POINT-OF-CARE TESTING FOR HIV AND TB INTEGRATION OF SERVICES

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DECLARATION

I, Natasha Myrna Gous declare that this thesis is my own work. It is being submitted for the Degree, Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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_____ day of _____, 2015

DEDICATION

This work is dedicated to my parents, Myrna and Elmar Gous for your endless love, support and patience.

“Our mission is to build a better world. To leave no one behind. To stand for the poorest and the most vulnerable in the name of global peace and social injustice.’

Ban Ki-Moon

United Nations Secretary-General

ABSTRACT

The United Nations Programme on HIV/AIDS (UNAIDS) have recently released challenging new Human Immunodeficiency Virus (HIV) treatment targets to be achieved globally by 2020; all of which require concentrated efforts in scaling up laboratory testing capacity for HIV diagnosis, treatment initiation and treatment monitoring. The Global Tuberculosis (TB) Strategy have also put forth a list of ambitious goals which include reducing the number of deaths due to TB by 95% and the number of new TB cases by 90%.

In South Africa, which has the highest national prevalence of HIV described globally and ranks fifth in the world in terms of TB incident cases, further integration of HIV and TB services will be needed to achieve these targets. A major challenge to successful integration of these programs however, will be the ability to diagnose and monitor the progress of both infections, a process that in South Africa, is hampered by lack of access to laboratory testing. Although public pathology laboratory service providers, such as the National Health Laboratory Service (NHLS), are responding to increasing testing demands by scaling up centralised laboratory capacity, limitations such as the need for expertise, infrastructure, space, cold-chain, maintenance, logistics and cost, are challenging full implementation and scale up.

Many international organisations believe that one of the ways to successfully achieve the global HIV '90-90-90' and TB targets, will be through the development and scaling up of innovative, simpler and more affordable technology approaches such as Point-of-Care testing (POCT), a view shared by the South African National Department of Health (NDoH). POCT refers to testing that is performed near or at the site of the patient with the result leading to a possible or immediate change in patient management or outcome and holds promise as a strategy to extend laboratory testing capacity. Prior to large-scale POC

implementation efforts can begin, defining the difficulties and potential solutions which are likely to arise, particularly in high disease burden clinical settings need to be addressed.

The main objective of this study was to investigate the feasibility, performance and operational considerations of multidisciplinary POCT in South Africa, including the development of a best practice framework to guide implementation efforts. This was achieved by performing a clinical needs assessment and engaging with government, evaluating POC technologies for HIV and TB diagnosis and/or monitoring and developing a framework for how to implement POCT in the field including quality, site and training requirements. The operational requirements for healthcare workers to perform multiple POCT in the South African clinical setting, was also determined. The assays required were based on the South African National Treatment guidelines in the period of review (2011-2014).

In July 2013, the South African NDoH called a meeting with various stakeholders to provide the context for POCT in South Africa and strong emphasis was placed on HIV and TB and how POCT could expand on existing laboratory infrastructure for these diseases. Outcomes from this meeting prompted a thorough literature review on the challenges likely to be faced by large-scale POC implementation efforts.

One of the key issues highlighted was the lack of evaluation data on numerous HIV and TB POC technologies available and/or in the pipeline. Even though viral load (VL) testing has been available in South Africa since 2004, the global treatment guidelines (World Health Organization) now recommend a VL test for HIV antiretroviral treatment (ART) monitoring and there are talks around the possibilities of a ‘test and treat’ strategy. In light of this, two potential POC plasma-based VL technologies available at the time were evaluated in the laboratory. The Liat™ HIV-1 Plasma Quant (IQuum Inc, MA, USA; now Roche Molecular, Branchburg, MJ, USA) and the Xpert® HIV-1 VL (Cepheid, Sunnyvale,

CA) assays both demonstrated good performance and were proven to be interchangeable with existing in-country high-throughput VL laboratory platforms. Both however, require centrifugation to obtain the plasma sample and thus may be more suited to a district level facility as opposed to a 'true' POC environment. In light of these operational challenges, two further blood-based POC VL platforms were also evaluated, the Liat™ HIV-1 Blood Quant VL assay (IQuum, Inc) and the Alere™ q HIV-1/2 assay (Alere Technologies GmbH, Jena, Germany). Both assays identified more patients as treatment failures at the 1000 copies/ml treatment failure threshold (WHO and South African treatment guideline recommended threshold) compared to plasma VL, due to their total nucleic acid extraction protocols. Thus, if either were implemented at POC, one could expect a significant upward misclassification, increasing the number of HIV-positive patients requiring follow up VL testing and programmatic costs. Application therefore, could be niched VL testing; utilising a blood-based POC VL assay in maternity wards to diagnose HIV in new-borns; plasma-based POCT for mothers to reduce risk of transmission.

POCT may not be the only solution to increasing access to laboratory testing services, and thus alternative strategies for improving access were also investigated. Dried blood spots (DBS) and PrimeStore media (a sample transport media; Longhorn Vaccines and Diagnostics, San Antonio, TX, USA) were shown to be as valuable as plasma VL for detecting HIV-positive patients failing ART at the 1000 copies/ml threshold and both solve logistical issues around sample transport and maintaining sample integrity for centralized testing.

For TB diagnosis, the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA) was evaluated to determine its appropriate placement within the South African setting. Although Xpert® MTB/RIF proved superior in performance to smear microscopy, it was originally modelled as too costly for POC placement in South Africa and was implemented into smear microscopy centres nationally. Subsequently, the complexity of the analyser maintenance

and power issues has reinforced the original decision. Further potential POC TB technologies are in the development pipeline, but only one other was available for evaluation, namely the EasyNAT® detection kit (Ustar Biotechnologies, Hangzou, China). Initial laboratory evaluation results look promising but the technology is still a long way from clinical evaluation due to its laborious procedure.

A further challenge identified for POCT is the lack of documented implementation science to ensure quality-assured multi-disciplinary POCT in the field. To address this, three key components of a quality testing framework were developed to ensure best practice for POCT; a clinic site readiness assessment tool, a POC training module and a quality monitoring program. The clinic site assessment checklist was developed to determine site readiness for POC placement. The POC training module included standard operating procedures, quick reference and workflow charts and a practical training component which was developed specifically with the non-laboratory trained user in mind. Both these components have been adopted and modified for use by the NHLS National Priority Program (NPP).

Certain POC assays already have External Quality Assessment (EQA) material, while others had to be developed. For quality management of HIV VL technologies, a standardized plasma panel was developed to ensure molecular VL platforms are ‘fit-for-purpose’ (verification, a requirement of the laboratory accreditation process). This panel, termed SAVQA, is being manufactured and supplied to aid POC assay developers in assessing their product for the South African market, and will also be further developed for use by healthcare workers at POC.

Due to the hurdles encountered with the biosafety regulations for transporting TB external quality assessment (EQA) material, a quality assessment program using dried culture spots (DCS) was also developed for TB diagnostic technologies consisting of two

components; a verification and an EQA program. The DCS technology has become a global product and as of 2015 is being supplied to 20 different countries. DCS were successfully shown to be suitable for use at POC by non-laboratory trained staff. The versatility of the material has been confirmed by its expansion to other molecular TB diagnostic tests, most notably the Hain Genotype MTBDr*plus* assay for TB drug susceptibility testing (Hain LifeScience GmbH, Nehren, Germany). This work has been acknowledged through the Research and Development team involved in the development of the DCS program, winning three awards: the NHLS Top Award for Innovation 2013, the Gauteng Accelerator Program (GAP) Biosciences Award in 2014 and a special Social Impact award for Africa Innovations held in Morocco in 2015.

Incorporating the quality components developed above, a clinical evaluation of nurse operated multidisciplinary POCT was performed. Although multiple POCT could be performed as accurately as laboratory testing on venepuncture specimens, it required dedicated staff and dramatically increased POC staff duties. It was further shown that multiple POCT could be accurately performed by a nurse on a single finger slice in order to obtain adequate blood volume to perform up to four POC tests, and that finger stick VL testing was also feasible by nurses at POC. Patients were also more willing to have up to three finger sticks performed than to have a single venepuncture specimen taken. The process of using finger sticks was further ratified by demonstrating that a single finger stick can provide up to 150µl of blood, which is sufficient to perform an array of POC tests.

In spite of the feasibility of nurse based POCT, limitations of current technologies using finger stick were also realised, such as the performance of the Liat™ Quant blood assay which generated increased VL misclassification at the 1000 copies/ml treatment failure threshold (70% misclassification). This would impact programmatic costs, but this technology may have value as a diagnostic tool in key populations.

The work described shows that multi-disciplinary POCT within a South African setting is achievable with appropriate clinic infrastructure, dedicated staff, training and stringent quality monitoring measures in place. The HIV and TB POC technologies evaluated were found to be as accurate as laboratory-based testing however, few meet the criteria of a 'true' POC device and thus further research and development is required. Based on South Africa's testing needs, a tiered hybrid model which expands on centralized laboratory capacity through incorporating POCT into very remote, hard-to-reach areas and innovations around linkage to care efforts, may help meet '90-90-90' targets but will require costing/modelling and future assessments of the impact and outcome of the intervention. Much of this work presented contributed towards the development of a draft National POCT policy document in support of the national strategic plan for POCT for the management of HIV and TB in South Africa.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Chapter 2: Assessing the clinical needs for POCT and engaging with government.

Publications

1. Stevens W, Gous N, Ford N, Scott L. Feasibility of HIV point-of-care tests for resource-limited settings: challenges and solutions. BMC Medicine 2014; 12(1): 173.

   Candidate contributed to literature review and drafting of the manuscript.

Conference Presentations

a) Natasha Gous on behalf of Wendy Stevens. 2013. Point of Care diagnostics: South Africa's experience. In: Laboratory Medicine Congress, Cape Town, South Africa, 28-31 July.

Chapter 3: The Evaluation of New Technologies for the Diagnosis and/or Monitoring of HIV and TB.

Publications

1. Scott LE, Gous N, Carmona S, Stevens WS. Evaluation of the Liat™ HIV Quant whole blood and plasma HIV-1 viral load assays for Point of Care testing in South Africa. J Clin Micro, May 2015. 53(5): 1616 -21.

