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**Field evaluation of the PIMA™ Point-of-Care CD4 machine
in a mobile clinic, South Africa**

By

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SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In partial fulfillment of the requirements for the degree

Masters in Public Health

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of submission: 14 February 2011

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DECLARATION

I, Nienke van Schaik, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work or any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ACKNOWLEDGEMENTS

Landon Myer: My Supervisor: Your guidance and support has been incredible. Thank you for teaching me to structure my thoughts and focus on the relevant issues. More cake and less icing for that reader in Timbuktu!

Linda-Gail Bekker: My Co-supervisor: It has been a privilege to work with such an inspirational leader in the HIV field. Thank you for having the confidence in me to manage the Tutu Tester and for your ongoing support. May this be the first of many publications on the use of point-of-care technology on the Tutu Tester.

Katharina Kranzer: My mentor, colleague and friend. I could not have done this without you!

Elizabeth Thebus, Nikki Davies and Eudoxia Raditlhalo: My dedicated team of nurses who performed all the PIMA™ CD4 counts

Shandré Malan: My data capturer who entered the data timeously and accurately

The Tutu Tester counsellors, educators and driver: without your important contribution there would be no Tutu Tester

ABSTRACT

Introduction

Universal access to CD4 counts could be improved through easy-to-use point-of-care CD4 systems such as the PIMA™ Analyzer (PIMA™). Validation studies performed in a laboratory and stationary clinic showed promising results. This aim of this thesis was to validate the PIMA™ in a mobile clinic in South Africa in 2010 and to review current literature regarding CD4 technology, with a focus on point-of-care technology in the HIV infected individual.

Methods

Consecutive HIV-positive individuals (both on ART and not on ART) had a capillary and/or venous sample analyzed using the PIMA™ and a venous sample analyzed externally (Beckman Coulter EPICS XL-MCL flow cytometer (XL-MCL)). Linear regression and Bland-Altman was used to assess PIMA™ performance. The impact of operator, machine, training and time delay to laboratory were evaluated.

Results

The median laboratory CD4 in the 349 participants was 405 cells/μl (IQR 277 – 600) with CD4 ≤200, 201-350 and > 350 cells/μl being 11%, 29% and 60% respectively. The mean difference between PIMA™ and XL-MCL CD4 counts was -4.5 cells/μl (95%CI -12.4; 3.40) for venous samples (n=325) and 29.7 cells/μl (95%CI 16.93; 42.49) for capillary samples (n=167). Regression coefficients were 0.999 ($R^2=0.909$) and 1.006 ($R^2=0.882$) respectively.

Misclassification using a 200 cells/μl cutoff was < 3.1% but ±10% with a 350 cells/μl cutoff for both.

Multivariable regression analysis, adjusting for operator and training, showed machine used and delay to be significant for venous but not capillary samples.

Conclusion

The PIMA™ performance in a mobile clinic compared favourably with previous studies, identifying most individuals with CD4 counts < 200 cells/ μ l. Capillary PIMA™ samples gave less reliable estimates than venous samples. Machine variability needs further investigation.

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PART A: PROTOCOL

Field evaluation of the PIMA™ Point-of-care CD4 machine in a mobile clinic,
South Africa

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SCHEMA

Field evaluation of the PIMA™ Point-of-care CD4 machine in a mobile clinic,
South Africa

- Design:** This study is a cross-sectional study to compare the PIMA™ to the Beckman Coulter Epics XL-MCL in assessing CD4 counts obtained from HIV-infected patients in a mobile clinic setting using both venous and capillary samples.
- Duration:** Total study duration of approximately 11 months
- Sample Size:** A minimum of 165 venous and 165 capillary PIMA™ samples
- Study participants:** Study participants will consist of HIV positive individuals (newly diagnosed, known positive not on ART and known positive on ART) identified in the mobile clinic who consent to having both PIMA™ and laboratory CD4 counts done.
- Primary Study Objectives:**
1. To assess the agreement between the CD4 count obtained when venous or capillary blood was analyzed by the PIMA™ Analyzer on a mobile clinic and the same sample analyzed by a Beckman Coulter EPICS XL-MCL™
 2. To assess the agreement between capillary and venous samples using the same individual, same operator and same machine.

PART I - INTRODUCTION

1.1 Background

Point-of-care CD4 testing has recently become available in South Africa. Same day, on site availability of CD4 count results enables appropriate post-test counseling and, when indicated, immediate referral for antiretroviral treatment. This is particularly important in a mobile clinic where it is difficult to retrospectively contact clients with their laboratory CD4 result.

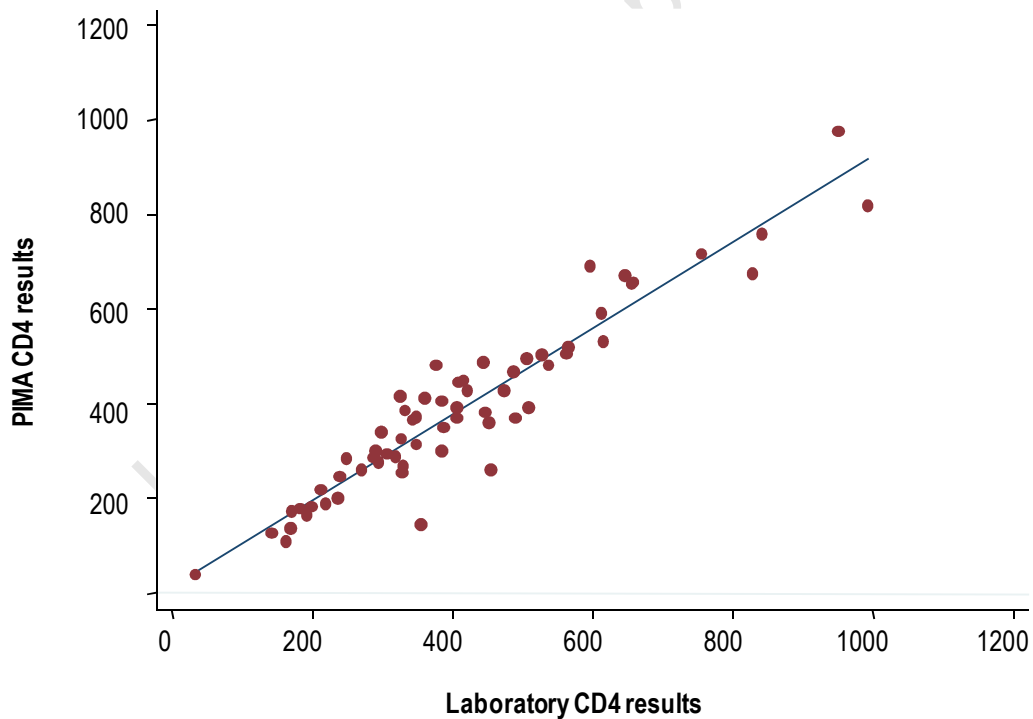
The PIMA™ Analyser, the latest point-of-care CD4 machine, is being used by the Desmond Tutu HIV Foundation in a mobile clinic setting on the Tutu Tester. It enables the determination of absolute counts of CD4 T- lymphocytes in whole blood without prior sample preparation¹ and is suitable for both finger-prick blood samples as well as venous blood samples. Full details are described elsewhere.^{1,2} In-laboratory validation by Glencross et al. showed good correlation using venous samples between the PIMA™ CD4 results and results determined by flow cytometry (Slope=0.96, R²= 0.98) on a Beckman Coulter XL. An overall bias of -12.02 cells/ μ l (\pm 38.65) was noted, with a slight under-estimation of absolute CD4 counts by the PIMA™ instrument.³ Excellent correlation of 165 PIMA™ capillary samples was found when compared to same day laboratory processing of specimens on a Becton Dickinson FACSCalibur in Zimbabwe. The overall bias was +7.6 cells/ μ l (95%CI:-6.5 to +21.8; P=0.72).¹ Greater bias was however found when the PIMA™ was evaluated in 2 other clinics in South Africa (D. Glencross[debbie.glencross@nhls.ac.za, Lecture, August 4, 2010).

The latter fact, together with our negative experience with another point-of-care CD4 technology, the POINTCARE NOW machine, has resulted in our decision to do an evaluation of the PIMA™ on our mobile clinic. In addition we feel that a validation in a mobile clinic is important in that the setting can be far from ideal: the machines are transported over large distances daily, operated on battery

power and used in varying environmental temperatures and conditions. It is likely that similar conditions will be encountered at sites where the PIMA™ will be most useful.

During the first month of using the machine all clients testing at the mobile clinic had both a laboratory and PIMA™ CD4 count done. Preliminary findings comparing PIMA™ results with CD4 count results determined on a Beckman Coulter Epics XL-MCL (XL-MCL) (Togatainer, Toga laboratories, Gugulethu) (n=63) showed good correlation (Slope=0.91, R2= 0.90) (Figure 1).

Figure 1: Comparison of point-of-care CD4 count results using the PIMA™ system with laboratory CD4 count results performed on the Beckman Coulter EPICS XL-MCL.



Staff also performed both dual capillary and venous samples on a small number of patients (n=12) to ascertain whether these methods give different results. A mean difference of 31.09 cells/ μ l (CI -16.42; 80.24) was found between capillary

and venous samples. This finding warrants further investigation. Ethical approval for this will be obtained as a sub-study on: Cross-Sectional Survey of HIV infection and CD4 counts in Masiphumelele township (REC REF 262/2010).

1.2 Aims and Objectives

Aim:

To perform a field evaluation of the PIMA™ CD4 Analyzer in a mobile clinic setting in South Africa

Primary Objectives:

1. To assess the agreement between the CD4 count obtained when venous or capillary blood was analyzed by the PIMA™ Analyzer on a mobile clinic and the same sample analyzed by a Beckman Coulter EPICS XL-MCL™
2. To assess the agreement between capillary and venous samples using the same individual, same operator and same machine

Secondary Objectives:

1. To assess factors associated with greater or lesser agreement: machine, user and the impact of training sessions
2. To calculate misclassification around the current CD4 based cutoffs of 200 and 350 cells/ μ l as used in South Africa

PART II - METHODS

2.1 Study design

Cross-sectional study

2.2 Study setting

The Tutu Tester is a nurse run, counsellor supported mobile screening clinic run by the Desmond Tutu HIV Foundation. It is a fully mobile vehicle with 3 rooms and also has tents which can be pitched to provide additional space. It operates 5 days a week mainly in underserved peri-urban areas in greater Cape Town and rotates through sites such as township shopping centres, taxi ranks and stations, as well as on the road side. This service is not formally advertised and hence attracts ambulatory clients who spontaneously access HIV testing. This client initiated HIV testing is offered in combination with screening for other chronic conditions such as hypertension, diabetes and obesity in an attempt to normalize testing and is performed according to the latest Western Cape HIV testing guidelines⁴.

Medical history, physical examination findings and risk reduction information is recorded on a client form (Appendix 1)

The standard of care for all Tutu tester clients is that if they test positive on their first HIV test, staff will obtain a second blood sample (either capillary, venous or both if necessary) and perform a confirmatory HIV test, syphilis test and a CD4 count. The latter is either a PIMA™ Point-of-care CD4 count (Alere, Waltham, USA) and/or laboratory CD4 performed at the Togatiner (TOGA Laboratories, Gugulethu). Clinical staging is performed and a pregnancy test is done if the client is female.

2.3 Subjects

2.3.1 Study population

Study participants will consist of HIV positive individuals (newly diagnosed, known positive not on ART and known positive on ART) identified in the mobile clinic that consent to having both PIMA™ and laboratory CD4 counts done.

They include:

1. Those from the preliminary field study
2. All HIV positive individuals who consent to taking part in the “Active Tuberculosis Case Finding in individuals accessing a mobile voluntary counselling and testing service” (REC REF: 507/2008) (Appendix 2). They will have a PIMA™ CD4 count done on the Tutu Tester as well as a laboratory CD4 count as part of study procedures.
3. Those who consent to taking part in the Cross-Sectional Survey of HIV infection and CD4 counts in Masiphumelele township (REC REF 262/2010) and give additional consent for providing both venous and capillary PIMA™ samples (Appendix 3: sub-study 2)

Inclusion criteria:

1. HIV–infected individuals
2. Age 18 years and older
3. Able and willing to provide the relevant informed consent

Exclusion criteria:

1. Individuals with indeterminate HIV results at the time of testing and who had CD4 counts done but are PCR negative
2. Age less than 18 years old
3. Unable to provide informed consent

2.3.2 Sampling strategy and size

Consecutive sampling will be used to select patients.

