Point-of-care CD4 devices for staging and monitoring of HIV infected individuals: what is the evidence?

Samantha Wilkinson, B.Sc.

Department of Epidemiology, Biostatistics and Occupational Health

McGill University, Montreal

December 2013

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Table of Contents

List of figures and tables

Abstract

Background: At the end of 2012, worldwide, approximately 35.5 million people were living with HIV. [1] HIV mortality rates, in resource limited settings are now starting to improve as antiretroviral therapy (ART) is becoming universally available. Now, these settings need to improve the care provided to patients. Good quality care requires timely detection, staging and initiation of therapy. CD4+ cell counting, point-of-care (POC) devices could improve the quality of care in resource limited settings, by allowing for the decentralization of HIV care. As a result, these POC CD4+ cells assays could circumvent patient barriers to care, and relieve building pressure on regional laboratories. Several POC CD4+ devices are available, but an independent comparison of performance has not yet been done. The aim of this thesis is to evaluate the current evidence for POC CD4+ cell counting technologies and determine whether their performance would allow them to be used interchangeably with the current gold standard.

Methods: To attain our objective we completed a systematic review and meta-analysis of the evidence relating to POC CD4+ cell assays. Our populations of interest were global populations of adults with a HIV+ status. Our outcome of interest was to the absolute Bland Altman mean bias, which represents the agreement between the POC device and the gold standard. We systematically searched 19 databases, relevant conferences and grey literature for the period 2000 to 2013. Of 4154 citations found, 16 articles were selected. A Bayesian hierarchical normal-normal model was used to meta-analyze data.

Findings: POC devices appear to perform best in capillary samples. Only sufficient data was available to allow for a meta-analysis of the PIMA device; a smaller BA mean bias in capillary blood vs. venous specimens was found (-3.0 cells/μL; 95% CrI: -28·2 to 22·8 vs. -26·5 cells/μL;

95% CrI: -46·7 to -6·8). Insufficient data was available for other POC devices (the MiniPOC and the MBio) to allow for a meta-analysis; however they also appear to perform well when considered graphically in a forest plot.

Conclusion: The PIMA CD4 device was comparable to flow cytometry as the estimated difference between the CD4+ cell count of the device and the reference fell within a range of acceptable accuracy (+/- 30 cells/μL). The miniPOC and the MBio devices also appear to perform well. Devices appear to better estimate capillary specimens compared to venous specimens.

POC CD4+ cell count devices are a rapidly developing field and so providers need reliable evidence for technology selection decisions. The synthesis of evidence relating to the accuracy of POC CD4+ cell counting devices may be of interest for initiatives that are scaling up the use of these devices globally.

Résumé

Contexte : À la fin de 2012, il y avait 35,5 millions de personnes à travers le monde vivant avec le VIH.[1] Le taux de mortalité du VIH dans des milieux à faibles ressources commence maintenant à s'améliorer avec la disponibilité de la thérapie antirétrovirale (TAR) qui est en train de devenir universelle. Maintenant ces milieux doivent améliorer les soins donnés aux patients. Les soins de bonne qualité nécessitent un dépistage, une détermination du stade et un début de traitement rapides. Les appareils de diagnostic qui comptent les cellules CD4+ au point de service (POC) pourraient améliorer la qualité des soins dans les milieux à faibles ressources en permettant la décentralization des soins VIH. Par conséquent, ces tests effectués par ces dispositifs POC de diagnostic et de comptage de cellules CD4+ pourraient contourner les obstacles aux soins des patients et atténuer la pression de devoir construire des laboratoires régionaux. Plusieurs dispositifs POC de diagnostic de CD4+ sont disponibles mais une comparaison indépendante de leur performance n'a pas encore été faite. Le but de cette thèse est d'évaluer les éléments dont on dispose déjà concernant les technologies POC comptage de cellules CD+4 et de déterminer si leur performance leur permettrait d'être utilisées de façon interchangeable avec la norme de référence actuelle.

Méthodes : Afin d'atteindre notre objectif nous avons procédé à un examen méthodique et à une méta-analyse afin d'évaluer les tests POC comptage de cellules CD4+. Notre résultat d'intérêt était selon le biais moyen absolu de Bland Altman, qui représente l'accord entre le dispositif POC et la norme de référence. Nous avons examiné systématiquement 19 bases de données, ainsi que des conférences et de la littérature grise pertinentes datant de 2000 à 2013. 16 articles ont été sélectionnés sur 4154 citations. Nous nous sommes servis d'un modèle bayésien hiérarchique de distribution normale-normale afin de faire la méta-analyse des données.

Résultats : les dispositifs POC semblent fonctionner mieux avec des prélèvements de sang capillaire. Il y avait seulement des données disponibles pour effectuer une méta-analyse du dispositif PIMA ; on a trouvé un plus petit biais moyen Bland Altman dans les prélèvements de sang capillaire par rapport aux échantillons de sang veineux (-3,0 cellules/ μL; 95% CrI : de -28,2 à 22,8 contre -26,5 cellules/ μL; 95% CrI : de -46,7 à -6,8). Les données disponibles pour les autres dispositifs POC (le MiniPOC et le MBio) n'étaient pas suffisantes pour permettre une méta-analyse, pourtant ces dispositifs semblaient bien fonctionner selon la représentation graphique en forêt.

Conclusion : Le dispositif PIMA CD4 était comparable à la cytométrie en flux parce que la différence estimée entre le comptage de cellules CD4 du dispositif et de la référence a donné une précision suffisante (+/- 30 cellules/ μL). Les dispositifs miniPOC et MBio semblent bien fonctionner aussi. Il semble que les dispositifs donnent une meilleure estimation de prélèvements de sang capillaire que pour les prélèvements de sang veineux. Les dispositifs POC de comptage de cellules CD4+ font partie d'un secteur en pleine croissance, donc les fournisseurs de soins de santé ont besoin de preuves fiables pour la prise de décision en matière de technologie. La synthèse des données probantes portant sur l'exactitude des dispositifs POC de comptage de cellules CD4+ pourrait intéresser les initiatives qui intensifient l'utilisation de ces dispositifs à l'échelle mondiale.

Acknowledgements

I would very much like to thank my supervisor, Dr Nitika Pant-Pai for such fantastic support and invaluable advice; both for my work here, and life in general. I would like to thank Dr Lawrence Joseph, for his support, statistical analysis and valuable feedback.

Thank you also to Dr Tiago Chiavegatti, who was the second reviewer for this work and gave me lots of help; his fantastic insights have really shaped this work. I am also very grateful for the support and work provided by Bénédicte Nauche, librarian at the McGill University Health Centre library. Bénédicte ensured that we completed a very thorough search of the literature. Thanks also to Patrick Bélisle for his help with the forest plots.

Thank you to the professors who introduced me to Epidemiological methods, and my Epi and Public health friends who got me through! Thank you to Anne-Marie Kelly for translating my abstract for me. And finally, thank you to my partner, Ant, for being so understanding.

Glossary of abbreviations and acronyms

AIDS	Acquired immunodeficiency syndrome
ART	Anti retroviral therapy
CD4+ cell	CD4 receptor positive T-lymphocyte cells
CDC	US Centres for Disease Control and Prevention
cells/µL	Cells per micro liter
CI	Confidence interval
CrI	Credible interval
FN	False negative
FP	False positive
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
PEPFAR	President's Emergency Plan for AIDS Relief
POC	Point of care
TN	True negative
TP	True positive
UNAIDS	Joint United Nations Programme on HIV/AIDS
VL	Viral load
WHO	World Health Organization

Chapter 1: INTRODUCTION

Globally 35.5 (32.2 - 38.8) million people were living with HIV in 2012.[1] The disease burden is felt disproportionately with countries of lower resource carry the greatest burden.[1,2] Much progress has been made in curbing the pandemic, recent figures show a downward trend in mortality linked to HIV.[1] The reductions in mortality have largely been attributed to low and middle income countries scaling up Anti Retroviral Therapy (ART) programs at the national level as well-delivered ART is an important tool in the fight against HIV, both for individuals and at the community level.[1,3] However, there are still challenges, many people drop out of care and clinics and laboratories in resource limited settings can be operating in very challenging environments.

Technological advances have allowed for the development of point of care (POC) devices that can be used to monitor HIV patients more effectively in rural settings, without being reliant on infrastructure which is often lacking. New CD4 and viral load POC tests can operate in the absence of a continuous electricity supply, laboratory equipment, specialist personnel or transport systems. And so POC devices could be used to develop better care systems.[4-6] Some CD4 POCs can provide CD4+ cell counts in just 8 minutes, at the point of clinical contact. With such fast turnaround times, these assays could allow for ART to be initiated at the same site; saving time and money for health systems. For patients, they will reduce the burden on patients by reducing the number of clinic visits, thereby improving treatment adherence speeding up linkages to care and reducing loss to follow up. There are many POC CD4+ cell counting devices that are currently being marketed or developed, using different underlying technologies. The PIMA (Alere, USA) device uses dual fluorescence image analysis. The MiniPOC (Partec,

GmbH), the PointCareNOW (PointCare, USA) and the HumaCount (Human Diagnostics, GmbH) are effectively miniaturized flow cytometers. The Daktari CD4 (Daktari Diagnostic, USA) device is based on microfluidics and the MBio CD4 analyzer (MBio Diagnostics, Inc, USA) uses optical technology. Two disposable devices are also in development: the Zyomyx's CD4 test (Zyomyx, Inc., USA) and the VISITECT CD4 (Omega Diagnostics group, UK).[6-8] Although the performance of some of these devices has been evaluated, there is a need to compare their performance across specimens; finger stick and venous blood specimens. So far a comparative evaluation of their performance has not yet been performed, and as POC CD4 assays are likely to be introduced across Sub Saharan Africa and Asia there is a need to summarise their performance across devices.

The objective of this thesis is therefore to address two key questions. First, we need to identify whether these devices are comparable to the gold standard, and which devices are superior. Secondly, investigate whether these POC devices perform well for both capillary and venous blood specimens alike. This is important as capillary specimens are likely to be easier to obtain where resources are limited. In order to achieve these aims we completed a thorough search of the literature, and then a meta-analysis of the available data.

This thesis is made up of five chapters. This first introductory chapter provides an overview of the topic and the rationale for completing the work, along with the objectives. The literature review provides some background to the field of HIV, the current treatment challenges as well as a detailed review of the current knowledge of CD4 point of care devices. The subsequent chapter provides detailed information of the methods that we used to conduct the research, along with a statistical description of the meta-analysis. The next chapter presents the results of the meta-analysis and further investigations of the data. The fifth and final chapter synthesises the findings

of my thesis work into a coherent package of work, further discusses the results as well as future prospects.

This thesis consists of a systematic review of the field of HIV and the current evidence related to CD4 Point of Care assays. In addition to this we completed a meta-analysis of the evidence. The results from the meta-analysis have been submitted for publication in JAIDS in December 2013, and the work is being peer reviewed.

Chapter 2: BACKGROUND AND LITERATURE REVIEW

This section will provide a review of the literature and includes some background to HIV, HIV care and tools for monitoring infections.

2.1 Historical context of HIV

AIDS (acquired immunodeficiency syndrome) was initially recognised in the USA by physicians in the early 1980s, in a published case study that highlighted an unusual clustering of Pneumocystis pneumonia. This was a condition only rarely seen and normally only apparent in severely immunosuppressed people.[2,9] The cause of the syndrome, the retrovirus HIV-1 was subsequently identified in 1983. [2,10] Today, the most prevalent lineage of HIV is HIV-1 and has a global distribution which accounts for 99% of infections; HIV-2 exhibits very different epidemic characteristics.[11] How HIV-1 emerged as a global pandemic is a very complex topic, not without controversy, therefore this section aims to provide only summary points to add some context for the reader.

Geographical data and molecular evidence indicates that HIV-1 originated from SIVcpz (simian immunodeficiency virus) in Africa.[11-13] The timing of the event that gave rise to the earliest HIV-1 (group M) has been estimated to 1853 (95% credible intervals 1799-1904).[14] Since the emergence of HIV-1, much diversification has occurred and each group has many subtypes; and variation still increasing, particularly in Africa.[12] As the virus was not recognised until 1981 in the US, the evolution of HIV was left largely unchecked in Africa until 2001 when ART became widely available.[15,17]

How and why HIV-1 escalated from an initial infection to pandemic proportions is still unclear. Current theories associate the growth of the epidemic to changes in central Africa in the early 20th century that allowed for rapid viral adaptation and increased transmission.[11,12] From 1905 colonial cities were established across central Africa, leading to dramatic changes in the structure of the society, including forced labour camps, poor living conditions, an increased reliance of villagers on bush meat, and widespread vaccination programs.[12,16,17] These changes may have led to increased opportunities for viral adaptation and transmission by increasing exposure to zoonotic diseases, bringing previously isolated groups into close contact, using unsterilized needles in new vaccination programs, more promiscuous sexual practices, more commercial sex workers and an increased prevalence of GUDs (genital ulcer diseases).[16-20] Despite these theories, what is clear is that HIV-1 is a major pandemic, which, since its emergence, has claimed the lives of millions of people.

2.2 Biology of HIV

HIV is a retro virus which infects activated CD4 T-lymphocytes and leads, over several years to the systematic destruction of T-lymphocytes, namely the CD4+ T-cells.[21] The HIV virus invades T-lymphocytes by binding with the cellular CD4 receptor, as such, it is the CD4+ T-lymphocytes that are selectively destroyed (either by cytotoxic T-cells or poor replication of CD4+ cells after the weakening of the immune system).[21,22] Eventually, the depletion of CD4+ T lymphocytes destroys the integrity and resistance of the immune system, leaving the infected individual frail in the face of opportunistic infections; at which point the disease is known as AIDS (Acquired Immunodeficiency Syndrome).[21] Generally, the progression of HIV can be categorised into stages. The first stage, 2-4 weeks after infection, is defined by very high viral loads and falling CD4+ cell counts. This acute stage ends 3 months following exposure.[23] As early stage HIV

infections (acute infections) are characterized by very high viral loads, the likelihood of transmission is also higher during this stage.[24] Following the acute stage, the disease usually becomes latent for a time, showing no clinical symptoms for up to 10 years.[23] The final stage is defined by high viral loads and the onset of multiple indicator infections, this stage is referred to as AIDS and usually leads to death within 2-3 years.[2,23,25] Technologies that provide CD4+ cell counts can therefore be used clinically to provide an estimate of the disease stage and can be used to track disease progression.[21,22] Another assay that is important for staging and monitoring disease progression is viral load (VL) which gives a direct measure of HIV RNA and is associated with CD4+ cell counts and clinical progression of the disease.[21,22,26]

2.3 Epidemiology of HIV and the developing world

The UNAIDS World AIDS Day report 2013 estimates that globally 35.5 (32.2 – 38.8) million people were living with HIV in 2012.[1] Global new infections during 2012 are estimated to be 2.3 (1.9-2.7) million. Worldwide, Sub-Saharan Africa has the highest prevalence of HIV; currently, approximately 4.7% of adults (aged 15-49yrs) are living with HIV amounting to approximately 23.5 million people.[1] Therefore Sub-Saharan Africa accounts for over 60% of the global burden of HIV.[1] Other low and middle income countries (LMIC) such as Eastern Europe, central Asia and the Caribbean are also heavily affected with approximately 1% of all people living with HIV.[1]

One of the UNAIDS targets is to "Reach 15 million people living with HIV with lifesaving antiretroviral treatment by 2015"[3] and the latest figures indicate progress towards this goal.[3] In 2012, ART had reached 9.7 million people in low and middle income countries, but this still represents just 34% of those people eligible according to the new WHO ART guidelines.[1,27]

Access to ART is highly country specific, some countries in eastern Europe, central Asia, the middle East and North Africa still only provide ART to 15-25% of people who are eligible.[3] Access to the most vulnerable populations is very important as vulnerable groups are facing their own challenges in society and are often carrying a disproportionate burden of HIV.

2.4 Diagnosis of HIV infection

Various laboratory assays are relevant to the diagnosis or care of HIV infections. These are outlined in table 1. Traditionally, diagnosis of HIV is predominantly completed in the laboratory and can be considered as either screening (highly sensitive to prevent false negatives) or confirmatory assays (highly specific at the expense of some sensitivity).[2] Laboratory based methods for HIV diagnosis use immunoassays (to detect antibodies) or molecular PCR-based assays (to detect HIV antigens directly).[2] Modern HIV immunoassays can detect known HIV-1 group M subtypes, group O and HIV-2, however, such assays can require advanced methodologies that are not always available in resource limited settings.[12]

Assay target	Methods	Use	Notes
HIV antibodies	Immunoassay	Diagnosis	Gold standard assays Detect human antibodies against HIV-1 and HIV-2 (detectable 3-4 weeks after infection) Identify recent or established infections
p24 antigen	Immunoassay	Diagnosis	Can detect infection approximately 2-3 weeks after infection
Detect specific HIV genes	PCR	Diagnosis	Useful for testing neonatal blood and donated blood
CD4+ cell assays	Flow cytometry	Monitoring	Measure of CD4+ cell count
Plasma HIV-1 RNA load (Viral Load)	Real-time PCR	Diagnosis Monitoring	Useful for diagnosis of acute infection Measure of viral load
Drug Resistance	Phenotypic (Recombinant virus assay) or Genotypic (PCR sequencing)	Monitoring	Phenotypic: Laboratory created recombinant viruses are tested for susceptibility to therapies. Genotypic: Test for specific known mutations that infer drug resistance.

Table 1 Assays for diagnosis and monitoring of HIV from blood samples Adapted from [2,28,29]

The success of HIV screening programs in low and middle income countries have been restricted by laboratory tests due to limited resources and minimal infrastructure.[30] Laboratory tests also

suffer from long test-to-result times and can be inconvenient for patients who need to find time to return to the clinic for the results which results in many people remaining unaware of their serostatus.[31,32] Now, many rapid POC tests for HIV are available and are widely used, these assays rely on detection of antibodies. POC tests are very useful in rural and resource limited settings and can identify HIV infection from capillary or saliva samples, allowing for decentralization of HIV diagnosis.[33] Self testing devices are now also being developed and implemented, particularly for the developing world. These new ways of testing for HIV could result in dramatic changes in HIV care pathways, could empower patients to take more control of their health and conquer some barriers to testing (such as privacy issues, sigma, and access).[33,34] Alongside these new screening strategies, the use of POC CD4+ cell assays could help to make the monitoring aspect of HIV care more accessible in rural and resource limited areas. With availability of treatment, improvements in monitoring tools are warranted. POC CD4 and VL assays promise to a) reduce waiting times for patients, b) cut down time to treatment initiation, c) improve the control of HIV infection with improved monitoring, and d) deliver the quality of care that is needed to bring the epidemic under control.

2.5 Treatment for HIV

Standard care for HIV usually relies upon synergistic combinations of the drugs detailed in table 2, with an aim of targeting multiple molecular sites, limiting toxic effects and reducing the risk of resistance; such an approach can be referred to as Highly Active Antiretroviral Therapy (HAART).[35,36]

Drug group	Mode of Action	Example agents
Nucleoside Reverse Transcriptase Inhibitors (NRTIs) [37]	Active portion of NRTI competitively binds to viral reverse transcriptase, halting DNA chain elongation [37]	Abacavir, Didanosine, Tenofovir disoproxil fumarate, Stavudine (cheaper and more commonly used in resource limited settings but leads to many adverse effects)[37]
Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Interaction with reverse transcriptase to inhibit catalytic site [36]	Efavirenz, Nevirapine, Delavirdine [36]
Protease Inhibitors (PIs)	Inhibits HIV protease, preventing cleavage of precursor polyprotein [36]	Saquinavir, Ritonavir, Indinavir [36]
Fusion Inhibitors	Disruption of proteins involved in fusion process [38]	Enfuvirtide [38]
Entry Inhibitors - CCR5 co-receptor antagonist	Blocks HIV interaction with CCR5, common co receptor, particularly for early stage infections. Current interest in use of entry inhibitors to prevent HIV infection.[39]	Maraviroc[39]
HIV integrase strand transfer inhibitors	Blocking the catalytic site of HIV integrase which acts to integrate HIV RNA into the cellular genome [40]	Raltegravir, Elvitegravir[40]

Table 2 Action of HIV drugs [22,36-40]

Now, single tablet drug combinations are available such as Complera (Emtricitabine, Tenofovir disoproxil fumarate and rilpivirine) and Stribild (elvitegravir, emtricitabine and tenofir with cobicistat [a pharmacoenhancer]).[41,42] Successful treatment is characterised by sufficient

suppression of viral replication in order to halt the destruction of CD4+ T-cells. With well delivered care CD4+ T-cell levels can recover to some degree.[43] The effect of ART on mortality is dramatic, this is exemplified by huge changes in HIV mortality rates in the USA, upon the introduction of HAART in 1996, death rates fell dramatically. [35,44-46]

In the early days of treatment, drugs were not cheap, costing upwards of US$20,000 per person per year.[47] Because of the restrictive cost, HIV programs in countries with lower resources relied upon a number of global interventions. The Doha declaration, for example, adopted in 2001, allowed developing countries to be flexible with Trade-Related Aspects of Intellectual Property Rights (TRIPS) when public health was a concern.[15] Such interventions eventually allowed many more people in those countries (where approximately 90% of people with HIV were living) access to ART by decreasing the annual ART cost from US$10,000, to less than $200 per individual.[45,48] Efforts to diagnose and treat HIV positive individuals in resource-limited settings only really began in 2001 with government initiatives such as the United Nations global fund and NGOs such as the Gates and Clinton Foundations. [45,49,50] In 2003, the US initiative, the President's Emergency Plan for AIDS Relief (PEPFAR) was created, pledging $15 billion over 5 years to 15 countries with the highest HIV burdens.[51] Since then PEPFAR has funded treatment and support for more that 15 million people through the development of partnerships and healthcare expansion.[51]

2.6 Monitoring in a resource limited setting

Success of HIV treatment not only relies on access to ART, but on highly effective monitoring of people with HIV. WHO guidelines dictate that not all HIV+ patients are immediately started on ART, instead treatment is delayed and carefully monitored.[27] This is because HIV resistance is a major challenge in HIV care and can affect up to 50% of people receiving ART.[12, 24] When treatment does fail due to the development of viral resistance, clinicians usually switch the patients onto another drug combination.[21,52] In resource limited settings where only two lines of treatment are typically available it is very important to maximise upon the available treatments. And if HIV is incompletely suppressed with ART, viral resistance can develop very quickly.[53] Therefore the initiation and switching of a patient's treatment regime is a fine balance; starting or switching too early will risk depleting effective treatment options and too late will risk the accumulation of resistance. [21,22,45,52,53]

To guide initiation and switching, and where resources allow, laboratory assessments of disease progression are used to guide HIV care. These laboratory assessments include direct measures of HIV viral load (VL) and genotypic resistance monitoring.[45] However, these assessments are expensive and so are not accessible where resources are limited, instead the WHO recommends CD4+ cell counts as a substitute marker in resource limited settings.[54] CD4+ cell counts have been shown to reflect immunosuppression, the likelihood of opportunistic infections and mortality.[54] However, CD4+ cell counts are an indirect measure (thus, called surrogate biomarkers) of the disease and as such are less sensitive than viral load assays, particularly for early detection of virological treatment failure.[55,56] Therefore CD4+ cell count technology represents an important minimal standard of care, used for both the initiation and monitoring of treatment of HIV in resource limited settings.

