and their partners are at risk for HIV infection was hypothesized in a study conducted in Hlabisa and KwaNongoma, KwaZulu Natal, between

October 1998 and November 2000. A total of 196 migrants and 130 of their rural partners were recruited and compared with 64 non-migrant men and 98 women with non-migrant partners. The results showed that 25.9% of migrant men were infected with HIV as compared to 12.7% of their non-migrant counterparts. Amongst women, being a partner of a migrant man was found not to be a risk factor but having multiple partners and the presence of other sexually transmitted diseases were found to be the risk factors in this group (Lurie et al., 2003).

Diedrich et al. 2011, provided an explanation of the immunological events which explains how HIV manipulates TB disease progression and vice versa. In their review, the authors put forward several hypotheses on the immunological relations of the two infections. One of the hypotheses is the effect that HIV has on the TB granuloma. The general presumption is that HIV reduces the number of CD4+ T cells within the granulomas, which are the key cells required for the containment of TB within the granulomas (Diedrich et al., 2011).

Another hypothesis is that HIV replication is often increased at the sites of TB infection and that this reduces the ability of the host to contain TB infection. The target cells for HIV are CD4+ T cells and macrophages, which also happen to be the major components of the granuloma. In their review, Diedrich et al. 2011, cite several studies which have presented conflicting evidence on whether *M. tuberculosis* influences HIV replication, with some studies reporting increases (Goletti et al., 1998; Hoshino et al., 2007; Hoshino et al., 2002) and some reporting decreases in viral replication (Goletti et al., 2004; Diedrich et al., 2011).

A different premise presented by Diedrich et al. 2011, is that the ability of HIV to manipulate macrophage function inhibits the killing of *M. tuberculosis*. The alveolar macrophages can be reservoirs for both HIV and *M. tuberculosis* and are also the first cells to be infected with *M. tuberculosis.* Monocytes migrate to the lungs following entry of the TB tubercle into the lungs and separate into different macrophages. These macrophages are susceptible to HIV infection as well as *M. tuberculosis* infection. It is postulated that HIV infects (HLA-DR) alveolar macrophages (CD14 + CD36+) as well as lymphocytes (CD26+), in the pleural fluid or airway of individuals coinfected with HIV and TB, thereby disrupting the function of *M. tuberculosis*-infected macrophages leading to the dysfunction of granulomas (Diedrich et al., 2011).

1.2.3 Diagnosis of TB

A patient who was or is in contact with someone who has infectious tuberculosis and has the following symptoms is most likely infected with TB. The following criteria are used to screen for suspected TB:

a. Persistent cough for more than 2 weeks (every patient presenting with this symptom should be regarded as a "TB suspect"
b. Production of sputum which may be blood-stained
c. Chest pain and short breath
d. Malaise
e. Tiredness
f. Fever and night sweats

According to the South African National TB Program 2004.

Sputum collection, labeling, storage and transport

a. All TB suspects should have an examination of their sputum samples. At least 2 sputum specimens should be taken from a TB suspect
b. **First specimen** - this is a "spot specimen", obtained immediately after a patient has a bout of coughing. This specimen is collected under the supervision of a healthcare worker
c. **Second specimen –** the patient is given a sputum container to collect an early morning specimen at home
d. The sputum container is labeled with the details of the patient, the date, the appearance of the sample and labeled with whether the sample is a pre-treatment, follow-up or end-of-treatment sample
e. Sputum is stored in a fridge if not transported immediately
f. If transported, the sputum should be transported in a cooler bag, as high temperatures may kill the bacilli

The approach to testing for TB depends on whether the aim is to diagnose active or latent disease. Active disease should first be excluded before an infection can be diagnosed as latent (Konstantinos 2010).

1.2.3.1 Laboratory methods used for TB testing in the diagnosis of TB.

Non-Molecular techniques

Table 1: Overall comparison of advantages and disadvantages of non-molecular methods of TB testing

Methods		Advantages	Disadvantages
Staining	Ziel-Neelsen (Z-N) (Kommareddi et al., 1984)	More specificity Faster than the culture method	Less sensitivity than the AO stain Less sensitivity in immuno-compromised patients
	Auramine O (AO) (Kommareddi et al., 1984)	Simpler than the Z-N stain Faster screening Greater sensitivity Greater predictive value of a negative result	Less specificity than the Z-N stain
Culture	-Lowenstein Jensen medium -Middle Brook 7H10/7H1 (South African National Tuberculosis Programme 2004)	More sensitive than smear microscopy Higher specificity	Slow diagnosis – May take up to 4 weeks to obtain result Expensive and not accessible to all patients

Molecular techniques:

Gene Xpert MTB/RIF

The Gene Xpert test is a fully automated nucleic acid amplification test for the simultaneous detection of MTB and resistance to rifampicin. It produces results in 2 hours and has high sensitivity in detecting TB in smear negative specimens (Cepheid, 2010 Sunnyvale, CA, USA.)

1.2.3.2 The need for rapid TB diagnosis

In order to appropriately manage HIV patients, not only are the CD4+ Tcell counts required but also the TB status. TB diagnosis, as discussed earlier, is difficult, particularly in HIV-1 infected individuals with low CD4+ T cell counts and therefore there is an urgent need for POC TB testing.

Until recently, there have been no rapid tests for TB diagnosis or its drug-resistant forms. This has posed a major challenge to the improvement of healthcare and the reduction of the global burden of TB (Boehme et al., 2011).

Only 30 000 of the 500 000 cases of MDR TB are diagnosed and reported, leading to perpetuation of TB disease through nosocomial and community transmissions (Boehme et al. 2011). In PHC clinics, smear microscopy is often performed for TB diagnosis, however, it misses 40-60% of TB cases and does even worse in patients with advanced HIV disease (Theron et al., 2013).

Effective diagnosis of TB requires standardized and vigorous, easy-to-use diagnostic tools that would allow rapid detection of TB and resistance to key antibiotics such as Rifampicin. The Gene Xpert MTB/Rif is an ideal machine to perform the above-mentioned functions (Chang et al., 2012). Several studies demonstrated high sensitivity and specificity using the Gene Xpert MTB/Rif in the diagnosis of TB (Boehme et al., 2011; Held et al., 2014; Rachow et al., 2011; Theron et al., 2011; Nicoll et al., 2011). Although the Gene Xpert MTB/Rif has been endorsed by WHO as the initial diagnostic test for MTB drug resistance and HIV-associated pulmonary TB, the use of the Gene Xpert in PHC clinics has not been evaluated (Lawn et al., 2013).

The feasibility and clinical effect of the Gene Xpert MTB/Rif was demonstrated in a multicentre, randomised, controlled trial conducted in South Africa, Zimbabwe, Zambia and Tanzania. Patients who had one or more symptoms of pulmonary TB as per the WHO criteria, were enrolled and randomly assigned to have their sputum tested using either the Gene Xpert/Rif or smear microscopy (Theron et al., 2013). The study found that the use of the Gene Xpert MTB/Rif did not reduce MTB-related deaths, however the use of the Gene Xpert MTB/Rif was found to be feasible for a PHC setting. An important finding in this study was that the Gene Xpert MTB/Rif allowed not only for same-day MTB diagnosis but also for same-day treatment initiation and an increase in the number of patients who initiated treatment, mainly because of the higher sensitivity and specificity of this machine, compared with smear microscopy (Theron et al., 2013).

CHAPTER 2 – STUDY OBJECTIVES AND METHODOLOGY

Conventional flow cytometry to determine CD4 counts usually requires that samples be sent to a central laboratory, which may be off-site. This could have significant consequences for the timing of initiation of treatment based on CD4 count, which could in turn influence the health of patients. A comprehensive and holistic approach to combating the HIV epidemic in South Africa will necessarily include the treatment and management of tuberculosis (TB), which is a serious cause of morbidity and mortality in HIV-1 infected people. The confirmatory test widely used in countries where TB is endemic is smear microscopy; however this test has low sensitivity and takes weeks to obtain the results. There is thus a strong indication for the use of

rapid tests in settings where a prompt reaction to test results may have a significant impact on the spread of TB and on co-morbid conditions such as HIV.

This study originally therefore set out to evaluate and compare two point-of-care tests viz PIMA^{TM} POC for the enumeration of CD4+ T cells and the GeneXpert for TB diagnosis against the standard routine NHLS (Beckman Coulter) and smear microscopy). The aim was to determine the accuracy, sensitivity and specificity PIMA^{TM} POC analyser as a predictor of ART eligibility and the GenXpert for TB diagnosis in our hands for future implementation in a PHC setting.

2.1 SPECIFIC OBJECTIVES

Main Objectives

a. To measure the agreement between the PIMA analyser and NHLS PLG/CD4 (n=268).
b. To assess the potential operational role of using the PIMA CD4+ T cell count as compared to the NHLS PLG/CD4 as a predictor of ART eligibility.
c. To analyse the correlation and bias between the FACS Calibur, National Health Laboratory Services (NHLS) PLG/CD4 and the PIMA analyser using a subset of 100 whole blood patient samples.
d. To perform quality control procedures on the 3 PIMA analysers by measuring the precision and reproducibility of the analysers using stabilized whole blood quality control material (Immunotrol (IM) low and normal values); reusable bead-filled cartridges with pre-defined CD4+ T cell counts, (low and normal values); patients' whole blood samples; and patient blood samples left overnight.
e. To determine the number of HIV infected individuals who return for their NHLS PLG/CD4+ T cell count result against those who are lost to follow up between ART eligibility and initiation.

Secondary Objective

f. To compare the Gene Xpert and smear microscopy results done in the NHLS laboratory and the Gene Xpert and culture done in the TB Biomedical Research laboratories, and time to linkage to care from diagnosis.

2.2 STUDY DESIGN

This is an experimental study comparing 2 different POC tests with conventional methodologies.

Sample size calculation was carried out using Stata V12 (StataCorp, 2011). It was determined that a sample size of 254 HIV positive patients would be required to detect a difference of 15 cells/µl between the results of the PIMA analyser POC and the conventional test with 95% probability and 80% power, assuming the standard deviation (SD) of difference in means is 85. In order to allow for potential problems with samples, the sample size was increased with an additional 14 patients giving a total sample size of 268.

The assessment of the Gene Xpert test was based on the sensitivity of the test compared to the gold standard of culture. The measure of sensitivity was based on the number of patients with definite TB found among patients surveyed. It was determined that a sample size of 110 patients with definite TB was required to estimate the sensitivity of the Gene Xpert test to within 7% with 95% probability assuming a sensitivity of 84%. Depending on the prevalence of TB in the sampled community, the number of patients needed to be screened could range from 138 (prevalence = 80%) to 185 (prevalence = 60%) - the sample size was therefore set at the upper limit of n=185.

2.3 STUDY SITE

The study took place at Lancers Road clinic, a busy primary healthcare clinic (PHC) under the jurisdiction of the eThekwini Health Unit. The Lancers Road PHC, is located in the hub of the city of Durban where all transportation routes merge, with an estimated 350,000 commuters passing through it daily. About 900 patients undergo HIV counseling and testing (HCT) per month, with an HIV prevalence, in the non-pregnant population, of approximately 20%. Hence

the geographic coverage of this study is the surrounding Durban functional region with a radius of 50 kilometers.

This PHC facility offers HCT to walk-in patients who receive pre- and post test counselling, emphasizing the importance of CD4 testing for the staging of HIV-1 disease to determine eligibility for ART. Patients who test HIV-1 positive are encouraged to undertake the standard CD4 testing immediately after post-test counselling by a venipuncture blood draw by a counsellor-trained phlebotomist. Patients are advised to return to the clinic after 2-5 days for their CD4 test result. Patients with a CD4+ T cell count ≤350 cells/µl upon returning for their results are medically assessed, and education and counselling undertaken prior to ART initiation as per South African HIV and AIDS guidelines (KZN Department of Health, 2013). Those not eligible for ART with CD4+ T cell counts > 350 cells/µl but ≤500 cells/µl are counselled to return to the clinic after 3 months for CD4+ T cell count re-testing. Patients with CD4+ T cell counts >500 cells/µl are counselled to return to the clinic at 6 months for a new CD4+ T cell count and for further medical assessment. Patients eligible for ART who did not return for the CD4 test result were contacted telephonically to ascertain whether they had been initiated on ART and if not, they were encouraged to return for further care and management.

2.4 STUDY POPULATION

The study population consisted of a convenience sample of HIV infected adults who presented to the Lancers Road PHC in Durban. A total of 268 patients were recruited between January – July 2013. The following information was collected for each patient: Clinic number, gender, contact details, telephone number, and identification document number.

2.4.1 Inclusion Criteria

a. HIV infected adults
b. Willing and able to provide informed consent for samples to be tested

2.4.2 Exclusion Criteria

a. HIV uninfected

b. Pregnant
c. Children under the age of 18 years

2.5 APPROVALS

Approval was obtained from University of KwaZulu-Natal Postgraduate Committee (Appendix 1) to conduct the study. Ethical approval for the study was obtained from the Biomedical Research Ethics Committee (BREC) - reference number BE212/11 (Appendices II and III) and the eThekwini Municipality Health Unit Research Committee (Appendix IV). All study participants signed written informed consent to participate in the study (Appendices V and VI – Informed consent documents, English and isiZulu respectively).

2.6 LABORATORY METHODOLOGY

2.6.1 Testing of Blood Samples

Routine CD4+ T cell enumeration is conducted at the Addington South African National Health Laboratory services (NHLS) one day after the blood draw via Beckman Coulter flow cytometry using panleucogated (PLG) methodology, the standard of care in this setting (Glencross et al., 2008; Myer et al., 2013). An extra 2 millilitres of blood was drawn from the same blood draw as for the routine CD4+ NHLS PLG/CD4 test into another EDTA tube for the comparison of the enumeration of CD4+ T cells using the Alere PIMA technology (Alere Health Care, Waltham, Massachusetts) versus the NHLS PLG/CD4-laboratory from January – July 2013. 3 PIMA analysers were used for this study. Originally the Alere PIMA analysers were to be placed at Lancers Road PHC clinic. However because the Overport PHC clinic merged with Lancers Road due to flooding and reconstruction , there was no extra room for us to place the PIMA analysers. They were therefore placed at the MRC Research laboratories where I performed all the CD4 testing using the PIMA cartridges. Furthermore the protocol did not allow for the provision of the PIMA POC CD4 test result to the client only the NHLS PLG/CD4 test result was used for clinical decision making.

2.6.2 Quality Control / Precision of Instruments

The use of daily quality control bead filled cartridges and routine PIMA analyser maintanance was followed as per manufacturer's guidelines.

The precision and reproducibility of CD4+ T cell counts of the 3 PIMA analysers were measured using stabilised whole blood Beckman Coulter quality control material (Immunotrol – IM - low and normal); PIMA reusable bead-filled cartridges with pre-defined counts (low and normal); patients' whole blood; and patient blood left overnight.

a. Beckman Coulter whole blood quality control material, IM low (62-206 cells/µl) and normal (394-754 cells/µl) was tested on each PIMA analyser for ten consecutive days.
b. PIMA low (115-235 cells/µl) and normal (719-1355 cells/µl) using re-usable bead cartridges as specified by the manufacturer was performed for the first ten measurements when a new cartridge was used as well as over a period of 165 days (23 January -25 March 2014).
c. The reproducibility of CD4+ T cell counts between the 3 PIMA analysers using whole blood (n=21) was also assessed.
d. Since the NHLS performs CD4+ T cell counts on the next day after blood draw due to transport and logistsics, whole blood samples were tested by PIMA analyser and the same sample was left overnight for testing again the next day on the same PIMA analyser. This allowed detection of any difference in CD4+ T cell counts done on the same day or kept overnight. A total of 30 samples were tested in this way.