   Candidate performed all the laboratory evaluations, assisted with data analysis and manuscript draft.

2. Scott LE, McCarthy K, Gous N, Nduna M, Van Rie A, Sanne I, Venter F, Duse A, Stevens W. Xpert MTB/RIF for the diagnosis of pulmonary and drug resistant TB in a

high HIV prevalence setting: Comparison to other nucleic acid technologies. Plos Medicine, July 2011. 8(7): e1001061.

Candidate coordinated project, performed all the laboratory evaluations, data collection and assisted with data analysis and manuscript draft.

Conference Presentations

a) Gous N. 2015. Experience with a polyvalent molecular platform: The Xpert MTB/RIF assay and the Xpert HIV-1 Quant assay. In: Cepheid Satellite Session on Your GeneXpert Can Do So Much More!! 7th SA AIDS Conference, Durban, SA, 9-12 June.

b) Scott LE, Gous N, Carmona S, Stevens WS. 2014. HIV viral Load Monitoring: Introducing New Technologies. In: Cepheid Lunch and learn. African Society of Laboratory Medicine, Cape Town, South Africa, 30th November – 4th December.

c) Scott LE, Gous N. 2014. Alere Q whole blood viral load assay (prototype) evaluation for ART monitoring. In: Symposium. African Society of Laboratory Medicine, Cape Town, South Africa, 30th November - 4th December.

d) Gous N, Scott LE. 2015. Longitudinal cohort analysis of dried blood spots for viral load monitoring. Oral presentation. In: 7th SA AIDS Conference, Durban, SA, 9th-12th June.

e) Stevens W, Gous N, Erasmus L, Coetzee G, Cunningham B, Scott L. 2011. Taking the first steps in national GeneXpert implementation: lessons learned from South Africa. In: IAS, Italy, 17th -20th July.

f) Gous N, Scott LE, Khan S, Reubenson G, Coovadia A, Cunningham B, Stevens W. 2012. The Reality of Xpert MTB/RIF at Point of Care (POC) for the Diagnosis of Childhood TB using Raw Sputum. In: CROI, Seattle, USA, 5th – 8th March.

g) David A, Gous N, Stevens W, Scott LE. 2014. Laboratory validation of Ustar EasyNAT™ Diagnostic test compared to GeneXpert MTB/RIF for qualitative detection

of Mycobacterium tuberculosis using Dried Culture Spots. In: African Society of Laboratory Medicine, Cape Town, South Africa, 30th November - 4th December.

Chapter 4: Developing the principle components to ensure best practise for multi-disciplinary POCT: Quality, Site readiness, Training.

Publications

1. Scott LE, Carmona S, Gous N, Horsfield P, Mackay M, Stevens W. Rapid scale up of a high throughput HIV viral load testing: Use of a pre-qualification panel. J Clin Micro 2012; 50(12):4083-6.

   Candidate was involved in initial study design, performed all laboratory experiments and assisted with manuscript draft.

2. Scott L.E, Gous N, Cunningham B.E, Kana BD, Perovic O, Erasmus L, Coetzee G.J, Koornhof H, Stevens W. Dried Culture Spots for Xpert MTB/RIF External Quality Assessment: Results of phase 1 pilot study from South Africa. J Clin Micro 2011. 49(12): 4356-60.

   Candidate performed all research and development, matrix preparation and laboratory evaluations, assisted with manuscript preparation.

3. Gous N, Cunningham B, Kana B, Stevens W, Scott LE. Performance monitoring of Mycobacterium tuberculosis Dried Culture Spots for use on the GeneXpert within a National Program in South Africa. J Clin Micro. Dec 2013; 51 (12): 4018 – 4021.

   Candidate designed and performed all experiments, analysed data and took the lead on manuscript preparation.

4. Gous N, Isherwood L, David A, Stevens W, Scott LE. External quality assessment of Genotype MTBDR*plus* version 1 and 2 using dried culture spot material. J Clin Micro. April 2015: 53 (4): 1365 – 7.

Candidate conceived and designed all experiments, performed laboratory evaluations, analysed data and took the lead on manuscript preparation.

Conference Presentations

a) Scott LE, Gous N, Carmona S, Stevens W. 2014. Performance of Xpert® HIV-1 Quant compared to Roche CAP/CTM v2 and Abbott RealTi*m*e HIV-1 on a prequalification plasma validation panel. In: African Society of Laboratory Medicine, Cape Town, South Africa, 30th November - 4th December.

b) Gous N, Scott LE, Stevens WS. 2012. Requirements for Point of Care testing: A checklist for implementation. In: Grand Challenges Diagnostic Meeting, Seattle, Canada, September.

c) Gous N, Scott L, Cunningham B, Stevens W. 2012. Site and training requirements for Xpert® MTB/RIF assay implementation in remote settings: Prepare for the unexpected. In: 3rd SA TB Conference, South Africa, 12th – 15th June.

Chapter 5: Determining the feasibility of multiple POCT for HIV and TB service integration in the field.

Publications

1. Gous N, Scott L, Potgieter J, Ntabeni L, Enslin S, Newman R, Stevens W. Feasibility of performing multiple point of care testing for HIV anti-retroviral treatment initiation and monitoring from multiple or single fingersticks. PLoS One. 2013 Dec; 8(12): e85265.

Candidate was involved in the design of study, coordinated project and study site set up, performed all training, performed laboratory validations, performed all data collection and analysis and manuscript preparation.

2. Maiers TJ, Gous N, Nduna M, McFall SM, Kelso DM, Fisher MJ, Palamountain KM,. Scott LE, Stevens WS. An Investigation of Fingerstick Blood Collection for Point-of-Care HIV-1 Viral Load Monitoring in South Africa. S Afr Med J 2015; 105(3): 228-231.

   Candidate was involved with study site set-up and training, assisted with manuscript draft.

3. Gous N, Scott LE, Potgieter J, Ntabeni L, Sanne I, Stevens W. Implementing multiple point of care testing in two HIV antiretroviral treatment clinics in South Africa. Submitted to JAIDS – under review.

   Candidate was involved in the design of study, coordinated project and study site set up, performed all training, performed laboratory validations, assisted with data collection and analysis and manuscript preparation.

4. Gous N, Scott LE, Nduna M, Stevens W. Evaluation of the Liat™ HIV Quant prototype Assay (IQuum) for HIV viral load monitoring using finger stick at point of care. Submitted to J Clin Micro – under review.

   Candidate was involved in conception and design of study, study coordination and site set up, training, data collection and analysis, manuscript preparation.

Chapter 6: Policy Development.

1. Point of Care Testing. Position paper Forum Report to support National strategic Plan for POCT for the management of HIV and TB in South Africa: An overview of the Point-Of Care Forum hosted by the National Department of Health. Held at the South African National Department of Health on the 24th and 25th June, 2013.

The candidates output from this thesis contributed to this policy document.

Permission has been granted from all co-authors to include publications in this thesis.

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ABBREVIATIONS AND SYMBOLS

ACTG	AIDS Clinical Trial Group
AFB	Acid-Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
ART	Antiretroviral Therapy/ Treatment
ARV	Antiretroviral
ASLM	African Society of Laboratory Medicine
CAP/CTM	COBAS® Ampliprep/COBAS® Taqman
CHAI	Clinton Health Access Initiative
CLIA 88	Clinical Laboratory Improvement Amendments of 1988
CLSI	Clinical Laboratory Standards Institute
Cr	Creatinine
CrAg	*Cryptococcus neoformans* antigen
CSF	Cerebrospinal Fluid
DBS	Dried Blood Spot
DCS	Dried Culture Spot

DNA	Deoxyribonucleic Acid
DST	Drug Susceptibility Testing
EID	Early Infant Diagnosis
ELISA	Enzyme-Linked Immunoassay
EPTB	Extra-pulmonary Tuberculosis
EQA	External Quality Assessment
FDA	Food and Drug Administration
FNA	Fine needle Aspirate
GCC	Grand Challenges Canada
Hb	Haemoglobin
HbA1c	Glycated Haemoglobin
HBV	Hepatitis B Virus
HCT	HIV Counselling and Testing
HIV	Human Immunodeficiency Virus
INH	Isoniazid
ISO	An International Organisation for Standardization that develop and publish standards
LAM	Lipoarabinomannan

LIS	Laboratory Information System
LPA	Line probe assay
LSHTM	London School of Hygiene and Tropical Medicine
MDR	Multi-Drug Resistant
MGIT	Mycobacterial Growth Indicator Tube
mHealth	Mobile Health
MTBC	Mycobacterium Tuberculosis Complex
NAAT	Nucleic Acid Amplification-based Tests
NDoH	National Department of Health
NHLS	National Health Laboratory Service
NPP	National Priority Program
NSP	National Strategic Plan
NWGHF	North Western Global Health Foundation
PCR	Polymerase Chain Reaction
PEPFAR	Presidents Emergency Plan for AIDS Relief
PHC	Primary Health Care
PITC	Provider Initiated Testing and Counselling
PLG	Pan*Leuco*gating

PMTCT	Prevention of Mother to Child Transmission
POC	Point-of-Care
POCT	Point-of-Care Testing
PPT	Plasma Preparation Tube
QC	Quality Control
RIF	Rifampicin
RNA	Ribonucleic Acid
rpoB	A gene which encodes the ß subunit of bacterial RNA polymerase and is associated with resistance to the drug Rifampicin
SANAS	South African National Accreditation System
SAVQA	South African Viral Quality Assurance
SMS	Short Message Service
STI	Sexually Transmitted Infection
TB	Tuberculosis
TNA	Total Nucleic Acid
TPP	Target Product Profile
µl	Microliter
UNAIDS	United Nations Programme on HIV/AIDS

VCT	Voluntary Counselling and Testing
VL	Viral Load
WHO	World Health Organisation
XDR	Extensively Drug Resistant

CHAPTER 1: INTRODUCTION AND RESEARCH OBJECTIVES

1.1 THE VISION FOR GLOBAL MANAGEMENT OF HIV AND TB

The Joint United Nations Programme on HIV/AIDS (UNAIDS) has set an ambitious new Human Immunodeficiency Virus (HIV) treatment target for 2020, termed '90-90-90'. This calls for 90% of all people living with HIV to know their HIV status, 90% of all HIV-positive persons to be placed on antiretroviral (ARV) treatment (ART) and 90% of all people on ART to have achieved viral suppression (2). All three of these goals place a strong emphasis on the need for scaling up laboratory testing capacity for HIV diagnosis, treatment initiation and treatment monitoring. Despite plans already underway globally to achieve these targets, it has been predicted that at the current pace of laboratory testing scale-up, the '90-90-90' goals are unlikely to be reached by 2020 (Figure 1.1) (2).