Our sample size calculation is based on the initial NHLS data for 50 patients with venous PIMA samples where the mean difference was -12.02 (+-38.65) cells/ μ l between PIMA and laboratory samples. Our hypothesis is that our mean difference will be 0. The estimated sample size for a one-sample comparison of mean to the hypothesized value of 0 is 109 using a power of 0.90 and a significance level of 0.05.

However a target size of 165 samples will be set for both the venous and capillary arms so that our sample is at least as large as the literature currently published¹.

For the dual venous and capillary PIMA sample group we will however aim to get a sample size of 109 to limit study costs.

2.4 Study procedures

PIMA™ CD4 testing

All staff operating the PIMA™ CD4 Analyzer will be trained by an Alere Healthcare representative on the use of the PIMA™ device and the correct sample collection techniques.

Materials will be stored and daily controls will be run according to the manufacturer's instructions^{2,5,6}. The required 25 μ l of blood will be collected into a PIMA™ cartridge from either a finger prick or venous sample or both. The cartridge will be capped and then inserted immediately into the Analyzer.

Nurses will use a 1.8mm blade lancet for the capillary samples. Venous blood will be collected by phlebotomy in a 4ml EDTA tube and transferred to a PIMA™ cassette by means of a pipette. The sample will be run on the PIMA™ Analyzer available for use on the mobile clinic.

The nurse will document if the sample is venous or capillary. The time, date, operator and machine number are all reflected on the result printout.

Where dual samples are taken, the nursing will first do a PIMA™ CD4 test using capillary blood and then using the venous sample. The nurse who takes the venous samples must be responsible for transferring the venous blood to the PIMA™ cartridge. The samples must be run on the same machine.

Laboratory testing

Venous blood collected in a 4ml EDTA tube will be kept at room temperature and transported to the Togatainer on the next working day. The specimens will be analyzed using their standardized protocols on a Beckman Coulter EPICS XL-MCL™ (XL-MCL) flow cytometer. Both internal and external quality control procedures are in place at the laboratory. Laboratory staff will be blinded to the PIMA™ results.

Results stating the date of receipt at the laboratory and the CD4+ T-lymphocytes and %CD4 (percentage of total lymphocytes) will be emailed to the investigator.

CD4 results

All HIV positive clients will be given their PIMA™ results at the time of their Tutu Tester visit and encouraged to attend clinics for either comprehensive HIV care (PIMA™ CD4 count > 201 cells/μl) or to start ART if eligible (PIMA™ CD4 count ≤ 200 cells/μl or WHO stage 4). Where dual samples were taken clients will be asked to wait for, and receive, their capillary result. Waiting for the venous PIMA™ result will be optional.

The client will be contacted by telephone with their laboratory CD4 result (usually within 72 hours) and, where applicable, their venous PIMA™ CD4 count result if permission to do this was given (see section 2.5 Data collection). Alternatively they can collect their result from the Tutu Tester.

The PIMA™ and laboratory results could differ in a way that would result in a change of the client's management. If we are unable to contact the client telephonically, a home visit will be attempted if permission was given to do so, to give the client their result. An additional sample for laboratory CD4 testing will be taken where possible to assist in the further management of the client.

2.5 Data Collection

Routine data is collected on all Tutu Tester clients (Appendix 1). CD4 results will be recorded on the study specific data collection form (Appendix 4).

HIV positive clients will be requested to give their name and surname and contact details (see attached client details form- appendix 5). This specifies if, and how, we can contact the client in order to provide them with their laboratory CD4 count result. The client is asked to verify that the details are correct by signing the form.

2.6 Data management

All forms are returned to the Tutu Tester office in the evening and locked in the data room. Forms, together with laboratory results, belonging to HIV positive clients are kept in a locked cupboard in the data room with only relevant staff members having access to the files. All Tutu Tester staff is required to sign a confidentiality agreement when they start working on the project

Data is entered anonymously into an Access database designed for the study. Data entry is performed by designated staff members of the Desmond Tutu HIV Foundation. The database is password protected with only limited designated staff having access to them. The database is stored on a data server located at the DTHC which is backed up daily. Queries are run for inconsistencies and data is corrected continuously.

Data required for the purpose of this analysis will be obtained by running a query on the database.

2.7 Staff training

All nursing staff are trained in the correct methodology for rapid HIV testing, the use of the PIMA™ Analyzer and Good Clinical Practice.

2.8 Biohazard containment

Study staff will follow universal precautions when handling the specimens. This includes wearing of gloves.

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PART III – DATA ANALYSIS

Statistical analysis will be performed using STATA (Version 11.0, College Station, Texas, USA).

Data will be explored via simple proportions, medians, means, cross-tabulations and chi square tests. A Wilcoxon Signed Tank test will be used to calculate differences in median results between PIMA™ CD4 and laboratory CD4 results.

The sensitivity and specificity for the PIMA™ CD4 results, as well as and Kappa statistics, will be calculated using laboratory cut-offs of 200 and 350 cells/ μ l.

The data will be analyzed using scatter plots and the correlation co-efficient will be calculated. We will then perform a linear regression analysis and attempt to predict factors impacting on the performance of the PIMA™. The factors of interest are operator, machine and training.

Bland-Altman analysis will be done to determine the systematic bias of the PIMA™ relative to the XL-MCL.

PART IV - ETHICS AND COMMUNICATION

4.1 Ethical Considerations

Ethics has already been obtained for reporting of programmatic data from the Tutu Tester (Appendix 6).

All the procedures described here will be reviewed and approved by the University of Cape Town Research Ethics Committee. This study also adheres to the declaration of Helsinki 2008.

a. Participant Withdrawal

Study participation may be discontinued for either of the following reasons:

- The participant withdraws her consent. Participants may withdraw from study participation at any time, for any reason. This will in no way alter their treatment at the Tutu Tester.
- The nurse determines it to be in the best interest of the subject for a medical reason or the study for a technical reason.

b. Informed Consent

Informed consent will be obtained from all study participants. This will be verbal in routine Tutu Tester clients as well as for patients taking part in the active TB case finding study. Written consent will be obtained by one of the Tutu Tester nurses where both a venous PIMA™ and capillary PIMA™ and laboratory sample are required (Appendix 7). The rationale of the study, procedural details and investigational goals will be explained to each participant together with potential risks and benefits via the informed consent form.

Consent forms will be available in English and Xhosa. All translations will be checked to verify content.

c. Risks and Discomforts

- Capillary and Venous blood samples are invasive and might cause some discomfort. All nurses are trained in finger-prick sampling and venous blood sampling. This study will in no way alter the clinical management of participating patients.

d. Benefits

- The direct benefit to the participant for taking part in this study is that they will be able to obtain have confirmation of their CD4 count both immediately and with a follow-up telephone call for their laboratory result.
- An indirect benefit would be the opportunity to help researchers find out if there is better correlation with one method versus the other to determine best practice on the Tutu Tester.

e. Confidentiality

Every effort will be put in place to maintain participant's confidentiality. The staff will conduct follow-ups in accordance with the Tutu Tester SOP for follow-ups. Confidential data will be managed as discussed in section 2.5 above.

4.2 Dissemination of results

An abstract will be submitted in October 2010 for the CROI conference in February 2011. A manuscript entitled: "Field evaluation of the PIMA™ Point-of-care Cd4 machine in a mobile clinic, South Africa" will be completed by Month 11. Results and feedback will also be made available to Alere Healthcare on an ongoing basis as well as to our funders.

PART V - LOGISTICS

	Month										
	1	2	3	4	5	6	7	8	9	10	11
	Mar 2010	April 2010	May 2010	June 2010	July 2010	Aug 2010	Sep 2010	Oct 2010	Nov 2010	Dec 2010	Jan 2011
1) Data collection & entry											
Routine Tutu Tester Clients											
Active TB Case Finding study											
Dual samples from Cross- Sectional Survey											
2) Data analysis											
3) Manuscripts											
CROI abstract											
Manuscript											

PART VI - BUDGET

The cost of the PIMA™ CD4 and laboratory tests will be covered as Tutu Tester operating costs.

PART VII – BIBLIOGRAPHY

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PART B: LITERATURE REVIEW

Background

South Africa continues to be home to the world's largest population of people living with Human Immunodeficiency virus (HIV)^{1,2 3}. Despite having more than 971 000 people on antiretroviral therapy (ART) in October 2009⁴, the ART coverage in this large, continually expanding programme is still incomplete and was estimated to be only around 28-40% in 2008^{5 6}.

Studies from South Africa have show that only 55-90% of individuals have their CD4 count done after testing HIV positive despite the time frames being generous in these studies and ranging from two to six months⁷⁻⁹. Furthermore only 46-65% of individuals receive their CD4 count results⁷⁻¹⁰.

This is concerning as the enumeration of absolute numbers of CD4-positive T-lymphocytes plays an essential part in the monitoring of those with HIV: determining when ART is needed, monitoring the effectiveness of ART and indicating possible treatment failure. Moreover the current South African treatment initiation guidelines¹¹ are CD4 based.

This high attrition rate between being tested and receiving a CD4 count result means that individuals eligible for ART either have lengthy delays before ART initiation or are lost to care. If insufficient emphasis was placed by health-care workers on the importance of knowing the CD4 count or the benefit of remaining in care despite feeling otherwise well, individuals may not understand the importance of returning for their result. Long waiting times due to high caseload may also be a reason why individuals fail to return for their results and subsequent appointments¹⁰.

Reductions in treatment delays would improve morbidity and mortality¹²⁻¹⁵, including reducing HIV-associated Tuberculosis (TB)¹⁶ and reducing transmission of HIV from mother to child^{17,18}. In this light the latest WHO guidelines²

recommend earlier diagnosis through expanded testing programmes and ART initiation for all HIV-infected individuals with a CD4 count of ≤ 350 cells/ μ l. The guidelines reiterate that the lack of laboratory monitoring should not be a barrier to initiating ART and those with WHO clinical stage 3 or 4 should be initiated on ART if CD4 testing is not available. The WHO guidelines also encourage CD4 testing in those with WHO clinical stage 1 and 2 to timeously identify those needing ART².

Furthermore a recent cost-effectiveness analysis¹⁹ recommended that countries with very limited resources and still only one line of ART available should prioritize access to CD4 count monitoring and ART initiation at CD4 ≤ 350 cells/ μ l to offer the best survival outcomes.

The anticipated escalation in numbers of people in South Africa requiring CD4 counts as more individuals are found to be HIV-positive following the launch of the National HIV testing campaign, and current up scaling of the ART programme, may overburden the current National Health Laboratory Services (NHLS). Alternative reliable methods of performing CD4 counts need to be evaluated to improve access to, and reduce overall costs of performing CD4 assays especially in more outlying or lower burden areas.²⁰

The objective of this literature review is to ascertain methods in which the ever growing need for CD4 counts for HIV-infected individuals can be addressed with a special focus on the benefits of point-of-care technology.

Literature search strategy: inclusion and exclusion criteria

A computerized search of electronic databases was performed to find available literature on Point-of -Care CD4 technology.

The search terms used for Medline were: (CD4 Lymphocyte Count [MESH] or CD4-positive T-lymphocytes [MESH]) AND (Point-of-care systems [MESH] or POC (text term) or point*of*care (text term)). This search was combined with a

search using: (CD4 Lymphocyte Count [MESH] or CD4-positive T-lymphocytes [MESH]) AND (HIV infections [MESH]) AND (Flow cytometry [MESH] or image cytometry [MESH] or flow cytometry (text term)). The search was restricted to humans and papers published in the last 10 years (2001 through to January 2011) as point-of-care technology is a new development in this field. Only papers published in English were reviewed. The 572 citations were screened by title to capture potentially suitable publications. The abstracts of the suitable papers were then reviewed and the full text of suitable original papers and scientific letters was then obtained.