2.6.3 Comparisons of CD4+ T Cell Enumeration in Whole Blood in 3 Different Instruments

Comparisons of CD4+ T cell enumeration was undertaken between flow cytometry instruments (PIMA analysers and the NHLS PLG/CD4 on 268 blood samples). Due to transport logistics the NHLS PLG/CD4 laboratory performs CD4+ T cell enumeration the day after the blood draw. Therefore a subset of 100 blood samples were tested by PIMA analyser; FACS calibur; and the NHLS PLG/CD4 in order to check if the differences observed between PIMA analyser versus NHLS PLG/CD4 was due to CD4+ T cell enumeration done on the next day on the NHLS PLG/CD4 test. CD4+ T cell enumeration was assessed on the same blood sample on the same day using the FACS calibur as well as the PIMA analyser.

2.6.4 Predictions of Benefit of PIMA CD4 Results for ARV Eligibility and Linkage to Care

Prediction of the potential benefits of the POC PIMA CD4 testing in terms of antiretroviral eligibility and decision making was undertaken. Additionally, an assessment was undertaken to determine whether HIV infected individuals return for their CD4+ T cell count result and how many are lost to follow up between ART eligibility and initiation.

2.6.5 Gene Xpert Testing in Two Laboratories

The comparison of routine testing for diagnosis of TB by the NHLS laboratory (Gene Xpert and smear microscopy) results with Gene Xpert and culture results done at the TB Biomedical Research laboratories was undertaken. We recruited 110 patients attending the Lancers Road PHC who were undergoing a screen for *M. tuberculosis* (TB). In these patients a second sputum sample was obtained at the same time as the sputum specimen that was sent for routine screening to NHLS, for smear microscopy and Gene Xpert. This second sputum specimen was sent to the MRC TB Biomedical laboratory for Gene Xpert testing and culture. These patients were only recruited for this part of the study and were not co-enrolled in the PIMA study.

2.7 Non-Laboratory Test Principles

2.7.1 The PIMA Test

The PIMA CD4 test consists of a disposable test cartridge, containing dried reagents, and the PIMA analyser. A low sample volume of approximately 25 microliter (µl) capillary or venous whole blood was collected into the test cartridge, which was then capped.

The PIMA test cartridge was then inserted into the PIMA analyser and the sample sealed within the cartridge and then processed. Once blood was applied to the PIMA cartridge, analysis was

performed within 15 minutes by insertion of the cartridge into the PIMA analyser. The detection window of the cartridge should not be touched prior to insertion of the catridge into the analyser. During the processing stage, data was recorded, analysed and interpreted using software embedded within the analyser.

The PIMA analyser is factory calibrated and did not require any further calibration and verification operations. However, a daily quality control check using PIMA Bead Standard was recommended by the manufacturer. The PIMA Bead Standard was an external control material for daily quality control (QC) on the PIMA analyser and controls instrument setup and performance, and data analysis. It comprised of 2 ready-to-use test PIMA cartridges which contain pre-determined CD4+ T cell counts. The bead standards used were normal PIMA beads with pre-determined CD4+ T cell range of 719 – 1355 cells/µl and low PIMA beads with a CD4+ T cell range of 115-235 cells/µl (www.alerepima.com).

Figure 2: The PIMA analyser

Credit: www.alere.com

2.7.2 The Beckman Coulter Flow Cytometer

The Beckman Coulter flow cytometer used IM cells which were positive process controls for flow cytometry made of stabilized human whole blood. They were assayed for lymphocyte, granulocyte and monocyte specific antigens and single platform absolute counts. Light scatter, population distribution, fluorescence intensity, and antigen density mimic those of whole blood. (Beckman Coulter 2014). The pre-determined CD4+ T cell ranges for IM were 394 - 754 cells/µl for the normal control and 62 – 206 cells/µl for the low control.

A cytosphere monocyte blocking agent was added to whole blood and incubated. A CD4 cytosphere reagent was then added to the mixture. A blood-latex spheres mixture was pipetted

and lysed. The lysed mixture was loaded onto the system. The diameter of the cytospheres for Common Differentiation antigen reagent is different from the cytosphere monocyte blocking reagent. Therefore CD4+ T cells rosetted with antibody-coated latex beads can be distinguished from the monocyte rosetted latex beads due to their large latex sphere size (Shapiro 2005).

Figure 3: Beckman Coulter XI-MCL and TQ Prep instruments for enumeration of CD4+ T cells

Credit: www.beckmancoulter.com (2014)

Instruction And Procedures

Routine CD4+ T cell enumeration was undertaken in the Addington NHLS laboratory by the NHLS personnel. We requested the standard operating procedure followed, from the Head of the laboratory (Mrs R. Naidoo) but was declined as this was confidential information although we did re-iterate that this would be acknowledged in the dissertation.We then asked for a package insert but again was told that they do not receive a package insert with their reagents as they receive stocks from the central NHLS laboratory in Johannesburg. With the respect to the equipment we have included the pictures (XI-MCL and TQ Prep) that were given to us by the Addigton laboratory of the Beckman Coulter instruments that were used. For these reasons no methodology of CD4+ Tcell enumeration for the Beckman Coulter can be included.

2.7.3 The FACS Calibur

Principle: Immunophenotyping involves the identification and enumeration of targeted cells in whole blood samples using immune cell markers expressed by the cell. Fluorochrome-labeled antibodies bind specifically to the leukocytes of the whole blood. The erythrocytes were

lysed prior to flow cytometry analysis on a FACS Calibur flow cytometer (Becton Dickenson Immunocytometry Systems, San Jose, CA).

When whole blood was added to the reagent, flourochrome-labelled antibodies in the reagent bound specifically to leucocyte surface antigens. During acquisition, the cells travel past the laser beam and scatter the laser light causing the stained cells to fluoresce. These scatter and fluorescence signals, detected by the instrument, provided information about the cell's size, internal complexity and relative fluorescence intensity.
MultiTEST reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate (Becton Dickinson 2014).

Figure 4: The FACS Calibur

Credit: www.bdbiosciences.com

Instruction And Procedures

The one hundred samples that were tested on the FACS Calibur were tested as part of the routine sample testing done by the staff of the Durban MRC laboratory.

Safety Issues: All blood specimens, reagents and controls were considered biohazardous and infectious. When working within the biosafety cabinets, a Howie lab coat including double gloves, were worn. When using the FACS calibur a lab coat, gloves and eye goggles were

worn when loading samples. The antibody reagents contain sodium azide as a preservative. The Lysing Solution contains diethylene glycol and formaldehyde.

Sample preparation: The BD MultiTEST™ CD3 fluorescein isothiocyanate (FITC)/ CD8 phycoerythrin (PE)/CD45 peridinin chlorophyll protein (PerCP)/CD4 allophycocyanin (APC) is a four-color direct immunofluorescene reagent used with the TruCOUNT Tubes which contains a freeze-dried pellet of fluorescent beads. This kit was stored at 4°C.

- The antibody and the required number of TruCOUNT tubes were removed from the fridge and allowed to stand for a few minutes at room temperature.
- A 1:10 dilution of working FACS Lyse solution was prepared (Appendix 2).
- The TruCOUNT tube was labeled with the sample identification number and the date.
- The antibody was vortexed for ~5 seconds and 20µL was pipetted into each of the TruCOUNT tube. The tube was gently tapped to allow the antibody to mix with the bead pellet.
- The whole blood was then vortexed and 50µL was pipetted into the respectively labelled TruCOUNT tube. The TruCOUNT tubes were capped, vortexed for ~3 seconds. The tubes were then covered with tin foil (to allow for the incubation for 15 min at room temperature in the dark).
- On completion of the incubation, 450µL of the working FACS Lyse Solution was added to each tube. The tubes were capped and vortexed for ~3 seconds. The tubes were then covered with tin foil for 15 min at room temperature (to allow for the Red blood cells to lyse).
- The remaining blood was kept in the BSC until the CD4 assay had been completed. On completion of the analysis, the samples were discarded appropriately.

Internal quality control: The IMMUNO-TROL™ cells from Beckman Coulter are an assayed, lysable whole blood quality control sample that was processed with every batch of test samples. The IMMUNO-TROL™ cells provided optimization of the instrument settings and performance.

Calibration of Equipment: The CaliBRITE^{TM} Beads from Becton Dickenson was used to calibrate the FACS Calibur flow cytometer. Calibration allowed the photomultiplier tube (PMT) voltages and the fluorescence compensation to be set and also checked the sensitivity of the instrument.

Analysis of samples: The samples were analyzed on the FACS Calibur flow cytometer with the MultiTEST SOFTWARE (version1.1.2). Manual gating of each sample was performed if necessary. A printout out for each of the test sample and control sample was generated.

Cleaning and shutdown of the FACS Calibur Flow Cytometer: Once all the samples had been analysed, the flow cytometer automatically goes into a cleaning program. On completion of the cleaning cycle, the flow cytometer is depressurized and a worklist summary report was printed and filed. The analyzed samples were removed from the flow cytometer, capped and discarded into the biohazardous waste disposal buckets. The tube with distilled water was replaced so that the electrode was always kept wet.

2.7.4 Gene Xpert as Performed at NHLS and MRC TB Biomedical Research Laboratories

The figure below depicts the steps using the automated Gene Xpert MTB/RIF protocol and the Gene Xpert instrument

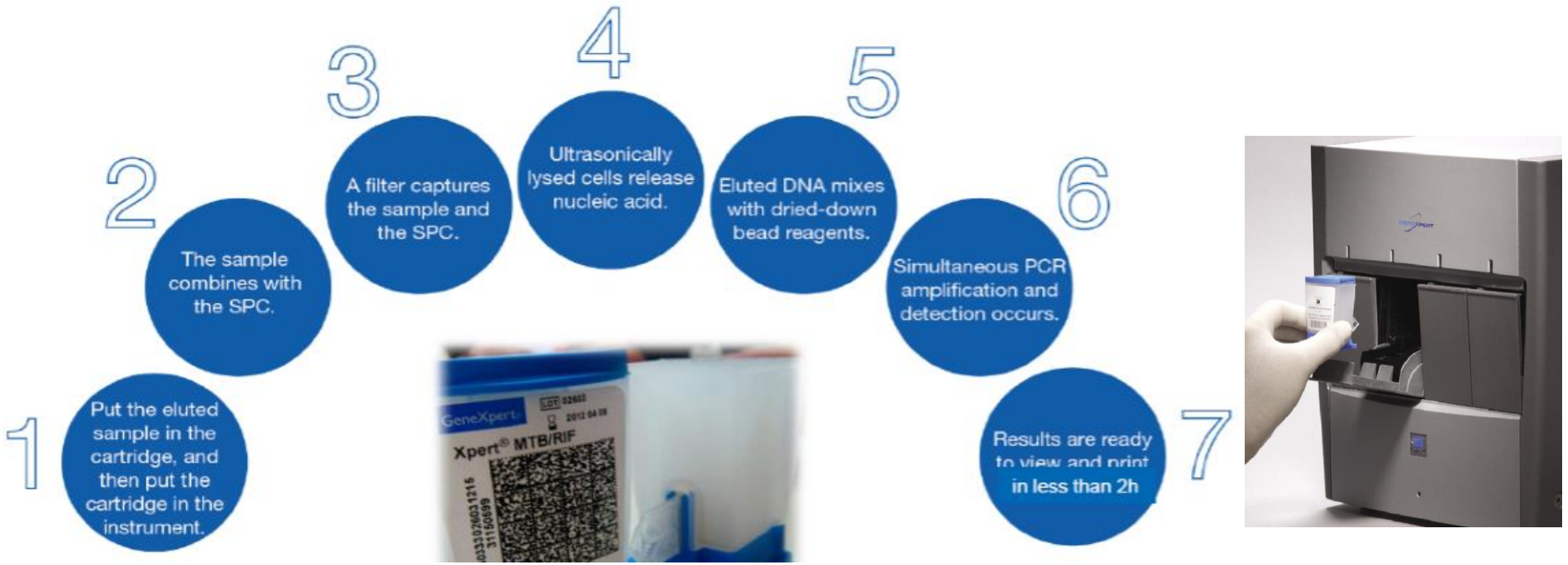

Figure 5: Gene Xpert instrument

Credit: Cepheid 2010

2.7.5 Smear Microscopy at the NHLS Laboratory

Acid fast bacilli are Gram positive bacilli but stain very poorly and are seldom seen in a Gram stain. This is due to the lipid content of the cell wall. Phenol crystals present in the Carbol fushin, stain dissolves the lipid sufficiently to allow the stain to penetrate, forming compound in the cell wall that is resistant to decolourising by acid/ alcohol.

Such organisms are termed Acid fast bacilli. A counter stain stains the background material that is not acid fast – thus highlighting the any acid fast bacilli.

The Ziehl Neelson stains are used routinely to stain all new specimens or to stain slides made from cultures and/or MGIT positive tubes. New smear stained by the Auramine fluorescent stain may thereafter be counter stained with ZN stain to confirm the presence of acid fast bacilli.

Specimens

The Ziehl Neelson stain was done on all smears made from specimens processed. Ziehl Neelson stain is also done on positive MGIT cultures and 7H11 cultures to confirm the presence of acid fast bacilli.

Quality Control slides were performed with each batch using a known MTB strain (H37RV) as a positive control, and Escherichia coli (E.coli), a Gram negative bacilli, as a negative control together with a reagent control. Controls were processed in the same way as a specimen, and were done on a daily basis and when new reagents were opened.

Instructions And Procedures

Ziehl Neelson Staining Method is a carbol fuchsin acid-fast stain.

1. MGIT slides ready for staining were removed from the biosafety cabinet and placed in a slide rack or a slide box in the order they were found on the slide warmer.
2. The slide rack or box was placed on a trolley and wheeled to the main lab where the slides were stained.
3. Slides previously stained with Auramine stain were transferred from the dark room to the staining bench in either a staining rack or slide box on a trolley.
4. Slides with flooded with filtered Carbol Fuchsin (0.3%) and left for 5 minutes.
5. Using a staining rod wrapped with non-absorbent cotton wool around an end, it was dipped in water to prevent soot being deposited on the underside of the slide.
6. Cotton wool was dipped in 70% alcohol, ignited and heat wa applied to the under surface of each slide.

7. Slides were heated until steam rose from the carbol fuchsin.
8. Slides were rinsed with distilled water, excess water was tipped off and decolorized with 3% acid alcohol for 2 minutes. Slides were rinsed again with distilled water and excess water tipped off.
9. The slides were counter stained with methylene blue for 1 – 2 minutes, thereafter rinsed with distilled water.
10. Stained slides were placed in a slide rack, and allowed to air dry in the hot room.
11. Slides were examined under oil immersion at 100 x.

Interpretation & Expected Results

Acid Fast Bacilli appear as red rods. The background appears blue.

The WHO/International Union Against Tuberculosis and Lung Disease (IUATLD) and national guidelines were followed as tabled below, for grading and reporting.

It was recommended that a minimum of 150 fields and a maximum of 300 fields be observed when reading Ziehl Neelson slides.

Quality Control expected results:

Positive Control (H37RV) – Must be positive + or greater

Negative Control (Escherichia coli) – Must be negative – no Acid fast bacilli present

Reagent Control – must be negative – no AFB seen

GRADING	REPORT
<2 bacilli per 150 fields	Not detected
2 – 9 AFB / 100 fields	Pos Scanty. Report actual number of AFB
10 – 100 AFB / 100 field	Pos +
1 – 10 / oil immersion field (50 fields)	Pos ++
>10 / oil immersion field (20 fields)	Pos +++

2.7.6. Culture as performed at the MRC TB Biomedical Research laboratory

Mycobacterium Tuberculosis (MTB) infection is confirmed by the isolation of tuberculosis from selective media containing antibiotic to prevent the overgrowth of contaminating organisms. Decontaminated sputa samples were inoculated into MGIT tubes which are incubated for 3 – 8 weeks allowing for the tuberculosis organism to grow.