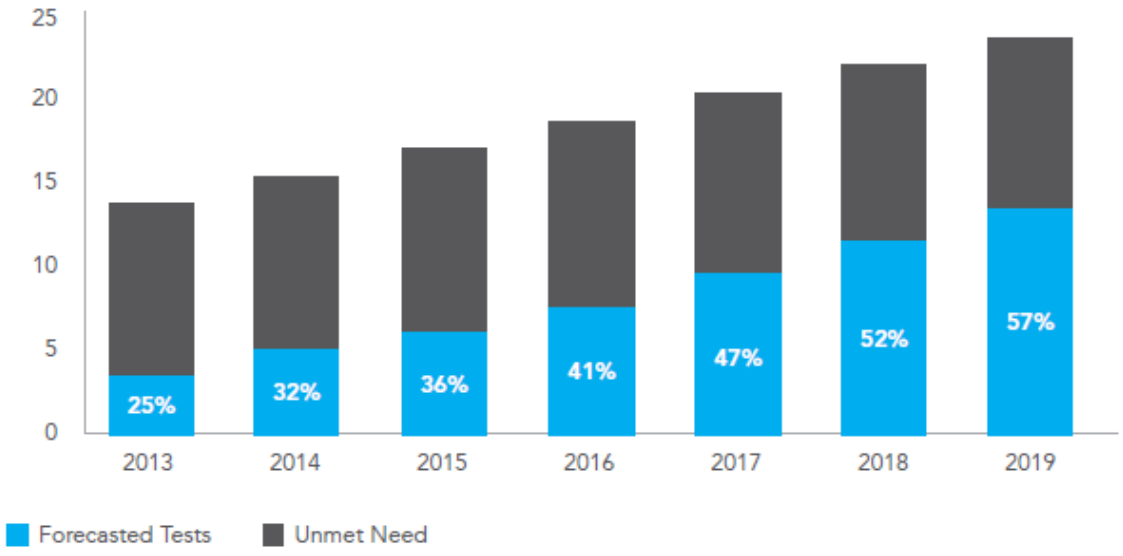

Figure 1.1: The Clinton Health Access Initiative (CHAI) performed data modelling to predict whether current scale up plans for laboratory testing capacity in 21 HIV high burden countries will be enough to meet global treatment demands. The graph shows that despite significant scale-up of viral load testing capacity, global demand for testing will not be met by 2020 (2).

The post-2015 Global TB Strategy has also set out a list of committed targets, which include reducing the number of deaths due to Tuberculosis (TB) by 95% and new cases by 90%, with the ultimate goal of ending the global TB epidemic (3).

Many international groups such as the World Health Organisation (WHO), UNITAID, Bill and Melinda Gates Foundation and CHAI (to name a few), also including the South African National Department of Health (NDoH), now believe that one of the ways to successfully achieve these goals will be through the development and scaling up of innovative, simpler and more affordable technologies (4, 5). This is driving the development of Point-of-Care (POC) tests, which promise to expand access to testing services (6). A strong focus is also being placed on collaborative HIV and TB activities, particularly in high-burden countries, in order to provide access through integrated services for prevention, diagnosis and treatment of both diseases (7).

1.2 THE PROBLEM IN CONTEXT: THE BURDEN OF HIV AND TB GLOBALLY

Identified over three decades ago, HIV infection, which leads to acquired immunodeficiency syndrome (AIDS), has been labelled the 'modern-day plague' (8). The global prevalence or number of persons living with HIV infection, increased from 34 million in 2011 [31.4 - 35.9 million] (9) to an estimated 35 million [33.2 - 37.2 million] in 2013 (10, 11). Although estimates of prevalence vary by country and region, the epicentre of the disease burden occurs in sub-Saharan Africa, that by 2013 had 24.7 million [23.5 - 26.1 million] persons living with HIV infection, a staggering 70% of the global reported cases (12). Despite increasing prevalence, AIDS-related mortality decreased from 2.4 million [2.2 - 2.6 million] in 2005, to 1.5 million [1.4 - 1.7 million] in 2013 (12), an effect which is largely attributable to the success of ART programs initiated in low- and middle-income countries (13).

The number of new HIV infections (or incidence) reported globally has been on a steady decline over the past few years. The global HIV incidence reported in 2013 stood at 2.1

million [1.9 – 2.4 million], 13% less than that reported in 2010 (14). Of the total incidence, 240,000 of those were reported in children deemed to be infected perinatally (12) which also represented a marked decline (58%) compared with that reported in 2002 (14).

The linkage between HIV infection and TB is well known; the risk of developing TB is 26 to 31 times greater in people infected with HIV (15). TB is therefore one of the most common, yet curable, opportunistic infections affecting people living with HIV. In 2013, the global estimate for TB prevalence stood at 11 million [10 – 13 million]; the majority of prevalent cases (81%) were reported in the 22 countries classified by the WHO as 'high burden' (16). TB incidence is defined as the number of new and relapse cases of TB over a certain period of time (usually a year) and as for HIV, is steadily declining. In 2013, 9 million [8.6 - 9.4 million] people developed TB disease; most (56%) were reported in Asia and the Western Pacific region and 25% were reported in Africa (16). Of these 9 million cases, 1.1 million were reported in HIV/TB co-infected persons and 550,000 were in children under 15 years of age (15, 16) (Figure 1.2). Global TB mortality figures stood at 1.5 million in 2013; 24% of these deaths were in HIV co-infected persons (16).

Although data is limited due to difficulty in diagnosis, it is estimated that 15-20% of all TB cases reported are actually of extra-pulmonary origin (EPTB) (17), meaning that TB has affected other organs of the body.

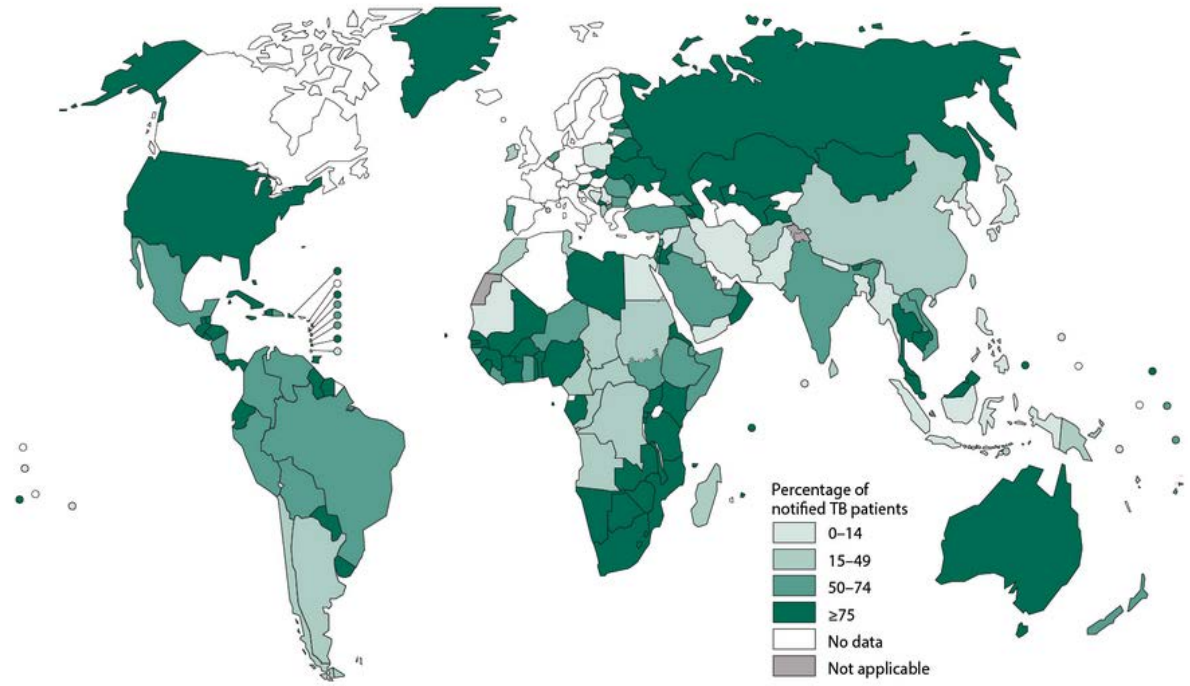

Figure 1.2: Diagram depicting the percentage of notified TB cases with known HIV status in 2013, based on data from the WHO (18).

Against the backdrop of the global TB epidemic and increased case detection and treatment, multi-drug resistant TB (MDR-TB) is on the rise. MDR-TB is defined as tuberculosis that is resistant to at least two of the widely used first-line TB drugs, rifampicin (RIF) and isoniazid (INH). An estimated 3.7% of TB cases reported worldwide have MDR-TB (19) and there are currently 27 countries considered to have high MDR-TB burdens. In 2013, 480,000 new MDR-TB cases were reported (16), 60% of the burden occurring in Brazil, China, India, Russia and South Africa (19). This figure may be underestimated however, as globally many cases remain unreported. Considerably more alarming is the fact that an estimated 9.6% of all MDR-TB cases are actually extensively drug resistant (XDR) (20), a term which defines drug resistance to both of the first-line TB drugs, any of the fluoroquinolones and at least one of the three second-line injectables (21). This greatly restricts treatment options. One hundred countries have reported XDR-TB cases.

1.2.1 THE BURDEN OF HIV AND TB IN SOUTH AFRICA

At the southern-most tip of Africa, South Africa had an estimated population of 54 million in 2014 (22), representing only 0.73% of the world's total population. South Africa nevertheless bears one of the highest HIV and TB burdens worldwide.

The WHO reported an HIV prevalence of 6.3 million [6.0 - 6.5 million] (12.1%) in 2013, 5.9 million of these cases occurring in persons 15 years and older (23). This was the highest national prevalence of HIV described globally. Women are the worst affected, carrying 23.3% of the country's burden (24) compared with 13.3% of men in the age group 15-49 years (24).