Reference lists of primary studies, reviews and editorials identified by the above methods were searched for additional articles. Experts in the field were contacted for additional literature, including abstracts from conferences. The manufacturers of the PIMA™ machine were able to provide additional evaluations of the machine but unfortunately none of these have been published to date and so were not used.

The current situation

Universal access to CD4 count monitoring, in South Africa and other resource-limited countries, is hindered by centralized laboratory services, the challenges of transporting samples to these laboratories, cost and delays in receiving results²¹.

Options to meet the need for improved access to CD4 count monitoring include finding alternatives to CD4 counts, decentralizing or addressing challenges faced by laboratories and point-of-care CD4 counts. These will be discussed in detail below.

Various studies have evaluated alternative methods to CD4 counts using laboratory measures which may be more readily available including total lymphocyte counts (TLC)²²; combining clinical staging with TLC, or combining TLC with haemoglobin levels, platelet counts and body mass index²²⁻²⁷. These have given conflicting results and are no longer recommended^{2,28}.

Clinical assessments alone may miss individuals who appear clinically well yet are severely immune-compromised²⁹. Relying solely on clinical staging according to WHO criteria requires that patients be seen by clinicians or experienced and trained nursing staff³⁰. Access to this cadre of healthcare personnel is often an issue in exactly those areas where access to CD4 counts is also lacking^{31,32}. POC CD4 counts would enable task shifting for HIV care and treatment to less skilled nurses and potential other health workers³⁰.

Currently flow cytometry remains the accepted gold standard for performing CD4 counts^{20,33}. It is an established laboratory technique that was available even prior to the HIV epidemic to confirm and monitor immune suppression³⁴. Flow cytometry simultaneously measures and analyses multiple physical characteristics of cells as they flow in a fluid stream through a light beam measuring the cell's relative size, granularity and fluorescence intensity. Lasers are used to excite fluorescent antibody probes specific for CD4 T-lymphocytes.³⁵ A simplified single platform (SP) PanLeucogated (PLG) CD4 methodology is now most commonly used in South Africa³⁶.

Most commercially available flow cytometers have automated procedures allowing many specimens to be tested simultaneously^{37 38}. The high output flow cytometers, performing from 250 to 350 samples per day³⁸, include the FACSCalibur (Becton Dickinson, Franklin Lakes, NJ)³⁹, EPICS XL-MCL(Beckman Coulter, Brea, CA)⁴⁰ and Cyflow SL-3 (Partec, Münster, Germany)⁴¹. Low-intermediate throughput machines can perform 30 – 100 tests per day³⁸ and includes the FACSCount (Becton Dickinson, Franklin Lakes, NJ)³⁹, Cyflow Counter (Partec, Münster, Germany)⁴¹, Easycyte (Millipore, Billerica, MA) (previously EasyCD4, Guava Technologies, Hayward, CA)⁴²and PointCare Now (PointCare Technologies, Malborough, MA)⁴³.

In addition non-flow cytometry techniques can be employed in laboratories. Existing non-flow cytometry techniques are typically manual or semi-manual assays and perform less than 50 tests per day³⁸. Manual magnetic isolation of

cells is done on a haematology analyzer with Sysmex Dynabeads (Sysmex)⁴⁴ or using a microscope count with Dynabeads (Invitrogen, previously Dynal SA)⁴⁵. Manual antibody-based identification of cells and microscope counts is done with Coulter® Manual CD4 count kit (previously Cytosphere) (Beckman-Coulter)⁴⁶

For all the above-mentioned laboratory-based CD4 testing techniques venous samples need to be obtained by phlebotomy. This requires the attending health care provider to have the necessary skills to do so which remains a limiting factor in many health care facilities.

Further issues with laboratory testing include:

- Delays in samples reaching the laboratory causes sample aging. Aging of blood samples collected in a K2EDTA tube causes changes in all white blood cells which affect the light scatter properties of the cells. This makes distinguishing monocytes from lymphocytes difficult in routine flow cytometry. Automated gating algorithms usually fail to identify the relevant populations and the technologist thus has to manually gate these samples. This can be challenging and, if monocytes are counted as T-helper cells, it could lead to an overestimation of the CD4 count (W.Pretorius [willem.pretorius@alere.com], email, May 16, 2011). Another factor that could influence the CD4 count in aged specimens is ongoing spontaneous apoptosis which appears to occur more in samples from patients with low CD4 Counts⁴⁷.

Unsatisfactory storage conditions of samples (temperatures more than 25 degrees Celsius), especially when transporting specimens from remote areas to a central laboratory, can also accelerate these changes.

Because there are multiple factors at play it is difficult to predict if aged specimens will over or underestimate the CD4 count. In order to get accurate CD4 counts specimens should be analysed no later than 48 hours after the specimen was collected.

- Adequate human resources are often lacking in resource-limited settings^{31,32} yet skilled and trained personnel are required to operate the machines available in the laboratories.
- Incorrect pipetting techniques cause errors in absolute cell counting especially when sample preparation is required. Cap piercing technology is not employed by all machines²⁰.
- Flow cytometers such as FACSCount require liquid format reagents⁴⁸. Cold-chain transportation and refrigeration are needed to maintain reagent integrity. Whilst this is not an issue in developed countries, resource-limited rural settings are faced with unreliable cold-chain transportation and inconsistent power supplies. Maintaining a cold chain adds additional cost to an already expensive test especially for remote laboratories with low patient flow.
- A lack of maintenance of the machines and hence non-functioning machines as well as procurement issues for reagents³⁰ are of concern especially for dedicated flow cytometers at remote sites.

Solutions

Suggestions for improving access to CD4 count results include:

- Reduced turnaround times for results by increasing laboratory capacity
- Making CD4 count results more broadly available such that results could be accessed at clinic-level rather than at hospital level where the patient may have been seen initially¹⁰. This would mean that patients would not need to make costly trips to obtain their results³⁰.

Suggestions for prolonging sample stability include:

- Transfix, a blood stabilizing compound⁴⁹
- BD CD4 stabilization tubes (ST [Vacutainer CD4 stabilization blood collection tubes; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ]).⁵⁰

Suggestions for maintaining reagent integrity:

Liquid reagents in remote laboratories could be replaced with dried reagent kits such as Rea T Count (Reamatrix, Bangalore, India)⁴⁸. It was found to be a suitable replacement for liquid reagents normally used with the FACSCount if adequate mixing of the dry reagent occurs⁴⁸.

Researchers worldwide are thus striving towards developing a point-of-care (POC) method for performing CD4 counts.

Point-of-care testing

POC testing has been defined as “any investigation carried out in a clinical setting or a patient’s home for which the result is available without reference to a laboratory and perhaps rapidly enough to affect immediate patient management”⁵¹

The ideal characteristics of a universal POC CD4 test for low-income countries are: (i) It should be simple to use^{20,30,38} so that it can be performed with minimal training. This could allow for task-shifting to less skilled health personnel. (ii) It should be able to be performed using non-venous blood³⁰: This means that phlebotomy skills are not required to perform the test. (iii) The result should be easy to read and interpret³⁰. (iv) Any equipment required should be robust^{20,52,53} so that it can withstand a wide array of environmental conditions such as hot, dry, dusty or humid climates. (v) It should be reliable³⁰ and give precise and reproducible results. This reduces the need to repeat tests and decreases the cost per patient. (vi) The test kit or reagents should not be cold chain dependent and should be able to withstand hot climates of up to 40 C^{20,30,34,38,52} (vii) The test kits should have a long shelf life^{20,53} of up to 15 months³⁰ (viii) Processing of the test should be short; about 10 minutes has been recommended.³⁰ (ix) It should be affordable; a cost of \$1-2^{20,30} would be acceptable to most low-income countries. (x) Bio-hazardous waste generated should be minimal^{30,52}. (xi) There should be some form of quality control and ideally the test should be suitable for

an external quality assurance programme^{30,34,38,52} such as QASI and AFREQAS. (xii) The maintenance for any machinery should be minimal as access to routine technological maintenance can be difficult^{38,52,53}. In addition replacement units should be readily available³⁸. (xiii) It should also not rely on basic services such as a consistent electricity supply^{20,52,53} or clean water²⁰.

The availability of point-of-care (POC) CD4 technology with characteristics such as those described above should increase the number of CD4 counts done as well as the number of individuals receiving their results, especially in mobile clinic settings or when transport costs and access to health care facilities (both through user fees and availability of staff) are barriers to returning to the facility.^{30,54,55}

When POC HIV testing was newly introduced, it was shown that 99% of individuals testing at mobile clinics in Zimbabwe chose same day HIV results over receiving their results at a future date at their local clinic.⁵⁶ The uptake of POC CD4 is unlikely to be less than this.

Ideally one would offer HIV testing in combination with POC CD4 testing³⁰. This would enable directed post-test counseling, based on the CD4 result, and would enable immediate referral for ART initiation. POC CD4 testing could potentially increase both the number of individuals remaining in HIV wellness programmes as well as the number of people initiating ART.

Concerns regarding POC CD4 testing

Concerns around providing POC CD4 tests include:

- The task of performing the POC CD4 test may become the responsibility of the nursing staff who typically already face high patient volumes and high workloads.³⁸ This change in job description may lead to low levels of motivation³⁸ and the increased level of responsibility may require a suitable salary increase³².
- Strict quality control procedures need to be in place to ensure that test are being performed correctly such that results produced are accurate

- True POC testing does not offer economy of scale. Consumable and labour costs, which make up the bulk of the overall cost of performing a POC test do not change regardless of how many tests are performed^{37,38}. This is in contrast to laboratory testing, where the most significant cost is the fixed cost of running the equipment whilst the cost of consumables remains insignificant³⁷
- Tests may be done unnecessarily due to the ease of having the test results available during the time-frame of the consultation

Current options for CD4 testing in mobile clinics in South Africa

Although several such innovative means of assessing CD4 counts using non-flow cytometry have been published over the past few years, they have either not been commercialized or not been rolled out on a large scale^{21,35,57-67}.

Three options were considered for use in our mobile clinic which is in a primary care setting and where nurses, rather than laboratory technicians, perform the CD4 counts. They were the Easycyte (Millipore, Billerica, MA) (previously EasyCD4, Guava Technologies, Hayward, CA)⁴², the PointCare Now (PointCare Technologies, Malborough, MA)⁴³ and the PIMA™ Analyser (Alere, Waltham, USA).

The Guava Easy CD4 uses microcapillary single flow cytometry. This eliminates the need for sheath fluid and thus requires smaller samples and fewer reagents, with minimal waste. Several evaluations have found that it compares well with flow cytometry both in laboratory and clinical settings^{52,68-70}. Although it is compact and ideally sized for small laboratories, it is not suitable for our mobile clinic setting. The sample requires pipetting and further preparation by a dedicated nurse, detracting from patient care. Furthermore the knocks sustained when driving the mobile as well as the vibrations as people step on and off the mobile are likely to cause misalignment of the laser technology⁵³.

No published literature is available on the Pointcare NOW⁴³. This compact and portable modified flow cytometer uses heat-stable colloidal gold-labelled reagents instead of monoclonal antibodies and fluorescence. This eliminates the need for fluorescence signal filter detectors²⁰ and should make it less likely to have optical alignment problems. Unpublished data from our mobile clinic however showed that results were poor and use of the machine was discontinued.

The PIMA™ Analyser (ALERE, Waltham, USA) is the only commercially available true POC CD4 machine in South Africa. It is suitable for performing POC CD4 counts using both finger-prick (capillary) and venous blood samples. CD4-lymphocytes are labeled with fluorescing monoclonal antibody labels which are detected using image cytometry^{71,72}. It can be readily transported to site in the carrier bag provided, can be battery operated and has no liquid bio-hazardous waste. In addition it is easy to operate, requires no specific technical skills to do so and requires little maintenance. The cartridges are also heat stable.