2.8 STATISTICAL ANALYSIS

Statistical Methods

The comparison of the mean CD4+ T cell count between PIMA analysers 1, 2 and 3 was assessed using ANOVA at low and normal lM respectively. The pairwise comparison of the PIMA analyser was conducted using unpaired t-tests. The percentage co-efficient of variation (%CV) was computed for each PIMA analyser at low and normal IM.

To assess the precision of the control cartridge at low and normal beads within each of the 3 PIMA analysers, the %CV was calculated for the 10 observations at low and normal beads.

Lin's concordance correlation coefficient was used to measure the correlation between observations on PIMA analysers 1 versus 2; 1 versus 3 and 2 versus 3. The reproducibility of each of the PIMA analysers on whole blood was assessed for 21 samples. Individual sample %CV and SD was computed.

To assess the agreement between blood samples run on the same day and the same blood samples left overnight, the mean bias was computed and results were presented graphically.

The percentage similarity model, Bland-Altman (BA) plots, limits of agreement (LOA) and Lin's concordance correlation coefficient were used to assess agreement between PIMA analyser and NHLS PLG/CD4 in 268 samples.

In a subset of 100 samples the same methodology as in the above paragraph was applied to assess the agreement between FACS Calibur versus NHLS PLG/CD4, PIMA versus NHLS PLG/CD4 and PIMA versus FACS Calibur instruments.

To assess the diagnostic accuracy of CD4+ T cell counts by PIMA analysers in identifying whether ART is required; sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was computed for the ART initiation cut offs of ≤200 cells/µl; ≤350 cells/µl and ≤500 cells/µl CD4+ T cells.

Standard measures of the efficacy of a diagnostic test were used to assess the Gene Xpert test: sensitivity, specificity, negative and positive predictive values using culture as the gold standard. A similar analysis was conducted looking at diagnosis of resistant TB. Percentage

agreement was assessed between the Gene Xpert test, smear microscopy and culture. Data were analysed using Stata V12 (StataCorp, 2011)

CHAPTER 3 - RESULTS

3.1 Patient characteristics of HIV positive individuals undergoing CD4+ T cell count enumeration

The majority (218/268) of HIV-positive individuals undergoing CD4+ T cell count testing were women in whom 28% were 25-29 years whereas the majority of males (36%) were in an older age category, 30-34 years (Table 2). There was no significant differences in the median CD4+ T cell count in males versus females performed by the NHLS PLG/CD4 versus the PIMA analyser POC although the median CD4+ T cell count was higher in the latter. Overall according to NHLS PLG/CD4 versus PIMA POC 61% versus 54% of HIV-positive individuals were eligible for ART initiation of whom 68% versus 62% were males and 60% versus 52% were females respectively.

Table 2: Patient characteristics of HIV positive individuals undergoing CD4+ T cell count enumeration

	Female n=218	Male n=50	Total n=268
Median age (IQR), years	32 (26-37)	33 (30-40)	32 (27 – 38)
18-24	39 (17.9%)	4 (8%)	43 (16.0%)
25-29	54 (24.77%)	8 (16%)	62 (23.1%)
30-34	46 (21.1%)	18 (36%)	64 (23.9%)
35-39	44 (20.2%)	7 (14%)	51 (19.0%)
>40	35 (16.1%)	13 (26%)	48 (17.9%)
Median (IQR) NHLS CD4 count cells/µl	292 (184 – 453)	254 (151 - 387)	286 (176.5 - 444.5)
Number (%) NHLS≤350 cells/µl	130 (60%)	34 (68%)	164 (61.19 %)
Median (IQR) PIMA CD4 count cells/µl	327.5 (204-451)	307.5 (179 - 419)	321.5 (204 - 449)
Number (%) PIMA≤350 cells/µl	114 (52.3%)	31 (62%)	145 (54.10%)

3.2 Comparison of the performance of the 3 PIMA analysers with whole blood quality control material (IM low and IM normal) over 10 consecutive days

We analysed commercially available stabilized product (IM) where the normal and low range as per manufacturers was 394-754/62-206 cells/µl respectively, over a period of 10 consecutive days on both the panleucogating (PLG)-CD4 Beckman Coulter instrument and the 3 PIMA analysers (Figure 6, Table 3). No significant differences were observed between the 3 PIMA analysers at normal IM (p value 0.2664) but differed significantly between NHLS PLG/CD4 and PIMA 2 and 3 at low IM (p-value 0.01 and 0.03 respectively), (Table 5).

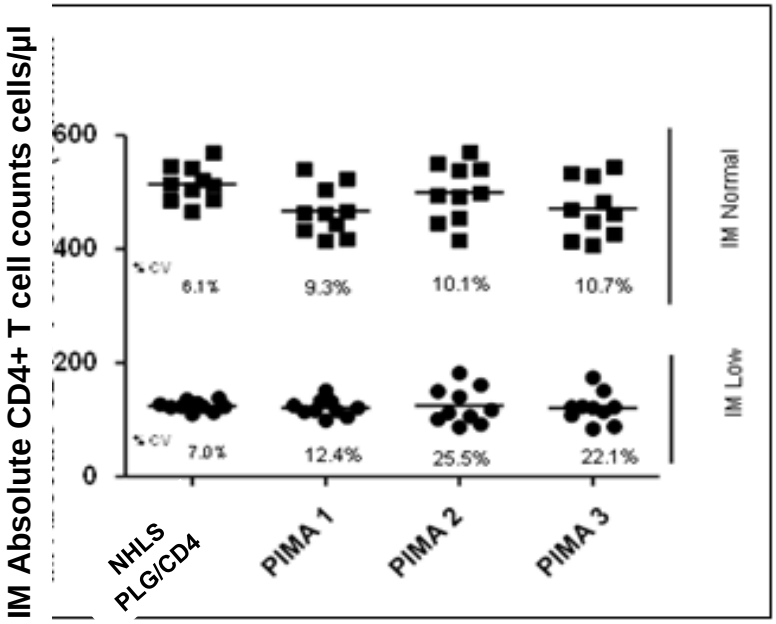

Figure 6: Reproducibility of PIMA analysers stabilized whole lood quality control material - normal and low Immunotrol (IM) (with % CV < 6% in both as per NHLS-Addington Laboratory personal communication

Table 3: Comparison of the performance of the 3 PIMA analysers with whole blood quality control material (IM low and IM normal) over 10 consecutive days

	LOW IM			NORMAL IM		
	Mean	**SD**	**% CV**	**Mean**	**SD**	**% CV**
Predicate	124.3	8.65	6.96	514.4	31.36	6.10
PIMA 1	121.2	14.98	12.36	466.5	43.35	9.29
PIMA 2	124.8	31.79	25.48	499.6	50.46	10.10
PIMA 3	120.7	26.65	22.08	471.00	50.32	10.68

3.3 Comparison of the performance of the 3 PIMA analysers and the agreement of control observations at low and normal beads over 10 consecutive days

Precision testing of the control bead-filled cartridge with pre-defined counts i.e. low (115-235 cells/µl) and normal (719-1355 cells/µl) beads within the 3 PIMA instruments was performed for each instrument over a replicate set of 10 bead analyses.

At low and normal beads the % CV of observations for each PIMA analyser were less than <5% which is within the manufacturer's specification. There was high reproducibility at normal beads with % CVs for PIMA analysers 1, 2 and 3 were 0.86%, 1.36% and 0.96% respectively (Table 4). As expected, higher CVs were observed at low beads. The %CVs for PIMA analysers 1, 2 and 3 were 2.13%, 1.28% and 1.41% respectively.

Table 4 : Quality control of bead-filled cartridges with pre-defined counts on each instrument over a replicate set of 10 bead analyses

	Low beads (115-235 cells/µl)			**Normal beads** (719-1355 cells/µl)		
	Mean cells/µl	**SD**	**%CV**	**Mean cells/µl**	**SD**	**%CV**
PIMA 1	187.2	3.99	2.13%	1075.2	9.29	0.86%
PIMA 2	187.2	2.39	1.28%	1118.7	15.24	1.36%
PIMA 3	179.8	2.53	1.41%	1101	10.58	0.96%

3.4 Comparison of the performance of the 3 PIMA analysers and agreement with control observations (normal and low beads) over a period of 165 days

There was high reproducibility at normal beads with %CVs for PIMA analyser 1, 2 and 3 at 1.14%, 1.67% and 1.30% respectively over a period of 165 days (Table 5). As expected, higher %CVs were observed at low beads with %CVs for PIMA analyser 1, 2 and 3 at 1.75%, 1.70% and 1.86% respectively. According to manufacturers' specifications %CVs should be below 5%.

Table 5: Quality control of bead-filled cartridges with predefined counts for each instrument over a replicate set of 165 days of bead analyses

	Low Beads (115-235 cells/µl)			**Normal Beads** (719-1355 cells/µl)		
	Mean cells/µl	**SD**	**%CV**	**Mean cells/µl**	**SD**	**%CV**
PIMA 1	186.16	3.25	1.75%	1074.97	12.28	1.14%
PIMA 2	187.59	3.19	1.70%	1123	18.86	1.67%
PIMA 3	182.31	3.40	1.86%	1117.39	14.50	1.30%

3.5 Reproducibility of the 3 PIMA analysers using fresh whole blood samples (n=21)

Figure 7 represents CD4+ T cell counts on each of 21 whole blood samples as determined by each of the 3 PIMA analysers. The within-sample variability, assessed through computation of the % CV, is presented in Table 8. The mean % CV was 15.4 % with range 1%-31%. Strong correlation was observed between the 3 PIMA analysers. In pairwise comparisons, no significant difference was observed between PIMA analyser 1 and 2, 2 and 3 and 1 and 3 (p-value 0.32, 0.43 and 0.61 respectively). High correlation was observed between PIMA analyser 1 and 2, 2 and 3 and 1 and 3 : R = 0.95, 0.96 and 0.97 respectively (data not shown).

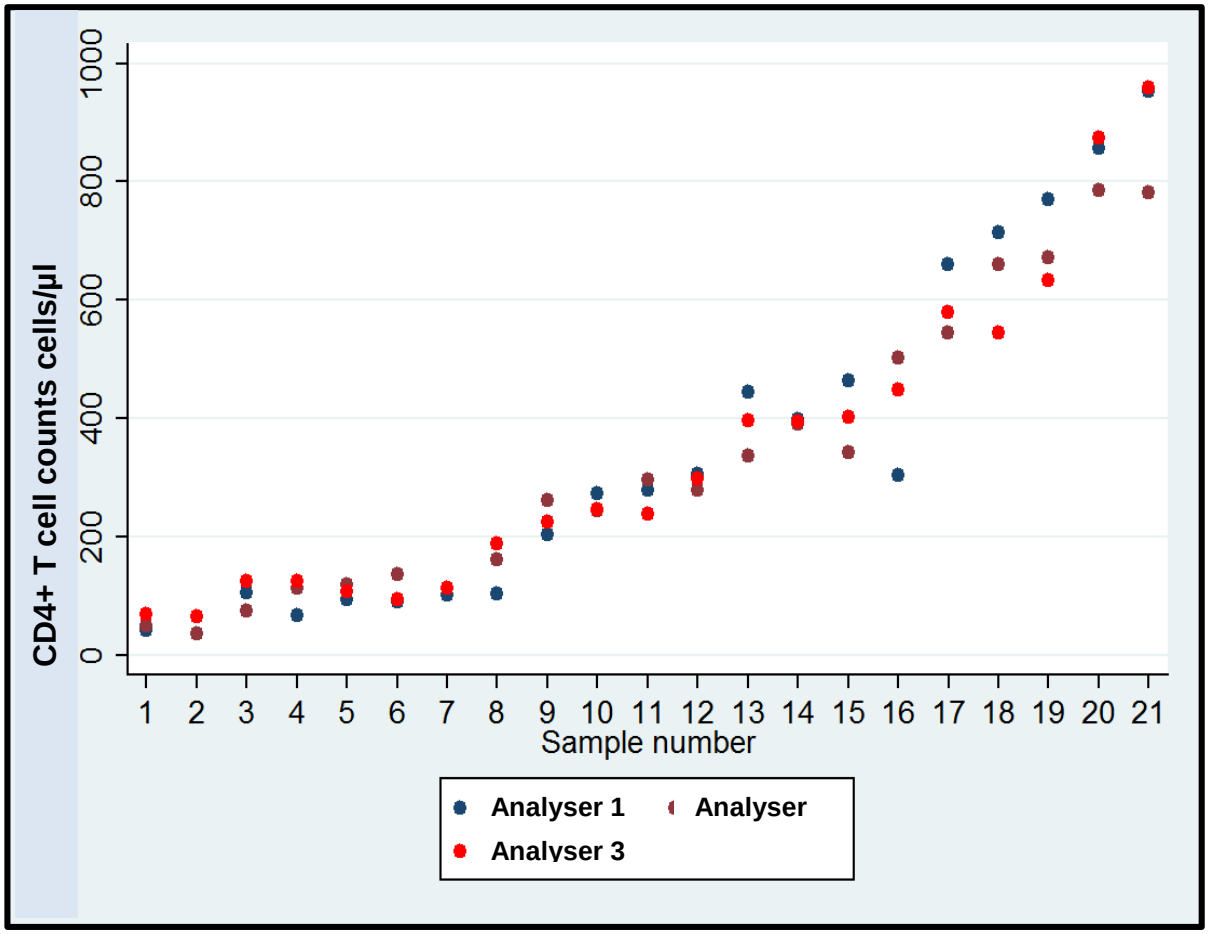

Figure 7: Reproducibility of CD4+ T cell counts between the 3 PIMA analysers using whole blood (n=21)

Table 6: Raw data of the reproducibility of CD4+ T cell counts between the 3 PIMA analysers using whole blood (n=21)

Sample Number	PIMA 1	PIMA 2	PIMA 3	SD	%CV
1	42	49	69	14.01	26
2	64	36	65	16.46	30
3	66	114	124	31.01	31
4	90	137	93	26.31	25
5	94	118	107	12.01	11
6	101	113	113	6.93	6
7	104	162	188	43.00	28
8	106	74	124	25.32	25
9	204	262	225	29.37	13
10	273	245	247	15.62	6
11	279	297	239	29.69	11
12	303	503	448	103.32	25
13	305	278	298	14.01	5
14	398	391	394	3.51	1
15	444	336	397	54.15	14
16	463	342	403	60.50	15
17	661	545	579	59.63	10
18	715	661	545	86.86	14
19	770	672	634	70.17	10
20	856	785	875	47.44	6
21	953	782	959	100.50	11
Mean	**347**	**329**	**339**	**40.38**	**15.4**

3.6 Reproducibility of overnight testing of same blood samples. (n=30)

Samples kept overnight tended to have slightly higher values than those analysed on the same day using the PIMA POC technology. The mean bias was 16.900 (CI -0.978 to 34.778), the correlation between day1 and day 2 results was 0.978. (Figure 8, 9). There was no significant difference in CD4+ T cell counts on the same blood sample between day 1 and 2 (Paired t-test p=0.063).