In terms of the number of TB incident case, South Africa currently ranks fifth behind India, China, Nigeria and Pakistan globally, with an approximate 1% of the population developing TB disease each year (16). In 2013, there were 328,896 new TB cases notified, 11.5% of which were of extra-pulmonary origin (25). South Africa also reports 40% of the global MDR-TB cases (20). Certain population groups have been identified as highly vulnerable to TB, for example miners (occupational health risk, at highest risk are gold-miners who frequently have Silicosis, in addition to HIV and TB) and offenders in correctional services. Years of neglect with regards to the health of these populations have resulted in exceptionally high incidence rates of TB in mines (3,000/100,000) (26) and a TB prevalence of approximately 3.5% -7.5% in correctional management centres (27).

The South African NDoH estimates that 60% of all HIV-positive people are also co-infected with TB (28).

1.3 THE ROLE OF LABORATORIES IN HIV AND TB DISEASE MANAGEMENT

South Africa has put forward a broad list of goals in their National Strategic Plan (NSP) for 2012-2016, which focuses on HIV and TB collaborative efforts such as reducing new HIV

infections by at least 50%, initiating at least 80% of eligible patients on ART and reducing the number of new TB infections, as well as deaths, from TB by 50% (29).

A major challenge to successful implementation of both antiretroviral and anti-tuberculosis therapy, however, remains the ability to diagnose, treat and monitor both infections. This process is limited by lack of laboratory infrastructure, technical skill and poor integration of HIV and TB services. Each phase of the diagnostic and monitoring process for both diseases is facilitated by a number of laboratory tests based on country-specific guidelines. The specific diagnostic clinical algorithms followed for HIV and TB in many high-burden countries is largely dependent on disease prevalence and available resources (human and financial) in clinics and associated laboratories. The latter has two extremes for service provision, namely

- high-throughput, centralized, placed testing, or
- low-throughput, decentralized POC testing (POCT).

1.3.1 CENTRALISED LABORATORY TESTING

In South Africa, diagnostic testing in the public sector is currently the mandate of the National Health Laboratory Service (NHLS). To meet NSP goals and local testing demands of expanded access to testing and treatment, the need for maximization of testing and screening strategies for HIV and TB is driving the development of next-generation, high-throughput diagnostic systems. The NHLS has provided significant investment in developing laboratory capacity through rapid expansion of a centralised laboratory infrastructure for CD4, Viral Load (VL), Early Infant Diagnosis (EID) and TB testing over the years.

1.3.1.1 CURRENT CENTRALISED TESTING STRATEGIES FOR HIV

This section details two of the main laboratory tests used for the management of HIV-positive persons, namely a CD4 count and a VL test.

Following on a positive HIV diagnosis, a CD4 count has traditionally been used to provide a measure of the immune system's response to infection, *i.e.* assessment of immune status, establish eligibility for ART and monitor patients on treatment (30, 31). A VL test, or the measure of the amount of HIV virus in the body in ribonucleic acid (RNA) copies/ml, has been used alongside a CD4 count to monitor response to treatment and detect appropriate timing for treatment switches (32-36).

The appropriate timing of ART initiation, based on the CD4 count threshold in HIV-positive people, has been hotly debated for the last 20 years. The CD4 treatment initiation threshold was initially set at less than 200 cells/µl by the WHO in 2002 (37). However, in 2009, results of a large clinical trial in Haiti (CIPRA HT 001) demonstrated alarming evidence suggesting that initiation of ART between a CD4 count of 200 and 350 cells/µl improved survival when compared with treatment initiation at less than 200 cells/µl (38). In light of these findings, the WHO lowered the CD4 count threshold to less than 350 cells/µl in 2010 (39). Since then, other studies have provided evidence to suggest the benefits of even earlier treatment initiation. One of the most well-known of these is the NA-ACCORD (a 'cohort of cohorts') study which found that patients who started treatment at greater than 350 or greater than 500 cells/µl, had a significantly lowered risk of death than patients who deferred treatment (40). In addition, a large retrospective study of African patients (n=24,037) receiving ART for at least 9 months demonstrated that higher CD4 counts during treatment were associated with lower mortality rates (41). The odds of survival were found to be significantly better if patients had a CD4 count of greater than 500 cells/µl than if they had a CD4 count of between 350 to 500 or between 200 to 349 cells/µl (41).

In 2013, the WHO advocated increasing the threshold for treatment eligibility to less than or equal to 500 CD4 cells/µl and recommended immediate treatment for sero-discordant couples, HIV-positive pregnant women, children younger than 5 years, and persons co-infected with TB (42). A growing body of evidence suggests that once a patient is virally suppressed on ART, a CD4 count adds little value for monitoring (43, 44).

HIV VL is becoming increasingly recognised and recommended by the WHO as the preferred measure for treatment monitoring, detection of treatment failure and prevention of inappropriate treatment switch (6, 45, 46). The 2013 guidelines made a recommendation for VL monitoring as more important than clinical monitoring using CD4 (42). In lieu of this, the guidelines were again updated in 2014 prompting a move towards VL monitoring as opposed to CD4 monitoring (6). The South African guidelines were also revised in 2015 to reflect these changes (47) (Figure 1.3). By mid-2014, more than 2.6 million people in South Africa were receiving treatment and requiring ongoing monitoring (48).

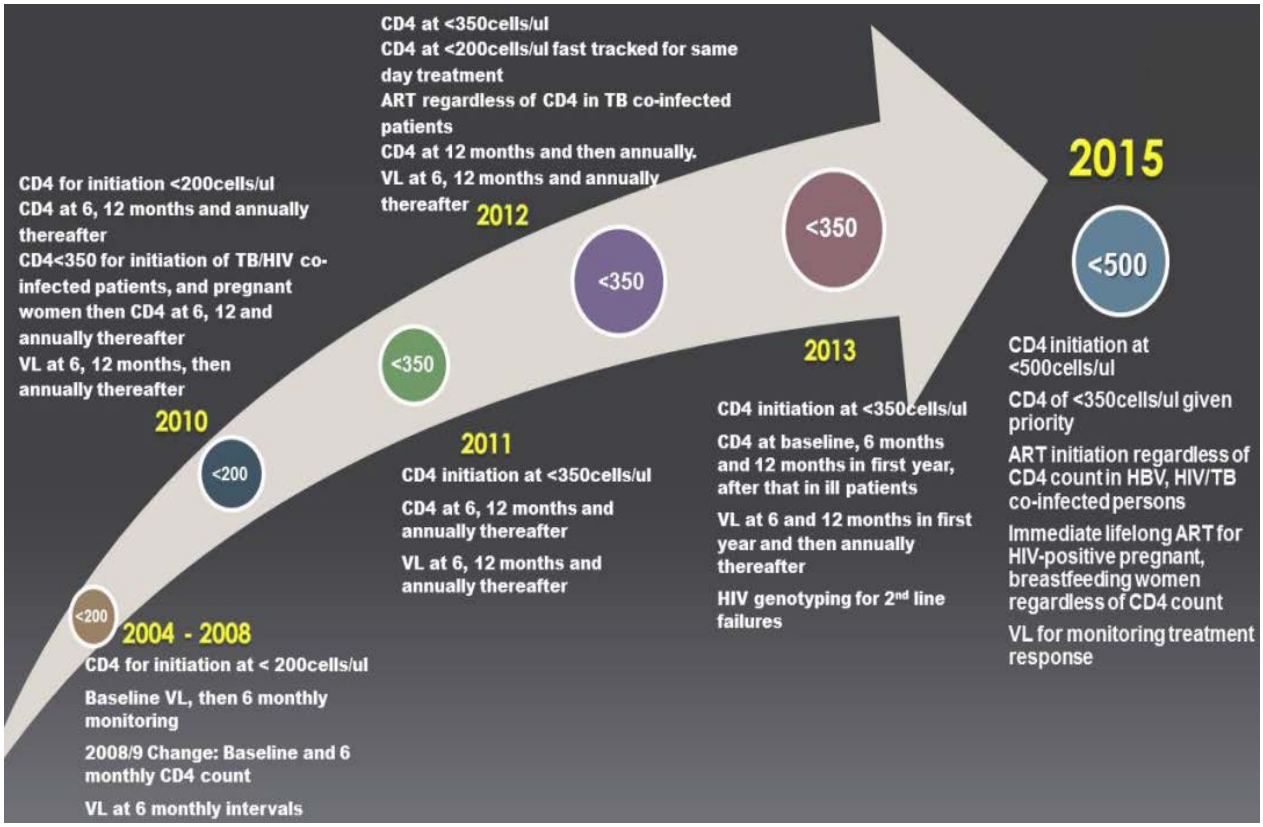

Figure 1.3: A graphical representation of the changing South African ART treatment guidelines from 2004 to 2015 (excludes assays other than CD4 and VL).

The debate on when is considered best to start ART continues and some parties have been advocating for a 'test and treat' strategy (49) whereby persons diagnosed as HIV-positive would be placed on treatment immediately, regardless of CD4 count (50). This strategy, based on patient benefits, has already been adopted by the San Francisco Department of Health since 2010 (51). A population level study in Canada also demonstrated that an increase in ART coverage was significantly associated with decreased community VL over a 15 year period (52). More recently, the START study ('Strategic timing of ART') showed a 53% reduction in AIDS-related illness or death in participants who were started on ART immediately as opposed to those deferred for treatment until their CD4 count dropped to below 350 cells/µl (53).

Currently in South Africa, both CD4 count and VL measurement rely on laboratory-based testing. A CD4 cell count is performed using flow cytometry, a procedure that allows a measure of single cells in terms of size, complexity, content and membrane-bound proteins (30). Within the NHLS, which services more than 80% of the population in the public sector, the routine method employed for CD4+ T cell enumeration is the Pan*Leuco*gating (PLG) technology, a cost-effective means of measuring CD4 cells in the total leucocyte population (54). PLG has the advantage of extending the window-testing period from six hours post-venepuncture (as most CD4 technologies require), to five days and is a single platform technique that measures bead flow rate thus, ensuring each prepared CD4 test has an internal control (55). This technology is reliant on daily-maintained, bench-top equipment, an air-conditioned environment, stable electricity, well-trained laboratory personnel, 4°C storage of reagents and in South Africa, is located in 60 laboratories (56).