Recent evaluations show good correlation between PIMA™ CD4 results and laboratory CD4 counts⁷³⁻⁷⁵. In-laboratory validation⁷⁵ at the National Health Laboratory Service (NHLS) in Johannesburg between PIMA™ CD4 results from venous samples and results determined by flow cytometry based PLG/CD4 showed a slight under-estimation of absolute CD4 counts by the PIMA™ Analyzer (Table 1). Capillary samples run on the PIMA™ Analyzer in Zimbabwe showed a slight over-estimation of absolute CD4 counts when compared to same day laboratory processing of venous blood specimens from the same individual⁷³ (Table 1).

Table 1. Comparison of CD4 results from evaluations of the PIMA™ Analyzer

	N	PIMA sample type	Laboratory CD4 range	Bias	Slope	R ²
Coetzee al. ⁷⁵	100	Venous	3 - 1332	-16.86 ± 36.7	0.913 ± 0.013	0.981
Mtapuri-Zinyowera et al ⁷³	165	Capillary	1 - 1297	+7.6 (CI -6.6 ; 21.8)	Not done	Not done

Variability of CD4 counts results

Various techniques for determining CD4 counts are currently available as described above, however they vary in accuracy and reproducibility as no definitive “gold standard” for CD4 counts exists. In addition physiological intra-subject factors can lead to variability in CD4 count measurements. CD4 counts vary by time of day^{76,77}, as well as by season, and may also be influenced by acute and chronic infections⁷⁸. The CD4 count can drop temporarily during pregnancy, infections (e.g. Tuberculosis) and during other systemic conditions⁷⁸. Treatment with corticosteroids can also cause a significant drop in the CD4 count⁷⁸.

Typically the CD4 cell count decreases progressively over time and decreases by 50-75 cells/μl per year in the absence of ARVs⁷⁸. The CD4 rises after ART initiation: typically by 50 cells/μl in the first weeks, and then 50-100 cells/μl if there is good viral suppression⁷⁸.

Opportunistic infections and well as the risk of developing immune reconstitution syndrome become more common with declining CD4 counts but are uncommon with CD4 counts over 500 cells/ μ l and most common below 200, with those below 100 and below 50 at increasing risk⁷⁸. Variations in CD4 count in those with counts greater than 500 have less clinical impact. However, the lower the CD4 count, the more important it is for the CD4 count to be accurate, regardless of the technology used.

Identification of gaps for further research

To date there is a paucity of data from sites such as mobile clinics where the PIMA™ CD4 analyzer is most likely to be used with the greatest benefit. Mobile clinics are different to standard laboratory environments and can be far from ideal: the machines are transported over large distances daily, operated on battery power and used in varying temperatures and conditions.

It is also unclear as to which method of blood collection for CD4 counts is most suitable as there is no data comparing venous to capillary PIMA™ samples taken from the same individual.

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PART C: MANUSCRIPT

This will be formatted for submission to JAIDS. Instructions for authors are available in Appendix 8. The Authorship Responsibility, Financial Disclosure, and Copyright Transfer form is also included.

Supplementary figures and tables are in Appendix 9.

The machines were donated by Alere Healthcare, but Alere had no part in design, conduct or analysis of the study.

University of Cape Town

Full title:

Field evaluation of the PIMA™ Analyzer Point-of-care CD4 machine in a mobile clinic, South Africa

Running head:

Field evaluation of the PIMA™ Analyzer

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Meetings at which part of the data were presented:

18th Conference on Retroviruses and Opportunistic Infections, Boston,
27February 2011- 02 March 2011(Abstract V144)

Sources of funding:

United States Agency for International Development (USAID): ANOVA funded
USAID programme
Agence Française de Développement (AFD)

Abstract word count: 250

Text word count: 3551

Tables: 5

References: 30

Abstract

Introduction

Universal access to CD4 counts could be improved through easy-to-use point-of-care CD4 systems such as the PIMA™ Analyzer (PIMA™). Validation studies performed in a laboratory and stationary clinic showed promising results. This study aimed to validate the PIMA™ in a mobile clinic in South Africa in 2010.

Methods

Consecutive HIV-positive individuals (both on ART and not on ART) had a capillary and/or venous sample analyzed using the PIMA™ and a venous sample analyzed externally (Beckman Coulter EPICS XL-MCL flow cytometer (XL-MCL)). Linear regression and Bland-Altman was used to assess PIMA™ performance. The impact of operator, machine, training and time delay to laboratory were evaluated.

Results

The median laboratory CD4 in the 349 participants was 405 cells/μl (IQR 277 – 600) with CD4 ≤200, 201-350 and > 350 cells/μl being 11%, 29% and 60% respectively. The mean difference between PIMA™ and XL-MCL CD4 counts was -4.5 cells/μl (95%CI -12.4; 3.40) for venous samples (n=325) and 29.7 cells/μl (95%CI 16.93; 42.49) for capillary samples (n=167). Regression coefficients were 0.999 ($R^2=0.909$) and 1.006 ($R^2=0.882$) respectively.

Misclassification using a 200 cells/μl cutoff was < 3.1% but ±10% with a 350 cells/μl cutoff for both.

Multivariable regression analysis, adjusting for operator and training, showed machine used and delay to be significant for venous but not capillary samples.

Conclusion

The PIMA™ performance in a mobile clinic compared favourably with previous studies, identifying most individuals with CD4 counts < 200 cells/ μ l. Capillary PIMA™ samples gave less reliable estimates than venous samples. Machine variability needs further investigation.

Key words: CD4 lymphocyte count, point-of-care, mobile clinic, HIV, PIMA™

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Introduction

South Africa is home to the world's largest population of people living with Human Immunodeficiency virus (HIV)^{1,2,3}. Despite having more than 971 000 people on antiretroviral therapy (ART) in 2009⁴, the programme must grow significantly to meet demand for ART^{5,6}.

The majority of treatment initiation guidelines, including the current WHO guidelines², are CD4 based. A recent cost-effectiveness analysis⁷ recommended that resource-limited countries with only one line of ART available should prioritize access to CD4 count monitoring and ART initiation at CD4 \leq 350 cells/ μ l to offer the best survival outcomes. However, universal access to CD4 count monitoring is hindered by centralized laboratory services, the challenges of transporting samples to these laboratories, cost and delays in receiving results. Recent studies in settings that rely on laboratory-based CD4 enumeration have shown that only 55-90% of individuals have a CD4 count within 2 to 6 months of testing HIV positive, and of those only 46-65% will return to receive their CD4 count result.⁸⁻¹⁰ Individuals who are potentially eligible for ART are thus lost to care.

The availability of point-of-care (POC) CD4 technology should increase the number of CD4 counts done and the number of individuals receiving their results. POC CD4 counts may be especially valuable in mobile clinics or when transport costs and access to health care facilities (both through user fees and availability of staff) are barriers to returning to the facility^{11,12}. For HIV testing services, the immediate availability of CD4 count results enables appropriate post-test counselling for HIV-infected clients and, when indicated, immediate referral for ART. This reduction in treatment delays would improve morbidity and mortality¹³⁻¹⁵ and decrease transmission of HIV from mother to child^{16,17}. The PIMA™ Analyser (PIMA™) (Alere, Waltham, USA) is one of the few commercially available POC CD4 machines. Recent evaluations show good

correlation between PIMA™ CD4 results and laboratory CD4 counts¹⁸⁻²⁰. To date there is a paucity of data from sites such as mobile clinics where the PIMA™ is most likely to be used with the greatest benefit. In addition, there are no published data comparing the two possible PIMA™ blood collection techniques (venous and capillary) on samples taken from the same individual.

We evaluated the PIMA™ by assessing CD4 counts obtained from HIV-infected patients attending a mobile clinic and determining factors which may impact on the performance of the PIMA™.

Methods

This cross-sectional study comparing PIMA™ and laboratory CD4 results was done in a primary care setting in Cape Town, South Africa, on samples obtained from patients who accessed a nurse-run, counsellor supported mobile clinic between March and November 2010..A CD4 count (PIMA™ and/or laboratory) was done as part of routine care for HIV infected individuals. Clients were referred for either ART or HIV care based on their CD4 result and clinical staging findings.

Study participants consisted of consecutive HIV positive individuals (newly diagnosed, known positive not on ART and known positive on ART) identified in the mobile clinic who consented to having both PIMA™ and laboratory CD4 counts done. A subset of individuals consented to having both capillary and venous PIMA™ CD4 counts and a laboratory CD4 count performed.

PIMA™ CD4 testing

The PIMA™ CD4 test enables the determination of absolute counts of CD4 T-lymphocytes in whole blood without prior sample preparation. Full details are described elsewhere¹⁸.

The PIMA™ is particularly suitable for mobile clinic settings as it can be readily transported to site in the carrier bag provided, can be battery operated and has no liquid bio-hazardous waste. In addition it is easy to operate, requires no specific technical skills to do so and requires minimal maintenance. The cartridges are heat stable and the cost per test (excluding the price of the machine) is no more expensive than a laboratory CD4 count.

Nine different PIMA™ machines were used over the course of the study. The manufacturer's instructions were followed for installation, operation, storage of test materials and running of daily controls. Three training sessions for operators were held: one before the study commenced (March 2010) and two additional refresher trainings (July and October 2010). The sessions were conducted by trained Alere Healthcare representatives and included instruction on the use of the PIMA™ device and the correct sample collection techniques.

The required 25µl of blood was collected into a PIMA™ cartridge from a finger prick and/or venous sample. Nurses were asked to use a 1.8mm blade lancet for the capillary samples. Venous blood was collected by phlebotomy in a 4ml EDTA tube and transferred to a PIMA™ cassette by means of a plastic non-calibrated pipette or an EDTA-filled capillary tube. Nurses self-selected the method in which the sample would be collected unless the individual had consented to both.

Laboratory testing

Venous blood collected in a 4ml EDTA tube was kept at room temperature and transported to a reference laboratory (Togatainer Gugulethu, Toga, Johannesburg, South Africa), usually the next working day. Specimens were analyzed using standardized protocols on a Beckman Coulter EPICS XL-MCL™ (XL-MCL) flow cytometer (Beckman-Coulter, Miami, FL). Both internal and external (AFREQAS through the NHLS) quality control procedures are in place at the laboratory. The laboratory personnel were blinded to the PIMA™ results.

Data analysis

Each PIMA™ sample was classified as being either “venous” or “capillary”, although some patients had “both”; in these cases, both a venous and capillary specimen were taken from the same individual, collected by the same operator and analyzed on the same machine. The time, date, operator and machine number were reflected on the PIMA™ result printout.

The variable, training, indicates the number of training sessions attended by the operator at the time of performing each PIMA™ test.

The number of days taken for the sample to reach the laboratory (days to lab) was calculated. A specimen was classified as delayed if “days to lab” was more than one day. We intentionally included all samples in our analysis as delays in reaching a laboratory are not an uncommon occurrence in Sub-Saharan Africa²¹ but repeated our analysis using only those XL-MCL specimens processed at the laboratory within one day.

The XL-MCL CD4 results, the “gold standard” were stratified into three groups (CD4 group) for further analysis in keeping with current cut-offs for ART initiation: XL-MCL CD4 count ≤ 200 ; 201 - 350 cells and >350 cells/ μl .

Statistical analysis:

Statistical analysis used STATA® Version 11 (StataCorp, College Station, USA). Data were explored via simple proportions, medians, means, cross-tabulations and chi square tests. A Wilcoxon Signed Rank test was used to calculate differences in median results. The sensitivity and specificity for the PIMA™ CD4 results, as well as Kappa statistics, were calculated using laboratory cut-offs of 200 and 350 cells/μl. Bland-Altman tests were used to determine the systematic bias of the PIMA™ relative to the XL-MCL.

Linear regression analysis was used to identify factors impacting on the performance of the PIMA™ for both venous and capillary samples. All models were adjusted for the corresponding laboratory XL-MCL value. We modeled individual predictors (delay, operator, machine and training) separately. All predictors were then included in a single multivariable model.

The reference group for “machine” consisted of all those machines on which there was a non-significant mean difference (i.e. the CI included 0) between the PIMA™ and XL-MCL CD4 values. This was done as statistical power was less if all machines were included individually. The composition of the reference group varied between venous and capillary samples.