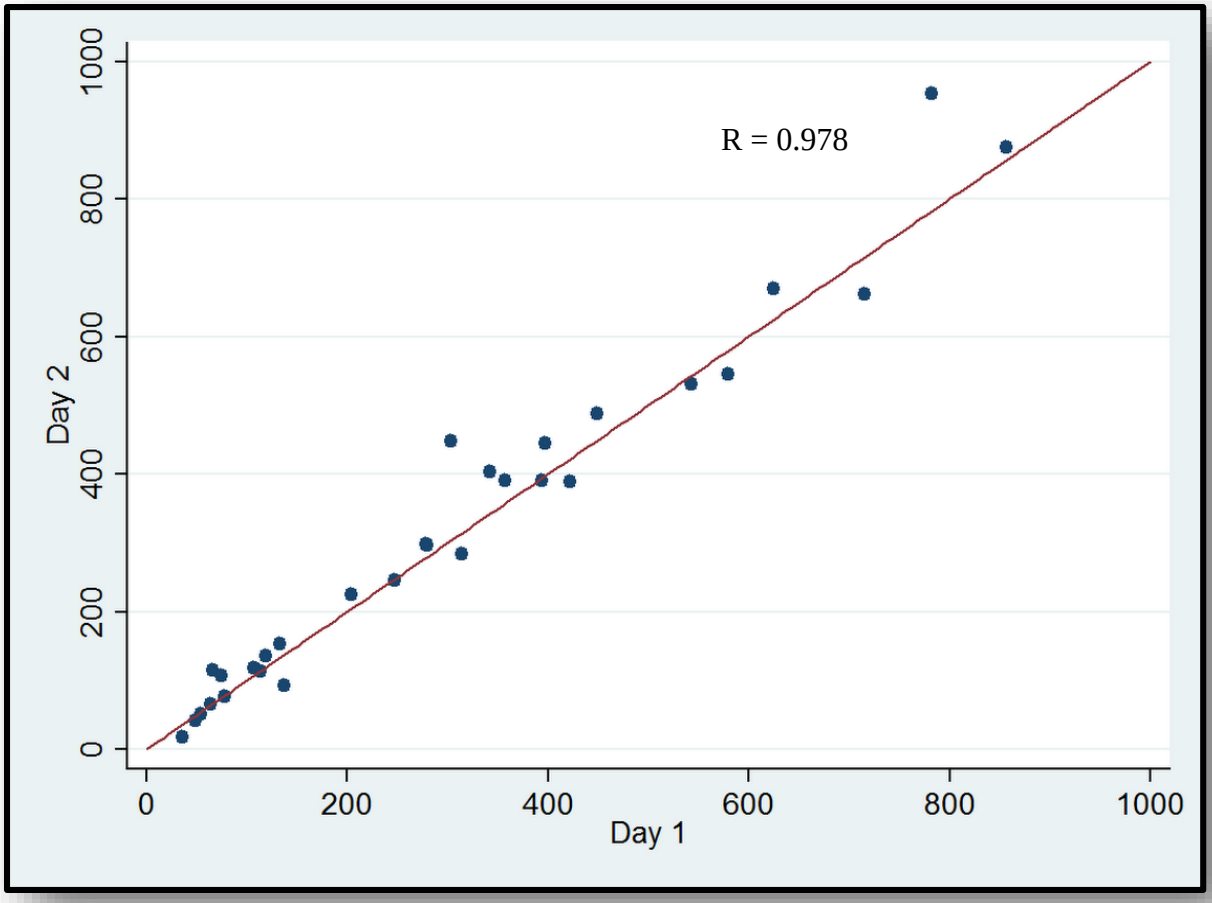

Figure 8: Scatter plot of the same blood samples (n=30) tested on day 1 and day 2

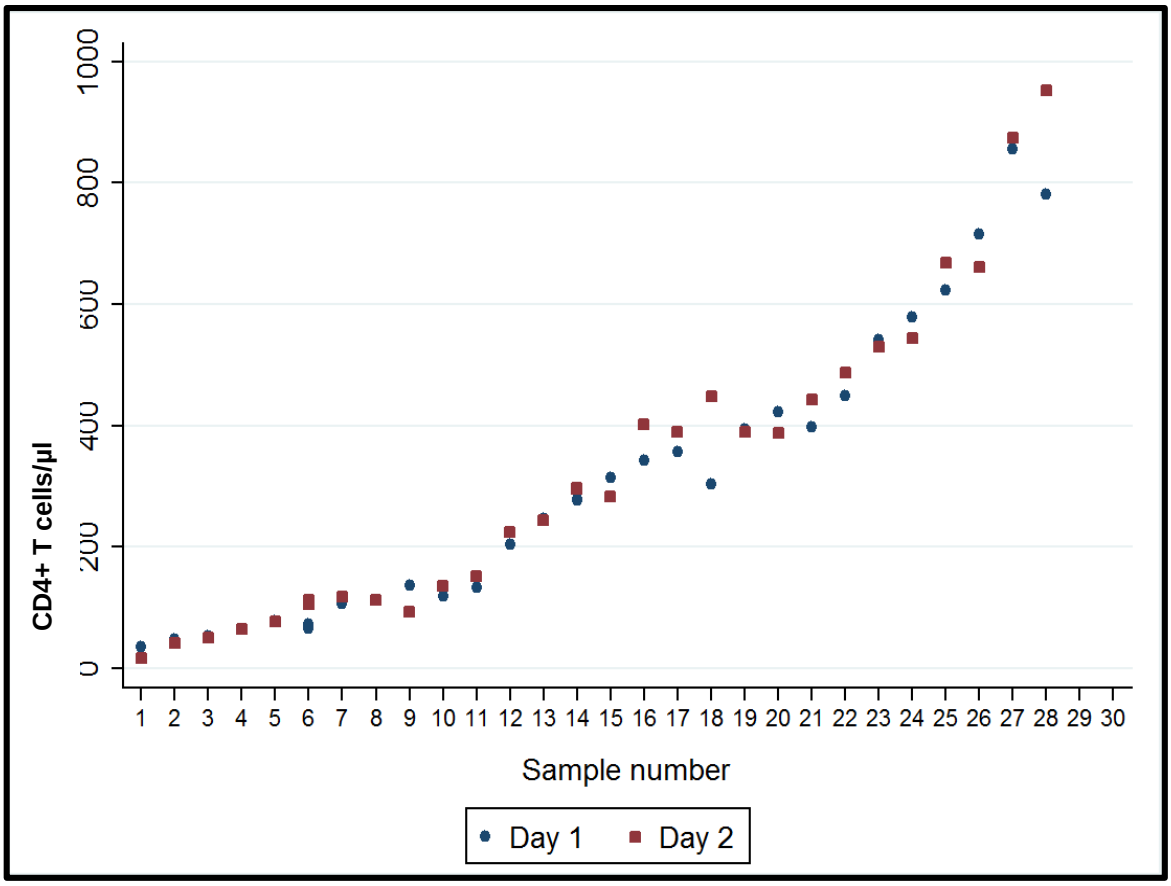

Figure 9: CD4+ T cell counts performed on the PIMA analyser on day 1 and day 2

3.7 Comparison between the PIMA analyser, NHLS PLG/CD4 , and FACS Calibur in a subset of blood samples (n=100)

The 100 patients were randomly selected from the cohort of 268 patients and had similar characteristics to the group as a whole and therefore separate baseline characteristics are not presented. The median (range) CD4+ T cell counts for the PIMA analysers, NHLS PLG/CD4 and FACS calibur was 323 (3-1151) cells/µl, 306 (5-1476) cells/µl and 293 (3-1261) cells/µl respectively (data not shown). Higher limits of agreement was observed between PIMA analysers and FACS calibur as evidenced by smaller mean bias of 7.52 and narrower Bland Altman limits of agreement -111 to 126 and a correlation of 0.97 (Table 7, Figure 10). Wider Bland Altman limits of agreement (-216 to 176 with mean bias -20.3) was observed between

the FACS calibur vs NHLS PLG/CD4 with a correlation of 0.92 (Table 7, Figure 11) compared to PIMA analysers vs NHLS PLG/CD4 (Bland Altman limits of agreement -226 to 200 mean bias -12.78) (Table 7) with a correlation of 0.90 (Table 7, Figure 12). For all 3 comparisons (PIMA analysers vs NHLS PLG/CD4, PIMA analyser vs FACS Calibur, and FACS Calibur vs NHLS PLG/CD4) a correlation greater than 0.90 was observed.

Table 7: Comparisons of PIMA analysers vs NHLS PLG/CD4 vs FACS Calibur (n=100)

	PIMA analysers – NHLS PLG/CD4	**PIMA analysers - FACS calibur**	**FACS calibur – NHLS PLG/CD4**
	n=100	n=100	n=100
Mean Bias ± 1 SD	-12.78 ± 106.63	7.52 ± 59.26	-20.3 ± 97.97
95% CI Bias	-33.94 to 8.38	-4.24 to 19.28	-39.74 to -0.86
BA 95% LOA	-226.04 to 200.48	-111.01 to 126.05	-216.23 to 175.63
% Similarity to predicate	101.3 ± 15	103.1 ± 12.7	98.7 ± 11.9

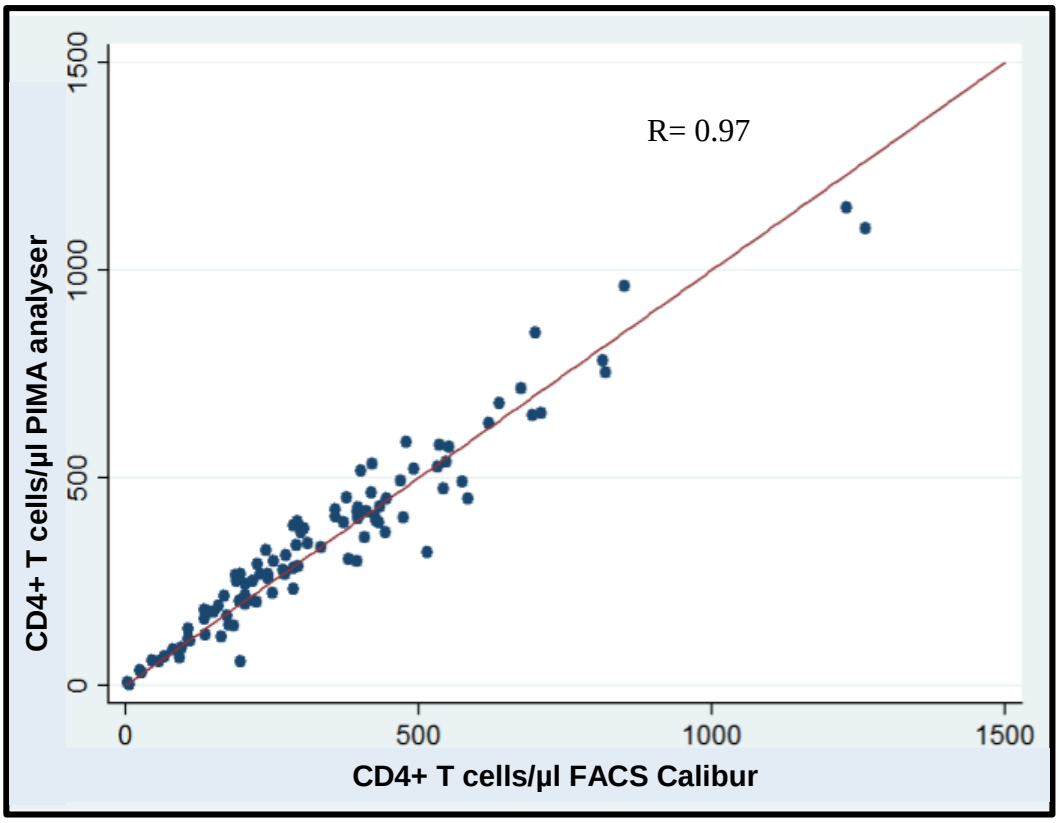

Figure 10: Correlation of CD4+ T cell counts on the PIMA analyser as compared to the FACS Calibur

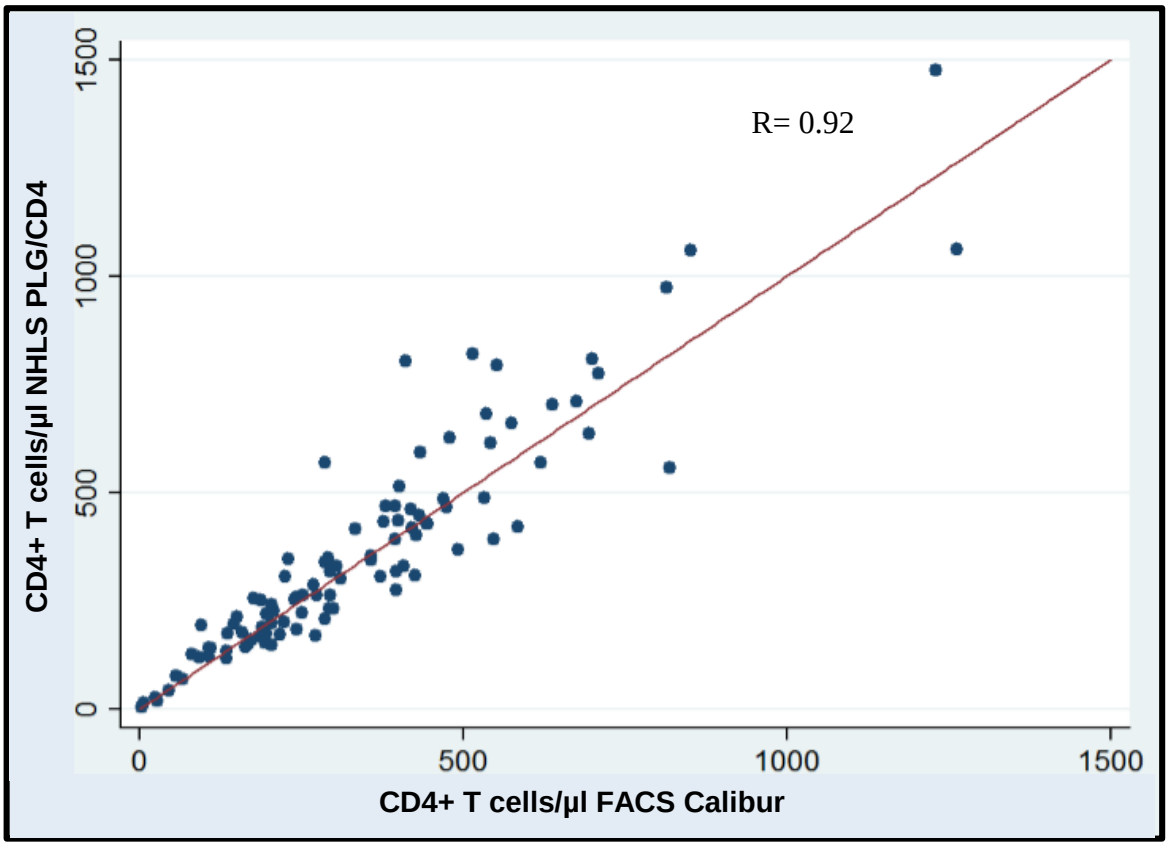

Figure 11: Correlation of CD4+ T cell counts on the NHLS PLG/CD4 as compared to the FACS Calibur