VL testing in South Africa is currently performed in 17 centralised facilities due to the requirements for infrastructure, expensive equipment, technical skills and the need for a blood draw, all of which serve to limit its access in resource limited settings (56-59). Two high-throughput VL platforms are used routinely within the NHLS (through a highly-competitive selection process), the COBAS® Ampliprep/COBAS® Taqman (CAP/CTM) version 2 (Roche Molecular Systems, Branchburg, NJ) and the Abbott RealTi*m*e HIV-1 assay (Abbott Molecular, Des Plaines, Illinois) (60). Both instruments are fully automated, real-time platforms, have throughputs of up to 96 samples per run (61) and facilitate turnaround times within 24 hours. To cope with high testing demands, a new system has been released into market by Roche, namely the COBAS® 8800, which can generate results for 960 specimens in an eight-hour shift (62) by a single laboratory technician (Figure 1.4). In 2014, Roche also announced their Global Access Program which will lower prices of VL tests in low- and middle-income countries in support of global goals (2, 63). Although this is a pivotal step towards achieving increased access to testing, many laboratories in Africa are

still underdeveloped (64). These technologies are reliant on phlebotomy and logistics around transport in order to maintain specimen integrity, which limits their geographic service catchment. Lack of available POC tests are therefore driving alternative ways to increase accessibility to laboratory testing for remote settings. Dried Blood Spots (DBS) can be collected from a finger or heel stick, thereby negating the need for phlebotomy skills and allows stable transport at ambient temperature to centralized testing facilities (45). Plasma preparation tubes (PPT) are also a simple and cost effective means of maintaining RNA integrity for transport of specimens to VL testing sites (65).

Figure 1.4 shows the current, centralized, high throughput HIV VL and TB diagnostic systems in South Africa. On the left is the Roche COBAS® 8800 VL testing platform and on the right, the GeneXpert® Infinity-80 system for TB diagnosis and RIF susceptibility testing.

Besides the two core tests mentioned, other diagnostic parameters such as haematology, chemistry and the diagnosis of opportunistic infections, are also required for management of HIV-positive persons in terms of treatment initiation and ongoing monitoring (Table 1.1).

Table 1.1: Tests required for initial HIV diagnosis, staging and treatment monitoring according to the current South African guidelines (47, 48).

Tests required	Purpose
Diagnosis	
HIV rapid test	HIV status
CD4	To assess ART eligibility <500 cells/µl To assess priority cases <350 cells/ul To assess fast-tracking cases <200 cells/ul
Pregnancy	Screen for pregnancy for women needing Prevention of Mother to Child Transmission (PMTCT)
Blood pressure and urine glycosuria	To identify chronic diseases such as hypertension and diabetes
***Cryptococcus neoformans* antigen (CrAg) screening**	To assess ART eligibility and in patients with a CD4 count <100 cells/ul
Screen for Hepatitis B virus (HBV)	To identify HBV infection
Screening for sexually transmitted infections (STI's) and syphilis	To identify and treat STIs
Active TB	Following symptom screening to identify TB
Haemaglobin (Hb) or full blood count	To initially detect anaemia/neutropenia
Creatinine (Cr)	To asses renal sufficiency if requiring Tenofovir
Alanine Aminotransferase (ALT)	To exclude liver dysfunction if requiring Nevirapine

Treatment monitoring	
CD4	At initiation and 1 year to identify response
Viral load	AT 6months, 12 months on ART and then every 12 months to monitor treatment failure and adherence
Cr and calculated Cr clearance	For monitoring Tenofovir toxicity
ALT	For monitoring Nevirapine toxicity
Full blood count	To identify Zidovudine toxicity
Fasting cholesterol and triglycerides	For monitoring of second-line lopinavir/ritonavir based regimes

1.3.1.2 CURRENT CENTRALISED TESTING STRATEGIES FOR TB

The increasing burden of TB, need for scale-up in testing requirements as well as challenges with low sensitivity and diagnostic delays with conventional TB detection methods (66-69), led to the WHO endorsement of a new molecular TB diagnostic, which has revolutionized TB testing; the GeneXpert® technology using the Xpert® MTB/RIF assay (70). Prior to this, sputum smear microscopy was the most widely used diagnostic test for TB detection in resource-poor countries for over 100 years due to its simplicity, specificity, low cost and rapid turnaround time. The sensitivity of smear for diagnosing TB is less than 60% (71-74) and decreases to between 38-54% in HIV co-infected individuals (75, 76) due to low bacillary load (smear – negative TB). Culture, which is highly sensitive, is currently the gold standard method for confirmation of TB, but has prolonged turnaround times, biosafety requirements, technical skill and laboratory infrastructure requirements (67, 77) thus limiting its availability and timeous utility in clinical patient management.

The GeneXpert® MTB/RIF assay (Cepheid, Sunnyvale, CA) was therefore designed for rapid and sensitive detection of *Mycobacterium tuberculosis* complex (MTBC) and simultaneous detection of resistance to RIF, which is also used as a surrogate marker for MDR-TB (78). The assay incorporates automated sample extraction, amplification of the 81-bp core region of the *rpoB* gene (a hot-spot for RIF mutations) and real-time detection, providing a result in just two hours once the cartridge is loaded into the instrument (78, 79). In a multi-centre study involving South Africa, Peru and India, with a cohort of 6648 participants, the Xpert® MTB/RIF assay showed a sensitivity of 76.9% in smear-negative, culture-positive patients and 98.3% in smear-positive, culture-positive patients (80). Use of the Xpert® MTB/RIF assay reduced the median time to TB treatment for smear-negative TB from 56 days to just 5 days (80). Based on these results and South Africa's high HIV/TB co-infection rates, the Xpert® MTB/RIF assay was implemented as the first-line TB screening diagnostic to replace smear microscopy in March 2011 by the NDoH and NHLS (81, 82). Based on a recent review of 27 studies, the pooled sensitivity of the Xpert® MTB/RIF was 98% in smear-positive, culture-positive TB and 79% in HIV co-infected people (smear-negative TB) (83).

Since its implementation in South Africa in 2011, the average national TB positivity rates among presumptively infected individuals has decreased from 16-18% nationally in the first year, to an overall 9% in the fifth year of using the Xpert® MTB/RIF (84). In some high burden districts however, the TB positivity rates in 2014 were still as high as 15.5% (Western Cape) (84). Cepheid has now launched the GeneXpert®-80, which has a throughput of more than 2,000 specimens in a 24 hour period by a single operator, to cope with increasing testing demands (see Figure 4). Two new assays, the Xpert MTB/RIF® ULTRA, which will have similar sensitivity to culture, and the XDR assay, a reflex test for Xpert RIF positive results, are also in development.

According to the South African National TB algorithm (Appendix A), all persons with one or more of the classic screening symptoms for TB (persistent cough or fever for more than two

weeks, night sweats and weight loss) should have a sputum specimen tested by the Xpert® MTB/RIF assay to rapidly determine the presence of TB disease and drug susceptibility. Clinical symptom screening has, however, been shown to be a poor indicator of TB infection (85). Liquid culture or MGITs (mycobacterial growth indicator tube) are used for confirmation of MDR-TB and detection of resistance to first- and second-line drugs through drug susceptibility testing (DST) (86). Phenotypic DST is based on addition of critical concentrations of drugs to the growth medium and then measuring bacterial growth. One of the main limitations is that it takes approximately two to three weeks over and above the two to four weeks for a positive culture (87).

A further molecular test for confirmation of first- and second-line drug resistant TB, assessing the need for triaging patients and as well as treatment initiation (86), is the Genotype® MTBDr*plus* line probe assay (LPA) version 1 and version 2 (Hain Lifescience, Gmbh). The LPA, endorsed by the WHO in 2008 (88), is a PCR-based probe hybridisation assay for use on smear positive specimens, liquid and solid culture isolates. The assay is able to simultaneously detect MTBC and resistance to the first-line TB drugs INH and RIF within 48 hours on direct sputum (89). The sensitivity of version 1 of the assay compared to culture was found to be 76%, but showed reduced sensitivity in HIV-positive persons (90). Version 2 can be used on smear-negative TB and has shown comparative sensitivity to MGIT (91).

Since its implementation, use of the MTBDR*plus* assay on all culture confirmed TB cases has substantially increased the proportion of new MDR-TB cases confirmed in South Africa (92). A further LPA for second line drug resistance detection, the MTBDr*sl* is also available and is currently undergoing evaluations (93). As with any molecular-based test, limitations exist such that LPA also requires infrastructure (Biosafety level II and PCR laboratory), resources and highly trained personnel for both operation and interpretation.

For EPTB diagnosis, the algorithm is much the same as for pulmonary TB using the Xpert® MTB/RIF assay, except that the specimen type will differ to include gastric washing, gastric lavage, lymph node fine needle aspirate (FNA), pleural biopsy or cerebrospinal fluid (CSF) (94).

1.3.2 DECENTRALISED POINT-OF-CARE TESTING

Limitations to centralized testing are steering diagnostics closer to the patient. A POC test is defined as any test which can be performed outside of a central laboratory, including home-based testing (95), and which provides rapid and accessible test results on minimally invasive specimen types, such as finger stick blood, sputum or urine (96). POCT can also be referred to as extra-laboratory or alternative site testing (97) as it may be seen as an extension of laboratory testing. One key difference between POCT and laboratory testing is that it allows the screening or diagnostic process to be potentially completed within a single clinical encounter (98) (Figure 1.5). More important than being simple and rapid, POCT should lead to increased access to testing and improved patient outcomes (98). Based on these criteria, the Clinical Laboratory Standards Institute (CLSI) defines the purpose of POCT as the provision of timely results that clinically and cost-effectively contribute to immediate patient management decisions (99).