The current WHO HIV guidelines for the treatment of HIV require that ART is started in asymptomatic individuals when the CD4+ cell count falls below 500 cells/μL.[3] This is a higher threshold for ART initiation compared to the previous WHO recommendations of 2010 and 2006 when the initiation limit was <350 cells/μL and <200 cells/μL respectively.[54,57] With these changes, at the end of 2012, an additional 9.2millioins people became eligible for ART compared to 2010, this is a figure bound to increase with a reduction in HIV-related mortality.[27] In addition, it appears that these changing guidelines may have impeded the development of POC CD4+ cell counting devices, specifically qualitative devices; this is described in later sections.

Increasing numbers of people eligible for ART will likely bring further challenges for resource limited countries where HIV treatment programs are already stretched; as such new technologies and care pathways must be developed in order to deal with these changes.

2.7 Current challenges and new care models

The challenge of ART delivery in developing countries doesn't just stem from a lack of resources to acquire the correct drugs. Wider problems are faced, as detailed in table 3. First-line therapies can fail for a number of reasons, mainly due to poor adherence to therapy and development of viral resistance.[43,53,54] The success of treatment therefore heavily depends upon the commitment by patients who, in many cases, could be facing their own challenges.

Patient challenges	Clinic and provider challenges	Laboratory and Health system challenges
• Social stigma and visibility that impairs access • Clinics difficult to reach fewer located in rural areas • Limited access to transport impedes regular visits • Limited time to visit clinic on multiple occasions	• Unstable drug supplies and device supplies • Limited infrastructure, electricity, transport • Must transport samples to central lab • Long turnaround time to test results • Unstable lab services • Poor providers to front line health care professional ratio	• Lack of infrastructure • Lack of specialist personnel • Loss to follow up of patients • High demand for lab services (e.g. in rural areas of Zambia, the number of CD4 tests required was 1.7 times greater than the laboratory capacity of the area. [58]

Table 3 Some of the barriers to HIV care [42,51,52,57,58]

Each contributor to HIV care, in a resource limited setting can face substantial barriers, leading to a disconnected and variable care pathway for patients. Laboratory staging of HIV typically takes approximately one week; patients are required to attend two appointments, first for the

blood draw and then for the result of the CD4+ cell count and appropriate care.[59,60] As a result many people drop out of care and remain oblivious of their eligibility for ART; only about 60% (range 35-88%) of individuals who receive an HIV diagnosis in sub-Saharan Africa receive a CD4+ count.[60-63] Many people also drop out of care once they have started on ART; in Africa this figure has been estimated to be around 40% over two years.[63]

Advances in molecular technology could hold the key to more successful linkage of eligible individuals with treatment, and help to reduce some of the barriers to care. Recent years have seen the development of a number of rapid assays that enable clinicians to determine CD4+ cell counts at the point of care (POC) allowing them to make treatment decisions right away.[7] These assays have recently shown the potential to improve patient retention, improve linkage to care, and relieve the pressure on regional laboratories by offering a decentralized alternative to laboratory assays.[4,5,60,64] POC devices that are less reliant on infrastructure such as electricity and highly skilled workers could mean that in the future, clinics can be based in rural communities more badly affected by HIV and deprivation.[5] This model of decentralized primary care could increase the number of people aware of their status, improve the initiation and monitoring of ART and ultimately generate more successful programs.[4,5,59,60,65]

The delivery of HIV care in resource limited settings is still relatively new and service delivery requires development, an urgent scale up of services is needed. This is seen in some countries, for example, in Kenya between 2000 and 2009, HIV testing centres increased from 3 to 900; and mobile clinics are still being implemented.[66] There is still a lot of development of ART clinic service delivery in a bid to overcome barriers to accessing care. For example, a project started in 2004 in Zambia, introduced HIV testing and care to primary care sites, delivered primarily by non physician clinicians.[67] This also included a home-based ART adherence monitoring

program.[67,68] The program has achieved mortality rates more aligned with a developed setting, showing that high quality treatment can be delivered in rural areas of limited resource, with fewer specialist personnel.[67,68] Despite much progress the program is still seeing a lot of people drop out of care, particularly in those not yet initiated onto ART.[68] Perhaps with the addition of POC CD4+ cell counting assays at the clinic, or as part of the home visit system, some enduring barriers could be overcome. Further research is needed to identify the reasons for these high drop outs, even when more convenient care settings are used.

Another interesting study, an international, randomized investigation of a multi-level intervention (mobile testing with community intervention and post-test support) compared to standard care, is called Project Accept.[69,70] The study sites were largely rural locations, in high burden countries (Thailand, South Africa, Tanzania, Soweto and Zimbabwe). Mobile clinics offered rapid tests in community setting such as transport venues, community centres and places of worship, on a schedule that suits working people.[69,70] Again these clinics were staffed with non-physicians clinicians, overcoming the lack of physicians in many countries. For the intervention group, more people were tested for HIV compared to the standard care group and identified four times as many HIV+ individuals.[69,70] This project demonstrates that empowering front line workers is key for the optimization of service delivery. In this model of decentralized care offered by front line health care workers, point of care CD4+ cell counting assays could very conceivably be incorporated to overcome barriers further down the patient care pathway.

Home based screening may be a feasible option in some settings and initial evidence suggests that CD4+ point of care assays could be applied in home-based settings; a pilot achieved 90% linkage to care.[71,72] It seems feasible therefore that POC CD4+ devices, alongside POC HIV testing could allow clinics to provide the full range of care (from screening through to

monitoring of ART) in decentralized settings, reducing the burden on patients and reducing subsequent losses to follow up. By being mindful of the needs of the HIV+ population, HIV care should be better tailored to groups that are vulnerable to drop out of care. More evidence and research will be important in this field to identify the most desirable pathways for patients, which will work around social barriers such as stigmatization.

In the future, amid people with HIV living longer, the development of new service delivery models will be crucial in order to deliver high quality care; CD4 POC assays could form a part of the solution. POC devices could help to create more choices for people while also allowing more efficient use of investment in ART treatment. Regular monitoring of treatment response and tailoring medications will help optimize treatment.

2.8 Systematic review of point of care devices for CD4+ cell counting

This section will outline the research relevant to point of care CD4+ cell counting technology, highlighting how the technology has developed and detailing the first implementation research that hints of the potential impact of these devices. Following this chapter, the focus of the data analysis will be the accuracy of the device, predominately the agreement of the POC device with the reference standard (flow cytometry).

The first concepts for POC micro fluidic devices were published between 2005 and 2007; these were mainly in the form of laboratory concepts. These articles described how advances in technology would allow for rapid CD4+ cell counting, with minimal sample preparation. Minimal sample preparation was pivotal for developing equipment free assays for resource limited settings.[73-77]

A range of POC CD4 devices are now being marketed or developed, with different underlying technologies. Prominent among them are the PIMA (Alere, USA) which uses dual fluorescence image analysis. The MiniPOC (Partec, GmbH), the PointCareNOW (PointCare, USA) and the HumaCount (Human Diagnostics, GmbH) which use miniaturized flow cytometry, whereas the Daktari CD4 (Daktari Diagnostic, USA) device is based on a electrical impedance microfluidics and the MBio CD4 analyzer (MBio Diagnostics, Inc, USA) uses optical technology. Two well known handheld disposable devices are also being developed, the Zyomyx's CD4 test (Zyomyx, Inc., USA) and the VISITECT CD4 (Omega Diagnostics group, UK).[5,6,8,78] To our knowledge no evaluations of the HumaCount, the Daktari CD4, the Zyomyx CD4 test or the VISITECT CD4 (test have been published. However, the VISITECT CD4 device is a qualitative assay that indicates that treatment is required when CD4 levels drop below the 350 cells/μL cut off, which was set in the WHO 2010 guidelines.[56,79] Perhaps this device will need to be updated prior to evaluative studies, given the new CD4 cell cut off of 500 cells/μL introduced in 2013.[27] The Zyomyx device was developed with funds from the Bill & Melinda Gates Foundation, and in summer of 2013, Zyomyx announced a new distribution agreement, with a focus on the developing world so it seems that this device is still at the development stage.[80]

Prior to regulatory approval, and after the concept has been developed, clinical assays must undergo laboratory and field trials to establish the performance of the device.[81] Such evaluations are predominately cross-sectional studies, that use leftover clinical samples or use a convenience based patient sample. Once the device is approved for use by regulatory bodies then further investigations of implementation and patient centred outcomes can be undertaken.[81] Currently, the stage of research for POC CD4+ cell counting devices appears to be at the evaluation of

performance stage, with only a few studies reporting additional outcomes such as patient-centred outcomes.

The first field evaluations of marketable CD4+ cell devices was published in 2010; the PIMA device was evaluated at a voluntary testing and counselling centres (VCT) in Zimbabwe.[82] This was a particularly interesting study as one of the few that investigated the use of point of care assays for newly diagnosed participants; a stage of the HIV care process where many people are lost to follow up.[82] The study appeared to be well designed, as a cross-sectional study using a convenience based sample; consecutive clinic attendees were approached during post HIV test counselling sessions. Convenience based sampling can introduce bias, as the participants included in the sample may not be representative of the general population. People attending the clinic for HIV testing and subsequent eligibility of the study may be systematically different to the general population with HIV, whom the device would be utilised in, introducing a spectrum bias.[83] However these tests are likely to be applied in these patients so probably represents a reasonable study group. The study reported that during the study, the service experienced a 13% increase in attendees at the clinic, perhaps indicative of patient interest in the device.

Many more studies followed in 2011, these included evaluations in hospital laboratories, clinical sites, primary care sites, and a mobile clinic.[84-88] These and all other studies included in the meta-analysis utilized a cross-sectional design; either specimens randomly chosen from leftover clinic samples or convenience based sample of clinic attendees, all of these studies are vulnerable to spectrum bias.

One study in 2011, completed by Jani et al investigated three POC assays for use in drug toxicity monitoring in a primary health clinics in Mozambique.[86] This is an important study for the basis

of the motivations for this thesis as this study concluded that POC devices could be applied in decentralized settings, with non laboratory staff completing the assay.[4] This study was a retrospective cohort study, whereby data from patient records were extracted before and after a CD4 device was introduced into care, to examine how long it took for patients to progress through the treatment cascade (CD4 counts, treatment initiation, etc.). The implementation of the PIMA device lead to a substantial reduction in the number of people lost to follow up prior to ART initiation, dropping from 64% to 33%, indicating the importance of POC CD4+ cell counting devices in these settings.[4] As a retrospective cohort study this is subject to bias from many sources. First, there was significant time difference between the two cohorts; approximately 1 year. In this year many significant changes may have been experienced in the clinic or area that could have impacted upon the rate of ART initiation. Although the two cohorts appear similar in characteristics it seems feasible that services could have progressed in this time; for example increased community education or changes to the way that data is recorded. Possible interventions such as these could have impacted on estimates of the outcome, potentially overestimating the effect size. Second, given that this is a retrospective study it would be very hard to blind observers to the cohort that they are observing. Therefore, as data is collected observers may be bias in their interpretation, leading to an information bias that could overestimate estimates of effect. A randomized study would be a better design for estimating the effect of POC testing in the field, and would handle some of these biases. However given the greater level of investment required in such a study, a retrospective study is a good first indicator of potential effect.

The first evaluation of the Partec Cyflow miniPOC device was also presented at a conference during 2011.[89] As an early evaluation, it was completed in a laboratory and used clinical samples from a hospital in Zimbabwe. However it is not clear how these patents were selected, which could introduce some selection bias (spectrum) as the patients may not be completely representative of the target population.[83] As this was a poster presentation, the information was lacking in some areas; apart from in the title it is not confirmed that all patients were HIV+, it only inferred in the source of the patient samples. However if the patients were not all HIV+, again spectrum bias could be introduced, the performance of the POC in this patient group may not be applicable to the target group. All samples were assessed using both the index test and the reference, which is important to reduce verification bias. This work was also completed by the manufacturers of the device and so would have a clear conflict of interest in the results of the study; whilst this may not exclude the study entirely, this should be recognised when interpreting the results. The paper also reported results for children, this is important as the miniPOC device is one of the only two POC CD4 devices that can currently provide a percentage CD4 count.[89] As the WHO recommends CD4% is used for children, this capability could be very important in the future.[27] Data missing from the study may impact on the assessment of CD4 devices; the most probable form of this bias is from device failure. If the device fails in a non-random way, this could be a source of bias. In this study however there are no missing data points making this bias unlikely.

Evaluations of the PIMA device in Africa, India and the western countries continued to be published during 2012.[90-95] One included HIV+ pregnant women from an urban HIV clinic in Johannesburg, South Africa.[96] The applicability of results from studies that included pregnant women is unclear. The current WHO guidelines for the initiation of ART from pregnant women

recommend that all women are initiated onto ART in order to prevent mother to child transmission of HIV.[97] Therefore CD4 devices could be used at baseline to stage women when they start on treatment, and with VL assays to predict response to treatment in women and control of HIV infection in their children. Again this was a convenience based sample, making the study liable to bias; the participants could differ from the population of interest if the participants arriving at the clinic change when the new intervention became available, although this will unlikely affect that Bland Altman statistic if the device is expected to perform well throughout the range of CD4.

The literature evaluated demonstrated several CD4 devices that could be used in various settings. Another study from Mbopi-Keou et al. based in Cameroon was, in 2012, able to implement a simplified CD4+ cell counting flow cytometer in a mobile therapeutic unit, running from a battery. The objective of this study was to evaluate the use of a simplified flow cytometer in a mobile clinic operating in settings far from a laboratory. Although this was not a POC device , the mobile unit was able to visit suburban areas in Cameroon providing care to people that do not usually have access to routine laboratory facilities.[98-100] The comparison device was a laboratory based flow cytometry. This was a convenience based study, the volunteers attending the mobile clinic were included, and chosen irrespective of their HIV status; although no details on how participants were approached is given. This could again introduce spectrum bias; the results may not be applicable to the target audience. Additionally, HIV- people were included in this study, this adds a potential for spectrum bias as the test is evaluated in a population that is not clinically relevant.[83,99] As the CD4 + cell counting devices are not likely to be used in HIV- populations there is potential for bias here.[82,98] HIV- people are likely to have much higher CD4+ cell counts and with much wider ranges; therefore the performance of CD4+ counting machines could differ

quite significantly. Blinding between the reference and mobile flow cytometer has not been explicitly stated introducing some uncertainty and potential for information bias to be introduced. However, the fact that the two devices were analysed at different times, in different places makes it seem unlikely that bias was introduced here. Results indicate that the mobile flow cytometer was able to work well in a mobile clinic, with Bland Altman mean difference of +7.6 cells/μL, with reasonable intra-run inter-run precision of 5.5% and 7.9%. Therefore, even though the device is not a point-of-care device in that it requires a mobile laboratory, this appears to be a good option for this particular setting.

The first evaluation of the PointCareNOW device was published in 2012.[101] This was an extensive study that reported the results of evaluations of the PointCareNOW device completed in separate and very different sites. The study included patients attending clinics at hospital in Mozambique, a university clinic in Mozambique, samples from the public health centre of Canada, patients attending a clinic in the US and anonymous samples from South Africa.[102] The CD4+ cell count was evaluated using the PointCareNOW device with flow cytometry as the reference standard. The data included in this article was limited, perhaps because so many sites are included in just one article. It is clear that different methods were utilized depending on the site, for example, some sites used different comparator flow cytometry machines. As some information was missing, limited information is available regarding patient flow. Patient flow is an important aspect of the study design which allows for readers to assess the likelihood of spectrum bias, as already discussed. In addition, there is no mention of blinding in the paper, and it is not clear whether the same individual read the results of the index and that of the reference standard. This again is of great concern as a potential source of information bias. Most notably for this evaluation, the manufacturer of the PointCareNOW device (PointCare) published two

letters to the editor in response to the findings of the paper, introducing some doubt when interpreting the results.[101] In addition, the results reported indicate that the device strikingly overestimated the CD4 count at all sites, with Bland Altman point estimates up to +180 cells/μL.[102] Considering the surprising results reported, the lack of clarity in the report and the concerns raised by the manufacturers, we have utilized the results of this study with caution in our meta-analysis.

At a conference in 2012, Barnabas et al presented details of a very relevant, field evaluation of the PIMA device.[93] The first study to evaluate the device in a home testing environment, the PIMA device was used in home-based counselling and testing in rural Vulindlela, Kwa-Zulu Natal, South Africa.[93] Patients were first tested for HIV, if positive; the CD4 count was completed straight away. This was a very interesting study, not only for the setting but also because the patients were given the results of the PIMA device, to later take to a HIV clinic for treatment. An analysis of the before and after effects showed that the number of people receiving CD4 staging after a positive HIV test, increased from 59% to 100%, and the number initiated onto ART rose from 68% to 85%.[93] Further, the number of people retained in care after 3 months increased from 72% to 88%.[93] These pilot results are very promising and suggest that point of care CD4 counting devices could make major differences to the care pathways of people receiving care in rural settings.[72,93] It is not very clear how participants were recruited into the study, what group was eligible to receive a home visit. If those people eligible for home based screening were not also those people included in the study a bias could be introduced. This study reported that 64% of participants were also already aware of their HIV+ status prior to the study.[93] This figure could perhaps indicate that people were motivated to take part in the study for other reasons; maybe they wanted to receive the CD4+ cell count and so represent a more

proactive population, more likely to seek care. The reasons are not clear, but it seems likely that these could lead to an overestimation of the likely rate of ART initiation when POC CD4+ cell counting devices are used.

An investigation by Herbert et al [95] in 2012 was the first study to be completed exclusively in a developed setting, and of particular interest are the results from their patient survey. The patients eligible for the study were adults attending a large, inner city London HIV outpatient clinic. The study included all patients accessing care and was split into two phases, first in chronically infected and newly diagnosed patients, and secondly in acutely unwell or people re-accessing care through a drop-in clinic. All participants underwent testing using the PIMA and the laboratory flow cytometer in parallel and were then asked to complete a five-point Likert questionnaire to collect their views. The paper reported accuracy (sensitivity, specificity and Bland Altman) alongside results from the patient survey. They asked whether the PIMA was preferable to the laboratory test and found that 54% of participants would prefer it. Results also indicated that 87% were willing to wait for 20 minutes for the results. Additionally, 49% of patients would be happy to have the POC test at their GP; preference for a GP test was associated with higher CD4 counts (compared to lower CD4 counts) and for those chronically infected (compared to newly infected). These results indicate that further investigations of patient preference are warranted as there may be a need to improve the patient experience. However, given the cross-sectional nature of the study, it is again subject to bias. It is unclear whether the people accessing care at this clinic are representative of the wider population. Those people accessing care at this clinic must already be happy with the care that they are receiving, this survey misses people that have been lost to follow up; it is those people unhappy with the standard care that could stand to benefit the most from POC CD4+ cell counting. As a result, the

patient preference could be underestimated, and will not reflect the potential true benefit to the population. Additionally, the questionnaire was not a validated questionnaire, and was only piloted on a small number of patients prior to use. As the survey is not validated it is unclear how well the questionnaire collects the intended information. Therefore, the results should be treated with caution, overall though, the study points out the need for using qualitative research for POC CD4 testing.

During 2013 the first evaluation of the point of care CD4 counting device from MBio from the US was published alongside a further evaluation of the Partec miniPOC CD4.[59,103] The MBio device was evaluated in HIV+ patients attending a university research HIV clinic in the US. The POC MBio SnapCount system was compared against a reference flow cytometer. The POC device was operated both in a laboratory setting for venous specimens and in the clinic for capillary specimens.[59] There is some concern regarding the patient flow, as again this is a convenience based sample. Additionally, exclusion criteria included anemia and contraindication to venipuncture. However it is not clear how many people were excluded based upon these criteria, if many people were excluded then this could be a problem. If very sick people were excluded based on these criteria the results may not generalize to the population. Additionally, as was the case for many of these studies, the blinding between the device and reference raters was not explicitly stated, however the device counts were completed at different sites making it less likely for information bias to be introduced here.[59]

Newer CD4+ cell counting concepts also are being developed, however, to date these technologies have not been developed into marketable devices, or have not been evaluated. Some of these developments could be very interesting and beneficial. A saliva based CD4+

enumeration technique is being developed that may lead to a device that can provide CD4+ cell counts from saliva, using serum levels of a protein that is correlated with CD4+ cell levels.[104]

Most recently a handheld battery powered device that relies upon electrical impedance is being developed, and early results from laboratory evaluations show promising results. The device appears able to provide accurate results within 10 minutes, with little sample preparation, making it a very option for the future.[105] [106] Handheld disposable devices could signal the next generation of POC CD4+ cell counting devices, further eliminating the need for electricity. [6,7,105,106] Hand held devices will be a big help for those physicians who work in rural areas-they could test, stage, initiate treatment in one-two visits, without waiting for a long time for lab results. This could help link patients to care and retain patients in care, preventing loss to follow up, reducing changes of viral rebound and development of resistance.