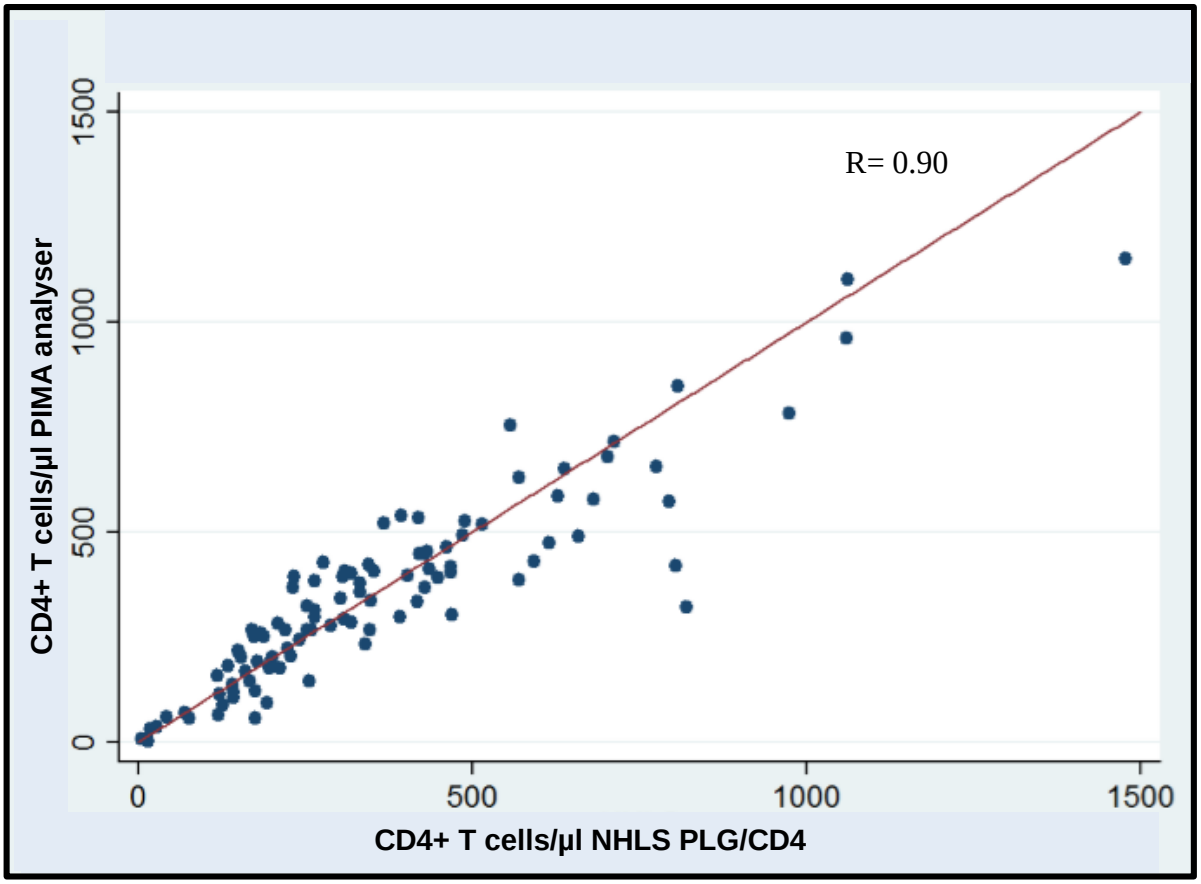

Figure 12: Correlation of CD4+ T cell counts on the PIMA analyser as compared to the NHLS PLG/CD4

3.8 Assessment of the correlation of the CD4+ T cell counts obtained between the PIMA analysers compared to NHLS PLG/CD4 (n= 268)

An overall correlation of 0.91 in CD4+ T cell counts between the PIMA analysers and NHLS PLG/CD4 was observed (Figures 13, 14). The number of samples tested on each PIMA analyser were as follows: PIMA 1 n=98; PIMA 2 n=97 and PIMA 3 n=73. The overall mean difference of PIMA analyser – NHLS PLG/CD4 was 17.5 cells/µl (95% CI 6.2 to 28.8) (Table 8). The %SIM Mean ± SD was 106 ± 15.5 indicative of acceptable agreement between the 2 tests. When categorised by the following CD4+ T cell counts of: ≤350 cells/µl; 351-500 cells/µl; ≤500 cells/µl and > 500 cells/µl, the mean difference of PIMA analysers – NHLS PLG/CD4 was 33 cells/µl (95% CI 23 to 42); 22 cells/µl (95% CI -3.5 to 47); 30 cells/µl (95%CI 21 to 39); and 36 cells/µl (95% CI -78 to 6.1) respectively. Acceptable mean percentage similarity in the range of 90 to 110 percent, with %SIM CVs<15% was observed at all CD4+ T cell count ranges.

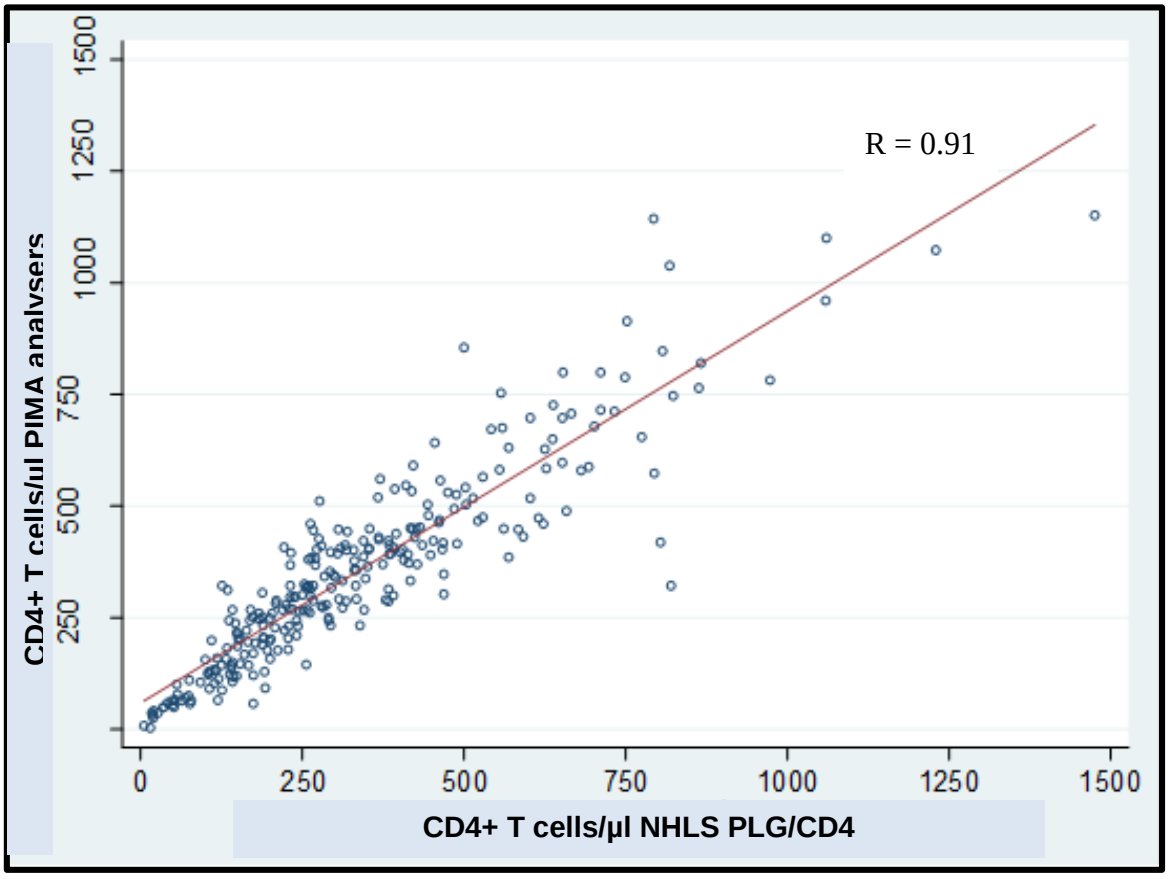

Figure 13: Correlation of CD4+ T cell counts on the PIMA analyser as compared to NHLS PLG/CD4 (n=268)

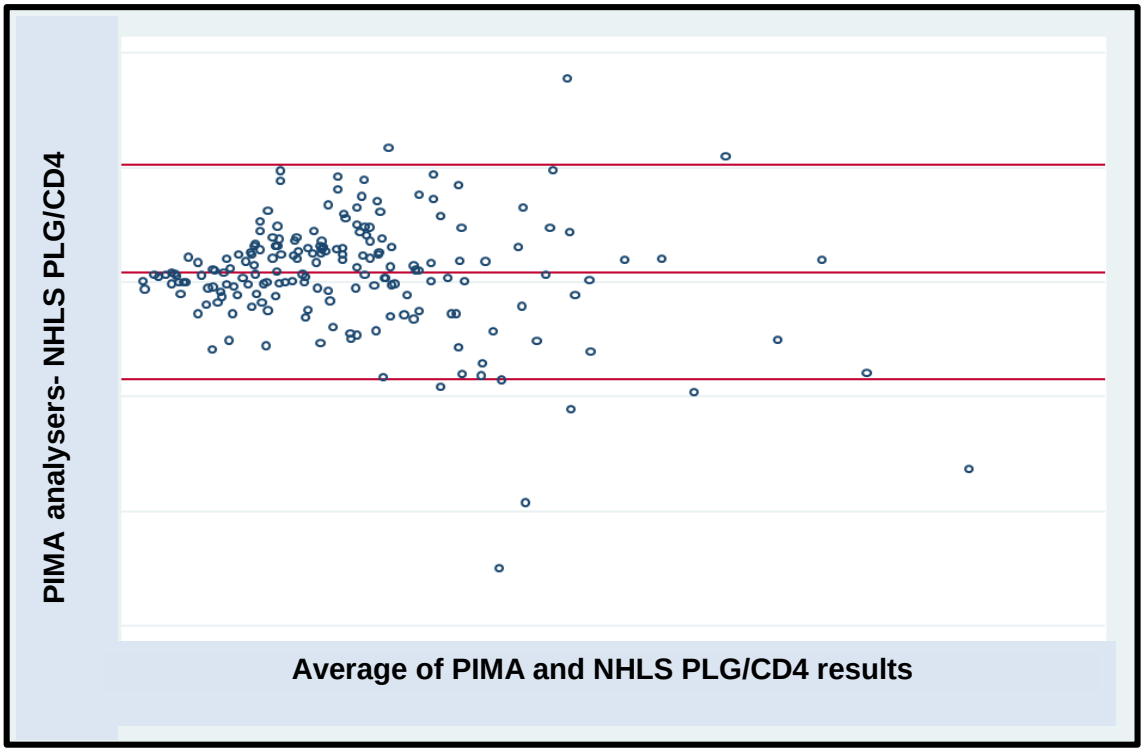

Figure 14: Bland-Altman comparison of PIMA analyser vs NHLS PLG/CD4

Table 8: Comparison of PIMA analysers vs NHLS PLG/CD4 as categorized by the following CD4+ T cell counts of ≤ 350 cells/µl, 351-500 cells/µl, ≤500 cells/µl and > 500 cells/µl

	≤350 cells/µl (n=164)	351-500 cells/µl (n=53)	≤500 cells/µl (n=217)	>500 cells/µl (n=51)	All CD4+ T cell counts (n=268)
Median bias	21	13	21	-23	18
BA Bias (± 1 SD)	32.9 ± 61.0	21.5 ± 90.8	30.1 ± 69.4	-36.1±150.04	17.5 ±93.8
95% CI Bias	23.4 to 42.3	-3.5 to 46.6	20.8 to 39.4	-78.3 to 6.1	6.2 to 28.8
BA 95% LOA	-89.2 to 154.9	-160.0 to 203.0	-108.7 to 168.9	-336.2 to 263.9	-170.0 to 205.0
% Similarity to NHLS PLG (% SIM Mean ± SD)	107.4 ± 15.2	102.7 ± 10.5	106.2 ± 14.35	97.97 ± 10	106 ± 15.5
%SIM CV	14.2%	10.2%	13.5%	10.2%	14.6%

3.9 Misclassification and eligibility for ART based on PIMA vs NHLS PLG/CD4 results

Under the previous treatment guideline threshold of ≤200 cells/µl CD4+ T cells, the PIMA analysers displayed a sensitivity of 73,5% and specificity of 98.4% in predicting predicate values ≤200 cells/µl (Table 9). Under the current South African guidelines of ≤350 cells/µl CD4+ T cells, the sensitivity of the PIMA analyser was higher (83.5%) and specificity a bit lower at 92%. At this threshold of ≤ 350 cells/µl there were 35 (13%) misclassifications, of which 27 were false negatives (FN). This implies that 27 patients would have been falsely deemed ineligible for ART according to the PIMA analysers. The mean difference between PIMA analyser and NHLS PLG/CD4 in this group of 27 patients was 112 cells/µl. The positive predicative value (PPV) at both these cuts off was high at 95% such that 95% of the patients eligible for treatment according to PIMA analysers would have also been deemed eligible for treatment on the NHLS PLG/CD4 test. Of the patients with CD4 + T cell count >350 cells/µl according to the PIMA analysers, 78% also had a NHLS PLG/CD4 CD4+ T cell count in the same range. Under future South African treatment guidelines at CD4+ T cell counts ≤500 cells/µl, a high sensitivity of 94% was observed at the sacrifice of lower specificity of 78%. In the 13 false negatives for the ≤500 cells/µl cut off, the mean bias observed was 149 CD4+ T cells/µl. Under all 3 guidelines, the overall performance of PIMA analysers as a diagnostic tool for treatment initiation was favourable, evidenced by the high proportion of patients correctly classified as eligible for treatment (91%, 87% and 91% respectively).

Table 9: Performance of PIMA analysers compared to NHLS PLG/CD4 at different CD4+ T cell cut offs

CD4+ T cells/µl	Sensi-tivity	Speci-ficity	% Mis-classified	% Correctly Classified	FP Rate	FN Rate	NPV	PPV
≤200	73.5%	98.4%	25 (9.3%)	90.7	3/25	22/25	85.2	95.3
≤350	83.5%	92.3%	35 (13%)	87	8/35	27/35	78.1%	94.5%
≤500	94%	78.4%	24 (9%)	91	11/24	13/24	75.5%	94.9%

3.10 Assessment of whether ART eligible patients (according to NHLS PLG/CD4 results) return for their CD4 result and whether they are initiated on ART

According to the NHLS PLG/CD4 laboratory CD4 test result, 164/268 (61%) of patients were eligible for ART (CD4+ T cell count ≤350 cells/µl) on the day of HCT compared to 145/268 (54%) with the PIMA analyser POC CD4+ T cell test, who should have been initiated on ART (Figure 15). The majority of patients (87%) returned to the Lancers Road PHC for their CD4 test result. However of those eligible for ART according to the ART register at Lancers Road PHC, 110/164 (only 67%) of these patients were initiated on ART. Of those who did not return 35/268 (13%) to the PHC clinic for their CD4 test result, 20/35 (57%) were eligible for ART according to the NHLS PLG/CD4 laboratory CD4 test result, all of whom were not initiated on ART. The median time to ART initiation was 25 days (IQR 9.5 to 48.5 days)

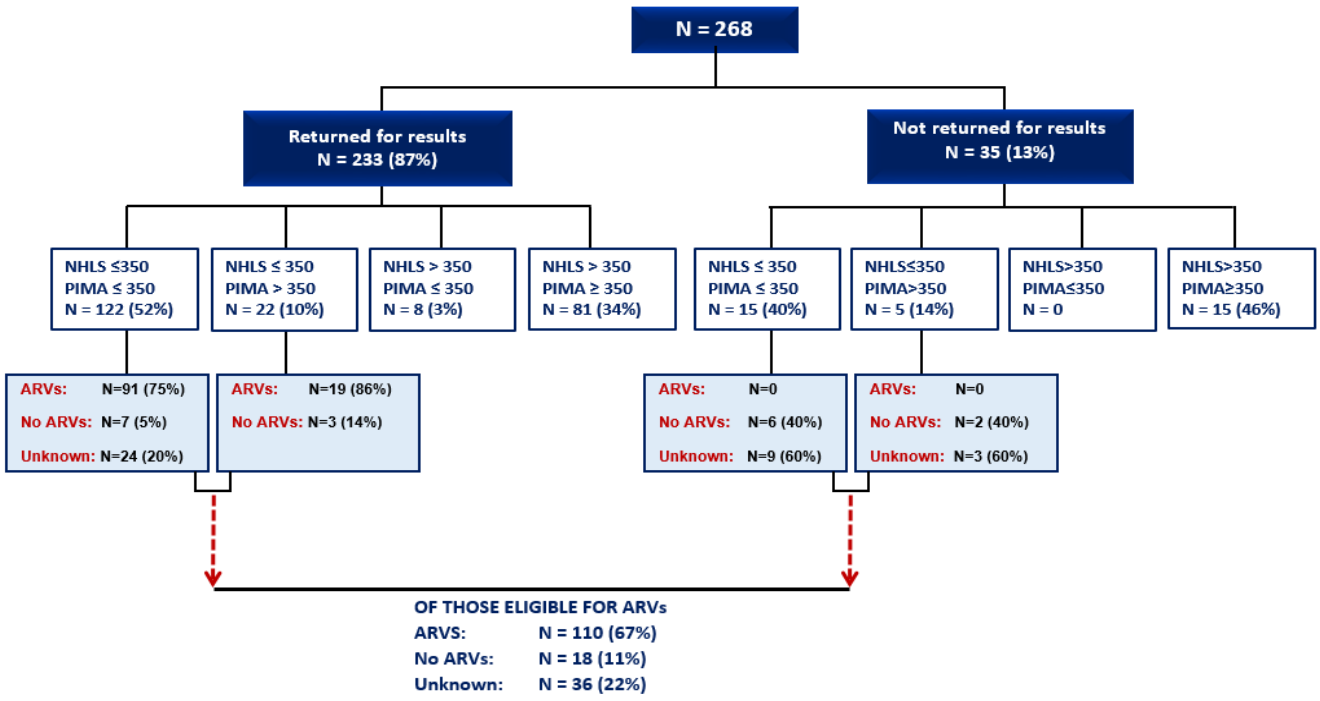

Figure 15: Comparison of CD4+ T cell counts with respect to ART eligibility of PIMA analyser vs NHLS PLG/CD4 in those HIV-1 infected patients who returned and did not return for their CD4+ T cell count results

3.11 Comparison of Gene Xpert results between the NHLS and TB Biomedical Research laboratories

In the original protocol the aim was to compare smear microscopy and culture as done routinely by the NHLS with Gene Xpert undertaken at the MRC TB Biomedical Research Unit laboratory. As we commenced our study the national algorithm for TB diagnosis as performed in the NHLS laboratories changed offering Gene Xpert as the first test for TB diagnosis. For this reason the study design was changed to allow us to compare the Gene Xpert assay performed in the NHLS laboratory compared to that in the MRC TB Biomedical Research laboratory. Smear microscopy was also routinely undertaken by the NHLS laboratory and culture was conducted in the MRC TB Biomedical Research Laboratory.