The reference operator was the nurse who had the least significant mean difference between the PIMA™ and the XL-MCL results. The reference operator was not the same for venous and capillary samples.

Ethics

Informed consent was obtained from all study participants. Data collection and analysis was approved by the University of Cape Town Research Ethics Committee. This study also adheres to the declaration of Helsinki 2008.

The machines were donated by Alere Healthcare, but Alere had no part in design, conduct or analysis of the study.

Results

Overall 349 HIV-infected individuals, seen between March and November 2010, had PIMA™ and laboratory CD4 results available. A total of 325 venous PIMA™ and 167 capillary PIMA™ samples were analyzed as 138 participants had dual venous and capillary PIMA™ specimens taken (Figure 1, Appendix 9), 182 had only venous PIMA™ and 24 only capillary PIMA™ samples.

The majority (88%) of the 349 study participants were black South African, female (63%) and not yet taking ART (82%). The median age was 33 years (IQR 27-40) (Table 1). The median laboratory CD4 was 405 cells/μl (IQR 277-600) (Table 2) with CD4 ≤200, 201-350 and > 350 cells/μl being 11%, 29% and 60% respectively.

Overall 270 samples (77%) were processed at the laboratory within one day whilst the remaining 79 were processed 2-3 days after the sample was taken (delayed). The numbers of samples performed by each user and on each machine was not consistent, neither was the number of training sessions received by the operators (range 0-3) (Table 1).

Venous samples

The median venous PIMA™ CD4 result (n=325) of 393 cells/μl (IQR 264-587) did not differ significantly from the corresponding median laboratory CD4 count of 409 cells/μl (IQR 277-609) (p=0.11) (Table 2). The Spearman rank correlation co-efficient was 0.96(p<0.0001). Our regression analysis using the gold standard revealed a regression coefficient of 0.999 (95%CI 0.964-1.034) (Table 3) (Figure 2A; appendix 9). Ninety-one percent of the variation in the PIMA™ result was explained by the laboratory result (R-square 0.909). Results were similar when excluding delayed specimens (Table 3).

The mean difference (Bland Altman) between PIMA™ CD4 counts and gold standard XL-MCL CD4 counts was -4.5 cells/μl (CI -12.5 to 3.4), and -11.4 cells/μl (CI -20.2 ; -2.6) when excluding delayed specimens (Table 3)(Also see Figure 2B in appendix 9).

The sensitivity of the PIMA™ in detecting CD4 counts≤200 cells/μl and CD4 counts≤350 cells/μl was 89% (95%CI 75-97) (Table 4) and 89% (95%CI 82-94) respectively. The specificity was 98% (95%CI 96-99) for CD4 counts> 200 cells/μl and 90% (CI 85 - 94) for >350 cells/μl.

In total ten samples (3.1%; CI 1.5 – 5.6) were misclassified around the threshold of 200 cells/μl; four samples above and six samples below the threshold. Those individuals misclassified above the threshold would potentially have been excluded from ART therapy and those below the threshold would have been given ART prematurely.

The overall misclassification was higher around the 350 cells/μl threshold at 10.5% (CI 7.4-14.3) with the misclassification of 14 samples above and 20 samples below the threshold.

Capillary samples

The median capillary PIMA™ CD4 result (n=167) of 429 cells/μl (IQR 296-596) differed significantly from the corresponding median laboratory CD4 count of 398 (IQR 269-572) ($p<0.0001$) (Table 2). The Spearman rank correlation co-efficient was 0.93 ($p<0.0001$). The R-square is 0.88 (Table 3) (Figure 2B in appendix 9). The mean difference was 29.7 cells/μl (95%CI 16.9 to 42.5) when compared to the gold standard, and 26.7 (CI 13.5 to 40.4) when delayed samples were excluded (Table 3) (Figure 2B, appendix 9).

The sensitivity of the PIMA™ in detecting CD4 counts ≤ 200 cells/μl and ≤ 350 cells/μl for capillary samples was 81% (95%CI 54-96) and 85% (95%CI 74-92) (Table 4) respectively. The specificity was 99% (95%CI 95-100) for CD4 counts > 200 cells/μl and 93% (95%CI 86-97) for CD4 counts > 350 cells/μl.

In a similar pattern to the venous samples, 3.0% (CI 1.0 – 6.8) of capillary samples were misclassified around the threshold of 200 and 10.8% (CI 6.5 – 16.5) around the threshold of 350. With capillary samples however, there were more misclassifications above the thresholds than below thus more individuals would potentially have been excluded from ART therapy than would have been started on treatment prematurely.

Comparison of venous versus capillary PIMA™ samples

A comparison was done between CD4 count results obtained when both venous and capillary PIMA™ samples from the same individual by the same operator were run on the same machine (n=138). There was a significant difference between the venous median CD4 of 406.5 cells/ μl (IQR 262-593) and the capillary median CD4 of 436.5 cells/ μl (IQR 299-618) ($p=0.0002$) in this group with a mean difference of 26.1 cells/ μl . As before, the results were similar when excluding all delayed samples.

The Kappa statistic using a cut-off of 200 cells/ μl was 0.76 and using a cut-off of 350 cells/ μl was 0.82

Regression Analysis

All linear regression models included the XL-MCL value.

In the regression analysis of venous samples, operator, machine and training and delay all significantly influenced ($p < 0.05$) the performance of the PIMA™ Analyzer (Panel I, Table 5). In the multivariable analysis (Panel II, Table 5), machine had the biggest effect estimate and delay remained significant ($p = 0.010$). Operator was borderline significant ($p = 0.086$).

In the regression analysis of the capillary PIMA™ samples (Panel III, Table 5) only machine and training were significant ($p < 0.05$). Delay was however not significant. In the multivariable analysis (Panel IV, Table 5) the machines were no longer of significance but training neared significance ($p = 0.058$).

Furthermore neither CD4 strata nor the presence/absence of ART contributed significantly to the model.

When doing a more rigorous analysis using only samples reaching the laboratory within one day (results not shown), machine remained a significant variable for venous samples. For capillary samples, none of the variables of interest were significant in this analysis.

Discussion

These data suggest that the PIMA™ performed well when used on a mobile clinic. The CD4 count estimates were more reliable with venous than capillary samples. There was an underestimation of CD4 counts using venous samples and an overestimation when using capillary samples which is consistent with previous studies,^{18,19,22} and was also consistent when comparing PIMA-based CD4 estimates using both venous and capillary samples from the same individual.

Although approximately 10% of CD4 results were misclassified using a threshold of 350 cells/μl, this was reduced to 3% when using a threshold of 200 cells/μl for both capillary and venous samples. These low misclassification rates would enable the mobile clinic staff to accurately and urgently refer individuals eligible for ART, as per the current South African guidelines. We could be referring individuals for ART prematurely, more so for venous than capillary results, but this is less serious than missing individuals who require ART.

Our field evaluation reinforces previous findings from both laboratory^{19,20} and facility based studies¹⁸ that POC CD4 testing, using the PIMA™ Analyzer, may be an acceptable alternative to laboratory based CD4 testing when absolute CD4 T- lymphocytes counts are required. Although several other innovative means of assessing CD4 counts have been published over the past few years, they have either not been commercialized, not been rolled out on a large scale or are have intricate components or operating requirements making them less suitable for a nurse-run mobile clinic such as ours^{21,23-28}. The latter can be far from ideal: the machines are transported over large distances daily, operated on battery power and used in varying environmental temperatures and conditions. Despite this the machine performed well.

Our sensitivity may have improved if our sample size was larger and included more individuals with CD4 counts below 200 cells/ μ l. Our previous study showed that individuals accessing this mobile clinic were healthier than those accessing hospitals and clinics²⁹. Our median CD4 counts were higher than these previous evaluations¹⁸⁻²⁰ with the majority (60%) of our XL-MCL CD4 counts being over 350 cells/ μ l compared to 40%²⁰ and 20%¹⁸. This may have led to a larger bias as typically the reliability of the CD4 estimate decreases as the CD4 count increases. However the clinical implications of a variation in CD4 cells at higher CD4 counts is less.

The PIMA™ machine used appeared to affect the test performance as machine remained significant in the multivariable analysis for venous samples. Some variation was noted amongst the operators, but after adjusting for the machine used and amount of training received, this was not statistically significant.

Training was significant in the multivariable model for capillary samples. Paradoxically, it appears that the more training received the greater the deviation from the laboratory result. Although it is unlikely that additional training results in poorer performance, the possibility of user fatigue (where operators using the machine for longer periods may be less likely to adhere to protocols) needs to be considered. In addition, training could be a proxy for calendar effect and needs to be understood better. We could have restricted the analysis to only the 2 operators who had undergone all three training sessions but this would have significantly reduced the power of the study.

Our study is subject to several limitations. Firstly our study is a field evaluation and the rigor normally associated with a research setting in terms of methodology is lacking. The exact time delays between sample collection, sample processing on the PIMA™ and sample processing at the laboratory were not calculated. The room temperature was also not recorded. The number of specimens performed on each machine and each user was not consistent. In addition the number of

training sessions attended by each user varied. This was however adjusted for in our multivariable analysis. The effects may also have been more apparent if the sample size was bigger for capillary samples and samples where the XL-MCL result was less than 200.

Secondly the study was run from March to November which are typically the cooler months in Cape Town. The impact of ambient temperature on machine performance has not been sufficiently evaluated.

Thirdly, poorly collected specimens can adversely affect the quality of CD4 enumeration. The collection of venous blood into an EDTA tube using a BD Vacutainer® system (Becton, Dickinson and Company, Franklin Lakes, NJ) and transfer of the sample with a pipette could have caused shearing, deterioration or loss of cells. Furthermore the blood-to-additive ratio may be altered through the additional use of EDTA-filled capillary tubes contributing to the slight underestimation of CD4 counts seen for venous samples.

Collection of the capillary samples also requires a well-trained individual. Despite the nurses being highly experienced and trained in finger-prick blood collection, the collection of blood into the PIMA™ cartridge was challenging initially. Insufficient volume or the presence of air bubbles in the sample adversely affects the accuracy of the PIMA™ result. If the PIMA™ is to be used by inexperienced or careless operators, the overall performance of the PIMA™ may not be as good as reported in our study or previous studies.

Fourthly, our nurses were not blinded to the PIMA™ results which may have been important when performing dual samples. This may have resulted in repeat specimens being performed.

Lastly, we had an imperfect gold standard. A universally accepted gold standard for CD4 counts does not exist but flow cytometers such as the FACScount and Beckman Coulter CD4 counts are often used. The gold standard in this study is

further influenced by a delay in reaching the laboratory which was found to be significant in our study. Delays in our study typically occurred because the reference laboratory does not provide an after-hours or weekend service. Both other evaluations^{18 19} used same day laboratory specimen processing. Whilst this gives a more accurate comparison, this does not take into account the fact that blood samples from most testing sites where POC would be most useful, could take days to reach the laboratory. Clinicians wanting to perform their own validations, especially at rural sites, should bear this in mind.

For accurate results, specimens must be analysed no later than 48 hours after collection as delays result in sample aging. This could induce apoptosis of cells resulting in an underestimation of CD4 counts. However aging can cause changed cell morphology altering light scatter properties. Distinguishing between monocytes & lymphocytes thus becomes difficult. Automated gating algorithms may fail to identify the relevant populations and manual gating may need to be performed. If monocytes are counted as T-helper cells it could lead to an increased CD4 count (W.Pretorius [willem.pretorius@alere.com], email, May 16, 2011). The latter could be a reason for finding that delay had a positive effect size for both venous and capillary samples in our study.

The potential advantages of giving CD4 results to the individual during the timeframe of their initial consultation are considerable. We were able to give 99% (n=346) of patients their PIMA™ result during their visit. This is a significant improvement over the 46-65% of individuals who return to their health care facility to receive their CD4 count result.⁸⁻¹⁰.

Future research may examine whether the use of POC CD4 results in improved referrals to care. An evaluation of the linkage-to-care of these individuals is warranted to assess the cost-effectiveness of POC CD4 counts.