2.9 Previous meta-analysis and systematic review

To date, a number of narrative reviews of CD4+ cell counting devices have focussed on technological aspects of devices.[7,8,94,107] To the best of our knowledge, no comparative evaluations of device performance have been performed, and there is a need to summarise the current evidence of performance across devices.

Chapter 3: METHODS

The methodologies employed in completion of this thesis are outlined in this section, along with background information for some of the methods used to assess the POC CD4+ cells assays.

2.1 Protocol

Before conducting the systematic review, a protocol was created, submitted and accepted by the Prospero register (No. CRD42013003666, appendix page 78). Prospero is an online repository of protocols submitted by prospective systematic reviewers; the aim is to avoid unnecessary duplication of work between research groups.[108]

Search strategy

To identify relevant research a search strategy was created with a specialist librarian from the medical library at the McGill University Health centre. The search was completed in April 2013 and included literature published from January 2000, up to April 2013. The search included 10 databases: Medline, Embase, BIOSIS, EBSCOhost, LILACS, African Index Medicus, Pubmed (excluding Medline), Web of Science, Scopus, and Central. Conferences were searched using 4 databases: Biosis, Embase, Web of Knowledge and Scopus. Relevant conferences were also manually searched: IAS 2011, AIDS 2012, IDSA, ISSTDR 2011, CAHR 2011, CAHR 2012, CROI 2013, CROI 2012, and IAS 2013. A manual search of the grey matter was also completed, this included relevant agencies and clinical trials registries: Health technology assessment agencies, Canadian Agency for Drugs and Technologies in Health (CADTH), Ontario Ministry of health and Long Term Care, World Health Organization (WHO), International Network of Agencies for Health Technology Assessment (INAHTA), Joanna Briggs Institute, metaRegister

of Controlled Trials (mRCT) and ClinicalTrials.gov. The search was updated in July 2013; this was run in Medline only. In addition, the bibliographies of selected studies and relevant reviews were then retrieved using Web of Science and Scopus. References retrieved from these studies were used to evaluate the validity of the overall search strategy; only one additional poster was retrieved via this search, indicating that the search strategy was valid.[109] In addition to the search of the published literature, the manufacturers of the point of care assays and related research groups were contacted to request the latest results of evaluations; no additional information was received.

Study eligibility

The ASSURED criteria are a set of benchmarks created by the WHO that define the minimal characteristics of POC devices. These criteria are outlined a being Affordable, Sensitive, Specific, User friendly, Robust and rapid, Equipment free, Deliverable to the people that need them. [110] However these criteria, were found to be either too restrictive or too subjective, and so instead a paper from 2012, by Pai et al. was used as the basis for defining POC.[111] Studies were eligible for inclusion if the device evaluated was commercially available, able to provide a rapid result within a patient visit, and if it could be applied in resource limited settings.[111] Concepts of new technology were not included as these are not currently available for use.

Flow cytometry is the current gold standard for determining CD4+ cell counts in the developing world. Studies were included only if the device was compared to a flow cytometry. Many different machines and methods are utilized when completing flow cytometry. As the technique should theoretically produce the same result across devices we did pool the results from different reference devices. Where a study reported results compared to multiple methods, the

FACSCalibur, the most commonly used machine across the developing world was chosen as the first choice and the FACSCount as the second choice where required. This rule allowed the results from all selected studies to be included in the analysis. Results from other machines were included if the FACSCount or the FACSCalibur were not used. Where studies reported sub grouped data or data from multiple sites, these were included as separate studies, but only if there was no cross-over between participants.

Selection criteria

The Bland Altman mean bias is the chosen outcome for this study, therefore only studies reporting an absolute Bland Altman mean bias, along with a measure of variance, were included in the analysis. Other outcomes, including sensitivity and specificity were also collected where available.

HIV- participants were excluded due to the very wide variation in the CD4+ cell counts of HIV- compared to HIV+ individuals. Including HIV- individuals would add unnecessary variability that would not be noted in the real world. Only results including HIV+ only patients were included in the analysis.

Children (younger than 16yrs) were excluded from the analysis as there is evidence of hemodilution in these patients, making absolute CD4+ cell counts unreliable for this patient group. Only percentage CD4+ cell counts are recommended for children. In addition, in an update to the WHO guidelines, children are now recommended for ART initiation immediately, regardless of CD+ cell counts.

All studies were included, regardless of language. Translations would have been requested had this been necessary, however this was not required. All citations, including abstracts, were included if they reported contained sufficient information for the meta-analysis.

Screening process

After electronically searching the literature, the results were imported into the citation management software, Endnote. Title and abstracts were screened simultaneously and independently by two reviewers (SW and TC). At this stage the selected studies from the two reviewers were compared, discrepancies resolved. Three (of 121: 2%) citations were different between the two reviewers, these were included. Full text articles were obtained for the relevant articles and both reviewers assessed for eligibility. This left a list of 16 identified studies that were of direct relevance to the evaluation of POC CD4+ cell assays.

Data extraction

The data abstraction form was created and piloted on a subset of the included studies; the final paper data abstraction tool is presented in the appendix, page 83. An Access database was created based upon the final paper abstraction tool, an example of the input form is on page 87 of the appendix. Later, data were directly entered into the database. Data was abstracted by reviewer one (SW), the second reviewer (TC) abstracted data for 50% of the studies, the data from the two reviewers was compared to check for discrepancies. In total from the 16 papers, 31 independent data points were included into our analysis. Where information was missing, authors were contacted by email. One author provided additional new data.[94]

The data points initially collected from papers included details on the study design, populations included, type of device, % similarity, measures of correlation, and measures of repeatability. Initially we had hoped to complete a meta-analysis on multiple measures of accuracy and repeatability. However it became clear that these outcomes were not consistently reported in the selected articles and so the only outcome of interest that we could reliably report on became the Bland Altman analysis.

Summary measures

Our selected outcome measure is the Bland Altman mean bias. This was the most consistently reported measure across the eligible studies. The Bland Altman is appropriate for a quantitative CD4 measure as it is a widely used and understood method that allows the agreement between two measures of a continuous variable to be assessed on the same scale.[112] The Bland Altman mean bias allows for assessment of systematic differences between two devices, the Bland Altman mean bias (d) is calculated as follows:

$$d = \frac{\sum_{i=1}^{n} x_i - y_i}{n}$$

Where, i represents the subject, n is the total number of measures, x is the count from the index device and y is the count from the reference device.[112]

The limits of agreement provide further details on the lack of agreement, by calculating the standard deviation (s) of the Bland Altman mean bias (d) 95% of the differences will be expected

to fall with 1.6 standard deviations of the mean. The limits of agreement (LOA) can therefore be summarised as:

$$LOA = d +/- 1.96\ s$$

Where, LOA= limits of agreement, d=Bland Altman mean bias, s= the standard deviation of the bias measurements.[112]

The LOAs are very important, in defining clinical equivalency for this analysis; we would expect that 95% of the measurements should fall within +/- 30 cells/μL of the reference standard. Therefore, devices that report differences outside of this range were not deemed to be equivalent to the reference. Multiple methods exist for the evaluation of quantitative tests such as CD4 counting devices. Of the studies identified through the systematic review the majority of studies used the Bland Altman measure to quantify the agreement between the CD4 device and the reference. However four citations were excluded from our meta-analysis as alternative methods were used to measure agreement.[95,113-115]

Other outcomes were also considered such as accuracy (sensitivity and specificity) as well as percentage similarity; however these measures were not systematically reported in the literature. Sensitivity and specificity would have been a useful measure for such analysis, however, the WHO guideline cut-off points for initiating treatment have changed from 200 cells/μL to 350 cells/μL in 2010, and then to 500 cells/μL in 2013. This meant that there was not consistent reporting of a clinically appropriate accuracy measure.

Other methods were also been used to assess the agreement of the devices, including linear regression correlation (correlation coefficient), % Similarity, and Passing Bablock, however there was either no consistency or the Bland Altman measure was deemed of better quality. The linear regression coefficient is a poor measure of agreement as the measure of bias from each device could be well correlated; i.e. both counts increase over the range, but the correlation coefficient does not account for systematic differences between the counts; the Bland Altman mean bias does.[112]

2.2 Data Analysis

Meta-analysis allows for data from multiple studies to be summarised, synthesising a single estimate of the effects. The benefit of a meta-analysis is that it provides an overall estimate of the parameter, potentially improving the precision of the estimate and providing a more powerful answer for the question posed.[116,117]

The aim of our meta-analysis is to produce an estimate of the average bias expected when point of care CD4 devices are used, compared to the current reference standard. We wanted to provide an estimate of the average Bland Altman mean bias for both capillary samples and for venous samples. We also wanted, where the data allowed, to compare the performance of devices.

Bayesian Hierarchical Analysis

Traditional methods for meta-analysis involve combining data from multiple studies; these methods can be built as fixed effects or hierarchical models (hierarchical models can also be referred to as random effects models). In a hierarchical model we assume that the studies included in our analysis are sufficiently similar to be pooled, but we want to maintain the

variability within and across studies.[117] This is an important aspect of the data analysis as we would like to estimate the likely range of the bias for POC devices. The Bland Altman data proved difficult to analyse using standard random effects models. We used a Bayesian approach to create a hierarchical model to analyse the data for this thesis.

To our knowledge, a previous meta-analysis of Bland Altman data has not before been attempted; the data analysis was therefore designed and implemented by Dr Lawrence Joseph. As the Bland Altman mean bias can be both positive and negative, pooling only the Bland Altman mean bias estimates could lead to erroneous results; the positive and negative results could cancel. In using a hierarchical model, the variance around the mean bias is maintained and provides a better estimate of the variability expected from these devices. The two parameters of interest are the average Bland Altman mean bias across studies and the expected variance across studies.

The model can be notated as follows:

$$Y_{[i]} = N(\mu_{[i]}, \sigma_{[i]}^2)$$

$$\mu_{[i]} \sim N(\mu_{[g]}, \sigma_{[b]}^2)$$

$$log(\sigma_{[i]}^2) \sim N(\mu_{\sigma[g]}^2, \sigma_{\sigma[g]}^2)$$

Where $\mu_{[i]}$ is the Bland Altman mean bias from each study Y, and $\sigma_{[i]}^2$ is the variance from each study. $\mu_{[g]}$ is the global mean, and $\mu_{\sigma[g]}^2$ is the mean variance of the log of the variances.

As shown in the notation above, this hierarchical model can be summarized as having two levels, allowing for variation at each level. The first level assumes that the Bland Altman mean bias

from each study ($\mu_{[i]}$) follows a normal distribution, with a study specific variance ($\sigma_{[i]}^2$). The mean from each study is then described at the second level as following a normal distribution, around a global mean ($\mu_{[g]}$) and a variance between studies ($\sigma_{[b]}^2$). The log of each study variance ($\log(\sigma_{[i]}^2)$) was also assumed to follow a normal distribution, allowing for variation.

The WinBUGS program was used to run the model. As we have systematically reviewed the evidence we assumed that all the information relevant to the field was included in the data, as such we wanted the data to drive the inferences and therefore non informative priors were used in the specification of the model.

Forest Plots

Forest plots provide a graphical way to explore the data. The results from each study with the 95% confidence intervals were plotted, along with the results from the meta-analysis where available.

Bland Altman Mean Bias

The Bland Altman mean bias result from each study was plotted in a forest plot, alongside the overall estimates from the meta-analysis. These plots give an indication of where the mean biases fall around zero; zero indicating no difference between the index and the flow cytometry (reference) result. In order to further investigate the clinical equivalence of the devices (compared to the reference) the forest plots were also plotted with very strict limits placed along the x-axis. By placing these limits the user is able to quickly identify the devices or situations where the devices may not be working to clinical equivalency, these forest plots are presented in the appendix, on pages 88 to 91. R was used to create the forest plots, using the forest.or.plot

command.[118] When 95% confidence intervals of the Bland Altman mean bias were not reported in the paper, these were derived from the available data (either limits of agreement, standard errors or standard deviations), the method is presented in the appendix, page 93.

Sensitivity and specificity

The sensitivity and specificity of a diagnostic test can be described using the following conditional probability statements:

$$Sensitivity = P(T+|D+)$$

$$Specificity=p(T-|D-)$$

Where P=probability, T+= testing positive, T-=testing negative, D+=disease positive, D-=disease negative

Only 9 data points in total were available. Due to insufficient reporting, use of bilateral inclusion ranges and changing definitions of a case, the data was difficult to pool. Instead, forest plots were created to investigate the available data. Full information regarding the sensitivity and specificity estimates for capillary samples were available from 6 studies and for venous samples only 3 studies reported information; data points were only available for the PIMA device only.

These forest plots, can be used to visually compare the sensitivities and specificities of CD4+ cell counting assays, when used at the different cut-off points. However with limited data the plots were not presented in the manuscript and are placed in the appendix on page 57. Forest plots were created in R, using the forest.plot.or command.[118] Where sensitivity, specificity or

95% confidence intervals were not presented in the paper, raw data was used to calculate them in R, example code is presented on page 92 of the appendix.

2.3 Analysis of Bias

Analysis of bias for evaluative studies

It is very important to consider the validity of the results included in the meta-analysis, unless valid data is used, the results of the meta-analysis will be flawed. Systematic sources of bias can arise in diagnostic accuracy studies, and can be broadly characterized as selection bias or information bias. Selection bias could arise in diagnostic accuracy studies if the population included in the study is not representative of the target population.[119] Information bias could arise if the reference test was inappropriate, if there was lack of blinding between the operator of the reference test and the POC device, or if there was a long delay between the reference and blood draw.

Spectrum bias is also assessed using the QUADAS-2 criteria, whether the results are applicable to the target population. Spectrum bias is introduced when a test is evaluated in a population that is not clinically relevant.[99] For this reason we have only included HIV+ adults in our study, and excluded a number of studies that reported results from HIV- populations.

To assess the quality of the methodology of each study, the QUADAS-2 criteria (Quality Assessment of Diagnostic Accuracy Studies) were included in the data abstraction form. Selection was considered to be appropriate if the participants were selected in a consecutive manner. Blinding between the index and the reference test results was considered to have taken place if explicitly stated in the methods, or if it was stated that these were completed in different

locations by different staff. QUADAS-2 assessment was completed by each reviewer for each study included, the results were compared and any discrepancies were resolved. The initial agreement between reviewer one and reviewer two was found to be 60% (6/16 were scored exactly the same by both reviewers), results are shown in the appendix, page 96. After comparison and discussion, a jointly agreeable score for the discordant studies was reached.

Analysis of bias in meta-analysis

By completing a comprehensive review of the literature, using a valid search strategy and multiple sources as we have done, will help to limit bias. However this will not protect against publication bias (selective reporting of results), time lag bias (results being reported at different speeds, according to the results), and outcome reporting bias (selectively reporting results dependent on outcomes). [116] We contacted all authors included in our review, as well as manufacturers of devices in an attempt to acquire unpublished work; however no additional data was identified in this way (one author submitted additional data for a study already published). As commercial interests will be related to the publication of evaluation studies of devices, publication bias is important, and likely to have impacted on our results.[116,120]

Funnel plots provide on method for identifying publication bias, along with methods to test for funnel plot asymmetry (such as the Egger test or Rosenthal's Fail-safe methods).[116,121] A funnel plot is a scatter plot of the effect measure, plotted against the study precision (inverse standard error).[121] In theory, the most precise studies should provide the most accurate estimate of the effect size, with less precise studies falling around this estimate, creating a funnel. In absence of publication bias, these plots should be symmetrical.[116,121]

Combining heterogeneous studies, chance, as well as reporting bias can lead to asymmetry of funnel plots.[121] In fitting a hierarchical model, we are assuming differences between studies, therefore a funnel plot will not add substantial information, and tests of asymmetry would likely be meaningless. [116,121]

Many of the devices included in our analysis are in development, and it is likely that the performance of these devices have changed over time. For this reason, this review will need to be repeated when more information is available, and as the devices are developed.

Chapter 4: RESULTS

Study Selection

Of the 4154 papers that were identified through the search, 16 studies were eligible for inclusion into the study. Figure 2 details the study selection process. For the PIMA meta-analysis by

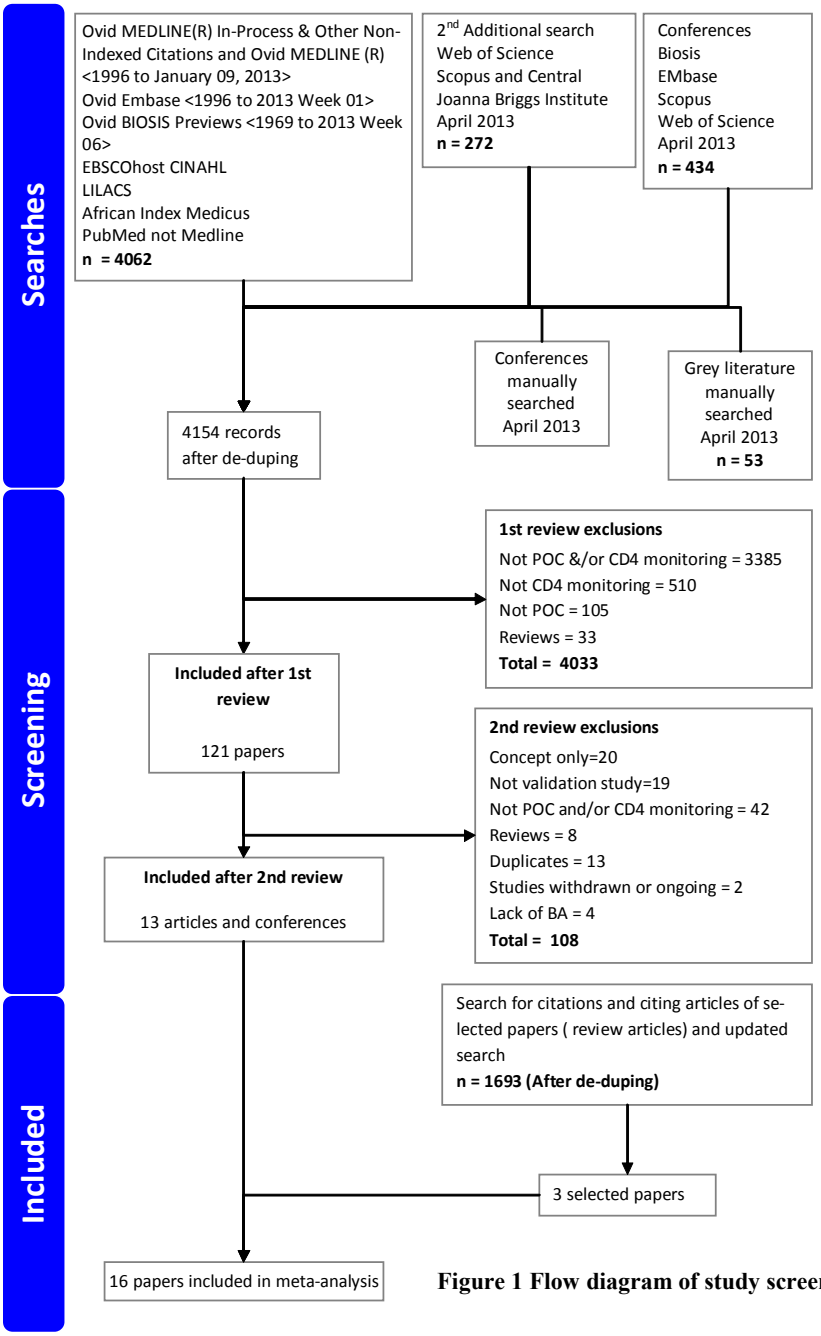


Figure 1 Flow diagram of study screening

specimens, a total of 10 studies contributed a total of 11 data points to the venous meta-analysis and 10 studies contributed 13 data points to the capillary meta-analysis (24 data points in total).

Summary of included studies

The mean sample size across studies was 223·7 (min: 52, max: 1790, SD: 310·89). Most of the included studies were completed in resource limited settings, of the 16 articles included in the analysis, only two reported results from developed settings. Of the 23 individual evaluations included, most (17/23, 74%) were completed in African countries. A table showing the characteristics for each study is included in the appendix, on page 98.

Quality of included studies

Both reviewers independently assessed the quality of each study using the QUADAS-2 criteria. [122] The scoring from each reviewer was compared (appendix page 96) and disagreements were resolved, overall results are shown in figure 2. In assessing the quality of our chosen studies, we found that all were of moderate or good quality (Figure 2).