A separate cohort was recruited from the CDC PHC clinic for TB diagnosis.

Table 10: Patient characteristics of patients undergoing a TB screen at Lancers Road PHC

	FEMALE n=86	MALE n=24	TOTAL n=110
MEDIAN AGE (IQR), YEARS	**33 (27-40)**	**35.5(33.5-42)**	**33(27-40)**
18 – 24	8 (9%)	2 (8%)	10 (9%)
25 – 29	22 (26%)	2 (8%)	24 (22%)
30 – 34	17 (20%)	6 (25%)	23 (21%)
35 – 39	17 (20%))	6 (25%)	23 (21%)
40	20 (23%)	8 (33%)	30 (27%)

The majority, 86/110 of individuals undergoing TB screening were women in whom 26% were 25-29 years whereas the majority of males were over 30 years of age (Table 10).

3.12 Comparison of TB results obtained by the NHLS (Gene Xpert and smear microscopy) vs the MRC TB Biomedical Research laboratories (Gene Xpert and culture) in patients undergoing a TB screen

There were 8/110 sputum samples that had a positive TB diagnosis by both laboratories in all or some of the tests performed (Table 11), while the remainder were negative by all tests in the two laboratories. All TB tests: Gene Xpert; smear microscopy and culture were concordant in 5/8 positive TB sputum samples while in each of the remainder the discrepancies were: Gene Xpert positive and smear negative (NHLS) but Gene Xpert negative and culture positive by the MRC TB Biomedical Research laboratory; Gene Xpert and smear positive by NHLS but Gene Xpert and culture positive by MRC TB Biomedical Research laboratory; and Gene Xpert and smear positive by NHLS but Gene Xpert and culture negative by MRC TB Biomedical Research laboratory. Only in 2/110 sputum specimens Gene Xpert results were discordant between the two laboratories. No Rifampicin resistance was detected by the Gene Xpert and/or culture in all 8 positive TB sputum samples done by both laboratories.

The median turn around time of Gene Xpert results performed by the NHLS laboratory was 2.5 days (range 1-10 days) while the median time to commencing antituberculous treatment in those diagnosed as TB positive was 5 days (range 2 days – 5 weeks).

Table 11: Comparison of laboratory tests for TB diagnosis performed by NHLS and the MRC TB Biomedical Research Laboratory

LABORATORY NAME	NUMBER DETECTED BY GENE XPERT AND/OR CULTURE AND/OR SMEAR MICROSCOPY
MRC TB Biomedical Research Lab. Gene Xpert + Culture + **NHLS laboratory** Gene Xpert + Smear microscopy +	**5**
MRC TB Biomedical Research L. Gene Xpert - Culture + **NHLS laboratory** Gene Xpert + Smear microscopy -	**1**
MRC TB Biomedical Research L. Gene Xpert - Culture - **NHLS laboratory** Gene Xpert + Smear microscopy +	**1**
MRC TB Biomedical Research L. Gene Xpert - Culture - **NHLS laboratory** Gene Xpert - Smear microscopy +	**1**

KEY: + positive - negative

CHAPTER 4 – DISCUSSION

Conventional HIV treatment and care services are largely unable to cope with the volume of patients entering the system, resulting in delayed and missed opportunities for treatment, and ultimately unacceptably high levels of morbidity and mortality. Conventional flow cytometry to determine CD4 counts usually requires that samples be sent to a central laboratory, which may be off-site. This could have significant consequences for the timing of initiation of treatment based on CD4 count, which could in turn influence the health of patients. A comprehensive and holistic approach to combating the HIV epidemic in South Africa will necessarily include the treatment and management of tuberculosis (TB), which is a serious cause of morbidity and mortality in HIV-1 infected people. The confirmatory test widely used in countries where TB is endemic is smear microscopy; however this test has low sensitivity and takes weeks to obtain the results. There is thus a strong indication for the use of rapid tests in settings where a prompt reaction to test results may have a significant impact on the spread of TB and on co-morbid conditions such as HIV.

This study originally therefore set out to evaluate and compare two point-of-care tests viz PIMA^{TM} POC for the enumeration of CD4+ T cells and the GeneXpert for TB diagnosis against the standard routine NHLS (Beckman Coulter) and smear microscopy). The aim was to determine the accuracy, sensitivity and specificity PIMA^{TM} POC analyser as a predictor of ART eligibility and the GenXpert for TB diagnosis in our hands for future implementation in a PHC setting.

The majority (218/268) of HIV positive individuals undergoing CD4+ T cell count testing were women in whom 28% were 25-29 years whereas the majority of males were older than 30 years. There were no significant differences in the median CD4+ T cell count in males versus females performed by the NHLS PLG/CD4 versus the PIMA analyser POC, although the median CD4+ T cell count was higher in the latter. Overall, according to NHLS PLG/CD4 versus PIMA POC 61% versus 54% of HIV-positive individuals were eligible for ART initiation of whom 68% versus 62% were males and 60% versus 52% were females respectively.

All 3 PIMA analysers POC performed well with high reproducibility using normal and low beads with coefficient of variation < 2% over time (10 and 165 days). However when the normal and low IM controls were tested in the 3 PIMA analysers and compared with the NHLS PLG/CD4 no significant differences were observed at normal IM in all 3 PIMA analysers (%CV <11%) but differences were observed in 2 PIMA analysers with low IM (%CV 22-26%) whereas the 3rd PIMA was more acceptable at 12.4%. Glencross et al. (2012) in their similar study found an average % CV of 6.2% with normal IM and 9.1% for the low IM. The poor %CV for the low IM control in the 2 PIMA analysers was difficult to explain considering that in all 3 PIMA analysers there was high reproducibility when using their own specific bead control and whole blood samples.

Although blood samples kept overnight and run the next day were found to have higher CD4+ T cell counts as compared to samples tested on the same day, there was excellent correlation with no significant differences.

The PIMA analyser POC slightly overestimates NHLS PLG/CD4 laboratory flow cytometry in CD4+ T cell enumeration in this study,which corroborates most studies using capillary or venous blood (Glencross et al. 2008, Sukapirom et al. 2011, Mtapuri-Zinyowera et al. 2010, Van Schalk et al. 2011). This overestimation is minimal (mean bias 17 cells/µl) and is of no major clinical significance. This is not unusual as differences have been reported of CD4+ T cell enumeration on conventional testing platforms between the BD FACS count versus the BD FACS Calibur (Mwau et al. 2013) where the mean bias between the 2 platforms was 76 cells/µl (95% CI LOA -316.0-163.0).

Although in this study there was better correlation between the PIMA analyser POC and FACS Calibur (0.97), a correlation >0.90 was found between all 3 instruments (PIMA analyser POC vs NHLS PLG/CD4, PIMA analyser POC vs FACS Calibur and FACS Calibur vs NHLS PLG/CD4). As we found no difference in CD4+ T cell enumeration when blood samples were left overnight, the most probable explanation for these observed differences is due to the platforms used, which have different instrument settings, different antibodies and fluorochromes used, different gating strategies and sample volumes used.

The overall sensitivity of the PIMA analyser POC CD4 test, in enumerating CD4+ T cell counts of HIV infected pregnant mothers to determine their eligibility for ART in the prevention of

mother to child transmission (PMTCT) programme has been reported at 92% (Myer et al. 2013). In another study Manabe et al (2013) found a sensitivity of 96.3% with a negative predictive value of 99.2% in individuals with CD4+ T cell count of ≤ 250 cells/µl. Our data corroborates with Myer at al, 2013, where we found 35 (13%) misclassifications, of which 27 were FN. The mean difference between PIMA analysers and NHLS PLG/CD4 in this group of 27 patients was 112 cells. In another study conducted in South Africa, 12.4% were misclassified as FP when multiple fingersticks were done for multiple POC tests on the same patient (Gous et al, 2013). Using future South African treatment guidelines at CD4+ T cell counts threshold of ≤500 cells/µl, 81% and 80% of individuals in this study would be eligible for ART by the NHLS PLG/CD4 and the PIMA analyser POC respectively. At this threshold a high sensitivity of 94% was observed at the sacrifice of lower specificity of 78% which corrobates similar findings in a Ugandan study (Galiwongo et al, 2014). In the 13 false negatives the mean bias observed was 149 cells/µl. This high sensitivity would fit in with these future South African guidelines to be introduced by 2015 where all those eligible for treatment will be initiated but it will come at a cost of low specificity whereby individuals not needing treatment will be commenced on ART. However in the light of ART resulting in lower viral loads and thus less horizontal transmission this downside is minimized. A recent study has demonstrated that as household ART coverage is increased there is a decrease in HIV acquisition (Vandormael et al., 2014). Under all three South African guidelines, the overall performance of the PIMA analyser POC as a diagnostic tool for treatment initiation was favourable, evidenced by the high proportion of patients correctly classified (91%, 87% and 91% respectively). It is therefore feasible to use the PIMA analyser POC for the provision of a rapid CD4+ T cell enumeration testing strategy. The agreement in these data between the PIMA analyser POC and NHLS PLG/CD4 laboratory based flow cytometry appears to decline with increasing CD4+ T cell count >500 cells/µl. This is not of concern as these HIV-1 infected individuals are ineligible for ART initiation.

From the operational perspective of the use of the PIMA analyser, we had an analyser reading error of 8% as found by others (Myer et al., 2013, Glencross et al., 2012), most of these were due to movement and vibration. The "operator" used was myself who is a trained medical technologist compared to health professionals e.g. nurse or counselor. Hence we had no air bubbles or debris in the sample nor damage during operation (Myer et al. 2013). Several studies

have also reported that the PIMA analyser is interchangeable with conventional platforms (Diaw et al., 2011, Mtapuri-Zinyowera et al., 2010, Sukpirom et al., 2011, Thakar et al., 2012, Herbert et al., 2012, Glencross et al., 2012), although one study (Mwau et al., 2013) has found it to be unreliable due to the high coefficient of repeatability and misclassification in favour of undertreatment compared to the FACS Calibur.

The PIMA analyser POC CD4 test has the potential role for CD4+ T cell enumeration in PHC settings. Although originally the Alere PIMA analysers were to be placed at Lancers Road PHC clinic this was not possible because of the Overport PHC clinic merging with Lancers Road due to flooding and reconstruction so there was no extra room for us to place the PIMA analysers. They were therefore placed at the MRC Research laboratories. Furthermore the protocol did not allow for the provision of same day PIMA results, so that clinical decision making was done on the NHLS PLG/CD4 result.

In a recent study Larson et al. 2013, reported that providing same day POC CD4 testing that is not rapid has no benefit in health outcomes. The uptake of POC testing using the FACS Calibur was sub-optimal due to the waiting time of 2-3 hours to get their CD4 test result (Larson et al., 2013). However using the PIMA analyser could significantly reduce the waiting time to 30 minutes. Furthermore, testing doesn't have to be "batched" and can be performed singly throughout the day particularly if there are several PIMA analysers available. If the results of the PIMA had been given to the patients immediately it would have prevented the 35 out of the 268 not having access to their results (through them not returning to collect). Of these approximately 50% were eligible for treatment. Using the normal route of patients coming in to collect their results and being initiated on treatment we observed a median time to ART initiation of 25 days (IQ 9.5-48.5 days) and we believe that with POC analysers this waiting time would be significantly reduced.

Of the patients eligible for ART who did not access treatment, the reasons given upon telephonic communication were economic (no transport costs), social (too busy to come to the clinic), structural (cannot take time off work) and emotional (were not ready to take ART as they were still feeling well). Even in those eligible patients who did return for their results we found 24% did not initiate treatment. It is possible that if they received their results immediately it may have helped by accessing immediate counselling and preparation for future pathway to

care. Provision of immediate CD4+ T cell count results has been shown to increase the number of patients linking into care (Faal et al., 2011, Larson et al., 2012b, Jani et al., 2011, Kranzer et al., 2011).

The high rates of "walk ins" patients found in this study as found by others (Larson et al., 2013), who are eligible for immediate ART at the time of the HIV test and the time lapse to ART initiation, undergirds the urgent need of the use of the rapid PIMA analyser POC technology. However it needs to be supported by changes in health systems (Larson et al., 2013), integrating HCT and detecting those eligible for ART into a single consultation by providing same day results through POC technologies and addressing psychosocial issues. Additionally, a comprehensive counselling package should be provided with information about HIV disease, ART, positive health-seeking behaviours, general guidelines to healthy living and encouraging patient ownership of their health. Other barriers found for continuum of HIV care such as transport costs and distance, long waiting times, fear of disclosure, drug toxicity, loss of work time and stigma (Govindasamy et al., 2012), if addressed, could have an impact in facilitating the next step in the pathway to care for ART eligible individuals in improving uptake of HIV care and treatment in South Africa. A study in Mozambique (Elul et al, 2014) reported on the challenges of the effectiveness of a combination strategy of intervention in offering clients non-cash financial incentives to offset the financial burden of clinic visits in order to transform clinic-based approaches to retention and care. In a recent systematic review (Govindasamy et al., 2014) has suggested that the streamlining of HIV services together with individual and combination interventions across the HIV cascade is important to address attrition and retention in care.

A family centred model of integrated health care incorporating all the above mentioned health system changes has previously been shown in a similar population to yield high adherence (94%) and retention in the care and management of HIV-1 positive individuals (Kwaan et al., 2010, Coutsoudis et al., 2010).

In the original protocol the aim was to compare smear microscopy and culture as done routinely by the NHLS with Gene Xpert undertaken at the MRC TB Biomedical Research Unit laboratory. As we commenced our study the national algorithm for TB diagnosis as performed in the NHLS laboratories changed offering Gene Xpert as the first test for TB diagnosis. For

this reason the study design was changed to allow us to compare the Gene Xpert assay performed in the NHLS laboratory compared to that in the MRC TB Biomedical Research laboratory. Smear microscopy was also routinely undertaken by the NHLS laboratory and culture was conducted in the MRC TB Biomedical Research Laboratory. A separate cohort was recruited from the CDC PHC clinic for TB diagnosis.