Pregnant patients and those with TB, where a cutoff of 350 applies, may need confirmatory CD4 count testing at their referral site due to the 10%

misclassification at this level. Further validation studies of the PIMA in these two populations may need to be done.

The significant variability between machines needs to be further explored.

Conclusion

Our field evaluation reinforces previous findings that the PIMA™ Analyzer may be an acceptable alternative to laboratory based CD4 testing for absolute CD4 T-lymphocytes counts. Misclassification rates are low when using a CD4 cutoff of < 200 cells/ µl enabling accurate identification of individuals requiring ART. In our evaluation venous samples underestimated and capillary samples overestimated the XL-MCL CD4 result. Operator, machine and training appeared to influence the performance of the PIMA™ and this finding needs to be further evaluated.

Acknowledgments

The authors thank the Tutu Tester staff for assisting with conducting the study. A special thank you to the nurses: Liz, Nikki and Eudoxia as well as Shandr  who captured all our data. The authors are also grateful for the excellent technical support provided by Alere Healthcare and the dedication of the staff at the Togatainer in Gugulethu.

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Table 1. Basic demographic data

Variables	Total N=349		Venous PIMA N=325		Capillary PIMA N=167		Both N=138		
	median	IQR (range)	mean	sd	mean	sd	mean	sd	
age	33	27-40 (16-72)	-	-	-	-	-	-	
	N	%	N	%	N	%	N	%	
HIV	newly diagnosed	183	52.4	173	53.2	76	45.5	64	46.4
	known not on ART	102	29.2	91	28.0	50	29.9	37	26.8
	known on ART	64	18.3	61	18.8	41	24.6	37	26.8
gender	male	129	37.0	124	38.1	51	30.5	44	31.9
	female	220	63.0	201	61.9	116	69.5	94	68.2
LAB CD4 (cell/ μ l)	\leq 200	38	10.9	38	11.7	16	9.6	15	10.9
	201-350	103	29.5	91	28.0	55	32.9	41	29.7
	$>$ 350	208	59.6	196	60.3	96	57.5	82	59.4
days to lab	0	8	2.3	8	2.5	8	4.8	8	5.8
	1	262	75.1	239	73.5	127	76.1	101	73.2
	2	51	14.6	50	15.4	19	11.4	16	11.6
	3	28	8.0	28	8.6	13	7.8	13	9.4
delay	yes	79	22.6	78	24.0	32	19.2	29	21.01
	no	270	77.4	247	76.0	135	80.8	109	78.99
operator	operator A			69	21.2	40	24.0	33	23.9
	operator B			37	11.4	3	1.8	3	2.2
	operator C			69	21.2	52	31.1	51	37.0
	operator D			150	46.2	72	43.1	51	37.0
machine	A			72	22.2	60	35.9	47	34.1
	B			24	7.4	23	13.8	22	15.9
	C			4	1.2	6	3.6	4	2.9
	D			19	5.8	5	3.0	4	2.9
	E			127	39.1	49	29.3	40	29.0
	F			50	15.4	10	6.0	8	5.8
	G			13	4.0	1	0.6	1	0.7
	H			15	4.6	13	7.8	12	8.7
	I			1	0.3	0	0.0	0	0.0
Number of training sessions	1			189	58.2	41	24.6		
	2			95	29.2	87	52.1		
	3			41	12.6	39	23.4		

Table 2. Summary of CD4 results

	Variables	N	mean	sd	median	IQR	Range
Overall	Laboratory CD4	349	448.7	232.6	405	277 - 600	7-1319
Venous sample	PIMA CD4	325	443.8	240.7	393	264-587	5-1259
	Laboratory CD4		448.3	229.6	409	277-609	7-1165
Capillary sample	PIMA CD4	167	466.6	227.3	429	296-596	12-1289
	Laboratory CD4		436.9	243.6	398	269-572	7-1319

Table 3. Summary of Bland-Altman and Regression results

	N	Mean PIMA	Mean XL-MCL	Mean diff	CI	Limits of agreement	Regression Coefficient	CI	R2
Gold standard (all samples)									
Venous PIMA™ samples	325	443.8	448.3	-4.5	-12.5 ; 3.4	-150.1 ; 141.0	0.999	0.964 ; 1.034	0.909
Capillary PIMA™ samples	167	466.6	436.9	29.7	16.9 ; 42.5	-137.6 ; 197.0	1.006	0.950 ; 1.063	0.882
Both subset (venous versus capillary)	138	-	-	26.1	9.9 ; 42.4	-166.4 ; 218.6	0.915	0.847 ; 0.984	0.837
Delayed samples excluded									
Venous PIMA™ samples	247	429.8	441.2	-11.4	-20.2 ; -2.6	-152.5 ; 129.7	1.006	0.967 ; 1.045	0.913
Capillary PIMA™ samples	135	462.5	435.5	27.0	13.5 ; 40.4	-131.4 ; 185.3	1.015	0.957 ; 1.073	0.900
Both subset (venous versus capillary)	109	-	-	29.5	11.8 ; 47.3	-157.4 ; 216.4	0.927	0.852 ; 1.001	0.851

Table 4. Misclassification of ART eligibility using PIMA™ based on CD4 thresholds of 200 cells/µl and 350cells/µl

	Lab ≤200	Lab 201-350	Lab > 350	Total
VENOUS				
PIMA ≤= 200	34 (89%)	4 (4%)	2 (1%)	40
PIMA 201 - 350	4 (11%)	73 (80%)	18 (9%)	95
PIMA> 350	0 (0%)	14 (15%)	176 (90%)	190
Total	38	91‡	196	325
CAPILLARY				
PIMA ≤= 200	13 (81%)	2 (4%)	0 (0%)	15
PIMA 201 - 350	3 (19%)	42 (76%)	7 (7%)	52
PIMA> 350	0 (0%)	11 (20%)	89 (93%)	100
Total	16	55	96	167

‡ Due to rounding differences this adds up to 99%

Table 5. Linear regression models predicting PIMA™ results according to selected covariates.

(Note that all models include XL-MCL CD4 values from the reference laboratory).

	Venous samples (n=325)						Capillary samples (n=167)						
	Panel I			Panel II			Panel III			Panel IV			
	co-efficient	95% CI	p-value	co-efficient	95% CI	p-value	co-efficient	95% CI	p-value	co-efficient	95% CI	p-value	
Operator A	ref						Operator A	26.0	-73.7 ; 125.7	0.607	17.7	-84.5 ; 119.9	0.733
Operator B	-21.4	-50.1 ; 7.4	0.144	-12.2	-41.0 ; 16.6	0.405	Operator B	ref					
Operator C	10.8	-13.2 ; 34.8	0.378	1.7	-26.3 ; 29.6	0.907	Operator C	22.1	-76.7 ; 120.9	0.659	-20.3	-130.4 ; 89.9	0.717
Operator D	-24.9	-45.4 ; -4.4	0.018	-18.6	-39.9 ; 2.6	0.086	Operator D	7.8	-90.4 ; 106.0	0.875	-19.3	-125.4 ; 86.8	0.720
Ref. group	ref						Ref. group	ref					
machine A	34.0	15.1 ; 52.9	0.000	28.3	7.3 ; 49.3	0.008	machine A	41.6	6.5 ; 76.7	0.020	23.8	-16.1 ; 63.7	0.241
machine B	34.0	4.2 ; 63.7	0.025	26.9	-6.6 ; 60.4	0.115	machine B	34.8	-9.2 ; 78.9	0.121	12.9	-41.2 ; 67.0	0.638
machine C	81.3	11.8 ; 150.8	0.022	65.0	-5.0 ; 134.9	0.069	machine E	40.4	3.9 ; 76.9	0.030	19.0	-25.9 ; 64.0	0.404
machine D	-46.8	-79.8 ; -13.8	0.006	-29.1	-63.5 ; 5.3	0.097	-	-	-	-	-	-	-
Delay	28.7	10.2 ; 47.1	0.002	24.4	5.9 ; 42.9	0.010	Delay	14.3	-18.3 ; 46.9	0.387	4.5	-30.4 ; 39.4	0.801
Training	21.4	10.4 ; 32.4	0.000	7.1	-8.4 ; 22.5	0.369	Training	21.3	2.9 ; 39.7	0.024	29.4	-1.1 ; 59.8	0.058

Panels I and III show the associations between individual predictors, modeled separately, and PIMA™ values (for venous and capillary values respectively). Panels II and IV show the associations when all predictors shown are included in a single multivariable model. Note that all models are adjusted for the corresponding XL-MCL values.

PART D: APPENDICES

University of Cape Town

Appendix 1: Client form (version 26 Jan 2010)

Insert sticker or add

Site: _____

Number: _____

Data _____ (for office use only)

DATE: _____

DD-MMM-YYYY

Client initials: _ _ _

DATE OF BIRTH: _____

DD-MMM-YYYY

GENDER: Male Female

RACE: Black SA Coloured Asian White Other _____

Black non SA

Tested before on our mobile clinic? No Yes (add details) date: _____ where _____

Weight: _____ **Height:** _____ **BMI =** _____

Waist circumference: _____

Pretest counselling session needed? Yes No

Couple counselling Yes No

NURSE: _____

HAVE YOU EVER HAD A HIV TEST?

- No / never
- < 3 months ago
- 3-6 months ago
- 6-12 months ago
- More than a year ago - Which year? _____

Most recent HIV Result: NEG POS → **Are you on ART?** yes no defaulted

When was your last CD4? _____ (month / year)

What was your last CD4? _____

MEDICAL HISTORY:

Have you ever had your Blood pressure checked: No yes unsure

If yes, Were you put on treatment? No yes Are you still on treatment? No yes

Have you ever had our glucose (diabetes) checked: No yes unsure

If yes, Were you put on treatment? No yes Are you still on treatment? No yes

Have you ever had a sexually transmitted Infection before? No yes unsure

Ever had a Pap smear?

- Not applicable (male)
- Yes < 5 years ago 5-10 years ago > 10 years ago(*refer*)
- Results:** Normal Abnormal Didn't get results
- No (*refer if > 30 years old*)
- too young (< 30)

Have you ever had Tuberculosis? No yes

 ↙
 If yes, Are you currently on treatment? No yes

 If yes, did you complete TB treatment? No yes

(if treatment not completed discuss and refer if necessary - especially if a recent defaulter)

Obtained verbal consent for HIV test? Yes No

Couple testing: Yes no

Bioline HIV result

- Positive
- Negative
- Known pos
- Not done
- Not applicable

Abbott Determine HIV result

- Not applicable
- Known pos
- Positive
- Negative
- Not done

- Overall HIV Result:**
- Negative
 - Newly diagnosed HIV Positive *****
 - Known HIV Positive - on ART***
 - Known HIV Positive - not on ART *
 - Indeterminate
 - Refused test
 - Not tested other reason
-

Blood pressure: ___ / ___ **If > = 140 / 90** new diagnosis on treatment defaulted treatment

DIABETES RISK SCORE

Glucose: ___ . ___ **If > 7.0:** (please circle) Random Fasting

If > 11.0 or > 7 (fasting) - new diagnosis on treatment defaulted treatment

Client Number: _____

TB symptoms? No Yes (refer)

STI symptoms? no yes (refer)

Needs a pap smear? Not applicable /male Yes(refer) No too young (< 30)

CNP: _____

If HIV Positive:

Blood taken for a CD4 count? yes* POINT OF CARE CD4 _____
 LAB CD4
 no _____
 n/a - on HAART (and regularly monitored)
 n/a - last CD4 was > 350 (done less than 6 months ago)
 n/a - last CD4 was >200 and <350 (done less than 3 months ago)
 refused

* take blood for CD4 if they are part of the TB study

Syphilis screen: negative positive not done n/a

Pregnancy test: negative positive not applicable not done

Clinical Stage: 1 2 3 4 on ART not done

Overall assessment: on ART Green Yellow Red  Referred for ARVs? no yes

Active STI screen: no yes not done

For patients with STIs:

Syphilis screen: negative positive not done not applicable

 Sample sent to lab for RPR? Yes No

STI treatment: no yes - code _____

CONDOM DEMONSTRATION (Must be done!. If not done by nurse MUST be done by counsellor)

Did patient demonstrate how they use condoms? no yes (initials of person observing:) _____

If no, document why not: _____

COUNSELLOR: _____

Have you had a sexual partner in the past 3 months?