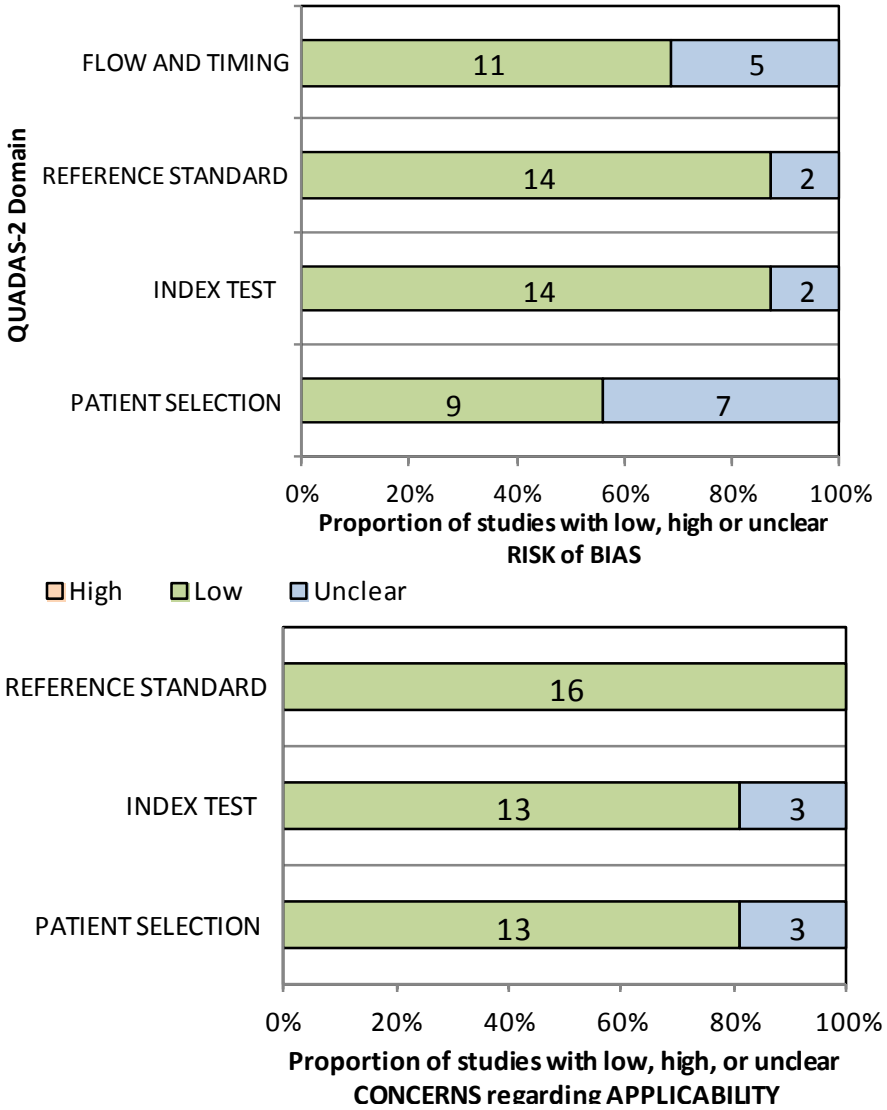


Figure 2 QUADAS-2 results

Analysis of agreement

Overall, there was only sufficient data to allow for a meta-analysis of the PIMA device. The results found that the PIMA device for capillary specimens had a small mean bias point estimate of -2·98 cells/μL (SD 12·84, 95% CrI: -28·20, 22·76), (Figure 3). For the PIMA venous subgroup the BA mean bias estimate was -26.45 cells/μL (SD 9.94, 95% CrI: -46·66, -6·81).

When assessing the performance of the MBio and the MiniPOC devices, the forest plot indicates that the results from these studies fall within the range of accepted equivalency. This early evidence indicates that these can also perform well, however, data is severely lacking in the literature for these devices.

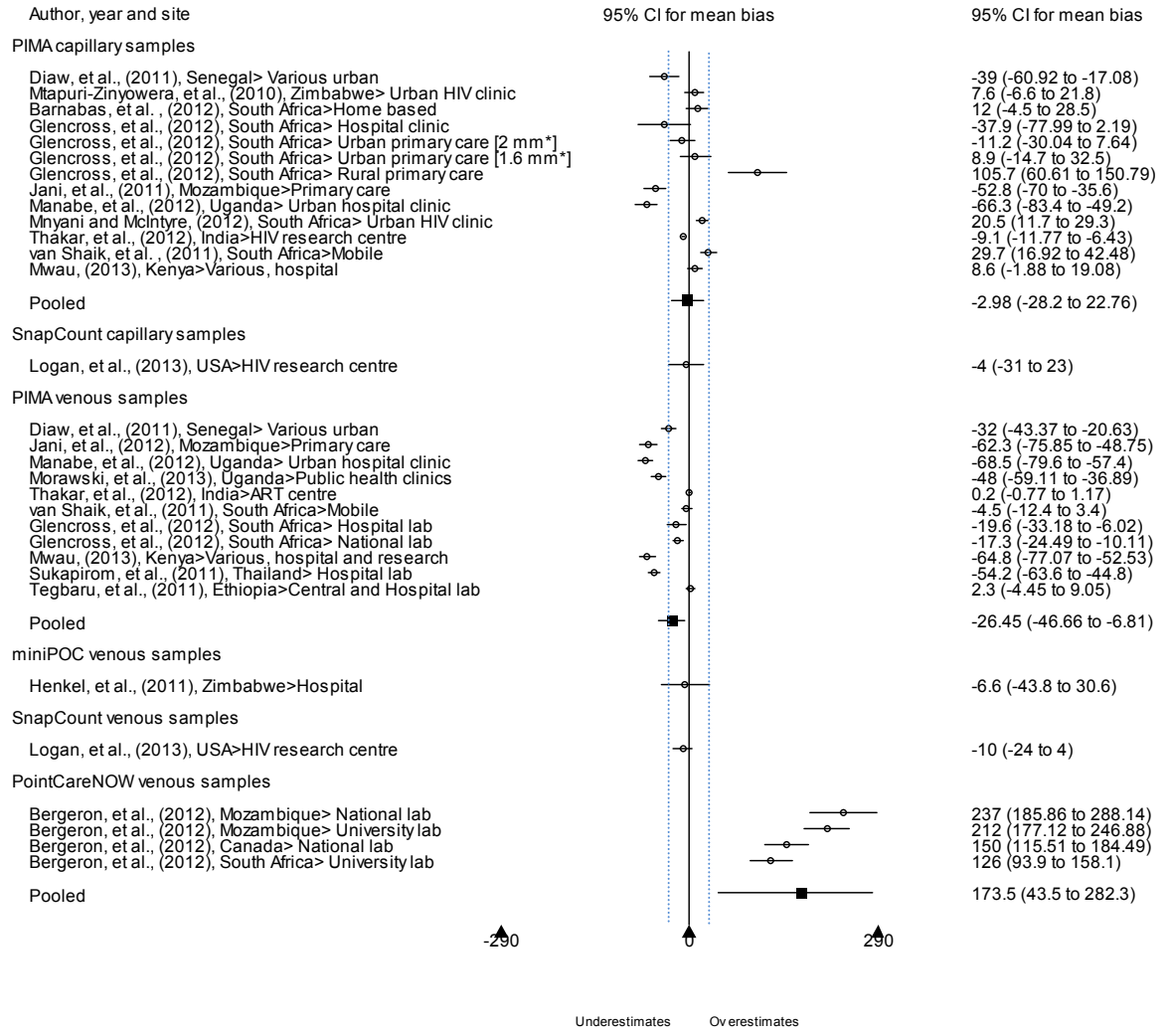


Figure 3 Forest plot and pooled results, grouped by substrate used and POC device

The limits of clinical equivalency (+/-30 cells/μL) have been indicated on the forest plot in blue, with these, it is clear which studies reported clinically equivalent results. Of the PIMA venous specimen results, 5/11 (45%) fell completely outside of the range of clinical equivalency. The PIMA capillary specimens were counted with better agreement, only 3/13 (23%) results fell completely outside of the clinically equivalent range. One paper reported results for the PointCareNOW device, the results indicated that the device over estimated the CD4+ cell count to well outside of the range of clinical equivalency, figure 3. However, the manufacturer of the

device, published letters to the editors has indicated that this study may not be reliable. We therefore considered the results from this study to inconclusive, but presented the evidence in the forest plots for completeness. [101,102]

We also observed that a majority (62%; 23/27) of the studies reported funnel-shaped BA plots. This indicates that the devices tend to be more accurate at lower CD4+ cell counts. In addition, some studies (22%; 6/27) showed asymmetrical BA plots. This observation indicates that the devices were liable to underestimate the CD4 counts, as also identified in our meta-analyses.

Sensitivity and specificity

Though sufficiently consistent data was lacking for a meta-analysis of the sensitivity and specificity data, there was some information for the PIMA device. Full data is presented in the appendix, page 99. In order to investigate the performance in a graphical way, the forest plot below was created (Figure 4).

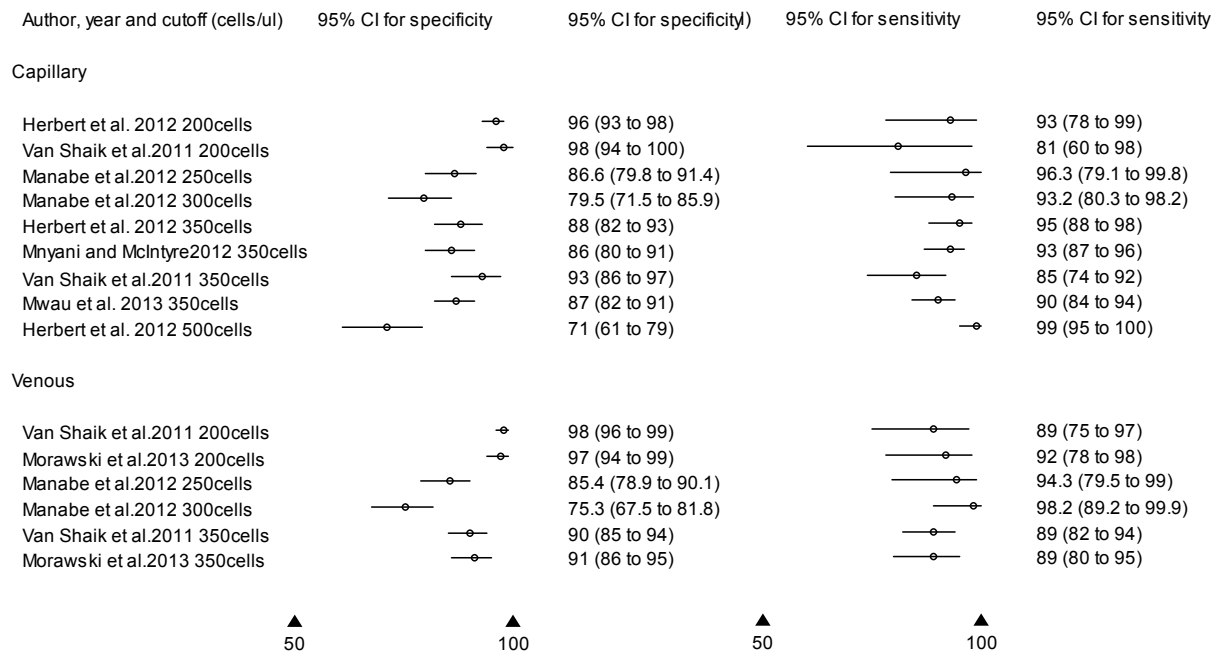


Figure 4 Forest plot: Sensitivity and Specificity

The data presented above does not represent the same cut-off, moving down the plot, the cut-off increases, ranging from 200 cells/μL to 500 cells/μL. Considering the data, the PIMA appears to perform well, with point estimates of specificities ranging from 71-98% and sensitivities ranging from 81-99%.

Publication bias

Funnel plots were created to investigate the potential for publication bias. The most precise studies should provide the most accurate estimate of the bias, with less precise studies falling around this estimate, creating a funnel.[116,121] In absence of publication bias, these plots should be symmetrical. The below funnel plots include data from all studies; the blue dots indicate where data could be missing, the black dots represent the included studies. Where there is not publication bias, these plots should be symmetrical.[116,121]

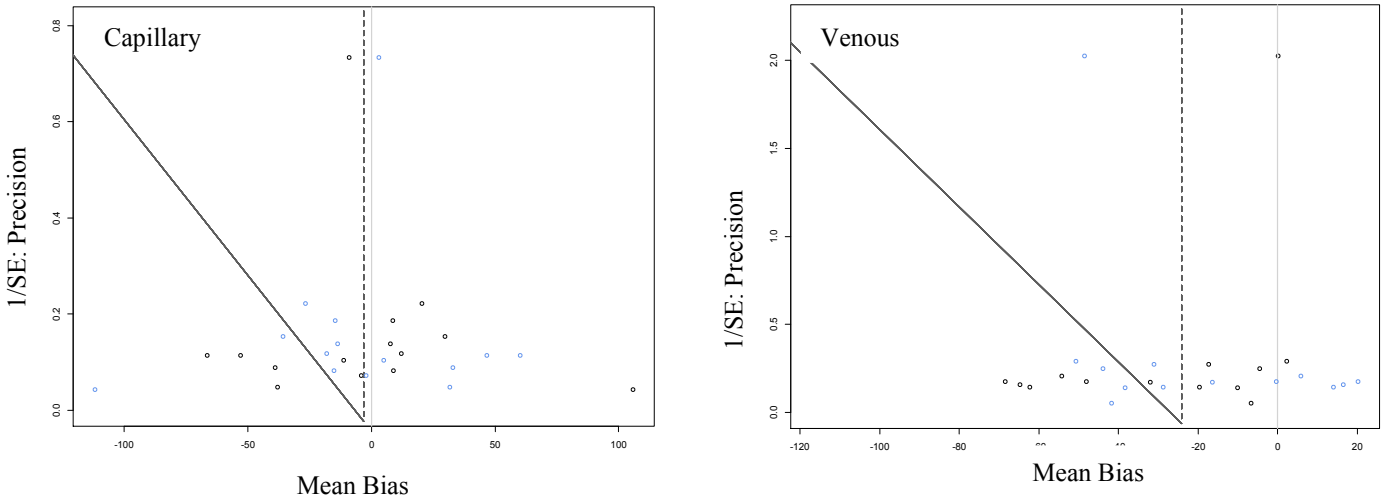


Figure 5 Funnel Plot, all capillary and venous specimens

The plot for the capillary samples appears to be more symmetrical than the venous plot. However, with such small amounts of data it is hard to make clear conclusions; we are already convinced that additional data is needed for POC CD4+ devices. Asymmetry could be a result of

chance, different underlying populations being studied or reporting bias, as we already accept that the studies are probably heterogeneous these funnel plots add very little, and tests for symmetry could be misleading.

Chapter 5: CONCLUDING CHAPTER

Summary of evidence

Our review suggests that overall, the POC CD4 devices included in the analysis (PIMA, MiniPOC and MBio CD4) were comparable in performance to flow cytometry. The PIMA device was the only device with enough data to allow for a meta-analysis. The meta-analysis indicates that on average the PIMA was comparable in performance to the conventional flow cytometer. Despite fewer data points and use of different underlying technologies, our forest plots indicate that the MBio and miniPOC devices fall within the ranges of clinical equivalency.

The PIMA device for now, and certainly other devices like the MiniPOC and the MBio devices in the future, offer the potential to be scaled up and operationalized in decentralized settings for the monitoring and staging of HIV+ infected individuals.

Our secondary findings suggest that current POC devices work best for capillary specimens (compared to venous specimens). This finding indicates that capillary blood could be safely used to monitor CD4+ cell counts. In interpreting our findings for the PIMA meta-analysis, it is vital that the mean bias results are not considered in isolation; the credible intervals (CrIs) are more informative (CrIs are the Bayesian equivalent term for the Frequentist 95% confidence intervals). These CrIs estimate the likely range of the difference between the CD4+ cell counts of the POC device and that of the reference. Given this, when considered in the context of the variance, the PIMA device performed well. With CrIs of -28·20 to 22·76 cells/µL, for capillary specimens we can be confident that these devices can be used interchangeably with flow cytometry. The evidence is less convincing for venous specimens, as the credible intervals fall outside of the range of clinical equivalency. For venous specimens, the credible intervals are more of a

concern, given the tendency for the CD4+ cell count to be more severely underestimated. This leads to 95% credible intervals of 46·7 to -6·8, which fall outside of the limits of clinical equivalency. Where resources are limited this finding is perhaps welcome; venous specimens require specialist phlebotomists and the disposal of clinical waste is more complicated for venous specimens.

Observations from the forest plots also support our finding. For the venous samples, 45% of studies fell completely outside of the range of clinical equivalency. Capillary specimens performed better, only 23% of studies falling completely outside of the range of clinical equivalency. Given this closer agreement with the reference standard, it is clear that most POC devices are best optimized for analysis of capillary specimens. This is an important observation for all international settings. Oftentimes, phlebotomists are not easily available on site to draw blood for venous specimens, but capillary specimens can be obtained easily by clinic staff after some training.

In terms of interpretation of our statistical findings for the PIMA, we found that the variance in the capillary specimens (SD: 12·84) is slightly larger than the venous specimens (SD: 9·94), this increased variance is also clear from the forest plot. This indicates more variability in capillary specimens that could perhaps be explained by variance in sampling technique. Meaning that whilst the agreement between the POC device and the flow cytometer is better for capillary samples, inconsistency in sampling technique could be increase the variability of results.

Sampling variability could further be explained by three key items: a) the type of lancet used to obtain a finger stick sample, b) the sampling technique used, c) training of technicians, and d) patient (blood flow should be optimum, in anemic patients it is sometimes hard to get a good

blood sample from the finger). All these factors are important because they potentially impact on the quality of the CD4 count.[90] On the other hand, when phlebotomists draw blood for venous specimens, a one-step procedure is used, and phlebotomists are well trained to take blood in a consistent way. This perhaps accounts for the reduced variability in estimates for the venous specimens. Typically, blade type lancets with a 1.8mm depth of penetration were used to obtain a good sample of capillary blood, for the PIMA, a 25µ L sample is required. [82,86,88,91]

Some field studies reported issues in obtaining sufficient blood (due to patients being Anemic), while some reported higher error rates when capillary blood specimens were used. [85,90,91,94,123] This supports the assertion that the training of staff in proper capillary sampling is important and that this training will standardize operating procedures, improve efficiency, translating to a better specimen and a more accurate result. So, we would emphasize training (and certification if needs be) to ensure the optimum performance of these devices that are hugely reliant on instruments. Indeed, built in quality control systems should, and have been incorporated into these devices. For example automatic control of cartridge expiry dates, sample volume control, as well as compatibility with external quality assessment specimens all help to improve quality assurance which is required for POC testing procedures.[7,124]

Limitations

As is always the case when pooling published data, the meta-analysis is vulnerable to bias. Because of the restricted data available, we were only able to fully pool data for the PIMA device. More evidence must exist for the full range of devices that are on the market, but these data have not yet been published. We have sufficient evidence for the PIMA device and we can only make some conclusions about the MBio CD4 analyzer, the MiniPOC and the

PointCareNOW. We were unable to find any published data relating to the performance of some high profile devices (Zyomyx, VISITECT, Daktari, and HumaCount).

We also noted that a majority of the BA plots were funnel shaped. The use of the absolute mean bias can make the BA plots appear to be funnel shaped, when in fact this is due to a large range of the CD4 count.[112] Because the ranges in the CD4 counts are wide, the mean bias is relative to the CD4 count, and should be reported as such. However, in our sample only two studies reported the relative mean bias.[91,102] Future evaluations should report the relative mean bias, which will take into account the fact that higher CD4+ cell counts are likely to differ more distinctly between the index and reference. This would be a more useful measure for comparison in future meta-analyses.

Transforming the delivery of care

POC assays could help to reduce the burden on patients in HIV care by halving the number of clinic visits required and making care pathway less disjointed. Such changes could be of most benefit to those individuals who face hardships when trying to access care; as they need to travel far for treatment and do not have access to transport. Dependence of clinics on centralized laboratories could be reduced and there will be no longer a need to transport bloods to laboratories (for CD4 counting). HIV diagnosis is the entry point into HIV care. If POC CD4+ cell counting assays could be paired with POC HIV assays, the counselling received by HIV+ individuals could be tailored according to the stage of the disease in the individual.

Researchers are currently appraising the use of primary care clinics, mobile clinics, and home based care as well as non physician clinicians for the provision of HIV care.[67-72] The prevailing aims of these projects are to decentralise care, function in areas where infrastructure is limited

and to overcome patient barriers. These programs have provided some very promising results, and the implementation of POC devices should be investigated to see if further gains can be made. It is seems feasible that POC CD4+ devices, alongside POC HIV diagnostic testing could allow clinics to provide the full range of care (from screening through to monitoring of ART) in a decentralized setting in order to access hard to reach individuals in a patient friendly way. ART-eligible patients could be triaged, or even started on ART immediately, without the need for a referral; this would dramatically reduce the burden on patients.

Evidence for patient-centred outcomes (such as acceptability and preference), feasibility, cost savings analyses and cost-effectiveness of POC CD4 devices is still very limited. These data need to be generated for different settings and programs. With the potential benefits, cost effectiveness analyses will be required that take into account test and equipment costs, staff costs, quality control as well as the financial burden or benefits for patients.[125] Patient-centred outcomes such as preference, acceptability of the device, acceptability of the waiting time, and preference for sample collection will need to be investigated

Assessing the clinical impact often requires more sophisticated study designs, and often greater investment. A randomised controlled trial (RCT) and patient questionnaire would be a rigorous way to compare POCs with flow cytometry, and could show whether the implementation of POCs deliver improvements in practice. The clinical impact on patient management of CD4 devices has been described in a few studies. The results of these studies are promising;

- A pilot of home based care (screening and CD4 count, then referral to a HIV clinic), in rural South Africa, this program improved the number of people visiting an HIV clinic, rising from 57% to 96%.[72]
- A POC CD4 device was successfully introduced to primary health sites in Mozambique. The total loss to follow up prior to ART initiation dropped from 64% to 33%.[4]

The estimates of the reduced loss to follow study from Mozambique have been used to estimate the 'cost per patient on ART', given the increased uptake on ART of 33-64%.[4,126] They found that POC CD4 devices were a cost effective intervention, the incremental cost per additional patient-year on ART was anticipated to be $221 over 10 years, compared to $223 without POC devices.[126] However, this forecast is based on very little information and further implementation studies will be required before accurate estimates can be made.

Future directions for research

In completing this work we have identified various aspects where research into CD4 POC devices needs to be strengthened. Future work should run along major themes; implementation research, further evaluations of newer devices (preferably a head to head comparison of top performing devices), cost effectiveness of devices in practice and update evidence on systematic reviews when more evidence is available.

For evaluative studies of CD4 devices, a measure of difference that is independent of the range of the CD4 count should be included in the study. We found that most studies reported funnel shaped BA plots, this could be due to the use of the absolute mean bias. An absolute mean bias is liable to bias when a large range of counts is included, as is the case in our included studies. When the range of the counts is wide, the mean bias starts to be correlated to the CD4 count, resulting in funnel shaped plots.[112] The relative mean bias can account for this, however, in our anlaysis only two studies reported the relative mean bias.[91,102] Future evaluations should report the relative mean bias, as this would be a less biased tool for comparisons in future meta-analyses. Education on the best measure to use must be presented to the device manufacturers and those involved in evaluating the performance of such devices which require a digression from sensitivity and specificity.