There were minimal discrepancies in the comparison of Gene Xpert; smear microcopy, culture and resistance results performed by the two laboratories. The most likely explanation for these discrepancies could be due to sputum quality and bacterial load as testing was done on two different sputum specimens taken on the same day from each patient.

The median turn around for Gene Xpert NHLS results was 2.5 days and the time to treatment was 5 days. All patients diagnosed with TB were linked to treatment and care. However, it has been reported that linkage to treatment was not achieved in 25% of drug sensitive smear positive TB patients (Claasens et al. 2013). Another study showed an average 5-6 week time lapse in Cape Town for MDR treatment initiation, although some patients were initiated onto treatment as little as 3 days after testing (Chang et al., 2012), compared to 75% of patients in KZN of delays up to 22 weeks (Narasimooloo and Ross 2012).

CHAPTER 5 - CONCLUSION

In summary the overall agreement between PIMA analyser POC and NHLS PLG/CD4 CD4+ T cell count enumeration in adult HIV positive individuals was acceptable with clinically insignificant mean bias. Together with high PPV and sensitivity and acceptable specificity the PIMA analyser POC lends itself to an excellent facilitator of improved healthcare. The operational role of the PIMA analyser POC CD4 test in provision of immediate CD4+ T cell count results combined with integrated health system changes, its impact in linkage to care, ART treatment initiation and retention in HIV care needs to be assessed in a PHC facility.

There was excellent correlation of Gene Xpert results between the two laboratories. Although Gene Xpert testing is the diagnostic test according to the South African guidelines for TB diagnosis, due to cost implications Gene Xpert testing is not performed at sites near to patient care provision. The Gene Xpert test has resulted in increased detection of multi drug resistant (MDR) TB. It would be important to investigate the magnitude of the non or delayed linkage to treatment pertaining to MDR TB patients in South Africa, and to assess the factors responsible for any delay or expedition to care. Several new POC technologies e.g. TB loop mediated isothermal amplification (TB LAMP) are undergoing evaluation to be used as a screening tool for the diagnosis of TB which are cheaper than Gene Xpert and have more rapid turn around times (Boehme et al., 2007).

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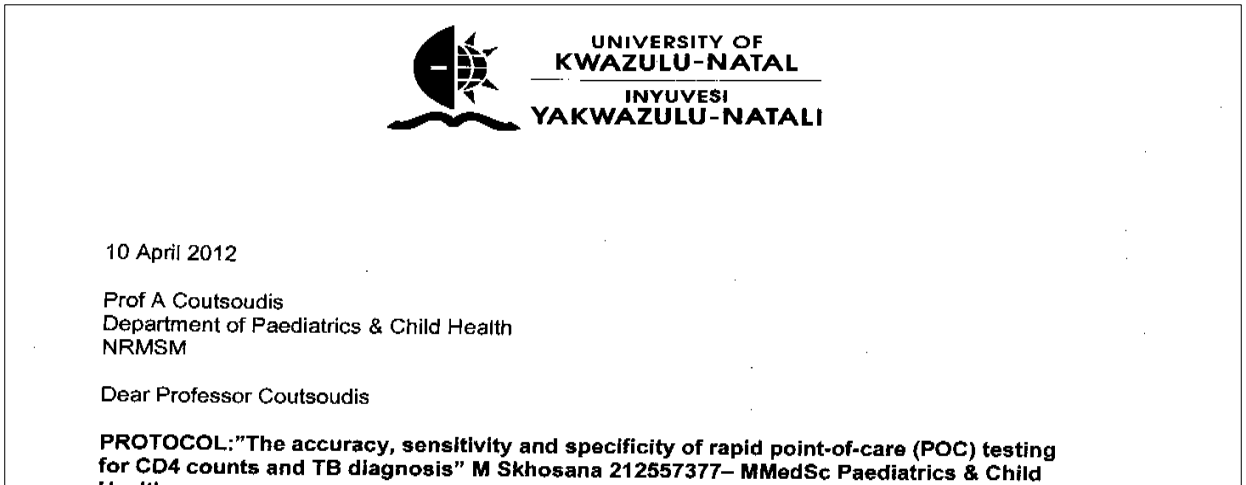
APPENDICES

Appendix I: UKZN Postgraduate Studies approval



UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

10 April 2012

Prof A Coutsoudis
Department of Paediatrics & Child Health
NRMSM

Dear Professor Coutsoudis

PROTOCOL:"The accuracy, sensitivity and specificity of rapid point-of-care (POC) testing for CD4 counts and TB diagnosis" M Skhosana 212557377– MMedSc Paediatrics & Child

Appendix II: Full BREC approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

13 December 2012

Ms. M Skhosana
Department of Paediatrics and Child Health
2nd Floor - DDMRI Building
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Ms Skhosana

PROTOCOL:The accuracy, sensitivity and specificity of rapid point-of-care(POC)testing for CD4 counts and TB diagnosis. REF:BE212/11

Appendix IV: Ethical approval from the Health and Social Services Health Unit

Appendix III: BREC approval for follow up calls

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

28 October 2013

Ms. M Skhosana
Department of Paediatrics and Child Health
2nd Floor - DDMRI Building
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Ms Skhosana

PROTOCOL: The accuracy, sensitivity and specificity of rapid point-of-care (POC) testing for CD4 counts and TB diagnosis. REF: BE212/11

I wish to advise you that your letter dated 09 October 2013 requesting permission from BREC to collect data from the phone calls made to 53 patients for the above study has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. Your response dated 17 October 2013 in response to BREC correspondence dated 16 October 2013 has been noted by BREC.

This approval will be noted at the next BREC meeting to be held on 12 November **2013.**

Yours sincerely

Ms A Marimuthu
Senior Administrator: Biomedical Research Ethics

Appendix IV: Ethical approval from the Health and Social Services Health Unit

28/11 2012 15:06 FAX 0313113530 HEALTH 002/005

0313113530

ETHEKWINI MUNICIPALITY

HEALTH, SOCIAL SERVICES
Health Unit

9 Archie Gumede Place
P O Box 2443, Durban, 4000
Durban, 4001
Tel: 031 311 3523, Fax: 031 311 3530
www.durban.gov.za

26 November 2012

Dear Ms Skhosana

RE: Permission to undertake research at Lancers Clinic.

Study Title: To test the accuracy, sensitivity and specificity of rapid point-of-care (POC) testing for CD4 counts and TB diagnosis.

The study site **has been approved** and the following to be noted:

- Submission of the indemnity form obtainable from the EThekwini Health Unit before commencement of the study.
- Prior arrangements to be made with the facility and an assurance that all services will not be disrupted.
- Staff will not be used for study.
- Progress reports to be provided and the final report of the study with an oral presentation to the eThekwini Municipality Unit.
- Obtain permission from the eThekwini municipality health department for press releases and release of results to communities/stakeholders.
- The department has to receive recognition for the assistance given.
- Any amended to the study to be communicated with the Health Unit, and the relevant amendment form obtainable from the unit to be submitted.
- Withdrawal of permission to conduct research will be left to the discretion of the eThekwini Health Unit.

Yours faithfully,

Dr NI GXAGXULA Date: 28-11-2012

~~Deputy~~ Head of Health: Clinical Support.

Appendix V: Informed Consent

PATIENT INFORMATION AND INFORMED CONSENT FORM

The accuracy, sensitivity and specificity of rapid point-of-care (POC) testing for CD4 counts and TB diagnosis

Version 1.0 Revision 2.0
10 January 2013

PRINCIPAL INVESTIGATOR: M. Skhosana

PHONE: +27 31 2423600

Short Title for the Study: PIMA/GeneXpert Study

INTRODUCTION

You are being asked to volunteer for the research study. This document gives you information about the study that will be discussed with you by the study doctor, nurse, research assistant or counselor. Before you decide whether to be in the PIMA/Gene Xpert study, we would like to explain its purpose, review the risks and benefits, what is expected of you, and what you can expect from the study site.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives information about the blood test(s) that will be done during this PIMA/ Gene Xpert study. Once you understand the study, and if you agree to take part, you will be asked to sign your name or make your mark on this form. You will be offered a copy of this form to keep.

PURPOSE OF THE STUDY

The main purpose of the PIMA/GeneXpert Study is to find out whether point-of-care (POC) diagnostic tests are as good as the testing that is done currently.

STUDY PROCEDURES

You have been recruited into the PIMA/GeneXpert study as you recently tested HIV positive. You will be post-test counseled and educated on how to protect yourself from sexually transmitted infections, and how to live positively with HIV and ways to avoid contracting HIV. If you are willing to participate in this study then you shall sign an informed consent form giving us permission to collect a blood sample not to exceed 5 ml (1 teaspoon); testing will be performed to run the following tests:

- A CD4-positive T cell count, which will measure the amount of damage HIV has done to your immune system by both the Pima analyser and the FACS calibur which is what is used currently. The immune system is the part of the body that fights off germs and infections

- If we suspect that you may have TB sputum specimens will also be collected to test whether you have TB and Rifampicin resistance by both the GeneXpert, microscopy and drug susceptibility tests

Your samples will never be sold or used to make products that could be sold.

You will continue to receive the standard of care and treatment you are receiving from your care providers at the clinic. You will always have the right to withdraw from the study at any time.

The Metropolitan Life Group and CU-CAPRISA AITRP scholarship programme is funding this study.

Approximately 370 HIV HIV positive individuals may be enrolled in this study. It may take up to about 2 years to enroll all participants.

By taking part in the PIMA/GeneXpert study, you are contributing to determining whether these POC tests which provide results on the same day can guide the doctor in making decisions to start treatment in order to impact your health. This is an important step towards attempting to reduce the epidemic.

RISKS AND/OR DISCOMFORTS

This is a minimal risk study. We do not expect you to experience any additional risks or discomforts above and beyond what you would normally experience as part of a routine doctor's visit.

Risk of Blood Draws

You may feel discomfort or pain when your blood is drawn. You may feel dizzy or faint. You may have a bruise, swelling, small clot, or infection where the needle goes into your arm.

Other Possible Risks

If you are HIV positive, knowing this could make you worried. Trained study counselors will help you deal with any feelings of anxiety or embarrassment or any questions you have.

NEW INFORMATION

You will be told about new information from this or other studies that may affect your health, welfare or willingness to be in this study. You will be told when the results of the study may be available and how to gain access to them.

BENEFITS

You will get no direct benefit from participating in this study however you will have made an important contribution to helping doctors decide how best to test CD4 and TB in order to help patients access care and treatment more quickly in order to help make you feel better. Therefore many others in the future may benefit from information learned in this study. . You may benefit from the advice of the research team/ and or test results you will receive as a result of your participation in the study. For example, you will receive HIV/sexually transmitted infections (STI) prevention education which may help to protect yourself and others from STI and HIV infection. If you contract a treatable STI during the study you will be referred to the appropriate hospital or clinic for treatment.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT

You may be removed from this study without your consent for the following reasons:

This PIMA/GeneXpert Study is stopped or cancelled

The study staff feels that this PIMA/GeneXpert Study would be harmful to you

Other administrative reasons

You are not able or willing to attend study visit or to complete the study procedures

ALTERNATIVES TO PARTICIPATION

There may be other studies going on here or in the community that you may be eligible for or other ways you could obtain the same kind of test results available through this study. If you wish, we will tell you about other studies and locations for testing that we know about.

COSTS TO YOU

There is no cost to you for being in the PIMA/GeneXpert Study.

REIMBURSEMENT

There is no re-imbursement as the specimens that we shall collect are routinely collected and offered to you as a service. The PIMA test will be done from the same blood that is collected to be used for the FACS calibur as well as the sputum specimen that is collected for microscopy and drug susceptibility testing will be also used for the GeneXpert test.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. However, absolute (complete) confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Any publication of this study will not use your name or identify you personally.

In order to protect your right to confidentiality, you will be assigned a code number. This will be used to identify you, rather than your name. Your personal information will not be released without your permission.

Your records will not be given to anyone without your permission except as needed for review by any or all of the following:

Biomedical Research Ethics Committee (BREC) of the University of KwaZulu Natal

Study staff

Dr. Photini Kiepiela 031-2423639

PROBLEMS OR QUESTIONS

If you have questions about whom to contact at the research site, you should contact:

Dr. P Kiepiela at +27312423639 (work) or +27823211180 (cell) or

Dr. Beth Spooner at +27312084634 (work) or +27723510284 (cell)

or the Medical Research Council Unit (HPRU) at 123 Jan Hofmeyr Road, Westville 3630.

If you have questions about your rights in the study, you should contact

Prof. Wassenaar, Chairperson, BREC Ethics Committee, at +27 31 260 4769/031 260 1074

or 031 260 2486/8350 (Mariette Snyman)

email: BREC@ukzn.ac.za or marimuthu@ukzn.ac.za or snymanm@ukzn.ac.za

SIGNATURES

If you have read this consent form, or had it read and explained to you, and you understand the information, and voluntarily agree to participate in the PIMA/GeneXpert Study, please sign your name or make your mark below.

_____	_____	_____
Participant Name (print)	Participant Signature (or Mark)	Date
_____	_____	_____
Study Staff Name	Study Staff Signature	Date
_____	_____	_____
Witness Name	Witness Signature	Date

Appendix VI: Zulu Informed Consent

IMVUME EQUKETHE ULWAZI

ULWAZI LWESIGULI NE MVUME UNOLWAZI

The accuracy, sensitivity and specificity of rapid point-of-care (POC) testing for CD4 counts and TB diagnosis

Version 1.0 Revision 2.0

09 January 2013

PRINCIPAL INVESTIGATOR: M SKHOSANA

PHONE NUMBER: 031 242 3600

Short Title for the Study: PIMA /GeneXpert Study

ISINGENISO

Uyacelwa ukuvolontiya kucwaningo olwaziwa nge PIMA/ GeneXpert. Lelifomu likunikeza ulwazi mayelana nocwaningo enizoxoxa ngalo nabasebenzi basocwaningweni namhlanje. Ngaphambi kokuba uthathe isinqumo sokuba yingxenye yocwaningo , sithanda ukucacisa inhloso yalo, sibukeze ubungozi kanye nemihlomulo, okulindeleke kuwena nawe ongahle ukulindele kulolucwaningo. Uma usuqondisise kahle ngalolucwaningo, futhi uvuma ukuba yingxenye yalo, uyobe usucelwa ukuba usayine iphepha lemvume equkethe ulwazi noma wenze uphawu lwakho phambi kukafakazi. Uyobe usunikwa elakho iphepha ukuthi uligcine ngakuwena.

Kungenzeka ukuthi lelifomu libe namagama angajwayelekile. Sicela ulifundisise kahle, ubuze nemibuzo lapho ungezwisisi kahle khona noma lapho udinga khona ulwazi oluthe xaxa. Akufanele uvume ukuba yingxenye yalolucwaningo ngaphandle kokuba uqondisise kahle ngalo futhi waneliseke ukuthi unolwazi olugcwele.

UYAVOLUNTIYA UKUBA YINGXENYE YALOLUCWANINGO

Lelifomu eliyimvume equkethe ulwazi likunikeza ulwazi ngokuhlolwa kwegazi okuzokwenziwa kulolucwaningo. Uma usuqondisise kahle inhloso yocwaningo, futhi uvuma ukuba yingxenye yalo, uyobe usucelwa ukuba usayine amagama akho kuleliphepha lemvume equkethe ulwazi

noma wenze uphawu lwakho kulelifomu. Uyobe usunikezwa elakho iphepha ukuthi uligcine ngakuwena.