- yes
- not yet sexually active
- not sexually active for > 1 year
- last partner 3 - 6 months ago
- last partner 6 - 12 months ago

Sexual orientation

- Straight (heterosexual)
- Homosexual / lesbian
- Bisexual

If HIV NEGATIVE:

HIV Result given to patient? yes no

Total Risk factors (0-10): _____ not done

Overall Risk assessment: low @ risk high

Next test date: 3 months 12 months before sexually active (again)

If HIV POSITIVE:

HIV Result given to patient? yes no

Post test counselling done: yes no n/a

Risk reduction done: yes no

"Road to HIV health" card given and explained? yes no

Any other information.....

TB testing: SPUTUM sent to the lab today? yes no unable refused

Client Number: _____

<u>RISK FACTORS:</u>		low risk	@ risk	Total
1. Have all your current sexual partners tested for HIV? (unknown partner status)	n/a	yes	No / don't know (1 point)	
2. Are any of your sexual partners HIV positive? (discordancy)	n/a	no	Yes/ Don't know (1 point)	
3. Did you use condoms every time you had sex in the past 3 months? (unprotected sex)	n/a	Yes	No (1 point)	
4. Have you had more than one sexual partner in the past 3 months or is your partner having other partners? (multiple partners)	n/a	No	Yes (1 point)	
5. is your partner more than 10 years older/ younger than you (intergenerational sex)	n/a	no	Yes (1 point)	
6. Did you have anal sex with someone in the past 6 months?	n/a	no	Yes (1 point)	
7. Did you have sex with someone for food, airtime, clothes, money, etc. in the past 6 months? (transactional sex)	n/a	no	Yes (1 point)	
8. Have you or your partner had a STI in the past 6 months?	n/a	no	Yes (1 point)	
9. Have you injected drugs/ had a tattoo/ needed blood products in the past 6 months?	n/a	no	Yes (2 points)	
			TOTAL	

0 = low risk = test yearly unless in window period

1 = @ risk = test in 3 months - 12 months (depending on risk factor and if in window period)

2 or more = high risk= test in 3 months time

Is there a risk factor which can be addressed? No Yes

Behaviour	Costs	Benefits

My specific goal is to: _____

Appendix 2: Ethical approval: Active Tuberculosis Case Finding

UNIVERSITY OF CAPE TOWN



**Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za**

4 February 2009

REC REF: 507/2008

Dr K Kranzer
Desmond Tutu
HIV Foundation
IIDMM
Medical School

Dear Dr Kranzer

**PROJECT TITLE: ACTIVE TUBERCULOSIS CASE FINDING IN INDIVIDUALS ACCESSING
A MOBILE VOLUNTARY COUNSELLING AND TESTING SERVICE.**

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 8th February 2010.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLÖCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

lemjedi

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix 3: Ethical approval Cross-sectional survey



Health Sciences Faculty
Faculty of Health Sciences Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: sumayah.ariefdien@uct.ac.za

13 September 2010

HREC REF: 262/2010

Dr K Kranzer
Desmond Tutu HIV Foundation
IIDMM Medical School

Dear Dr Kranzer

**PROJECT TITLE: CROSS-SECTIONAL SURVEY OF HIV INFECTION AND CD4 COUNTS
IN MASIPHUMELELE TOWNSHIP**

Thank you for your letter to the Faculty of Health Sciences Research Ethics Committee dated 31st August 2010.

It is a pleasure to inform you that the Ethics Committee has **approved** the following documentation with reference to the above mentioned study:-

- CD-4 Survey Sub-study 1, Version 1.0
- CD-4 survey Sub-study 2, Version 1.0
- Adult informed consent form. Cross-sectional survey version 3.0

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Appendix 4: Data collection form for PIMA™ results

TUTU tester PIMA study: capillary versus venous blood

Tutu Tester number	
Date of test	

Capillary blood	
If no print out available, please fill in by hand:	
CD4 result	_____
PIMA machine	_____
Operator	_____

Venous blood	
If no print out available, please fill in by hand:	
CD4 result	_____
PIMA machine	_____
Operator	_____

Toga lab result

Entered by: _____

Date: _____

Entered by: _____

Date: _____

Appendix 5: Client details sheet



DESMOND TUTU HIV FOUNDATION

Level 1
Wernher Beit North Building
Faculty of Health Sciences
Anzio Road, Observatory,
Cape Town, South Africa

P O BOX 13801, MOWBRAY, 7705
Cape Town, South Africa
(T) 27 021 6506966
(F) 27 021 6506963
VAT No. 4750185565

Client number #: _____

Date of birth: _____

Participant Name: _____

Other Names: _____

Personal Cell No: _____

May we sms you? Yes / No

Language: English / Afrikaans / Xhosa

Messages: Bland / HIV-specific

OK to leave a voicemail message? Yes / No

If so, who shall we say is calling? _____

OK to leave a message if someone else answers your phone? Yes / No

If so, who shall we say is calling? _____

Home Tel: _____

OK to leave Message Y/N: _____

If so, who shall we say is calling? _____

Work No: _____

OK to leave Message Y/N: _____

If so, who shall we say is calling? _____

Home address: _____

Postal address (if different): _____

May we come to your home? Y/N _____

If yes, with whom do you live? _____

Formal or Informal Dwelling: _____

Nearest landmark (e.g. next to church): _____

Clinic you are most likely to use: _____

Nearest Hospital: _____

Clinic folder # (if known): _____

Hospital folder # (if known): _____

Client Signature: _____

Date: _____

Staff Signature: _____

Date: _____

Protection of Confidentiality

Note: When we leave phone messages, we will never reveal information without your prior approval.

PASSION | INNOVATION | PROGRESS

Association Incorporated under Section 21: Registration No: 1999/005072/08

Public Benefit No: 18/11/12/51

Directors: Prof R Wood; Prof L-G Bekker; Prof G Kaplan;

Prof D Martin; Ms Z Ebrahim; Mr P-D Uys; Mr T Papier; Ms T Tutu-Gxashe

www.desmondhutuhivfoundation.org.za

**Appendix 6: Ethical approval for reporting of programmatic data from Tutu
Tester**



Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: lamees.emjedi@uct.ac.za

03 June 2010

Prof LG Bekker
Desmond Tutu HIV Centre
IIDMM
Medical School

Dear Prof Bekker

RE: PERMISSION TO REPORT PROGRAMMATIC DATA

Thank you for your letter to the Health Science Faculty Human Research Ethics Committee dated 28 May 2010.

1. Permission is granted to publish the data anonymously collected in the Tutu Tester.
2. Do the researchers feel it is worthwhile establishing a data registry with a formal HREC REF number for these data? The protocol application would include the information already described in this letter and would need an application form.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

PP

Appendix 7: Cross-sectional Survey Consent form

Adult informed consent form

Plot number _____
HH number _____
Sex _____
Age _____

Cross-Sectional Survey of HIV infection and CD4 counts in the Masiphumelele Township

INTRODUCTION

You are being invited to take part in this research study because you are a resident of Masiphumelele. The doctor in charge of this study at Masiphumelele is: Dr. K Kranzer. Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The research staff will talk with you about this information. You are free to ask questions about this study and discuss any worries you may have with the research staff. If you agree to take part in this study, you will be asked to sign this consent form. You will be given a copy of the form to keep.

WHY IS THIS STUDY BEING DONE?

We want to know how many people are infected with HIV in Masiphumelele and we want to know how many people need antiretroviral treatment (treatment of HIV). This is a non-interventional study. This means that you will not be provided with treatment on this study.

We also want to know if we get better (more accurate) CD4 counts if we take blood from your finger than if we take blood from your arm to do a CD4 count on the PIMA Analyzer. This is a machine which can do your CD4 count immediately rather than sending it to the laboratory.

In the future we may want to do a "viral load" (counting how much virus there is) on the blood of those testing positive. We will ask if we can freeze your blood sample in the mean time.

WHO CAN TAKE PART IN THE STUDY?

We have randomly chosen 1300 people living in Masiphumelele. "Random" means that these people were chosen out of a hat. This means we do not know anything about them, if they have HIV or if they take antiretroviral therapy or not. All randomly chosen people 15 years and older, who have lived in Masiphumelele for longer than one week, can be screened to take part in the study. The whole study will take about four months to complete.

Anyone testing HIV positive can give permission for more than one CD4 count to be done and can give permission to have their blood stored.

WHAT DO I HAVE TO DO IF I TAKE PART?

You have been visited at your home. At this visit a study staff member explained the study to you and answered any questions you may have had about the study. If you are interested in taking part, the field worker will invite you to attend the TUTU tester, a mobile HIV testing service, any time it is parked on Xhosa square.

When you arrive at the TUTU tester you will be offered a choice:

1. You can register as a client of the TUTU tester and undergo HIV testing and counselling and screening for other chronic diseases. You will be informed about your HIV-test result and you will be given post-test counselling.

Adult informed consent form

If you test HIV-positive, we will take blood from your arm (venous sample) for a CD4 count. As part of a sub-study we would like to ask if we can do an additional finger prick and do a CD4 count using this blood. We would also like to take an extra tube of blood to do a CD4 count at the TOGA laboratory. You will get this result if you give us your telephone number or you can come back to the TUTU TESTER and we will give it to you.

2. You can register as a client of the TUTU tester and undergo screening for chronic diseases and to provide a venous blood sample. An HIV test will be done on the blood, but your name will not be written on the specimen. In this way, we will be able to see how many people have HIV, but we will not know your individual HIV status. We will not know if YOU are HIV positive or negative. A CD4 count test will be done on your blood sample if it is HIV positive. You will not know your HIV-test result.
3. You can provide a venous blood sample. An HIV test will be done on the blood, but your name will not be written on the specimen. In this way, we will be able to see how many people have HIV, but we will not know your individual HIV status. We will not know if YOU are HIV positive or negative. A CD4 count test will be done on your blood sample if it is HIV positive. You will not know your HIV-test result.

We will also ask you some questions about your age and if you are taking antiretroviral therapy.

We would also like to obtain your consent to store your blood sample once we have done a CD4 count on it. Your blood sample will be labelled only with your study number and stored in a freezer specially designed for storing blood samples. We would like to do a "viral load" test on your blood in the future. This test will determine how much virus there is in your blood. You will not receive these results.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?

There are no significant risks to participating in this study.

WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

If you undergo HIV testing and counselling and you are found to have HIV we will refer you to Masiphumelele clinic where treatment is available free of charge. If you chose not to be registered as Tutu tester client we will not give you your HIV-test result and there will be no further action.

WHAT ABOUT CONFIDENTIALITY?

The study team will keep your personal information confidential. You will be given a study number. The questionnaire and blood specimens will be labelled with this study number and NOT with your name. All information will be kept in a locked cupboard. We will make every attempt to ensure confidentiality. Any publication of this study will not use your name or identify you personally.

If your blood is sent to the laboratory and you have asked us to give you your results, your contact information will be kept in a locked cupboard. This information is only available to study staff.

WHAT ARE THE COSTS TO ME?

There are no costs to you if you are involved in this study.

WILL I RECEIVE ANY PAYMENT?

You will not be paid for taking part in this study. However you will receive a 70R gift voucher to compensate your time.

Adult informed consent form

WHAT ARE MY RIGHTS AS A RESEARCH SUBJECT?

Taking part in this study is completely voluntary. You may choose not to take part in this study. You will not be giving up any of your legal rights by signing this consent form.

INVOLUNTARY WITHDRAWAL OR EARLY WITHDRAWAL

If at any time during the study you refuse to complete one of the study procedures or the study team deems it unsafe to proceed, you will be withdrawn from the study. You may also decide at any point that you no longer wish to participate in the study and may then withdraw from the study by informing the study staff about your decision.