Further, these technologies are developing fast and many manufacturers are equipping their devices with data storage and GPS capabilities, which can allow data to be transferred to online cloud storage; encouraging technology-assisted quality control systems. For example a service in Mozambique is using GPRS (general packet radio service) enabled PIMA devices to make use of the already available cellular communication networks.[127] Such capabilities allow for regional

and national level supervision of performance, tracking test results, and rapid resolution of problems. These modern, high quality integrated care systems are sensitive to patient needs with data storage and quality capability.

For global use, the absolute CD4 count has been shown to be unreliable in pediatric populations, making many CD4 POC devices unsuitable for staging in this patient group.[128] New generations of POC CD4 devices will need to incorporate the ability to provide CD4% for pediatric populations.[27] Currently, only two devices (PointCareNOW and the miniPOC) offer that option and so further improvements and evaluations of these devices would be welcomed.

Looking beyond the next five years, the standard of care delivered in the developed world should provide a model for aspiration for resource limited clinics. As technology becomes more affordable and better quality assays become more accessible, CD4+ cell counting devices are likely to be working side by side with viral load assays. Viral load assays provide a direct measure of the viral burden and are much more sensitive to treatment failures.[129] Viral load assays are used in developed settings as standard care. As such these CD4+ cell counting devices will be used less frequently, (once in 6 months) as is now the trend in more developed settings.[129] Therefore, the development of affordable POC viral load assays will be needed in tandem with CD4 POC's so as to provide the best care in global settings.

References

1. UNAIDS. Global Report: UNAIDS Report on the Global AIDS Epidemic 2013. 2013 2013.

2. *ABC Series, Volume 40 : ABC of HIV and AIDS (6th Edition)*. Somerset, NJ, USA: Wiley; 2012.

3. UNAIDS. Global Report: UNAIDS Report on the Global AIDS Epidemic 2012. 2012 2012.

4. Jani IV, Sitoe NE, Alfai ER, et al. Effect of point-of-care CD4 cell count tests on retention of patients and rates of antiretroviral therapy initiation in primary health clinics: an observational cohort study. *Lancet.* Oct 29 2011;378(9802):1572-1579.

5. Varughese JK, Rosenberg MG, Kim K. HIV in the tropics: staging in the resource-limited setting. *Current opinion in infectious diseases.* Oct 2012;25(5):477-483.

6. Murtagh M. UNITAID Technical Report: HIV/AIDS Diagnostic Landscape. In: Secretariat U, ed: World Health Organization; 2011.

7. Boyle DS, Hawkins KR, Steele MS, Singhal M, Cheng X. Emerging technologies for point-of-care CD4 T-lymphocyte counting. *Trends Biotechnol.* Jan 2012;30(1):45-54.

8. Glynn MT, Kinahan DJ, Ducree J. CD4 counting technologies for HIV therapy monitoring in resource-poor settings--state-of-the-art and emerging microtechnologies. *Lab Chip.* Jul 21 2013;13(14):2731-2748.

9. Gottlieb MS, Schroff R, Schanker HM, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med.* Dec 10 1981;305(24):1425-1431.

10. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science.* May 20 1983;220(4599):868-871.

11. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med.* Sep 2011;1(1):a006841.

12. Hemelaar J. The origin and diversity of the HIV-1 pandemic. *Trends Mol Med.* Mar 2012;18(3):182-192.

13. Gao F, Bailes E, Robertson DL, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. *Nature.* Feb 4 1999;397(6718):436-441.

14. Wertheim JO, Worobey M. Dating the Age of the SIV Lineages That Gave Rise to HIV-1 and HIV-2. *PLoS Comput Biol.* 2009;5(5):e1000377.

15. Organization WT. Declaration on the TRIPS Agreement and Public Health. In: Organization WT, ed. *WT/MIN(01)/DEC/2*2001.

16. Chitnis A, Rawls D, Moore J. Origin of HIV type 1 in colonial French Equatorial Africa? *AIDS Res Hum Retroviruses.* Jan 1 2000;16(1):5-8.

17. Pepin J. The origins of AIDS: from patient zero to ground zero. *J Epidemiol Community Health.* Jun 2013;67(6):473-475.

18. Marx PA, Alcabes PG, Drucker E. Serial human passage of simian immunodeficiency virus by unsterile injections and the emergence of epidemic human immunodeficiency virus in Africa. *Philos Trans R Soc Lond B Biol Sci.* Jun 29 2001;356(1410):911-920.

19. Pepin J, Labbe AC, Mamadou-Yaya F, et al. Iatrogenic transmission of human T cell lymphotropic virus type 1 and hepatitis C virus through parenteral treatment and chemoprophylaxis of sleeping sickness in colonial Equatorial Africa. *Clin Infect Dis.* Oct 1 2010;51(7):777-784.

20. de Sousa JD, Muller V, Lemey P, Vandamme AM. High GUD incidence in the early 20 century created a particularly permissive time window for the origin and initial spread of epidemic HIV strains. *PloS one.* 2010;5(4):e9936.

21. Volberding PA, Deeks SG. Antiretroviral therapy and management of HIV infection. *Lancet.* Jul 3 2010;376(9734):49-62.

22. De Clercq E. Chapter Nine - The Nucleoside Reverse Transcriptase Inhibitors, Nonnucleoside Reverse Transcriptase Inhibitors, and Protease Inhibitors in the Treatment of HIV Infections (AIDS). In: Erik De C, ed. *Advances in Pharmacology.* Vol Volume 67: Academic Press; 2013:317-358.

23. Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature.* Mar 25 1993;362(6418):355-358.

24. Brenner BG, Roger M, Routy JP, et al. High rates of forward transmission events after acute/early HIV-1 infection. *J Infect Dis.* Apr 1 2007;195(7):951-959.

25. Bristow C. The Two Principal Viremias of HIV. In: Ugen K, Bendinelli M, Friedman H, eds. *Human Retroviral Infections*: Springer US; 2002:1-16.

26. Rodriguez B, Sethi AK, Cheruvu VK, et al. Predictive value of plasma HIV RNA level on rate of CD4 T-cell decline in untreated HIV infection. *JAMA.* Sep 27 2006;296(12):1498-1506.

27. Organization WH. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection2013.

28. Kozal M. Overview of HIV drug resistance testing assays. 2013; http://www.uptodate.com/contents/overview-of-hiv-drug-resistance-testing-assays. Accessed Nov 2013.

29. Bartlett J. Diagnostic assays for HIV infection. 2013; http://www.uptodate.com/contents/diagnostic-assays-for-hiv-infection?source=search_result&search=hiv+testing&selectedTitle=1%7E109#H28. Accessed Nov 2013, 2013.

30. Reid SD, Fidler SJ, Cooke GS. Tracking the progress of HIV: the impact of point-of-care tests on antiretroviral therapy. *Clin Epidemiol.* 2013;5:387-396.

31. Pai NP, Pai M. Point-of-care diagnostics for HIV and tuberculosis: landscape, pipeline, and unmet needs. *Discov Med.* Jan 2012;13(68):35-45.

32. Pai NP. Rapid oral fluid-based point-of-care HIV testing: applicability in developing countries. *Indian J Med Res.* Sep 2007;126(3):171-173.

33. Pai NP, Balram B, Shivkumar S, et al. Head-to-head comparison of accuracy of a rapid point-of-care HIV test with oral versus whole-blood specimens: a systematic review and meta-analysis. *The Lancet infectious diseases.* 2012;12(5):373-380.

34. Napierala Mavedzenge S, Baggaley R, Corbett EL. A review of self-testing for HIV: research and policy priorities in a new era of HIV prevention. *Clin Infect Dis.* Jul 2013;57(1):126-138.

35. Bartlett JG. Ten Years of HAART: Foundation for the Future. 2006; http://www.medscape.org/viewarticle/523119. Accessed 8th Nov 2013, 2013.

36. De Clercq E. Strategies in the design of antiviral drugs. *Nat Rev Drug Discov.* Jan 2002;1(1):13-25.

37. Fletcher CV. Pharmacology of nucleoside reverse transcriptase inhibitors. 2013; http://www.uptodate.com/contents/pharmacology-of-nucleoside-reverse-transcriptase-inhibitors. Accessed 07/11/2013, 2013.

38. Hardy H, Skolnik PR. Enfuvirtide, a New Fusion Inhibitor for Therapy of Human Immunodeficiency Virus Infection. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy.* 2004;24(2):198-211.

39. Henrich TJ, Kuritzkes DR. HIV-1 entry inhibitors: recent development and clinical use. *Current Opinion in Virology.* 2013;3(1):51-57.

40. Raffi F, Wainberg MA. Multiple choices for HIV therapy with integrase strand transfer inhibitors. *Retrovirology.* 2012;9:110.

41. Belavic JM. Complera for the treatment of HIV. *Nurse Pract.* Jul 10 2013;38(7):8-10.

42. Horn JR, Hansten PD. Stribild--A New Approach to Pharmacoenhancement. *Pharmacy Times.* 2013;79(2):48-48.

43. HIV Treatment and Prevention Technologies in International Perspective. Basingstoke: Palgrave Macmillan; 2010: http://dx.doi.org/10.1057/9780230297050. Accessed 2013/11/04.

44. Broder S. Twenty-five years of translational medicine in antiretroviral therapy: promises to keep. *Science translational medicine.* Jul 7 2010;2(39):39ps33.

45. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. *The Lancet infectious diseases.* Mar 2010;10(3):155-166.

46. Pomerantz RJ, Horn DL. Twenty years of therapy for HIV-1 infection. *Nat Med.* 2003;9(7):867-873.

47. Vella S, Schwartlander B, Sow SP, Eholie SP, Murphy RL. The history of antiretroviral therapy and of its implementation in resource-limited areas of the world. *Aids.* Jun 19 2012;26(10):1231-1241.

48. WHO. Access to AIDS medicines stumbles on trade rules. *Bulletin of the World Health Organization.* May 2006 2006;84(5):337-424.

49. McCutchan JA. Management of HIV in resource limited settings. *Current opinion in infectious diseases.* Oct 2009;22(5):464-470.

50. Quinn TC. Global burden of the HIV pandemic. *Lancet.* Jul 13 1996;348(9020):99-106.

51. Lima VD, Granich R, Phillips P, Williams B, Montaner JS. Potential Impact of the US President's Emergency Plan for AIDS Relief on the Tuberculosis/HIV Coepidemic in Selected Sub-Saharan African Countries. *J Infect Dis.* Dec 2013;208(12):2075-2084.

52. Perrin L, Telenti A. HIV treatment failure: testing for HIV resistance in clinical practice. *Science.* Jun 19 1998;280(5371):1871-1873.

53. Bartlett JA, Shao JF. Successes, challenges, and limitations of current antiretroviral therapy in low-income and middle-income countries. *The Lancet infectious diseases.* Oct 2009;9(10):637-649.

54. Organization WH. Antiretroviral Therapy for HIV Infection in Adults and Adolescents. Vol 2010 revision. Switzerland: WHO Press; 2010.

55. Mermin J, Ekwaru JP, Were W, et al. Utility of routine viral load, CD4 cell count, and clinical monitoring among adults with HIV receiving antiretroviral therapy in Uganda: randomised trial. *Brit Med J.* Nov 9 2011;343.

56. Organization WH. Antiretroviral Therapy for HIV Infection in Adults and Adolescents. Recommendations for a public health approach - 2010 rev.2010.

57. Siegfried N, Uthman OA, Rutherford GW. Optimal time for initiation of antiretroviral therapy in asymptomatic, HIV-infected, treatment-naive adults. *Cochrane database of systematic reviews.* 2010(3):CD008272.

58. Nyambe C, Mwamba F, Nsama C, et al. Urgent call for laboratory services strengthening for the provision of quality ART services: the gap between the number of CD4 test required and the current laboratory capacity in three rural districts, Zambia. Paper presented at: IAS Conference on HIV Pathogenesis, Treatment and Prevention2011; Rome, Italy.

59. Logan C, Givens M, Ives JT, et al. Performance evaluation of the MBio Diagnostics point-of-care CD4 counter. *J Immunol Methods.* Jan 31 2013;387(1-2):107-113.

60. Micek MA, Gimbel-Sherr K, Baptista AJ, et al. Loss to follow-up of adults in public HIV care systems in central Mozambique: identifying obstacles to treatment. *J Acquir Immune Defic Syndr.* Nov 1 2009;52(3):397-405.

61. Gauthier R, Livrozet JM, Prevoteau du Clary F, et al. Feasibility and acceptability of rapid HIV test screening (DEPIVIH) by French family physicians. *Medecine et maladies infectieuses.* Nov 2012;42(11):553-560.

62. Fox MP, Rosen S. Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: systematic review. *Tropical medicine & international health : TM & IH.* Jun 2010;15 Suppl 1:1-15.

63. Rosen S, Fox MP, Gill CJ. Patient retention in antiretroviral therapy programs in sub-Saharan Africa: a systematic review. *PLoS medicine.* Oct 16 2007;4(10):e298.

64. Tucker JD, Bien CH, Peeling RW. Point-of-care testing for sexually transmitted infections: recent advances and implications for disease control. *Current opinion in infectious diseases.* Feb 2013;26(1):73-79.

65. Amuron B, Namara G, Birungi J, et al. Mortality and loss-to-follow-up during the pre-treatment period in an antiretroviral therapy programme under normal health service conditions in Uganda. *BMC public health.* 2009;9:290.

66. Grabbe KL, Menzies N, Taegtmeyer M, et al. Increasing access to HIV counseling and testing through mobile services in Kenya: strategies, utilization, and cost-effectiveness. *J Acquir Immune Defic Syndr.* Jul 2010;54(3):317-323.

67. Morris MB, Chapula BT, Chi BH, et al. Use of task-shifting to rapidly scale-up HIV treatment services: experiences from Lusaka, Zambia. *BMC Health Serv Res.* 2009;9:5.

68. Stringer JS, Zulu I, Levy J, et al. Rapid scale-up of antiretroviral therapy at primary care sites in Zambia: feasibility and early outcomes. *JAMA.* Aug 16 2006;296(7):782-793.

69. Khumalo-Sakutukwa G, Morin SF, Fritz K, et al. Project Accept (HPTN 043): a community-based intervention to reduce HIV incidence in populations at risk for HIV in sub-Saharan Africa and Thailand. *J Acquir Immune Defic Syndr.* Dec 1 2008;49(4):422-431.

70. Sweat M, Morin S, Celentano D, et al. Community-based intervention to increase HIV testing and case detection in people aged 16–32 years in Tanzania, Zimbabwe, and Thailand (NIMH Project Accept, HPTN 043): a randomised study. *The Lancet infectious diseases.* 2011;11(7):525-532.

71. Lugada E, Levin J, Abang B, et al. Comparison of Home and Clinic-Based HIV Testing Among Household Members of Persons Taking Antiretroviral Therapy in Uganda: Results From a Randomized Trial. *JAIDS Journal of Acquired Immune Deficiency Syndromes.* 2010;55(2):245-252 210.1097/QAI.1090b1013e3181e1099e1069.

72. van Rooyen H, Barnabas RV, Baeten JM, et al. High HIV testing uptake and linkage to care in a novel program of home-based HIV counseling and testing with facilitated referral in KwaZulu-Natal, South Africa. *J Acquir Immune Defic Syndr.* Sep 1 2013;64(1):e1-8.

73. Rodriguez WR, Christodoulides N, Floriano PN, et al. A Microchip CD4 Counting Method for HIV Monitoring in Resource-Poor Settings. *PLoS medicine.* 2005;2(7):e182.

74. Cheng X, Irimia D, Dixon M, et al. A microfluidic device for practical label-free CD4(+) T cell counting of HIV-infected subjects. *Lab Chip.* Feb 2007;7(2):170-178.

75. Moon S, Keles HO, Ozcan A, et al. Integrating microfluidics and lensless imaging for point-of-care testing. *Biosensors and Bioelectronics.* 2009;24(11):3208-3214.

76. Huh D, Gu W, Kamotani Y, Grotberg JB, Takayama S. Microfluidics for flow cytometric analysis of cells and particles. *Physiol Meas.* Jun 2005;26(3):R73-98.

77. Mishra NN, Retterer S, Zieziulewicz TJ, et al. On-chip micro-biosensor for the detection of human CD4(+) cells based on AC impedance and optical analysis. *Biosens Bioelectron.* Nov 15 2005;21(5):696-704.

78. Boyle DS, Hawkins KR, Steele MS, Singhal M, Cheng X. Emerging technologies for point-of-care CD4 T-lymphocyte counting. *Trends in Biotechnology.* Jan 2012;30(1):45-54.

79. PLC ODG. CD4 device product page. 2013; http://www.omegadiagnostics.com/cd4/. Accessed Dec 2013.

80. Mylan. Mylan and Zyomyx Enter into Exclusive Distribution Agreement for Innovative Point-of-Care CD4 Diagnostic Test, Enhancing Mylan's Leadership Position in HIV Care. 2013; http://www.zyomyx.com/wp-content/uploads/2013/06/Mylan-and-Zyomyx-Distribution-Partnership.pdf. Accessed July 2013, 2013.

81. Peeling RW, Smith PG, Bossuyt PM. A guide for diagnostic evaluations. *Nat Rev Microbiol.* Dec 2010;8(12 Suppl):S2-6.

82. Mtapuri-Zinyowera S, Chideme M, Mangwanya D, et al. Evaluation of the PIMA point-of-care CD4 analyzer in VCT clinics in Zimbabwe. *Journal of Acquired Immune Deficiency Syndromes: JAIDS.* Sep 2010;55(1):1-7.

83. Knottnerus JA, van Weel C, Muris JW. Evaluation of diagnostic procedures. *Bmj.* Feb 23 2002;324(7335):477-480.

84. Sukapirom K, Onlamoon N, Thepthai C, Polsrila K, Tassaneetrithep B, Pattanapanyasat K. Performance evaluation of the Alere PIMA CD4 test for monitoring HIV-infected individuals in resource-constrained settings. *Journal of Acquired Immune Deficiency Syndromes: JAIDS.* Oct 1 2011;58(2):141-147.

85. Diaw PA, Daneau G, Coly AA, et al. Multisite evaluation of a point-of-care instrument for CD4(+) T-cell enumeration using venous and finger-prick blood: the PIMA CD4. *Journal of Acquired Immune Deficiency Syndromes: JAIDS.* Dec 1 2011;58(4):e103-111.

86. Jani IV, Sitoe NE, Chongo PL, et al. Accurate CD4 T-cell enumeration and antiretroviral drug toxicity monitoring in primary healthcare clinics using point-of-care testing. *AIDS.* Mar 27 2011;25(6):807-812.

87. Tegbaru B, Messele T, Abebe A, et al. Evaluation of a point of care-CD4 testing in Ethiopia. *IAS Conference of HIV Pathogenesis, Treatment and Prevention.* Vol WELBD05 - Oral Abstract Session. Rome, Italy2011.

88. van Schaik N. *Field evaluation of the PIMA™ Point-of-care CD4 machine in a mobile clinic, South Africa*. Cape Town: Faculty of Health Sciences, University of Cape Town; 2011.

89. Henkel T, Göhde R, Ost V, Göhde W. True Point-of-Care CD4 and CD4% Testing with Highest Accuracy and Precision for Adult and Pediatric HIV/AIDS Patients. 2011.

90. Glencross DK, Coetzee LM, Faal M, et al. Performance evaluation of the Pima point-of-care CD4 analyser using capillary blood sampling in field tests in South Africa. *Journal of the International AIDS Society.* 2012;15(1).

91. Thakar M, Mahajan B, Shaikh N, et al. Utility of the point of care CD4 analyzer, PIMA, to enumerate CD4 counts in the field settings in India. *AIDS Res Ther.* 2012;9(1):26.

92. Chen JL, Ford T, Metcalf R, et al. Validation of point-of-care CD4 tests in pregnancy. *HIV Medicine.* 2012;13(Supplement 1: Abstracts os the 18th Annual Conference of the British HIV Association (BHIVA)):61-62.

93. Barnabas R, van Rooyen H, Joseph P, et al. Home Point-of-Care CD4 Test Provides Accurate Enumeration of CD4 Count in South Africa. *CROI*2012:Abstract #: V-114.

94. Manabe YC, Wang Y, Elbireer A, Auerbach B, Castelnuovo B. Evaluation of portable point-of-care CD4 counter with high sensitivity for detecting patients eligible for antiretroviral therapy. *PLoS ONE.* 2012;7(4):e34319.

95. Herbert S, Edwards S, Carrick G, et al. Evaluation of PIMA point-of-care CD4 testing in a large UK HIV service. *Sex Transm Infect.* Oct 2012;88(6):413-417.

96. Mnyani CN, McIntyre JA, Myer L. The reliability of point-of-care CD4 testing in identifying HIV-infected pregnant women eligible for antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes: JAIDS.* Jul 1 2012;60(3):260-264.

97. Organization WH. Global Update on HIV Treatment 2013: Results, Impacts and Opportunities. 2013.

98. Mbopi-Keou FX, Sagnia B, Ngogang J, et al. Validation of a single-platform, volumetric, flow cytometry for CD4 T cell count monitoring in therapeutic mobile unit. *J Transl Med.* 2012;10:22.

99. Ransohoff DF, Feinstein AR. Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. *N Engl J Med.* Oct 26 1978;299(17):926-930.

100. Mulherin SA, Miller WC. Spectrum bias or spectrum effect? Subgroup variation in diagnostic test evaluation. *Ann Intern Med.* Oct 1 2002;137(7):598-602.

101. PointCare. A letter to the editors regarding the article "Performance of the PointCare NOW System for CD4 Counting in HIV Patients Based on Five Independent Evaluations". 2012; http://www.plosone.org/annotation/listThread.action?root=53465, 2013.

102. Bergeron M, Daneau G, Ding T, et al. Performance of the PointCare NOW system for CD4 counting in HIV patients based on five independent evaluations. *PLoS ONE.* 2012;7(8):e41166.

103. Nasi M, De Biasi S, Bianchini E, et al. Reliable and Accurate CD4 T Cell Count and CD4 Percent of the New Portable Flow Cytometer Cyflow Minipoc. Paper presented at: ISAC2013.