INHLOSO YOCWANINGO

Ihloso ngqangi yocwaningo ukuhlolisisa ukuthi ukuhlolwa okwenziwa emtholampilo bese igazi liyohlolisiswa kwenye indawo kukhipha imiphumela efanayo nokuhlolwa bese igazi lihlolisiswa khona lapho ngalesosikhathi (POINT-OF-CARE)

IZINHLELO ZOCWANINGO

Uma usanda kuhlola wathola ukuthi unegciwane lesandulela ngculaza, noma awunalo, ungaba yingxenye yalocwaningo. Nokho usazophinda uhlolwe, walulekwe uphinde ufundiswe ngezindlela zokuzivikela ezifeni ezithathelana ngokocannsi, ikakhulukazi uma ungenalo igciwane lesandulela ngculazi nokuthi ungaphila kanjani uma usunalo leligciwane. Ngokusayina lelifomu lemvume equkethe ulwaziuyobe usinikeza imvume yokuba sidonse elinye isampula legazi elingu 5ml (ukhezo lwetiye olulodwa) kuwena ukuze sikwazi ukuhlola ukuthi amasosha akho omzimba angakanani (CD4 count). Lokhu sizokwenza ngokusebenzisa umshini obizwa nge PIMA ngaphezu kokusebenzisa lona ojwayelekile umshini obizwa ngokuthi i FACS CALIBUR. Ngaphezu kwalokho, uma kutholokala ukuthi unezimpawu ze TB, siyocela ukuthi usinike isikhwehlela ukuze sihlole ukuthi unayo ngempela yini i TB nokuthi iTB yakho ayiwanqabi yini amaphilisi iRifampicin asetshenziselwa ukwelapha iTB. Lokuhlola kuzokwenziwa ngomshini obizwa ngokuthi i GeneXpert bese kuthi imiphumela yalapho iqhathaniswa nemiphumela ezotholakala uma kusetshenziswa umshini obonakalisa izinto ezincane (microscope), okuyiyona ndlela esetshenziswayo njengamanje ukuhlola i TB.

Labo abayingxenye yalolucwaningo, bayohlolwa kabili ngoDokotela, bese benikezwa nezincwadi zokudluliselwa esibhedlela, uma kutholokala ukuthi kukhona izifo abanazo. Unelungelo lokuhoxa kulolucwaningo noma ngasiphi isikhathi.

Lolucwaningo luxhaswe uhlelo lomfundaze lakwaMetropolitan Life Group, kanye no CU-CAPRISA ARTRP. Cishe kungabhalisa abantu abawu 370 abangenalo/noma abanalo igciwane lengculaza kulolucwaningo. Lokubhalisa kungathatha cishe iminyaka emibili.

Ukuba yingxenye yalolucwaningo uzobe ubamba iqhaza ekutholeni isiqiniseko sokuthi ukuhlolwa okwenziwa emtholampilo ihlaziywe ngalesosikhathi (POINT-OF-CARE) kungamsiza uDokotela ukuthatha izinqumo zokuqala imshanguzo engasindisa impilo yakho. Lesi isinyathelo esibalulekile sokuzama ukunciphisa lolubhubhane.

INQUBO YOCWANINGO

Uma ususithathile isinqumo sokuba yingxenye yalolucwaningo, wafunda, waxoxa wasayina noma wenza umaka wakho kulefomu, uyobe usuthathwa igazi nesikhwehlela, ngalo lelolanga. Uma kutholakala ukuthi unegciwane lesandulela ngculazi, ungaphinde ubizwe futhi ngolunye usuku. Abasebenzi bocwaningo bayokuchazela baphinde baphendule imibuzo ongaba nayo ngaphambi kokusayina noma wenze umaka wakho kulelifomu. Uma ususithathile isinqumo sokubayingxenye yocwaningo, uyocelwa ukuthi unikeze abasebenzi bocwaningo ngemininingwane mayelana nendawo ohlala kuyona nendlela abangakuthinta ngayo. Uma kudingeka, olunye usuku lokuvakasha kwakho luzobekwa abasebenzi basocwaningweni. Uyobe usuxoxisana nabeluleki ngesandulela ngculaza, ukuphila naso nezindlela zokugwema ukusithola.

Amasampula akho awasozwe adayiswa noma kwenziwe ngawo umkhiqizo ongahle udayiswe.

Uma unesandulela ngculaza noma ungenaso isandulela gculaza, uyokwalulekwa ngokwengqondo.

Abangenalo igciwane lesandulela ngculaza, kodwa benezimpawu zeTB, bazothathwa isikhwehlela siyosihlola ukuthi awunayo yini iTB nokuthi iTB yakho ayiyinqabi yini iRifampicin, sisebenzisa i GeneXpert, umshini obonakalisa izinto ezincane (microscope) esetshenzisela ukuhlola ukuthi imiphi imishanguzo engayidambisa iTB yakho.

Kwabanegciwane lesandulela ngculaza: Siyodonsa isampula legazi elingeqile ku 5ml (ukhezo lwetiye olulodwa) bese sihlola loku okulandelayo:

- Sizobala amasosha omzimba wakho (CD4 count) ukuthola ukuthi igciwane selenze umonakalo ongakanani ohlelweni lokuzivikela komzimba. (Uhlelo lokuzivikela yingxenye yomzimba elwa namagciwane nezifo).
- Isampula lwesikhwehlela lizothathwa ukuze sihlole ukuthi ingabe unaso yini isifo seTB nokuthi iTB yakho ayiwunqabi yini umshanguzo iRifampicin sisebenzisa i GeneXpert, umshini obonakalisa izinto ezincane (microscope) nezindlela zokuhlola ukuthi imiphi imishanguzo engayidambisa iTB yakho.
- Kuyodingeka ukuthi uphinde ubuye futhi izihlandla ezimbili ukuze abasebezi bocwaningo bakwazi ukukucacisela imiphumela yokuhlolwa okulandelayo osekwenziwe, lemiphumela ingathatha isikhathi esingangenyanga eyodwa:
  - Ukubalwa kwamasosha omzimba wakho (CD4 count)
  - Imiphumela yokuhlolwa ngomshini obonakalisa izinto ezincane (microscope) nokuhlola ukuthi imiphi imishanguzo engadambisa igciwane le TB.

- Uma unegciwane lesandulela ngculazi, lolucwanigo alungeke likunike imishanguzo kodwa abasebezi bocwaningo bazosheshe bakunikeze ukwalulekwa futhi bakudlulisele kweminye imithombo yokwelashwa, nokwelulekwa kanye nolunye usizo ongase ulidinge. Ngenxa yokuthi imiphumela yokuhlolwa kwasocwaningweni ingasiza oDokotela ukuthi benze izinqumo ezisemqoka ngempilo yakho, abasebenzi basocwaningweni banganikeza oDokotela imiphumela yokuhlolwa kwakho uma uthanda futhi unikeza imvume.

UBUNGOZI KANYE/NOMA UBUHLUNGU

Lolu ucwaningo lunobungozi obuncane kakhulu. Asilindele ukuthi ungahle ubhekane nobungozi noma ubuhlungu obungale kwalokhu obhekana nabo uma uyobonana nodokotela ngokwenjwayelo.

- INGOZI YOKUDONSWA KWEGAZI
  - Kungenzeka uzwe ubuhlungu uma uma udonswa igazi.
  - Ungaba nesiyezi noma uquleke
  - Ungaba nesihlisa, ukuvuvuka, ihlwili elincane noma uthole amagciwane lapho inaliti ingene khona engalweni yakho.

- OBUNYE UBUNGOZI ONGAHLE UBUTHOLE
  - Uma unegciwane lesandulela ngculaza, ukwazi ngalokhu kungakwenza ukhathazeke. Abeluleki bocwaningo baqeqeshiwe ukukusiza ukubhekana nalemizwa yembandezeko nokuphoxeka neminye imbuzo ongaba nayo.
  - Sizokwenza konke okusemandleni ukuvikela imfihlo nesifuba sakho ngesikhathi usocwaningweni. Ukuvakasha kwakho lana kuzokwenzeka ngokufihlakala. Nokho kungenzeka ukuthi bezwe ngokuba yingxenye yocwanongo kwakho bese bekuphatha kabi, bakucwase bakubandlulule. Mhlawumbe kubenzima ukuthola noma ukuhlala emsebenzini noma ukwamukelwa umndeni nomphakathi wakho. Ukwazi ngesimo sakho sesandulela ngculaza kungadala izinkinga phakathi kwakho nowakwakho. Ukubandlululwa kungenziwa abanye uma bezwa ukuthi uyingxenye yalolucwaningo. Abasebenzi bocwaningo bazoqinisekisa ukuthi imiphumela ingafinyeleli kunoma ubani omunye ngaphandle kwalabo ababhaliswe kwingxenye eyimfihlo yalelifomu. Uma kwenzeka uba nalezizinkinga, abeluleki bosebenzisana nawe ukuzama ukuzixazulula.

ULWAZI OLUSHA

Uyokwaziswa ngemniningwane emisha mayelana nalolucwaningo nanoma yiluphi olunye ucwanongo othintekayo kulona ngandlela thile. Uyokwaziswa ukuthi ulwazi uzoluthola kubani futhi kanjani.

INZUZO

Ayikho inzuzo oyitholayo ngokuba yingxenye yalolucwaingo, kodwa uzobe ubambe iqhaza elibalulekile ekusizeni odokotela ukuba bakwazi ukuthatha izinqumo ezisemqoka uma behlola i amasosha omzimba (CD4 count) neTB, ukuze bakwazi ukusiza iziguli ukuthi zifinyelele ekwelashweni masinyane ukuze zibengcono. Ngalendlela baningi abanye abazozuza ngolwazi oluzotholakala kulolucwaningo. Ungazuza nawe ngokuthola izeluleko kubasebenzi bocwaningo /nemiphumela yokuhlolwa kwakho ngenxa yokuba yingxenye yocwaningo, isibonelo, ungathola imfundo mayelana nokugwema ukutheleleka ngesandulela ngculazi/izifo ezithathelani ngocansi. Uma utheleleka ngesifo socansi eselaphekayo ngenkathi usocwaningweni uyodluliselwa esibhedlela noma emtholamilo ofanele.

IZIZATHU EZINGENZA UKUTHI UHOXISWE OCWANINGWENI NGAPHANDLE KWEMVUME YAKHO

Ungahoxiswa ocwaningweni ngaphandle kwemvume yakho ngalezizizathu ezilandelayo:

1. Uma ucwaningo lungamiswa noma luchithwa.
2. Uma abasebenzi bocwaningo bebona ukuthi ucwaningo lungaba yingozi kuwena.
3. Noma yiziphi izizathueziphathelene nengqubo yocwaningo.
4. Uyahluleka noma awuthandi ukuvakashela ocwaningweni noma ukulandela inqubo yocwaningo .

ONGAKWENZA ESIKHUNDLENI SOKUBA YINGXENYE YOWANINGO

Kungenzeka kubekhona olunye ucwaningo oluqhubekayo lapha noma emphakathini ongahle kutholakale ukuthi ufanele ukubayingxenye yalo noma kubekhona ezinye izindlela ongathola ngazo ukuhlolwa okufana nalokhu okuthola kulolucwaningo. Uma ufisa, singakwazisa ngolunye ucwaningo nezindawo zokuhlolwa esizaziyo.

IZINDLEKO EZIZA NGAKUWENA

Ayikho indleko eza ngakuwena ngenxa yokuba yingxenye yocwaningo.

INHLAWULO

- Akukho nhlawulo ozoyithola ngoba amasampula esizowathatha kuwena azothathwa ngokujwayelekile futhi lokhu sikwenzela ukukunika usizo kuphela. Ukuhlolwa kwegazi nge PIMA kuzokwenziwa egazini lakho ebelikade lizodonselwe ukuhlolwa nge FACS CALIBUR kanye nesikhwehlela sakho esithathelwe ukuyohlolwa ngomshini obonakalisa izinto ezincane (microscope) nokuhlola ukuthi imiphi imishanguzo engadambisa igciwane le TB, sizohlolwa nge GeneXpert. Emva kwalokho silindele ukuthi uphinde usivakashele isihlandla ezimbili ukuze uma unesandulela ngculaza uzothola imiphumela ye CD4 count neye TB. Ngokunjalo, asikho isinxephezelo ozosithola kulokukuvakasha . Uma ungenaso isandulela ngculaza kuzodingeka uphinde uvakashe yikhona uzothola imiphumela yakho yeTB.

IMFIHLO

Kuzokwenziwa konke okusemandleni ukuze imininingwane yakho igcinwe iyimfihlo. Kodwa-ke asikho isiqiniseko sokuthi imininingwane yakho izofihlwa ngokuphelele. Imininingwane yakho ingadalulwa uma ifunwa abomthetho. Ukusakazwa kwalocwaningo kungeke kuphathe igama lakho noma kukhombe wena uqobo lwakho.

Ukuze sikhusele ilungelo lakho lobumfihlo uzo nikezwa inombolO ezosetshenziselwa ukukhomba wena. Imininingwane ephathelene nawe ayingeke idalulwe ngaphandle kwemvme yakho. Abaqondisi bocwaningo kuphela abazoqinisekisa ukuthi uthola imiphumela yokuhlolwa eqondile nokuthi uyakhunjuzwa ngezinsuku onqunyelwe zona. Abaqondisi abangeke badalula igama noma inombolo yakho kubanye abasebenzi beqembu lwasocwaningweni.
Amarekhodi akho awangeke anikezwe muntu ngaphandle kwemvume yakho ngaphandle uma kudingeka abuyekezwe yinoma yibaphi kwabalandelayo:

- i University of KwaZulu Natal Ethics committee
- Abasebenzi bocwaningo.

UKULIMALA OCWANINGWENI

Mancane amathuba wokuthi ungalimala ngenxa yokuba yingxenye yocwaningo ngoba lolucwaningo lunombungozi obusenzingeni eliphansi. Uma kwenzekile walimala ngenxa yokuba socwaningweni, i MRC HIV Prevention Research Unit izokunikeza ukwelashwa okusheshayo

ngokulimala kwakho. Awuzukukukhokhela lokwelashwa. Uzodluliselwa esibhedlela noma e clinic eseduze ukuze uthole ukwelashwa okwengeziwe ngokulimala kwakho. Awuwalahli amalungelo akho asemthethweni ngokusayina lelifomu. Uma kwenzeka ulimale ocwaningweni, thintana no: Dr Photini Kiepiela : 031 242 3639.

IZINKINGA NOMA IMIBUZO

Uma unemibuzo mayelana nokuthi ubani ongamthinta, ungathinta uDr P Kiepiela ku 031 242 3639 (emsebenzini)/ 082 321 1180 (cell) noma u Dr Beth Spooner 031 208 4634 (emsebenzini)/072 3510 284 noma ushayele e Medical Research Council (HPRU) e 123 Jan Hofmeyr Road, Westville, 3630.

Uma unemibuzo mayelana namalungelo akho ocwaningweni, thinta u Prof. Wassenaar,

Chairperson, BREC Ethics Committee ku 031 260 4769/ 031 260 1074/ 031 260 2486/8350

(Marriette) email:

BREC@ukzn.ac.za/ marimuthu@ukzn.ac.za/ snymanm@ukzn.ac.za

ISISHICILELO

Uma usulifundile noma ulifundelwe lelifomu lemvume, wachazelwa, wawuqondisisa umbiko futhi uvuma uvolontiya ukuba yingxenye yocwaningo i PIMA/GeneXpert, uyacelwa ukuba usayine noma wenze umaka wakho ngezansi.

_____	_____	_____
Igama lombambiqhaza	Isishicilelo sombambiqhaza	Usuku
(Hlukanisa)		
_____	_____	_____
Umsebenzi wocwaningo	Isishicilelo somsebenzi wocwaningo	Usuku
ophethe izingxoxo		
_____	_____	_____
Igama likafakazi	Isishicilelo sikafakazi	Usuku