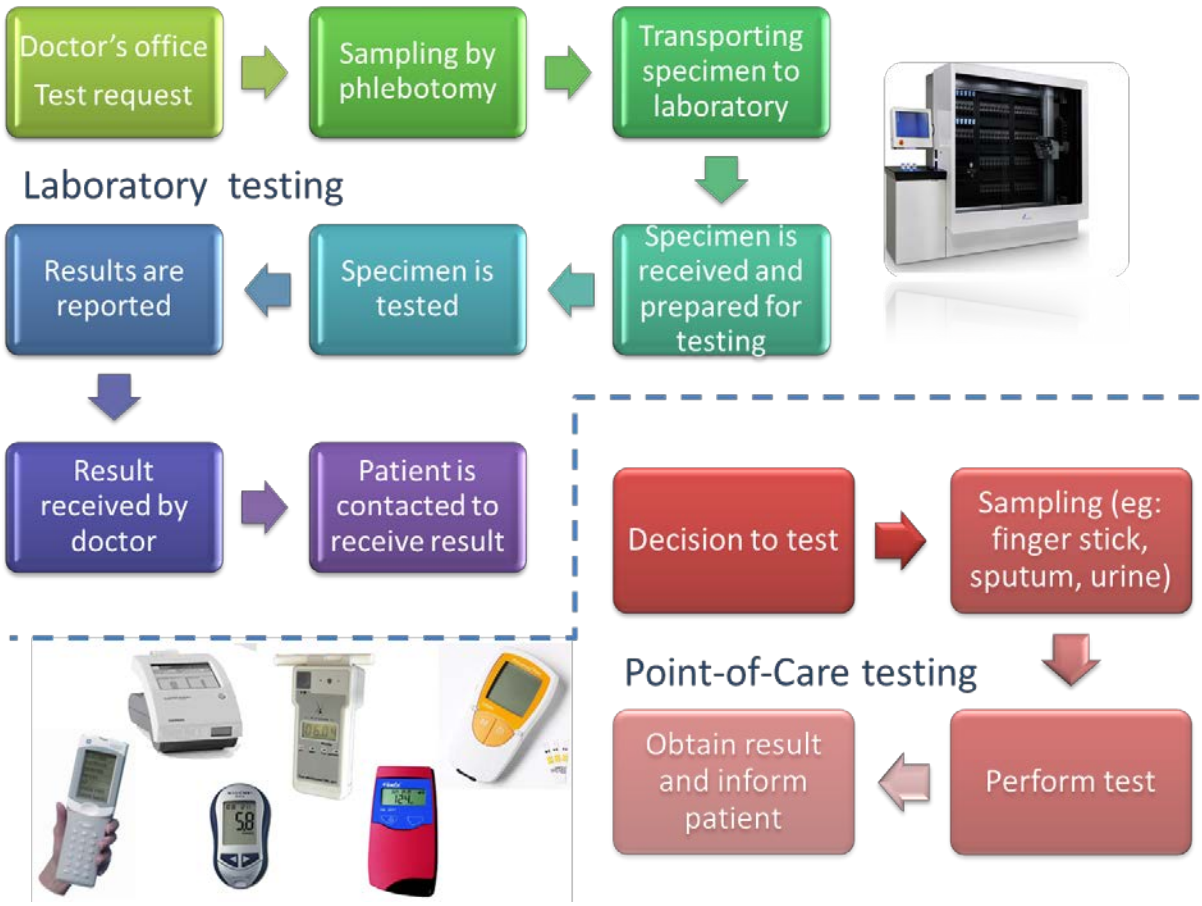

Figure 1.5: Diagram showing the essential difference between conventional laboratory testing and POCT in terms of number of steps required to obtain a result (adapted from (100)). POC can encompass different test types or equipment such as non-instrumental systems (disposable devices or strip-based testing) or small hand-held analysers and desktop analysers (101).

1.3.2.1 RAPID HIV TESTING IN SOUTH AFRICA

In a 2010 population survey of 10 sub-Saharan countries, the WHO found that more than 69% of people were unaware of their HIV status and that gaps existed between testing and counselling needs and existing practice at the time (102). In light of this, the 'National HIV counselling and testing policy guidelines' were published in 2010 in which voluntary

counselling and testing (VCT) programs (now called HIV Counselling and Testing – HCT) were expanded to include provider initiated testing and counselling (PITC) in order to extend access to testing services (103).

Currently, diagnosis of HIV in adults can be made on the basis of several different tests; the appropriate diagnostic test used depends largely on the stage of HIV infection and the age of the individual (61). In South Africa, HIV diagnosis in adults and children (more than 18 months old) is conducted frequently at the primary health care (PHC) level, using two HIV rapid strip-based tests sequentially (one for diagnosis and one for confirmation) (Appendix B) (48). Discordant results between the rapid tests are reflexed to laboratory-based antibody assays, namely enzyme-linked immunosorbant assay (ELISAs). HIV rapid testing, although fast and accessible, has many limitations and is plagued by quality assurance dilemmas (104). Rapid testing also does not address gaps, such as the need for detection of acute infection, diagnosis of babies born from HIV-positive mothers, and monitoring of treatment effectiveness and failures (105).

1.4 THE PROMISE OF POINT-OF-CARE TESTING: BRIDGING THE DIVIDE

An old approach to testing, POC has been available for several years in areas like hospitals, emergency rooms, doctors' offices, clinics and even self-monitoring at home (106). The first POC device to be developed was a urine dipstick in 1957; a simple, instrument-free, rapid diagnostic test which was later followed by the development of a handheld glucose meter in 1970 (107). Some of the most common and long established examples of POC tests available today include blood gas analysers, glucose meters (108, 109), urine analysers (108), HIV rapid tests and pregnancy tests. POC tests were initially used exclusively in the physician's office or at home but have since expanded to four main areas; self-monitoring, community testing primarily in pharmacies, general practice, mobile vehicles and the emergency department (110). Due to their simplicity, users of POC tests can be laboratory

trained personnel, non-laboratory trained healthcare professionals or lay individuals, depending on the regulatory setting in which the testing is conducted.

Initially, the ASSURED criteria, developed by the WHO, were used to describe first-generation POC tests (strip or dipstick-based) as ‘affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and delivered’ (111). With advancements in the field of POC to hand-held devices and Nucleic Acid based Testing (NAAT), the Target Product Profile (TPP) is now being used to define POC tests based on the diversity of POC technologies available and the spectrum of users and settings in which they are used (112). The Clinical Laboratory Improvement Amendments of 1988 (CLIA88) which was introduced in the United States of America to ensure the accuracy, reliability and timeliness of patient results regardless of where the test is performed, distinguishes POC tests into two groups according to their degree of complexity: CLIA-waived and Food and Drug Administration (FDA) cleared POC tests which are suitable for home use, due to their simplicity and accuracy and limited risk of harm to the patient if performed incorrectly (95); and non-waived tests which are more complex to perform and require training and increased responsibility (113).

Today, a set of POC connectivity standards have also been developed, namely POCT01-A1 and POCT01-A2, which consist of specifications for the manufacture of POC diagnostic devices to allow integration of the laboratory information system (LIS) to POC devices (114).

1.4.1 GLOBAL DRIVERS FOR POINT-OF-CARE TESTING

When POCT was first introduced, it was considered by central laboratories as supplemental testing which was seen as a competitor to laboratory services and of sub-standard quality to laboratory testing (115). This viewpoint is changing rapidly as POC developers continuously introduce advancements to the technology and improve required specifications. The main

driver for POC development in low resource settings where laboratory facilities may not be readily available, has been the need to diagnose high burden diseases such as HIV and TB (95). High rates of loss to follow up and poor retention in care are also driving funders, suppliers and users to seek alternatives to centralised testing (116).

Strong advocacy for POCT is now emerging from various groups, such as the WHO, the Bill and Melinda Gates Foundation, Clinton Foundation, President's Emergency Plan for AIDS Relief (PEPFAR) and African Society of Laboratory Medicine (ASLM), who maintain that universal access for HIV and TB care requires the use of POCT for earlier testing and improved retention in care.

Today, POCT is one of the fastest growing segments of laboratory medicine, increasing by an average of 10-12% per year (up to 30% in some areas) (117) and accounts for 1 in 4 tests used in the developing world (118, 119). But demand is set to grow even more, with an estimated worth of $27.5 billion by 2018 (120). This is mainly attributable to the increasing prevalence of infectious diseases and lifestyle-based diseases (for example diabetes) and a strong development pipeline (120).

Additionally, new strategies by groups such as UNITAID are also being proposed to help reach the more than 50% worldwide who do not yet know their HIV status (6). There is now an increasing interest in oral self-testing, whereby a person wanting to know his/her HIV status can screen themselves using a rapid saliva-based HIV test using a saliva specimen, in the privacy of their own home (121). Some countries have already approved the sale of over-the-counter HIV rapid tests, whereas countries such as South Africa are considering its adoption into national policy in order to meet NSP goals (121, 122). Although this approach could potentially be beneficial in terms of increasing access and uptake of HIV testing, it also has many ethical implications which raise concerns (123).

1.4.2 POINT-OF-CARE TESTING PIPELINE FOR HIV

An exciting developmental pipeline exists for POCT in the HIV arena and enormous leaps have been made in terms of the available POC diagnostic market (Table 2). Since 2009 the Pima™ Analyser (Alere Technologies GmbH, Jena, Gemany) has been used in many countries (124), and other promising analysers are the CyFlow® CD4 miniPOC (Sysmex Partec GmbH, Germany) and the BD FACSPresto™ (BD Biosciences, San Jose, CA) (4). Numerous evaluation studies have been published detailing the performance of the Pima™ analyser against predicate technologies both on capillary samples (finger stick) (125-131) and venous blood (venepuncture) (125, 128, 131, 132). Limited performance data is available for other CD4 technologies in the pipeline (128, 131, 133-135) but all have shown good performance with predicate technologies, although capillary sampling shows increased variability as concluded in the Pima™ CD4 meta-analysis and summarized from this manuscript (124) in Table 1.2.

Table 1.2: Summary of CD4 meta-analysis results showing the misclassification, sensitivities and specificities for the Pima™ CD4 on venous and capillary samples [Adapted from (124)].