104. Bristow CL, Babayeva MA, Modarresi R, et al. The alpha-test: rapid cell-free CD4 enumeration using whole saliva. *J Vis Exp.* 2012(63):e3999.

105. Watkins NN, Sridhar S, Cheng X, et al. A microfabricated electrical differential counter for the selective enumeration of CD4+ T lymphocytes. *Lab Chip.* Apr 21 2011;11(8):1437-1447.

106. Watkins NN, Hassan U, Damhorst G, et al. Microfluidic CD4+ and CD8+ T lymphocyte counters for point-of-care HIV diagnostics using whole blood. *Sci. Transl. Med.* 2013;5.

107. Varughese JK, Rosenberg MG, Kim K. HIV in the tropics: staging in the resource-limited setting. *Current opinion in infectious diseases.* Oct 2012;25(5):477-483.

108. York TUo. Prospero: International prospective register of systematic reviews. *Centre for Reviews and Dissemination* 2013; http://www.crd.york.ac.uk/prospero/, 2013.

109. Sampson MaM, J. . Inquisitio validus Index Medicus: A simple method of validating MEDLINE systematic review searches. *Res. Synth. Method.* 2011;2(2):103–109.

110. Peeling RW, Holmes KK, Mabey D, Ronald A. Rapid tests for sexually transmitted infections (STIs): the way forward. *Sexually transmitted infections.* December 1, 2006 2006;82(suppl 5):v1-v6.

111. Pai NP, Vadnais C, Denkinger C, Engel N, Pai M. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. *PLoS medicine.* 2012;9(9):e1001306.

112. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* Feb 8 1986;1(8476):307-310.

113. Herbert S, Edwards S, Carrick G, et al. Evaluation of PIMA point-of-care CD4 testing in a large UK HIV service. *Sexually transmitted infections.* October 2012;88(6):413-417.

114. Jani I, Sitoe, N., Quevedo, J., Lehe, J., Peter, T. Cost comparison of point-of-care and laboratory CD4 testing in resource-limited settings. Paper presented at: 6th IAS Conference on HIV pathogenesis, treatment and prevention2011; Rome, Italy.

115. Tobaiwa O, Bollinger T, Sitoe N, et al. Implementation of a wireless GPRS-based monitoring system for point-of-care CD4 testing at rural primary health facilities in Mozambique. *Journal of the International AIDS Society.* Oct 2012;15(Suppl. 3):244-246.

116. Cochrane Handbook for Systematic Reviews of Interventions In: Higgins JPT GS, ed: The Cochrane Collaboration; 2011: www.cochrane-handbook.org.

117. Gelman A, Carlin J, Stern H, Rubin D. *Bayesian Data Analysis, Second Edition (Chapman & Hall/CRC Texts in Statistical Science)*: Chapman and Hall/CRC; 2003.

118. Joseph L, Belisle P. [R] Drawing a forest plot. 2013; Version 1.6 (September 2013):http://www.medicine.mcgill.ca/epidemiology/Joseph/PBelisle/forest-plot.html.

119. Schmidt RL, Factor RE. Understanding Sources of Bias in Diagnostic Accuracy Studies. *Archives of Pathology & Laboratory Medicine.* 2013/04/01 2013;137(4):558-565.

120. Lexchin J, Bero LA, Djulbegovic B, Clark O. Pharmaceutical industry sponsorship and research outcome and quality: systematic review. *Bmj.* 2003-05-29 17:48:55 2003;326(7400):1167-1170.

121. Sterne JAC, Sutton AJ, Ioannidis JPA, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *Bmj.* 2011-07-22 15:04:01 2011;343.

122. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med.* Oct 18 2011;155(8):529-536.

123. Logan C, Givens M, Ives JT, et al. Performance evaluation of the MBio Diagnostics point-of-care CD4 counter. *J Immunol Methods.* Jan 31 2013;387(1-2):107-113.

124. Frontières MS. Putting HIV Treatment to the Test. In: Campaign MA, ed2013.

125. Larson B, Schnippel K, Ndibongo B, Long L, Fox MP, Rosen S. How to Estimate the Cost of Point-of-Care CD4 Testing in Program Settings: An Example Using the Alere Pima™ Analyzer in South Africa. *PloS one.* 2012;7(4):e35444.

126. Lehe J, Wayda B, Quevedo J, Sitoe N, Peter T, Jani I. Impact of point-of-care CD4 testing on anti-retroviral therapy scale-up and patient treatment costs in resource-limited settings. *IAS 2011*. Rome, Italy2011.

127. O. Tobaiwa1 TB, N. Sitoe2, J. Lehe1, T. Peter1, I. Jani2, J. Quevedo1. Implementation of a wireless GPRS-based monitoring system for point-of-care CD4 testing at rural primary health facilities in Mozambique. *19th International AIDS Conference*2012.

128. Shearer WT, Rosenblatt HM, Gelman RS, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol.* Nov 2003;112(5):973-980.

129. Gale HB, Gitterman SR, Hoffman HJ, et al. Is frequent CD4+ T-lymphocyte count monitoring necessary for persons with counts >=300 cells/muL and HIV-1 suppression? *Clin Infect Dis.* May 2013;56(9):1340-1343.

130. Pai M, McCulloch M, Enanoria W, Colford JM, Jr. Systematic reviews of diagnostic test evaluations: What's behind the scenes? *ACP J Club.* Jul-Aug 2004;141(1):A11-13.

131. UNAIDS. Report on the Global AIDS Epidemic. 2010.

132. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. *Lancet Infect Dis.* Mar 2010;10(3):155-166.

133. WHO. Towards Universal Access: Scaling up priority HIV/AIDS interventions in the health sector: Progress Report. 2010.

134. WHO. Towards Universal Access: Scaling up priority HIV/AIDS interventions in the health sector: Progress Report 2009. 2009.

135. Hirnschall G. Towards 2015: reorienting the health sector response to HIV. WHO's strategic directions and priorities. 2010.

136. Malaria TGftfATa. The Global Fund Results Report 2010: innovation and impact. 2010.

137. Bartlett JA, Shao JF. Successes, challenges, and limitations of current antiretroviral therapy in low-income and middle-income countries. *Lancet Infect Dis.* Oct 2009;9(10):637-649.

138. Murtagh MM. HIV/AIDS Diagnostic Landscape 2nd Edition. *UNAIDS Technical Report.* 2011.

139. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol.* Nov 10 2003;3:25.

140. Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Clinical Chemistry.* Jan 2003;49(1):7-18.

141. Macaskill P GC, Deeks J. eds. Chapter 10:Analysing and Presenting Results: The Cochrane Collaboration. *Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy Version 1.0.* 2010.

142. Henkel T, Göhde R, Ost V, Göhde W. True Point-of-Care CD4 and CD4% Testing with Highest Accuracy and Precision for Adult and Pediatric HIV/AIDS Patients. *6th IAS Conference on HIV Pathogenesis and Treatment*. Rome2011.

143. Morawski BM, Meya DB, Boulware DR. Accuracy of Pima Point-of-Care CD4 Analyzer in Routine Use in Public Health Clinics in Uganda. *Journal of Acquired Immune Deficiency Syndromes: JAIDS.* Jul 1 2013;63(3):e113-115.

144. Mwau M, Adungo F, Kadima S, et al. Evaluation of PIMA(R) Point of Care Technology for CD4 T Cell Enumeration in Kenya. *PLoS ONE.* 2013;8(6):e67612.

145. Mwau M. Information request for POC CD4 assay systematic review. In: Wilkinson S, ed. Adult-only Bland Altman mean bias ed2013.

APPENDICES

Prospero protocol

Review methods

15 **Review question(s)**

State the question(s) to be addressed / review objectives. Please complete a separate box for each question.

What is the state of the art evidence on performance of point-of-care CD4 assays, from accuracy through to impact, in global settings?

What is the state of the art evidence on performance of point-of-care Viral Load (VL) assays, from accuracy through to impact, in global settings?

16 **Searches**

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

DATA SOURCES AND SEARCHES
The systematic review will be conducted according to the road map for diagnostic reviews. [130]

Our search strategy will be comprehensive and performed to the highest standards laid out by the PRISMA guidelines. Our search strategy will cover the time period from 1st January 2000 to 1st October 2012.

SOURCES OF DATA
We plan to search 6 electronic databases (i.e., PubMed, Embase, BIOSIS, Web of Science, Cochrane Central, CINAHL) seeking to identify all original studies. Our search will also include screening of bibliographies in relevant primary studies and review articles. We will contact authors and experts for additional data and unpublished work. A hand search of citations from selected studies will also be done.

INCLUSION AND EXCLUSION CRITERIA
Any study that has utilized a CD4 point-of-care test will be included. In case of non-availability of full-text articles, abstracts will be included if they provide sufficient information. Brief reports and grey literature will also be included.
English and Non-English articles will be included. Studies conducted in human populations will be included. Perspective pieces, editorials, opinions and narrative and other reviews will be shortlisted, but not included in the systematic review.

SEARCH STRING
The search strings will be generated with the help of a librarian, who will also be consulted on the search of the databases.

STUDY SELECTION
Two reviewers will independently screen the citations retrieved from all six data sources. After pooling citations from all databases, and after removing duplicates, identified citations will be reviewed for a final selection. A list of excluded studies will be made available by the authors upon request. All full-text articles, abstracts, letters, brief reports published will be included, provided complete information can be elicited from them. A third reviewer will help resolve disagreements between the two reviewers.

18 **Condition or domain being studied**

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.

The global impact of HIV/AIDS has been widely acknowledged. An estimated 34 million (95%CI: 31.6-35.2 million) people globally are infected with HIV, of which a vast majority (68%) reside in Sub-Saharan Africa.[131] Although developed countries like Canada and the United States have benefited greatly from the introduction of antiretroviral therapy (ART) beginning in 1996, in contrast, ART became widely available in African countries only recently (2004). [132]

With universal access to treatment, 400,000 people with HIV in low and middle income countries were on ART in 2003; at the end of 2009, this number had increased to approximately 5 million.[133 134] As of 2011, about 7 million people in low and middle income countries were on ART, an expansion from 39 to 47%.[135 136] However, global estimates of the number of individuals with HIV requiring ART is expected to exceed 14 million by 2012. With expanded universal access, countries and programs are bound to face additional challenges of bringing people to test, effectively treating and monitoring them, and efficiently scaling up infrastructure to cope with the increasing numbers on treatment. ART consists of a combination of antiretroviral drugs and is the standard treatment for people living with HIV/AIDS. ART is highly effective in controlling HIV replication, improving a patient's immune status and prolonging the life of individuals with HIV/AIDS, as evidenced by the increase in life expectancy of HIV-1 infected individuals in the developed world since the introduction of ART.[21] A major challenge in managing individuals on ART is monitoring its effectiveness with the overarching goal of preserving treatment options and preventing treatment failure.[137] In resource rich settings, laboratory based CD4, CD8 and viral load (VL) RNA assays are ubiquitous and are widely used to: a) assess eligibility for ART, b) initiate ART, c) monitor ART response (i.e., virological and immunological failure), and d) initiate treatment switches. These stepwise processes are intended to expedite clinical decision making to optimize/maximize the response to ART. In contrast, in resource limited settings (RLS), conventional laboratory based CD4 and VL assays are expensive to procure, maintain and operationalize. Due to high costs of monitoring, patients are sub-optimally monitored for resistance (due to lack of inexpensive VL assays) in many RLS settings. Further, clinical monitoring based on presenting symptoms (WHO clinical staging), although widely used in settings where diagnostic facilities are limited, is suboptimal and oftentimes ineffective in controlling and managing HIV infection.[21] Surrogate markers such as CD4 cell count and VL are the best prognostic predictors of ART response, and have been used extensively for clinical staging, ART initiation, and effectively detecting ART failure in developed settings for decades. Within this context, there is a huge push to fund, develop, evaluate, monitor and scale up use of point-of-care (POC) CD4 and VL assays, since these biomarker based assays are known to play a huge role in monitoring disease progression and response to ART. Several POC CD4 and VL assays are being used at point-of-care or being evaluated, but the evaluations are currently ongoing and evidence on their global performance and diagnostic accuracy, patient-centreed outcomes, impact on clinical decision making and economic outcomes has, to date, not been synthesized.

19 **Participants/population**

Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.

HIV infected populations that are confirmed positive, being staged, initiated or monitored for treatment, worldwide (resource rich and RLS settings). These will include patients studied as part of established HIV positive cohorts, cross sectional studies, and clinical trials.

20 **Intervention(s), exposure(s)**

Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed

POC CD4 assays include strip based tests and handheld devices, below lists the test currently available:
Zyomyx & Visitect (Omega Diagnostics, Burnet Institute UK)
Coulter CD4 Count kit (Beckman Coulter Inc., USA)
Dynal T4 Quant Kit (Dynal Biotech, Oslo, Norway)
PointCare NOW™ (PointCare Technologies Inc., USA)
Alere PIMA™ CD4 (Alere Inc., Germany)
CyFlow® miniPOC (Partec Germany)
Daktari™ CD4 Counter (Daktari Diagnostics, Inc., USA)
MBio™ Diagnostics CD4 system (MBio Diagnostics, Inc., USA)
CD4 Point of Care Technology (BD Biosciences, USA) [138]
[78]

VL point-of-care and rapid tests:
SAMBA (Diagnostics for the Real World, UK)
Liat Analyzer (IQuum Inc., USA)
NAT system (Alere Inc., Germany)
GeneXpert System (Cepheid, USA)
EO-NAT HIV Rapid RNA Assay system (Wave 80 Biosciences, USA)
Benchtop Analyzer (Advanced Liquid Logic, Inc., USA) under development
[138]

21 **Comparator(s)/control**

Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).

For CD4 POC assays the reference standard of choice for the evaluation of diagnostic accuracy is the flow cytometer. The reference standard for Viral load assays is viral RNA detection by nucleic acid amplification technologies, which are costly and require sophisticated laboratory infrastructure.

22 **Types of study to be included initially**

Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.

No type of study will be excluded

23 **Context**

Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

Global populations, including both resource rich and resource-limited settings. Please see "19 Participants/populations" for further details.

24 **Primary outcome(s)**

Give the most important outcomes.

Primary objectives: to determine the diagnostic accuracy parameters (i.e., sensitivity, specificity) and precision (i.e., coefficients of variation, inter-rater and intra-rater reliability) of CD4 POC assays.

25 **Secondary outcomes**

List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.

Secondary outcomes - Patient centred outcomes: In line with the GRADE recommendations, we will assess the impacts of tests on patient centred outcomes such as time to staging, time to clinical decision making, time to treatment initiation, and loss to follow up

Tertiary outcomes (if studied) will include feasibility and cost effectiveness.[4] However, given that the field is in its infancy, we expect it to be unlikely that these outcomes will be reported.

Conflict of interest: In this review, we will also collect data to assess the influence of conflicts of interest in industry-sponsored studies compared to studies sponsored by government health agencies.

26 **Data extraction, (selection and coding)**

Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

DATA ABSTRACTION
Each study will be assigned a unique ID. A master list linking IDs and authors will be created. Where necessary, authors will be contacted for additional information. Data abstraction will be conducted independently by two reviewers. Disagreements will be resolved by consensus and in consultation with a third reviewer.

DATA ABSTRACTION FORM
A pre-piloted data abstraction will be created in Excel in consultation with the review committee. Data on the following will be abstracted: study setting, country, year, study design, sample size, participant characteristics, eligibility criteria, objectives of testing, index test, reference tests, primary outcomes such as diagnostic accuracy parameters (i.e., sensitivity, specificity), secondary outcomes, prevalence, feasibility, preference, impact, and cost. To evaluate the association between reporting of conflicts of interest and reporting of positive results, a section on the role of study sponsors, declaration of funding, and study results (i.e., positive, negative, neutral) will be included. A section on quality assurance and quality control of laboratory reporting will evaluate test conduct in field settings.

27 **Risk of bias (quality) assessment**

State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.

Elements for critiquing and reporting of methodological quality will be adapted and incorporated from the QUADAS-2 that are domain based and give a high, low and unclear risk of bias, and STARD reporting checklists [139,140]

i) Publication Bias: We will attempt to minimize publication bias by searching several databases, by contacting companies and experts for unpublished and ongoing studies and by including English and Non-English studies.

ii) Selection Bias: We will minimize bias in study selection by ensuring that two reviewers independently screen and select the relevant studies; reviewers will be blinded to article authors.

iii) Data Extraction Bias: Data extraction bias will be reduced if two reviewers independently extract data using a pre-piloted standardized data extraction form. Disagreements will be resolved in consultation with a third reviewer. Authors will be contacted if further data are necessary.

iv) Bias in Synthesis: We will avoid the common pitfalls of simple pooling of sensitivity and specificity, and instead analyze data using SROC and bivariate random effects regression methods. These methods are more meaningful and are also recommended by the Cochrane Diagnostics Reviews Group as the preferred approach.[141]

28 **Strategy for data synthesis**

Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.

We will establish the diagnostic accuracy parameters and the precision of CD4 POC assays when utilized in research, implementation and as part of roll out projects for the monitoring of HIV infected individuals. Our search will be systematic, in line with established guidelines and PRISMA checklist. Our review will be registered with PROSPERO.

29 **Analysis of subgroups or subsets**

Give any planned exploration of subgroups or subsets within the review. ‘None planned’ is a valid response if no subgroup analyses are planned.
None planned. Our plans will depend on the data available from studies.

Review title and timescale

1 **Review title**

Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.

Evaluation of diagnostics and overall performance of point-of-care CD4 and Viral Load assays for monitoring HIV infected individuals being treated with Antiretroviral Therapy (ART)

2 **Original language title**

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3 **Anticipated or actual start date**

Give the date when the systematic review commenced, or is expected to commence.

12/1/2012

4 **Anticipated completion date**

Give the date by which the review is expected to be completed.

12/3/2013

5 **Stage of review at time of this submission**

Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

Paper data abstraction tool

Systematic Review and Meta-Analysis: CD4 Point of Care Assays | 2013

Record ID: ☐

Reviewer:	☐ 1Samantha Wilkinson	☐ 2Tiago Chiavegatti	
Language:	☐ 1English ☐ Other.....	**Published?**	☐ 1Yes ☐ 0No
Country:	.....	**Year published:**	.....
Recruitment period:	.....	**Sample size:**	.....
Author contacted?	☐ 2Yes, non response ☐ 0No ☐ 1Yes, response ↓	**Study design:**	☐ 1Diagnostic test evaluation ☐ 2RCT ☐ 3Observational ☐ 4Other .....
Further information provided by the authors:	.....		
Setting:	☐ 1 Rural ☐ 2Urban ☐ 3Mixed	☐ 4 Academic med centre ☐ 5 Community centre ☐ 6 Mobile clinic	☐ 7 Hospital ☐ 8 Dedicated HIV clinic ☐ 9 Primary care .....
Resources of area:	☐ 1Low income ☐ 2Middle income	☐ 3High income	*Choice from...* ☐ 4Paper ☐ 5Other .....
Environment:	☐ 1Temperate ☐ 2Humid	☐ 3Extreme heat ☐ 4Extreme cold	*Choice from...* ☐ 5Paper ☐ 6Other .....
Type of publication:	☐ 1Full paper ☐ 2Conf. abstract	☐ 3Letter ☐ 4Unpublished	☐ 5Thesis .....
Participants			
Mean CD4 of sample pop	.....	☐ 1 Absolute ☐ 2 Percentage	☐ 3Other..... ☐ 4Not reported
Participant characteristics *Tick all that apply*	☐ 1Adults ☐ 2Children (<18yrs) ☐ 3Neonates (<1 mon)	☐ 4Male ☐ 5Female ☐ 6Pregnant women	☐ 7Not stated ☐ 8Other .....
Age range	.....		
HIV status	☐ 1All HIV positive ☐ 2Mixture	☐ 3All HIV negative ☐ 4Not stated	☐ 5Other .....
Exclusion criteria	Please list → → → ☐ 1None stated	....	
Patient sampling strategy:	☐ 1Nonzero probability ☐ 2Convenience based	☐ 3Retrospective ☐ 4Prospective	☐ 4Not stated .....
Missing data			
Any missing data?	☐ 1Yes ☐ 0No	**Missing data in analysis:**	☐ 1Included in analysis ☐ 2Excluded from analysis .....
Ethical considerations			
Conflict of interest stated?	☐ 1Yes	☐ 0No	.....
Declaration of funding	☐ 1Industry	☐ 2University	☐ 3Public .....
Study sponsor:	.....		☐ 3Not stated
Reviewer concerned about conflict of interest?	☐ 1Yes	☐ 0No	.....

Record ID: ____

Point of care device

Company name:		**Test name:**	
Type of technology:	☐ 1Flow cytometry ☐ Other.....	**Type of CD4 test:**	☐ 1Quantitative ☐ 2Qualitative
Time from sample to test:		**Marketable product?** *i.e. not concept/technique*	☐ 1Yes ☐ 0No

ASSURED criteria

Costs reported:	Device: ..... Per test:	Total per test: ..... ☐ 66Not stated	☐ Designed as low cost
User-friendly?	☐ 1Yes ☐ 2No	*Operated by...* ☐ 1Clinic staff ☐ 2Technicians	☐ 3Not stated ☐ 4Other .....
Time to result:	.....		
Robustness:	☐ 1Mobile ☐ 2Battery powered	☐ 3Water not required ☐ 4Other →	.....
Type of equipment:	☐ 1Lab test ☐ 2Handheld device	☐ 3Table top device ☐ 4Unclear →	.....
Type of substrate used:	☐ 1Venous blood ☐ 2Capillary blood	☐ 3Saliva ☐ 4Other →	.....
Amount of substrate required:	.....		
QC measures taken?	☐ 1Yes	☐ 0No	☐ 2Not stated
Device limitations:	.....		☐ 2None stated
Condition of specimen	☐ 1Fresh ☐ 2Frozen	☐ 3Not stated ☐ 4Other →	.....
Training provided by device manufacturer?		☐ 0No ☐ 1Yes	☐ 2Other .....
Ambient conditions required for sample prep?		☐ 3Not stated	

Reference test

Company name:		**Test name:**	
Type of technology:	☐ 1Flow cytometry ☐ Other.....	**Type of CD4 test:**	☐ 1Quantitative ☐ 2Qualitative
Time from sample to test:		**Marketable product?**	☐ 1Yes ☐ 0No
Time to result:	.....		
Type of substrate used:	☐ 1Venous blood ☐ 2Capillary blood	☐ 3Saliva ☐ 4Other →	.....
Amount of substrate required:	.....		
Condition of specimen	☐ 1Fresh ☐ 2Frozen	☐ 3Not stated ☐ 4Other →	.....