In either event, you will be free to withdraw from the study with no further obligations.

CAN I REFUSE TO TAKE PART IN THE STUDY?

Yes, you may refuse to take part in the study. If you decide not to take part in the study, this will not affect any medical care or treatment you may require now or in the future.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about this study or a research related injury, you may contact:

Dr Katharina Kranzer at (021) 633 6599

This study has been approved by the the Human Research Ethics Committee of the University of Cape Town and the Ethics Committee of the London School of Hygiene and Tropical Medicine.

Adult informed consent form

SIGNATURE PAGE

If you have read this consent form (or have had it explained to you), all your questions have been answered, and you agree to take part in this study, please indicate which study you agree to take part in and sign your name below. If you are unable to sign, you can make a thumb print in the designated space.

I agree to take part in the CD4 count survey **yes** **no**

- I will test as a TUTU tester client and receive my HIV result
- I will test as a TUTU tester client but do not want to receive my HIV result
- I will give venous blood but do not want to receive my HIV result

I agree to have my blood stored for viral load testing in the future if I test HIV positive

- yes** **no**

For TUTU tester clients receiving HIV test results only:

I agree to give an extra venous sample and a fingerpick sample for CD4 count testing if I test HIV positive

- yes** **no**

Participant's Name (Print)

Participant's Signature

Date

Study Staff Conducting
Consent Discussion (Print)

Study Staff's Signature

Date

Witness' Name (Print)
(As appropriate)

Witness' Signature

Date

Appendix 8: Instructions for authors (JAIDS) including Authorship, Responsibility, Financial Disclosure and Copyright Transfer

Instructions for Authors

SCOPE

JAIDS: Journal of Acquired Immune Deficiency Syndromes is a peer-reviewed, multidisciplinary journal directed to an audience of physicians and researchers. The journal publishes original work in the form of Original Articles, Implementation and Operational Research*, Rapid Communications, Critical Reviews, Brief Reports, and Letters to the Editor*. **JAIDS does not publish case reports.** (*published online only)

MANUSCRIPT SUBMISSION

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plications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr*. 2002;31:257–275.

Book Chapter

- Wortmann RL, Bentzel CJ. Renal handling of uric acid. In: Massry SG, Glasscock RJ, eds. *Massry and Glasscock's Textbook of Nephrology*. Philadelphia: Lippincott Williams & Wilkins, 2001;90–92.

Entire Book

- Mandell GL, Mildvan D, eds. *Atlas of AIDS*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001.

Software

- Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention, 1994.

Online Journals

- Friedman SA. Preeclampsia: a review of the role of prostaglandins. *Obstet Gynecol* [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database

- CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute, 1996. Updated March 29, 1996.

World Wide Web

- Panel on Clinical Practices for the Treatment of HIV Infection. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Department of Health and Human Services and Henry J. Kaiser Foundation, January 28, 2000. Available at: <http://www.hivatis.org/guidelines/AA599.pdf>.

Paper Presented at a Conference

- Koenig L, Ellerbrock T, Pratt-Palmire M, et al. Prospective predictors of medication adherence: a study of the first six months of highly active antiretroviral therapy (HAART) using electronic monitoring [WePeB5818]. Presented at: XIV International AIDS Conference; 2002; Barcelona.

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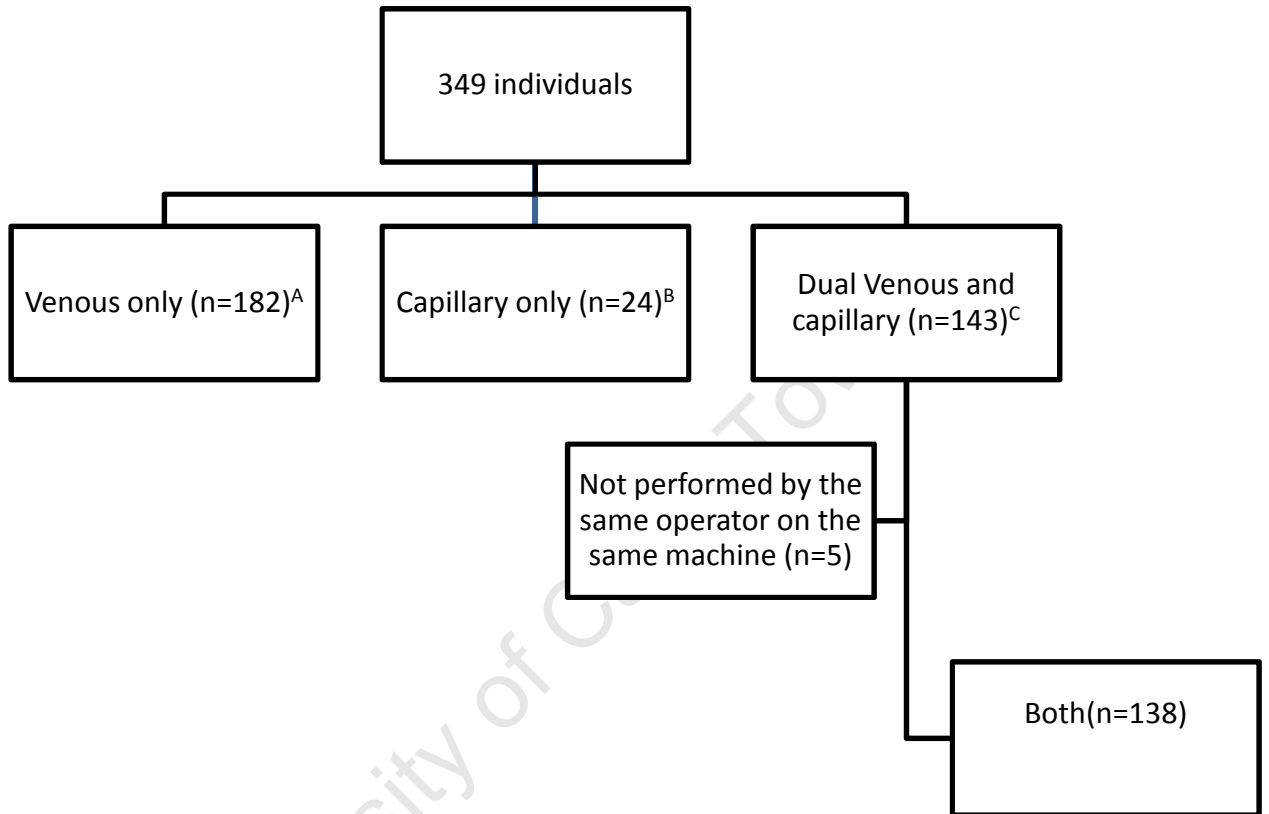
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Appendix 9: Supplementary figures and tables

Figure 1. Flow diagram of study participants.



Venous samples (A+C)=325

Capillary samples (B+C) = 167

Both=138

Figure 2 A. Scatter plots with regression slopes for venous and capillary samples

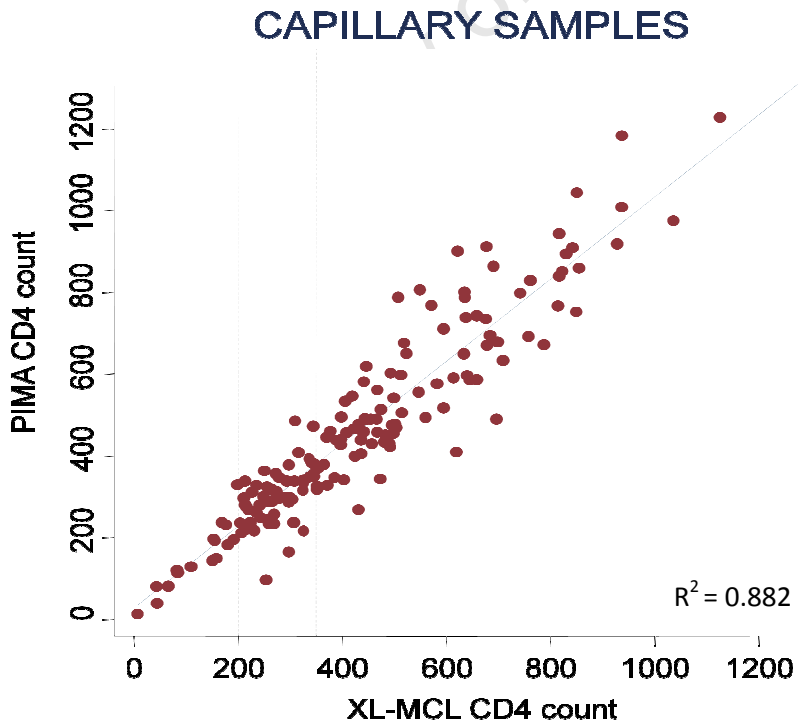
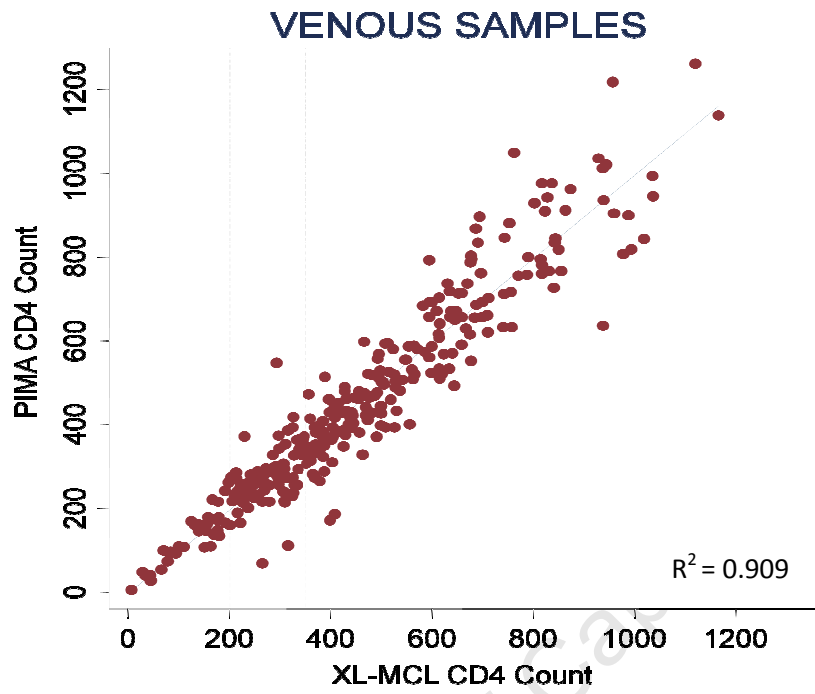
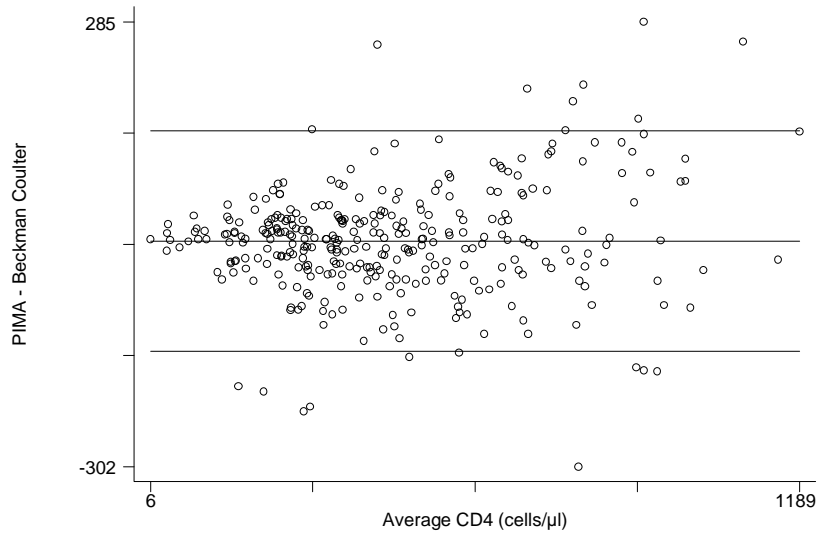


Figure 2B. Bland Altman Plots

Venous samples



Capillary samples