	Overall N=11803	Venous N=7648	Capillary N=4155
Reference technology			
Mean (absolute range	428 (402–453)	436 (418–474)	411 (384–437)
Median (IQR)	383 (249–555)	390 (254–565)	371 (241–537)
Pima™			
Mean (absolute range	404 (373–425)	416 (388–444)	382 (351–412)
Median (IQR)	363 (234–524	373 (242–534)	342 (221–507)
Total Misclassification 350 cells/µl	11.0% (9.6 - 12.5%)	9.2% (7.5 - 11.1%)	13.8% (12.1 - 15.8%)
Total Misclassification 500 cells/µl	9.5% (8.3 - 10.8%)	8.3% (7.0 - 9.8%)	11.3% (9.6 - 13.2%)
Sensitivity at 350 cells/µl	93.3% (91.4 - 94.9%)	94.3% (92.1 - 95.9%)	91.8% (88.8 - 94.1%)
Sensitivity at 500 cells/µl	96.1% (95.2 - 96.9%)	96.9% (95.8 - 97.7%)	95.0% (93.5 - 96.1%)
Specificity at 350 cells/µl	86.3% (82.8 - 89.1%)	89.1% (85.4 - 92.0%)	82.1% (77.5 - 85.9%)
Specificity at 500 cells/µl	78.2% (73.9 -82.0%)	81.3% (76.6 -85.2%)	73.7% (67.2 -79.3%)

Until recently, VL testing has been almost exclusively laboratory-based, as no POC device/assay existed. This is however, rapidly changing with the market release of the SAMBA (Simple Amplification Based Assay) by Diagnostics for the Real World, Ltd (DRW; Chesterford, UK) in 2014 (136) and many other fast followers undergoing external evaluations (Table 1.3). Due to challenges in the collection of clinical data, particularly in PHCs (frequently manual or paper-based), the need for new POC technologies to have connectivity capabilities has become imperative (137).

Table 1.3: Current HIV POC VL technology landscape (4).

Technology	Description	Time to result	Connectivity capabilities
SAMBA (Diagnostics for the Real World, Ltd.)	A semi-quantitative measurement of HIV-1 RNA. Two systems, the semi-automated SAMBA I system and the fully automated SAMBA II system. Both based on a single-use disposable cartridge (strip-based detection).	90 to 120min	Yes
COBAS® Liat Analyser (Roche Molecular)	Automated (extraction, amplification, detection) quantitative or qualitative measurement of HIV-1. Single-use disposable cartridge.	15 to 35min	Yes
Alere q (Alere)	Automated (extraction, amplification, detection) quantitative detection of HIV-1 and HIV-2. Based on a single-use disposable cartridge.	55min	Yes
EOSCAPE-HIV™ HIV Rapid RNA Assay System (Wave 80 Biosciences	A qualitative HIV-1 RNA test (*EOSCAPE-HIV-D)* and a quantitative viral load test (*EOSCAPE-HIV-Q)*. Both based on a single-use disposable cartridge.	Processing time = 45min. Result read = 2 min.	Yes

Truelab™ (Molbio Diagnostics Pvt Ltd)	Quantitative HIV-1 viral load detection through a Truenat™ micro PCR chip.	< 60min	Yes
GeneXpert® System (Cepheid)	Automated (extraction, amplification, detection) quantitative HIV-1 viral load test. Based on a single-use disposable cartridge	95 min	Yes

*Was previously called the Liat Analyser (IQuum, Inc).

The SAMBA I semi-q, a qualitative test which distinguishes patients as either above or below 1000 copies/ml has been validated against the Roche COBAS® CAP/CTM version 2 (138). Initial results look promising with 99% overall accuracy on patient specimens (138). When the SAMBA semi-q was tested on 284 specimens from patients on ART from Uganda and Malawi, the SAMBA incorrectly misclassified 2.5% as above the 1000 copies/ml treatment failure threshold (139). The SAMBA has already received in-county approvals in Uganda, Malawi and Kenya. Field experience using the SAMBA I (Malawi and Uganda) has suggested that a laboratory trained operator, such as a technician, will be needed due to the manual steps involved in testing; a single operator will be able to perform between 32 and 42 samples in a 7 hour working day with a low invalid rate, but electricity is required (140). DRW are also developing a fully automated instrument, the SAMBA II that is suitable for operation by lay persons and is currently undergoing field evaluations.

The Liat™ platform was originally developed by IQuum, Inc (Marlborough, MA, USA) but has recently been acquired by Roche Molecular Diagnostics and renamed the COBAS® Liat System, in order to take it to market (141). The Liat™ platform provides automated sample preparation, nucleic acid extraction, PCR amplification and real-time detection on a platform which weighs a mere 3.8kg and provides an interpreted result in 30 to 35 minutes. An initial laboratory evaluation of the platform showed a limit of detection of 57 copies/ml and high concordance with Siemens Versant HIV-1 RNA b-DNA (deoxyribonucleic acid) version 3.0

and Roche COBAS Amplicor high throughput VL platforms (92% and 88% respectively) (142). In its current 'lab-in-a-tube' format, the Liat™ reagent tube requires cold-chain storage, which is less than ideal for POC, however, Roche are continuing research and development on the assay and anticipate market release by 2016 (143).

Alere™ (Waltham, MA, USA), a company well known for their POC CD4 platform, is also evaluating a POC platform for VL that is currently only available for research use. The platform named the Alere™ q Analyser and HIV-1/2 assay, is a multiplex real-time PCR with fluorescence-based detection on competitive reporter probe hybridisation on an integrated microarray (144). The assay provides a quantitative VL result in 55 minutes on 25µl of whole blood making it ideal for finger or heel stick specimens. Performance studies are still ongoing and limited data is available, but the assay does seem to show better performance than DBS VL testing (145).

Cepheid (Sunnyvale, CA, USA) (the company that developed the Xpert® MTB/RIF assay), is also in the process of evaluating a new qualitative and quantitative VL assay for their GeneXpert® platform. Performance data is also limited as external evaluation studies are ongoing, but interim results on 390 clinical specimens, show 100% specificity and a limit of detection of 21 copies/ml (146).

In addition to the above mentioned assays which have limited evaluation data, there are a number of other POC VL assays still in various phases of development, such as the North Western Global Health Foundation (NWGHF, Chicago, Illinois) Savanna VL Test and Platform, EOSCAPE-HIV™ HIV rapid RNA Assay system (Wave 80 Biosciences, San Francisco, USA), Viral Load Assay using BART Technology (Lumora Ltd. Cambridgeshire, UK), RT CPA HIV-1 Viral Load Test (Ustar Biotechnologies, Hangzhou, Ltd), Gene-RADAR® Platform (Nanobiosym® Diagnostics, Inc, Cambridge, MA), ZIVA™ (Cavidi, Sweden), and the Genedrive™ (Epistem Ltd, Manchester, UK) (4, 143).

1.4.3 POINT-OF-CARE TESTING PIPELINE FOR TB

Although the GeneXpert® platform has revolutionized TB testing globally, it is still a laboratory-based platform that requires computer literacy, electricity and some technical skill (147, 148). The POC molecular technology landscape for TB is still in development and a number of POC TB assays are now becoming commercially available such as the EasyNAT™ TB (Ustar Biotechnologies), GeneDrive™ Mycobacterium test kit (Epistem, UK) and Truelab™ Uno real time microPCR (MolBio Diagnostics Pvt, Ltd, India) (5), although limited data has been published on their use. Others are in the early market entry phase (Table 1.4). The Foundation for Innovative New Diagnostics (FIND) are also leading efforts to develop a series of TTP's to better inform developers of the core requirements for TB dignostics.

Much interest is also now being placed on polyvalent POC platforms such as the GeneXpert® (149), which can detect multiple diseases on a single platform.

Table 1.4: TB POC diagnostic landscape - early market entry products (5).

Technology*	Description	Time to result	Connectivity capabilities
EOSCAPE TB and RIF-FQ (Wave-80)	TB detection on a chip with Android phone operating system	1 hour	Yes
Alere q Near TB assay (Alere)	PCR based TB detection. Cartridge based	30 minutes	Yes
TB assay (NWGHF)	Fully automated Qualitative TB detection	Not known	Yes
QuantuMDX Q-TB	Smartphone-like device to detect TB and drug resistance	15-20minutes	Yes

*Not an exhaustive list

1.4.4 ADVANTAGES AND DISADVANTAGES OF POINT-OF-CARE TESTING

Many benefits have been reported after introduction of POCT into non-laboratory environments such as emergency departments, doctors' rooms and patients' homes in developed countries (Table 4). Self-monitoring of glucose and glycated haemoglobin levels (HbA1c) have long been described to provide significant benefits to patients with diabetes (150-152). Several clinical studies are available that highlight advantages for assays such as blood gases, electrolytes, cholesterol and lipids (153), glucose (154), glycated haemoglobin (155) and haemoglobin in specific clinical scenarios (156-159). In general faster turnaround times, better patient management (160) and reduced patient length of stay (161) have all been documented. Reduced hospital admissions have also been found with POCT compared with laboratory testing due to the ability of POCT to provide a rapid result allowing faster decisions with regards to patient care and subsequent earlier discharge (162). As an example, implementation of a bedside hand-held lactate device for determination of sepsis in the emergency department reduced time to obtains results and administering of fluids in the intensive care unit, ultimately leading to a reduction in patient mortality (163). Similarly, use of a lateral flow strip-based assay for detection of cryptococcal infection (an opportunistic infection) was positively associated with predicting the development of meningitis in HIV infected hospitalized patients (164).

Implementation of POCT may also improve resource utilization in PHC facilities (127, 165). A decrease in tests ordered per patient, reduced number of patient return visits and decreased phone calls, all resulted in improved efficiency and cost savings after POCT was introduced (166).

Diagnosing TB at the POC also has advantages. Use of the Xpert® MTB/RIF as a POC test at a PHC facility has shown to increase the number of patients starting same day treatment and decrease time to treatment (167, 168). Provision of a rapid strip test namely the urine

LAM (lipoarabinomannan) assay, used to diagnose HIV-associated TB in adults about to initiate ARVs, was found to be a good predictor of poor prognosis and mortality risk (164, 169). It was also a useful and rapid rule-in test for TB in patients with advanced immunosuppression (170).