Record ID:

Primary Outcomes

	% Similarity				
Data pair	Mean similarity	SD Mean similarity	MPD Mean % difference	Standard deviation MPD	CV Correlation coefficient

Notes

.....

.....

	Bland Altman		
Data pair	Bias *Mean difference*	95% CI of mean *Or* SD	Limits Of Agreement

Notes

.....

.....

Accuracy of POC test									
	Number of true/false +ves/-ves								
Cut off point	TP	TN	FP	FN	LR+	LR-	OR	Sens	Spec

Notes

.....

.....

	Other results reported				
Group	.....	.....	.....	.....	.....

Notes

.....

.....

Record ID:

Patient centered outcomes

Patient Centered Outcome reported?	☐ 1Yes	☐ 0None reported
Outcome reported	**Findings**	

QUADAS

Where there inappropriate exclusions?	☐ 1Yes	☐ 0No .....
Concern that included patients do not match the review question?	☐ 1Low ☐ 2High	☐ 3Unclear .....
Delay between sample and test acceptable?	☐ 1Yes	☐ 0No .....
Blinding between reference and POC?	☐ 1Yes	☐ 0No .....
Difference between test and review question?	☐ 1Yes	☐ 0No .....
Could conduct or interpretation have introduced bias?	☐ 1Yes ☐ 0No	☐ 2Unclear .....
Is the reference standard likely to correctly classify the target condition?	☐ 1Yes ☐ 0No	☐ 2Unclear .....
Were the reference standard results interpreted without knowledge of the results of the index test?	☐ 1Yes ☐ 0No	☐ 2Unclear .....
Could the reference standard, its conduct, or its interpretation have introduced bias?	☐ 1Low ☐ 2High	☐ 3Unclear .....
Any concern that the target condition as defined by the reference standard does not match the review question?	☐ 1Low ☐ 2High	☐ 3Unclear .....
Was there an appropriate interval between index test(s) and reference standard?	☐ 1Yes ☐ 0No	☐ 2Unclear .....
Did patients receive the same reference standard?	☐ 1Yes ☐ 0No	☐ 2Unclear .....
Did all patients receive a reference standard?	☐ 1Yes ☐ 0No	☐ 2Unclear .....
Were all patients included in the analysis?	☐ 1Yes ☐ 0No	☐ 2Unclear .....
Could the patient flow have introduced bias?	☐ 1Low ☐ 2High	☐ 3Unclear .....

Notes

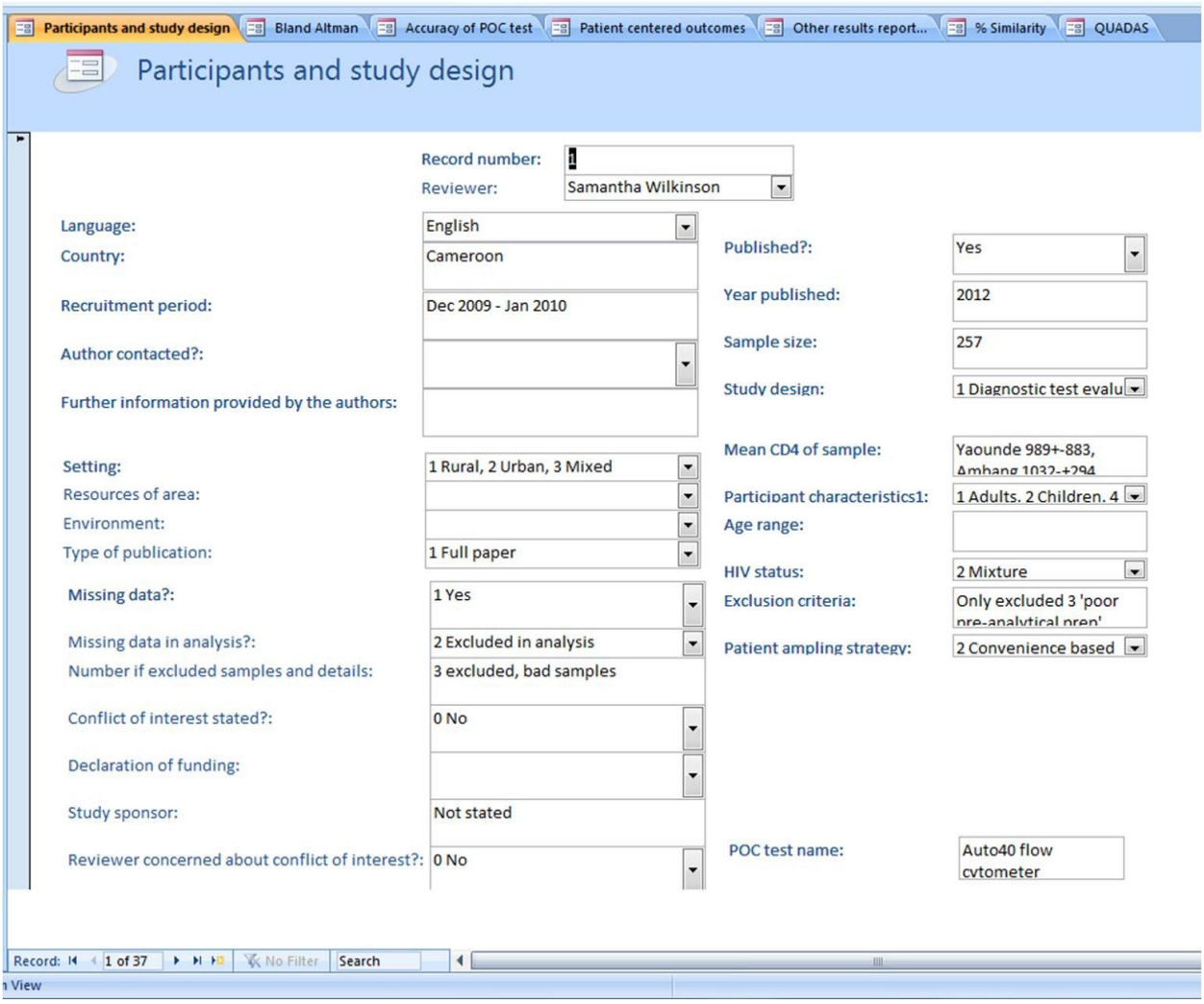
.....

.....

.....

Final decision: ☐ Include paper
☐ Exclude paper

Access database data abstraction tool (screenshot)



Forest Plots

Figure 6 Forest plot: Bland Altman by sample, clinical equivalence limits

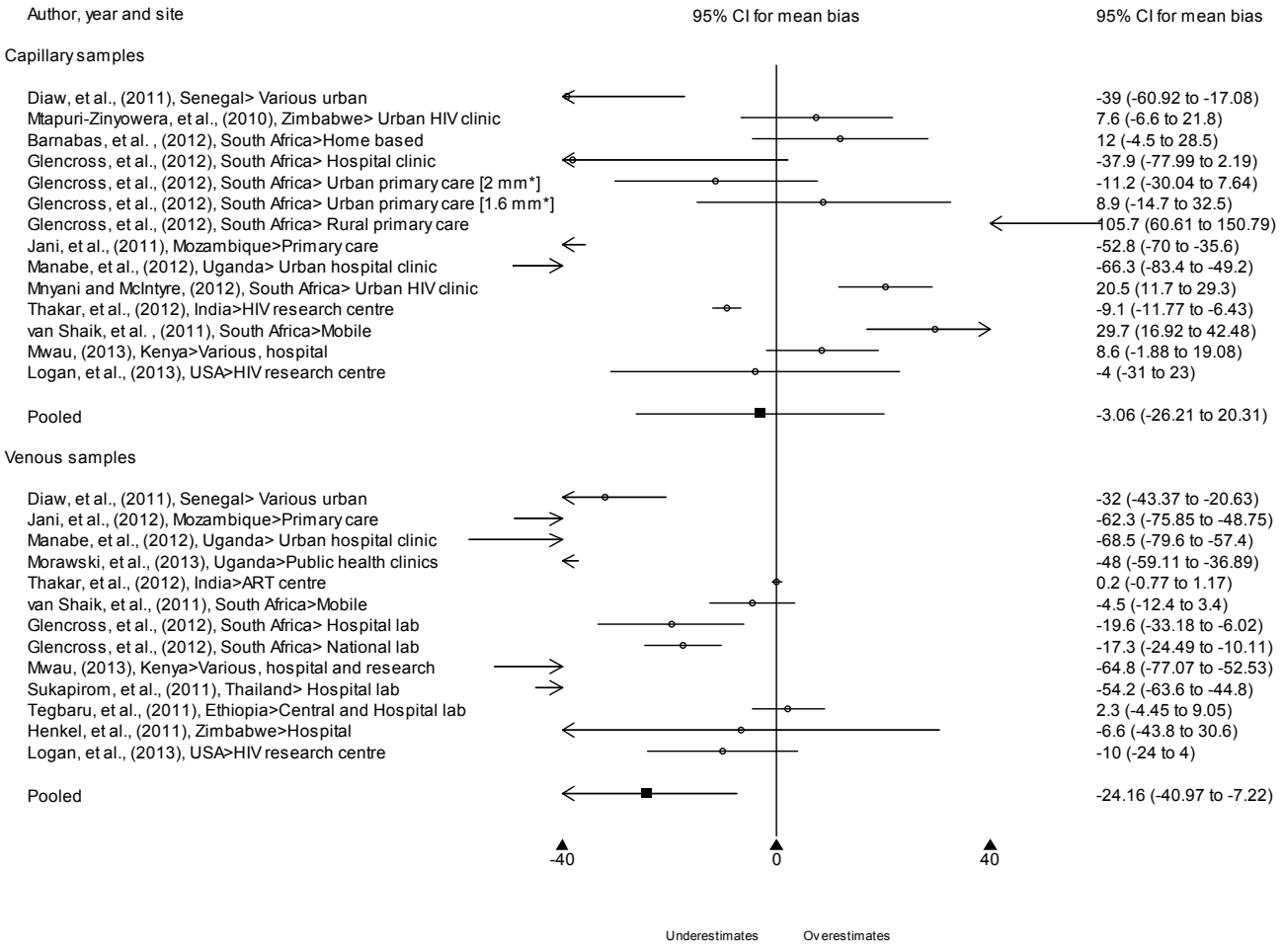


Figure 7 Forest plot: Bland Altman by sample and device, clinical equivalence limits

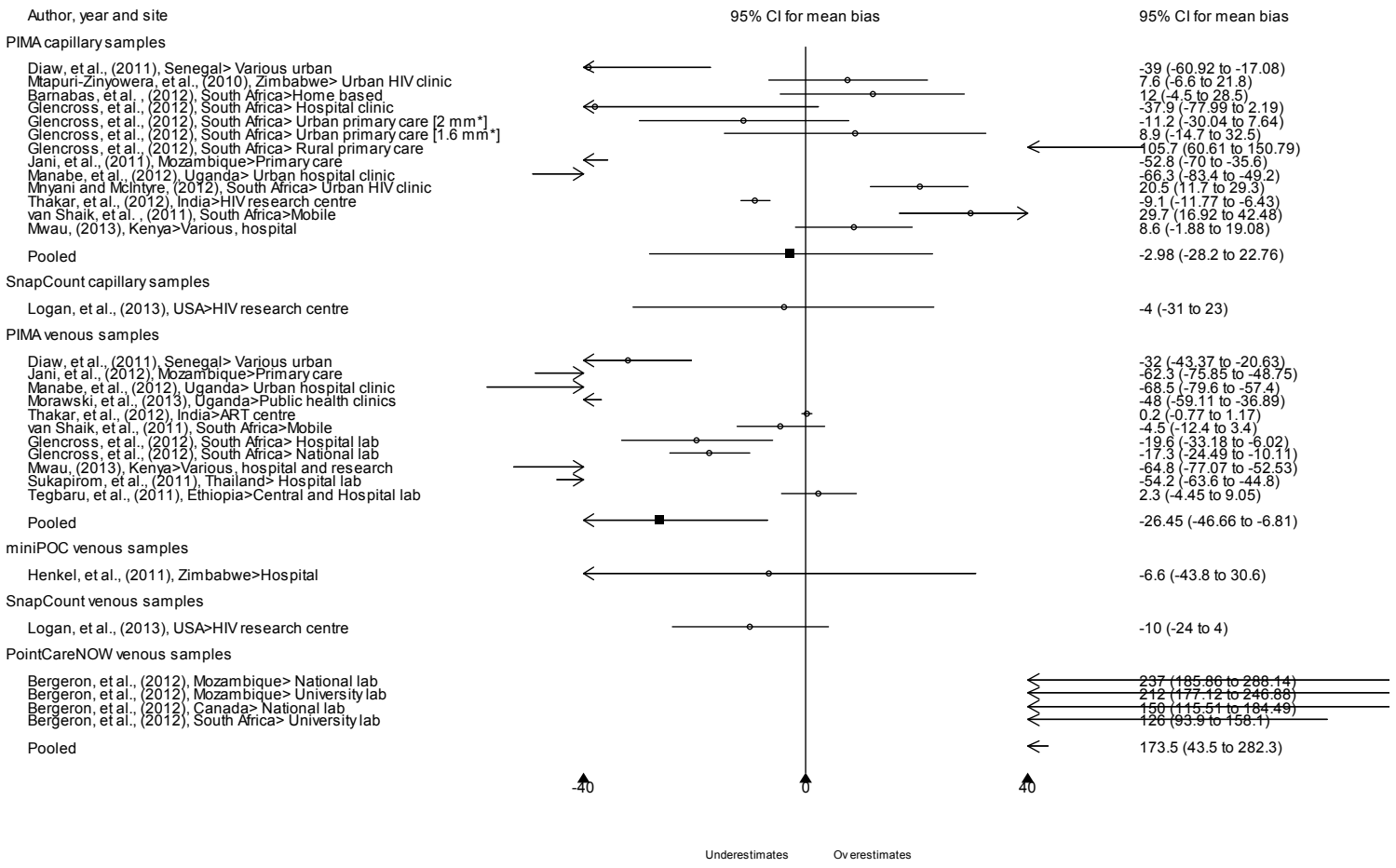


Figure 8: Forest plot: Bland Altman by staff group, with clinical equivalence limits

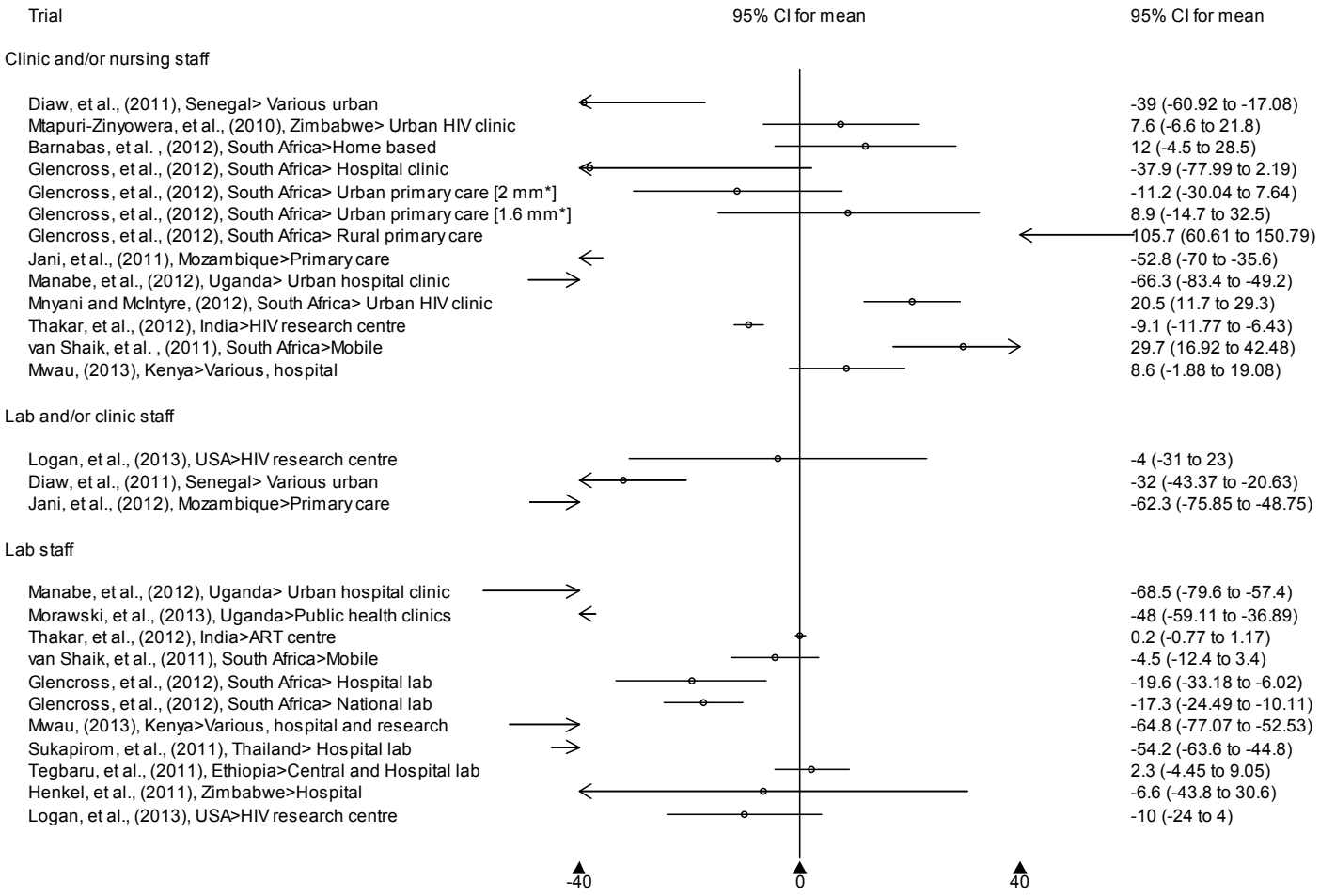
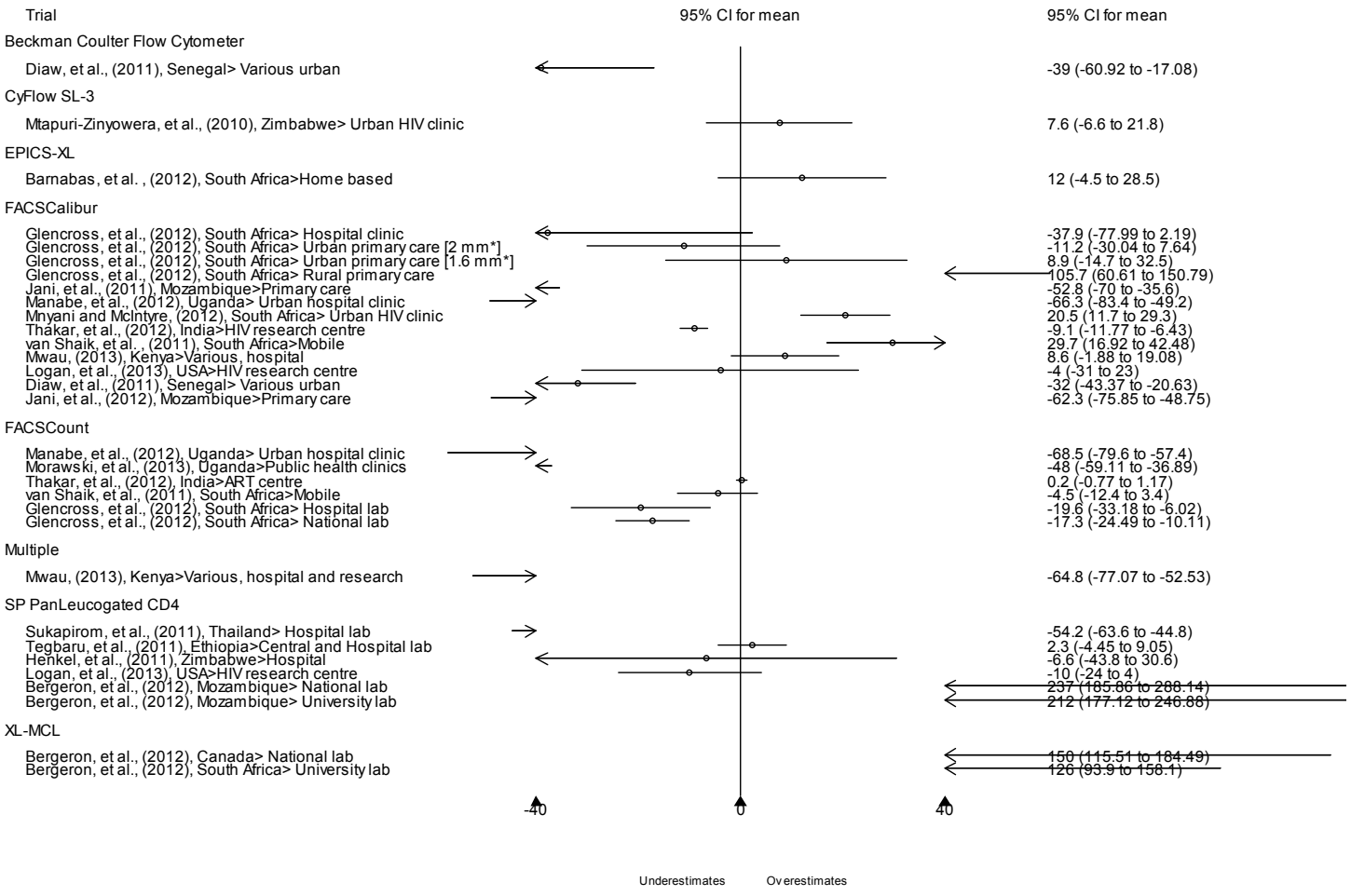


Figure 9 Forest plot: Bland Altman by reference, clinical equivalence limits



Example code for calculating sensitivities, specificities in R

```
> Her.200<- as.table(matrix(c(28,9,2,215), nrow = 2, byrow = TRUE)) ; epi.tests(Her.200,
conf.level = 0.95, verbose = FALSE)
          Disease +    Disease -      Total
Test +           28            9         37
Test -            2          215        217
Total            30          224        254

Point estimates and 95 % CIs:
-------------------------------------------------------
Apparent prevalence                   0.15 (0.1, 0.2)
True prevalence                       0.12 (0.08, 0.16)
Sensitivity                           0.93 (0.78, 0.99)
Specificity                           0.96 (0.93, 0.98)
Positive predictive value             0.76 (0.59, 0.88)
Negative predictive value             0.99 (0.97, 1)
-------------------------------------------------------
```

R-code used for calculating 95% CIs, and forest plots

```
#Download and rearrange BA data

BAdata <- read.table("C:/Users/samantha.wilkinson/Documents/Sam's work/CD4 POC Assays/Results and
data/CSV files/SR POC CD4 Unique_HIV+ BlandAltman 28Augv1.csv", sep=",", head=T)
head(BAdata)

#Summary statistics
#Mean and SD of n
mean(BAdata$n) #223.7097
sd(BAdata$n) #310.893
summary(BAdata$n) #median 140.0

# Rename some headings
names(BAdata) [1] = "ID"
names(BAdata) [3] = "Yr"
names(BAdata) [4] = "Cntry"
names(BAdata) [6] = "POC"
names(BAdata) [9] = "Ven"
names(BAdata) [10] = "BMD"
names(BAdata) [13] = "SD"
names(BAdata) [18] = "Ref"
names(BAdata) [19] = "POCop"
BAdata
# Drop out some of the useless fields
colnames(BAdata)
#Remove POC substrate, notes and new columns
BAdata <- BAdata[c(-8,-16, -17)]
colnames(BAdata)

# # Calculate SE, depending on the info available from the study # # Do for all studies, for
completeness

# Method 1: Using the SD and the z quantile
BAdata$SE<-NA
BAdata$SE<-BAdata$SD/sqrt(BAdata$n)
BAdata$SE

# Method 2: Using the CI
BAdata$SEci<-NA
BAdata$SEci<-(BAdata$UCI-BAdata$LCI)/(2*1.96)
BAdata$SEci

#SE2 SD
BAdata$SDci<-NA
BAdata$SDci<-BAdata$SEci*(sqrt(BAdata$n))
BAdata$SDc

# Combine calculated SE into SE
BAdata$SE<-ifelse(is.na(BAdata$SE), BAdata$SEci, BAdata$SE)
BAdata$SE<-ifelse(is.na(BAdata$SE), BAdata$SEci, BAdata$SE)
BAdata$SE

# # Calculate missing SD # #
# Method 1 using LOA
BAdata$SDloa<-(BAdata$ULOA-BAdata$BMD)/2
BAdata

# Use SDloa to fill in blanks from SE
BAdata$SE<-ifelse(is.na(BAdata$SE), BAdata$SDloa/sqrt(BAdata$n), BAdata$SE)
BAdata$SE

# Now calculate all sds to create a final SD2
BAdata$SD<-BAdata$SE*sqrt(BAdata$n)
BAdata

# # Create a subgroup only including useful fields # #
```

```
BAdata$Study<-paste(BAdata$Author,", (", BAdata$Yr,"), ", BAdata$Cntry,">",BAdata$Site, sep="")

# Only keep useful fields
colnames(BAdata)
# Remove Author, Yr, Cntry, Site, LCI, UCI, LLOA, ULOA, POCop, SE, SEdi, SDci, SDloa
BAdata<-BAdata[-c(2,3,4,5,10,11,13,14,16,18,19,20,21)]
colnames(BAdata)

# Compare against reported CIs where possible
#Export to Excel for comparison
write.table(BAdata, "C:/Users/samantha.wilkinson/Documents/Sam's work/CD4 POC Assays/Results and
data/BAdataexport1508.xls", sep=";")
#Data OK compared to Dr Joseph

## Use Lawrence Joseph Forest Plot code
#http://www.medicine.mcgill.ca/epidemiology/Joseph/PBelisle/R/forest-plot.R

# Create median, Upper and lower CI category
#BAdata$UCI.1<-BAdata$BMD+c(1)*1.96*(BAdata$SD/sqrt(as.numeric(BAdata$n)))
#BAdata$LCI.1<-BAdata$BMD+c(-1)*1.96*(BAdata$SD/sqrt(as.numeric(BAdata$n)))
BAdata$ci.all<-matrix(c(BAdata$BMD,(BAdata$BMD+c(-
1)*1.96*(BAdata$SD/(sqrt(BAdata$n)))),(BAdata$BMD+c(1)*1.96*(BAdata$SD/(sqrt(BAdata$n)))) ),
ncol=3)
BAdata$ci.all
head(BAdata)

# Create meta-analysis data points, LJospeh analysis #
PIMA.c.bias<--2.975
PIMA.c.sd<-12.84

PIMA.v.bias<--26.45
PIMA.v.sd<-9.942

PC.bias<-173.5
PC.sd<-59.13

# Create categories for POC
categoryci<-matrix(c(
round(PIMA.c.bias, digits=2)
, -28.20
, 22.76
,"NA", "NA", "NA"
, round(PIMA.v.bias, digits=2)
, -46.66
, -6.81
, "NA", "NA", "NA", "NA", "NA", "NA"
, round(PC.bias, digits=2)
, 43.50
, 282.30)
, byrow=T, ncol=3)

# Data should already be sorted from the .csv file
#indexBA<-with(BAdata, order(Ven, POC))
#BAdata[indexBA,]
#BAdata

# Forest plot for all data
BAdata$Study<-round(BAdata$Study, digits=2)

BAdata$ci.all<-round(BAdata$ci.all, digits=2)
categoryci

length(BAdata$Study)
length(BAdata$ci.all)/3

forest.plot.or(
, authors=BAdata$Study
, studies.ci=BAdata$ci.all
, standard.or.plot=F
)
par(mai=c(1,1,1,1)
```

```
ci.all

BAdata

forest.plot.or(
log.scale=F
, authors=BAdata$Study
, cex=0.75
, studies.ci=BAdata$ci.all
, ci.txt="95% CI for mean bias"
, plot.lim=c(-290, 290)
, ref.vline.at=0
, standard.or.plot=F
, or.side.labels.cex=0.6
, category.labels=c("PIMA capillary samples", "SnapCount capillary samples", "PIMA venous
samples", "miniPOC venous samples", "SnapCount venous samples", "PointCareNOW venous samples")
, study.categories=rep(seq(6), c(13,1,11,1,1,4))
, blank.after.category.label=T
, blank.before.category.subtotal=T
, blank.between.categories=T
, blank.after.total=T
, or.side.labels=c("Underestimates", "Overestimates")
, categories.ci=categoryci
, study.txt="Author, year and site"
, plot.categories.ci=c(T, F, T, F, F, T)
)

forest.plot.or(
log.scale=F
, authors=BAdata$Study
, cex=0.75
, studies.ci=BAdata$ci.all
, ci.txt="95% CI for mean bias"
, plot.lim=c(-40, 40)
, ref.vline.at=0
, standard.or.plot=F
, or.side.labels.cex=0.6
, category.labels=c("PIMA capillary samples", "SnapCount capillary samples", "PIMA venous
samples", "miniPOC venous samples", "SnapCount venous samples", "PointCareNOW venous samples")
, study.categories=rep(seq(6), c(13,1,11,1,1,4))
, blank.after.category.label=T
, blank.before.category.subtotal=T
, blank.between.categories=T
, blank.after.total=T
, or.side.labels=c("Underestimates", "Overestimates")
, categories.ci=categoryci
, study.txt="Author, year and site"
, plot.categories.ci=c(T, F, T, F, F, T)
```

Agreement between reviewers for QUADAS-2 analysis

Reco		PATIENT SELECTIO	INDEX TEST	REFERENCE STANDARD	FLOW AND	Record number		PATIENT SELECTIO	INDEX TEST	REFERENCE STANDARD	FLOW AND		% Agreeme
7	Study 1	Unclear	Low	Low	Unclear	7	Study 1	Unclear	Unclear	Unclear	Low	Check	60
16	Study 2	Unclear	Low	Low	Unclear	16	Study 2	Unclear	Low	Low	Low	Check	
33	Study 11	Low	Low	Low	Low	33	Study 11	Unclear	Unclear	Unclear	Unclear	Check	
35	Study 10	Unclear	Low	Low	Unclear	35	Study 10	Unclear	Low	Low	Low	Check	
40	Study 8	Low	Low	Low	Low	40	Study 8	Unclear	Low	Low	Low	Check	
41	Study 9	Low	Low	Low	Low	41	Study 9	Unclear	Low	Low	Low	Check	
45	Study 15	Low	Low	Low	Low	45	Study 15	Low	Low	Low	Low	OK	
46	Study 7	Low	Low	Low	Low	46	Study 7	Low	Low	Low	Low	OK	
47	Study 6	Low	Low	Low	Low	47	Study 6	Low	Low	Low	Low	OK	
48	Study 12	Low	Low	Low	Low	48	Study 12	Low	Low	Low	Low	OK	
52	Study 5	Unclear	Low	Low	Unclear	52	Study 5	Unclear	Low	Low	Unclear	OK	
60	Study 3	Low	Low	Low	Low	60	Study 3	Low	Low	Low	Low	OK	
61	Study 4	Low	Low	Low	Low	61	Study 4	Low	Low	Low	Low	OK	
67	Study 14	Low	Low	Low	Low	67	Study 14	Low	Low	Low	Low	OK	
68	Study 13	Low	Low	Low	Low	68	Study 13	Low	Low	Low	Low	OK	
69	Study 16	Low	Low	Low	Low	69	Study 16	Low	Low	Low	Low	OK	
		SVW						TC					

Search strategy: run in Medline Jan 2013

Database: Ovid MEDLINE(R), Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid OLDMEDLINE(R) <1946 to Present>

1 exp HIV/ (75915)
2 exp HIV infections/ (212454)
3 HIV Antibodies/ (9058)
4 exp HIV Antigens/ (13090)
5 HIV Long-Term Survivors/ (499)
6 Lymphoma, AIDS-Related/ (2214)
7 hiv*.mp,jw,in. (248593)
8 (acquired adj2 immun$ adj2 syndrome$1).mp,jw,in. (89260)
9 (acquired immun$ adj3 deficiency).mp,jw,in. (11940)
10 (human adj2 immun$ adj2 deficiency).mp,jw,in. (1671)
11 human immun?deficiency.mp,jw,in. (71891)
12 ((aids or lymphadenopathy) adj5 (virus* or viral or retrovirus or lentivirus or infection* or syndrome*)).mp. (38348)
13 aids.jw,in. (56728)
14 Anti-HIV Agents/ (29492)
15 Antiretroviral therapy, highly active/ (14880)
16 ((ART or HAART or anti?retrovir* or anti retrovir*) adj3 (therap* or regimen* or initiat* or program* or start* or naive or monitor* or treatment* or test* or result* or administ* or clinic* or service* or respon* or patient* or drug$1 or medicat* or agent$1)).mp. (43472)
17 or/1-16 (310489)
18 ("PointCare NOW" or CyFlow or Coulter or ((dynal or dynamal) adj2 (t4 or cd4 or quant)) or mBio or daktari or zyomyx or Burnet or "dried blood spot*" or (pima* adj2 (cd4 or analyzer$1 or poc or point or alere))).mp. (4355)
19 exp CD4 Lymphocyte Count/ (18931)
20 serologic tests/ (15626)
21 AIDS serodiagnosis/ (5766)
22 Flow Cytometry/ (95708)
23 cytomet*.mp. (147276)
24 cell analys*.mp. (2616)
25 exp CD4-Positive T-Lymphocytes/ (76248)
26 Antigens, CD4/ (12499)
27 25 or 26 (85448)
28 (test* or count* or monitor* or screen* or assay* or measur* or analys* or technol* or diagnos* or marker* or biomarker* or response or estimat* or enumerat* or quantif* or percentage or ratio).mp. (9993082)
29 27 and 28 (58352)
30 (cd4 adj3 (test* or count* or monitor* or screen* or assay* or measur* or analys* or technol* or diagnos* or marker* or biomarker* or response or estimat* or enumerat* or quantif* or percentage or ratio)).mp. (36205)
31 18 or 19 or 20 or 21 or 22 or 23 or 24 or 29 or 30 (247196)
32 17 and 31 (40637)
33 Point-of-Care Systems/ (5995)
34 Physicians' Offices/ (1408)
35 (point of care or POC or POCT).mp. (9847)
36 point of service.mp. (312)
37 ((rapid* or quick* or express or fast or prompt* or instant* or immediat*) adj2 (test* or count* or monitor* or screen* or assay* or measur* or analys* or technol* or diagnos* or marker* or biomarker* or response or estimat* or enumerat* or quantif* or percentage or ratio or answer*)).mp. (79566)
38 (handheld or (hand adj held) or portable or mobile or remote* or bedside or (bed adj2 side)).mp. (129829)
39 device*.mp. (242643)
40 strip$1.mp. (32622)
41 microchip$1.mp. (3990)
42 (near adj2 patient*).mp. (1559)
43 (physician* adj2 office*).mp. (4631)
44 ((extra* or outside) adj2 laborator*).mp. (878)
45 decentr*.mp. (5574)
46 ancillary.mp. (8892)
47 (alternat* adj2 site*).mp. (2751)
48 or/33-47 (500272)
49 32 and 48 (1109)
50 limit 49 to yr="2000 -Current" (854)
51 limit 50 to humans (793)
52 limit 50 to animals (38)
53 50 not (51 or 52) (55)
54 51 or 53 (848)
55 remove duplicates from 54 (835)
56 letter/ (775604)
57 comment/ (517963)
58 editorial/ (318375)
59 or/56-58 (1207686)
60 55 not 59 (817)

Summary of selected studies

Author	Year	Population	Intervention, type of study	POC test name	Reference flow cytometer	Site	Country	n	POC Operator	Reference
Mtapuri-Zinyowera, et al.	2010	Newly diagnosed HIV+ male and female participants	Paired blood samples	PIMA	FACSCalibur	Urban HIV clinic	Zimbabwe	165	Nurses and lab technicians	82
Mnyani and McIntyre	2012	Consecutive HIV+ pregnant women in a prevention of mother-to-child transmission of HIV service in Johannesburg	Parallel CD4+ cell count testing was done using capillary specimens for the PIMA and venous samples for flow cytometry.	PIMA	Beckman Coulter Flow Cytometer	Urban HIV clinic	South Africa	296	Clinic staff	96
Bergeron, et al.	2012	Five sites independently conducted studies primarily using samples mostly from HIV+ patients	Samples were tested with both the PointCare NOW and reference flow cytometry	PointCareNOW	FACSCalibur	National lab	Mozambique	143	Clinic staff	102
						University lab	Mozambique	114		
						National lab	Canada	89		
					EPICS-XL	University lab	South Africa	71		
Sukapirom, et al.	2011	HIV+ blood samples, at various stages of HIV-1 infection.	Simultaneous testing on PIMA and reference	PIMA	FACSCount	Hospital lab	Thailand	203	Lab technicians	84
Manabe, et al.	2012	Adults attending an infectious disease clinic a Hospital, for a routine clinic visit.	Evaluation of the PIMA compared to the BD FACSCalibur	PIMA	FACSCalibur	Urban hospital clinic	Uganda	176	Clinic staff	94
Glencross, et al.	2012	Coordinated through an Academic Hospital CD4 reference laboratory, located in Johannesburg	Baseline accuracy, followed by field testing in primary care sites	PIMA	SP PanLeucogated CD4	National lab	South Africa	100	Lab technicians	90
						Hospital lab		91	Lab technicians	
						Hospital clinic		77	Nurse	
						Rural 1° care		96	Nurse	
						Urban 1° care (1.6mm lancet)		87	Nurse	
						Urban 1° care (2mm lancet)		52	Nurse	
Diaw, et al.	2011	Patients presenting for HIV follow-up or other laboratory examinations presented in 4 different clinical sites	CD4 counts were measured by PIMA and by FACSCount considered as the reference	PIMA	FACSCount	Various urban	Senegal	100	Lab and clinic staff	85
								95		
Barnabas, et al.	2012	Home-based counseling and testing	POC CD4 testing and a venous blood draw by Facs Calibur	PIMA	FACSCalibur	Home based	South Africa	185	Nurse	93
Tegbaru, et al.	2011	Evaluation of a point of care-CD4 testing in Ethiopia	Evaluation study	PIMA	FACSCalibur	Central and Hospital lab	Ethiopia	316	Lab technicians	87
Logan, et al.	2013	HIV+ individuals were recruited for this study from a research centre in the US	Venous and capillary and tested using the MBio system and conventional flow cytometry.	MBio CD4, SnapCount™	FACSCalibur	HIV research centre	USA	52	Lab technicians	123
					FACSCalibur	HIV research centre	USA	94	Lab technicians	
Henkel, et al.	2011	Blood samples were collected from an infectious disease Hospital	Each sample was processed twofold for the miniPOC and the reference instrument	miniPOC	CyFlow SL-3	Hospital	Zimbabwe	125	Lab technicians	142
Jani, et al.	2011	Adult HIV+ patients enrolled consecutively at primary healthcare clinics in Mozambique	Paired samples of finger prick and venous blood tested on the POCT CD4 device and with laboratory instruments	PIMA	FACSCalibur	Primary care	Mozambique	135	Nurses	86
				PIMA	FACSCalibur	Primary care	Mozambique	140	Nurses	
Thakar, et al.	2012	Participants at 21 ART centres from different parts of the country	Evaluation study against the reference methods (FACSCalibur, FACSCount and CyFlow SL3).	PIMA	FACSCount	HIV research centre	India	175	Clinic staff	91
					Multiple	ART centre	India	1790	Clinic staff	
van Shaik, et al.	2011	Consecutive HIV+ individuals (both on ART and not on ART) had a capillary and/or venous sample in a mobile clinic	Cross-sectional, convenience based sampling. Both PIMA™ and laboratory CD4 counts done	PIMA	XL-MCL	Mobile	South Africa	325	Nurses	88
Morawski, et al.	2013	Testing in a multisite real-world setting at 7 Kampala city health facilities under general clinic conditions.	Venous samples were run on the PIMA, excess portions were sent to the University laboratory for reference testing.	PIMA	FACSCalibur	Public health clinics	Uganda	225	Clinic staff	143
Mwau, et al.	2013	Nine health facilities offering CD4+ T cell enumeration. All patients attending the facilities for HIV treatment.	Comparison study, venous and capillary blood specimens were collected consecutively patients presenting	PIMA	FACSCount	Various, hospital and research	Kenya	822	Lab technicians	144 145
							Kenya	521	Lab technicians	

Summary table of accuracy data

Author	Year	POC test name	Substrate Ven: Venous Cap: Capillary	Cut off point cells/µL	Sensitivity %	Specificity %	Total misclassified %	Misclassified above %	Misclassified below %	Notes	Reference
Diaw, et al.	2011	PIMA	Ven	200	90	98				10% bilateral inclusion range used	85
Diaw, et al.	2011	PIMA	Cap	200	91	96				10% bilateral inclusion range used	85
Diaw, et al.	2011	PIMA	Ven	350	98	79				10% bilateral inclusion range used	85
Diaw, et al.	2011	PIMA	Cap	350	91	80				10% bilateral inclusion range used	85
Herbert, et al.	2012	PIMA	Cap	200	93·3	96					95
Herbert, et al.	2012	PIMA	Cap	350	94·8	88					95
Herbert, et al.	2012	PIMA	Cap	500	98·6	70·5					95
Jani, et al.	2011	PIMA	Cap	350			17	2·2	14·8		86
Jani, et al.	2011	PIMA	Cap	200			5·2	0	5·2		86
Thakar, et al.	2012	PIMA	Cap	350	96	91					91
Mtapuri-Zinyowera, et al.	2010	PIMA	Cap	200			6·6	2·4	4·2		82
Mtapuri-Zinyowera, et al.	2010	PIMA	Cap	350			6·6	4·2	2·4		82
Tegbaru, et al.	2011	PIMA	Cap	200			9·2				87
Logan, et al.	2013	MBio CD4, SnapCount™	Cap	350			3·8	3·8	0		123
Logan, et al.	2013	MBio CD4, SnapCount™	Ven	350			5·3	2·1	3·2		123
Manabe, et al.	2012	PIMA	Cap	250	96·3	86·6					94
Manabe, et al.	2012	PIMA	Cap	300	93·2	79·5					94
Manabe, et al.	2012	PIMA	Ven	250	94·3	85·4					94
Manabe, et al.	2012	PIMA	Ven	300	98·2	75·3					94
Mnyani and McIntyre	2012	PIMA	Cap	350	93	86					96
Mnyani and McIntyre	2012	PIMA	Cap	350	82	94					96
Bergeron, et al.	2012	PointCareNOW	Ven	350	53	94				10% bilateral inclusion range used	102
Bergeron, et al.	2012	PointCareNOW	Ven	200	39	94				10% bilateral inclusion range used	102
Van Shaik, et al.	2011	PIMA	Ven	200	89	98					88
Van Shaik, et al.	2011	PIMA	Ven	350	89	90					88
Van Shaik, et al.	2011	PIMA	Cap	200	81	99					88
Van Shaik, et al.	2011	PIMA	Cap	350	85	93					88
Mwau, et al.	2013	PIMA	Cap	350	89·6	86·7					144
Morawski, et al.	2013	PIMA	Ven	200	100	>99					143
Barnabas, et al.	2012	PIMA	Not reported								93
Henkel, et al.	2011	miniPOC	Not reported								142
Sukapirom, et al.	2011	PIMA	Not reported								84
Glencross, et al.	2012	PIMA	Not reported								90