POCT also lowers the required skill of workers, as non- laboratory trained staff, such as nurses, have been shown to accurately and feasibly perform POCT (171-174). Most healthcare workers and POC operators report that POC tests are easy to perform (175) and lead to improved staff satisfaction (176). Management of HIV and TB will, however, require a multidisciplinary testing approach and few studies (177) have investigated the feasibility of a non-laboratory trained worker performing multiple POC tests simultaneously.

Table 1.5: General listed advantages and disadvantages of POCT (115, 178).

Advantages	Disadvantages
• Easy to obtain sample type • Small sample volumes • Limited pre-analytical processing • Easy to use • Rapid patient results • Portable • Negates sample transport • Improved access to testing • Patient satisfaction • Healthcare worker satisfaction • Convenience	• Reliability of results • Increased errors • Management of errors • Potential increased workload • Comparability to predicate methods • Lack of standardised quality control • Potential increased cost • Management of results and interfacing • Potential over-use of tests due to convenience

In general, the performance of POCT when compared to laboratory testing has been acceptable (179-182) despite some incidents and complaints involving patient harm by CLIA-waivered tests (183-185). There seems however to be a general distrust in POCT, which may lead to over-use of laboratory testing to confirm POC results or duplication of

POCT due to ease of access. A survey looking at the practices of clinicians using blood gas analysers found that more than half would wait for laboratory confirmation rather than use the obtained POC result (186). A further study in a busy Johannesburg hospital that provided critical care staff with a HemoCue (Hemocue AB, Angëlholm, Sweden) instrument for rapid haemoglobin measurements, showed similar results. During the study period, there was a sharp increase in laboratory haemoglobin testing which effectively doubled hospital testing volumes (187).

This study highlights the need for strict control measures and quality assurance practice systems to be in place, which are often lacking or unsuitable for POCT (105, 188). This lack of quality control cannot be more apparent than the use of HIV rapid tests in South Africa. Although HIV rapid testing has been instrumental in ensuring wide-scale diagnosis and access to HIV care, it has also highlighted a number of issues around quality of testing. When a detailed evaluation of practices and processes for HIV rapid testing was conducted in 38 clinical sites, the testing process was found to be fraught with difficulties. Despite having over 10 years' experience with HIV rapid tests in public sector clinics, fewer than 13% of clinics followed recommended quality assurance testing guidelines (189).

The widespread availability of POC tests in recent years has led to concerns over quality and reliability of results. Various factors in the pre-analytic, analytic and post-analytic testing cycle have the potential to affect results and their interpretation that the POC user may not be aware of (190). The current standard for quality testing in South African medical laboratories is governed by the South African National Accreditation system (SANAS, ISO15189, (191)). SANAS have released a further International Organisation for Standardisation (ISO) guideline governing the use of POC assays (ISO/FDIS 22870), entitled 'Point-of -Care testing - Requirements for quality and competence' (192), to be used in conjunction with ISO15189. Most POC platforms have built in Quality Control (QC) checks, such as checks for sample volume and integrity, extraction, amplification and

detection. Regardless, an external oversight will be needed as part of the QC procedure (193). This poses a unique challenge as traditionally, quality control material has been designed for laboratory-use with a technician or technologist in mind. Providing material which is simple, robust and cost effective should be the gold standard for designing a QC material for POC.

To ensure quality, centralised data monitoring and the ability to interface with the LIS needs to also be taken into consideration (194). Centralised laboratory infrastructure relies heavily on data management systems for interfacing, quality monitoring and result reporting. With pressure from users, manufacturers of newer POC instrumentation now realise the importance of these systems and are attempting to automate the collection, review and transmission of patient data that is generated with computer-based software packages (195). As suppliers use their own propriety solutions and communication protocols, there is no common industry standard for interconnecting multiple POC platforms (195).

Another disadvantage of POCT is that in general, it is considered expensive in terms of the cost per test, which is higher than that of laboratory testing costs, but may offer financial savings through rapid delivery of results and reduced facility costs (196). Most of the economic analyses for POCT come from costing and modelling projects. In South Africa for example, the cost of different placement models for GeneXpert® were investigated prior to wide scale implementation. Placing the GeneXpert® at the POC was found to be 51% more expensive than placing it into laboratories, mainly due to low test volumes, high human resource requirements and high number of POC sites required (197). The Xpert® MTB/RIF assay was also found to be more costly for healthcare providers compared to smear microscopy and culture, even though it was an effective tool for diagnosing smear-negative TB (198). The ‘XTEND’ trial in South Africa showed no reduction in initial loss to follow up or increase in patients initiating TB treatment following implementation of the Xpert® MTB/RIF

assay, although Xpert® did yield more confirmed positives versus smear microscopy (199, 200).

An Australian clinical trial to address clinical effectiveness, cost effectiveness and safety of POCT in diabetic patients, patients with hyperlipidaemia and patients receiving anticoagulant therapy, was conducted in a large number of general practices throughout Australia (201). Findings from this study concluded that POCT was non-inferior to laboratory-based testing but at a substantially higher cost, which would need to be weighed against overall health benefits (201). Numerous other studies have also found increased costs for POCT in self-monitoring, emergency departments and general practice (202-204). Zarich and colleagues (205) however, found that providing a POC test to suspected myocardial ischemia patients in an emergency department reduced overall hospital costs.

A recent cost effectiveness analysis of POC CD4 versus laboratory CD4 testing in Mozambique, found that even though POC CD4 testing has higher costs, it had the potential to be more cost effective than laboratory CD4, if it improved linkage to care efforts (206).

In addition, many challenges with POCT relating to poor regulatory control (207, 208), lack of policy documents and guidelines (187) have also been documented. Even when POC technologies are developed and become available, regulatory and bureaucratic issues are delaying their uptake. Before POC implementation can happen, a policy needs to be developed detailing a single, strategic national plan for multi-disciplinary POCT, as was advocated by the Maputo declaration (209). Appropriate policy and guideline development will ensure that the most beneficial POC technologies can be rolled out on a wide scale to improve access to services (210).

1.4.5 FURTHER CONSIDERATIONS: THE 'LEAKY' CASCADE

Besides the many advantages and disadvantages of POCT listed, and even though POCT will help alleviate many of the logistical problems faced by current centralised laboratory-based testing, translation into access to care is not always guaranteed (211). To be truly advantageous in terms of achieving successful treatment outcomes, the patient needs to successfully pass through each step of the treatment cascade: recruitment for testing, testing and diagnosis of infection, determining ART/TB treatment eligibility, linkage to clinical care and retention in care (122, 210, 212).

In reality, laboratory testing frequently results in long turnaround times and delays in results, leading to attrition of patients in the testing and treatment pathway (112) at each stage of the treatment cascade (Figure 1.6) (212).

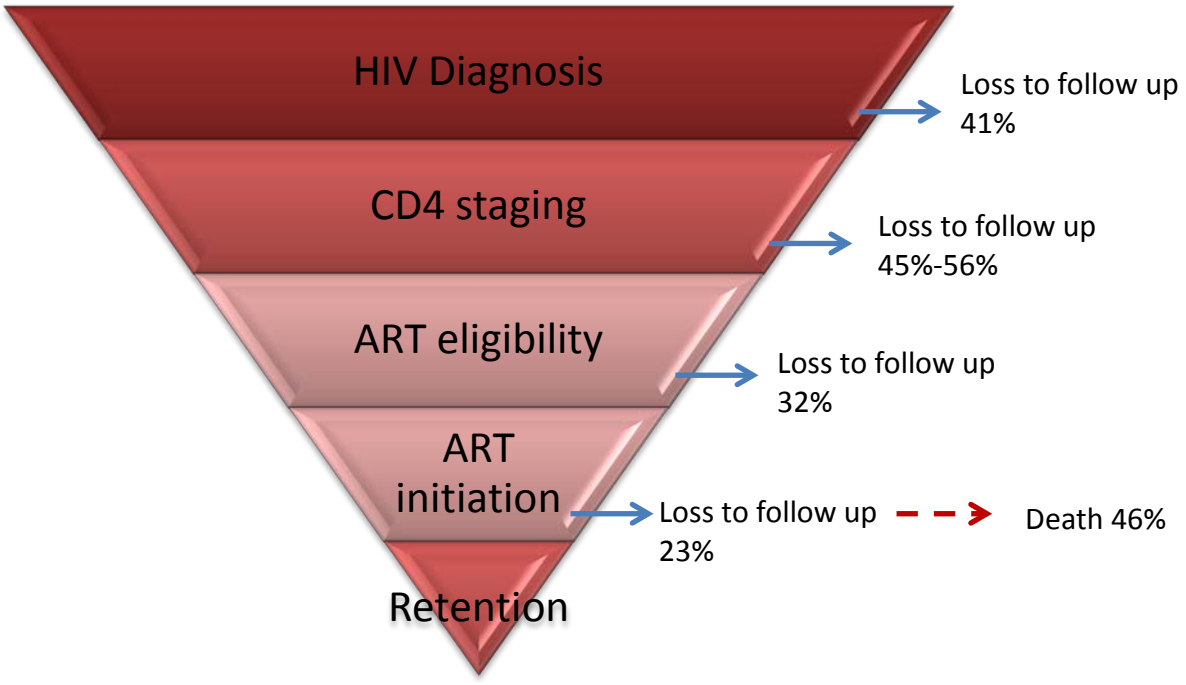

Figure 1.6: The 'leaky' HIV treatment cascade through which patients are lost to follow up at different stages (213-215). Adapted from (216).

A review of pre-ART linkage to care studies showed that at every step of the process, from HIV testing to ART initiation, there is a substantial loss of patients, highlighting the need for enhanced, innovative linkage-to-care efforts (211, 217-221). Data from South Africa demonstrated that nearly half of patients diagnosed as HIV-positive at two clinic sites in Durban, failed to have a CD4 test within 8 weeks of diagnosis (222). Only 39% of patients, who had a CD4 test done and were eligible to start ART, did so within 12 months (223). More recently a 'Links to Care' program in Limpopo and Gauteng provinces was able to link only 51% of the 1,096 participants to care and the mean time to linkage was 31 days (224).

An analysis of patient outcomes after the first year following HIV diagnosis at a PHC clinic in Johannesburg, South Africa, found that overall retention in care was only 37.9% (225). A systemic review quantifying the losses of patients in the continuum of care in sub-Saharan Africa estimated that of those patients eligible to start ART, only 66% initiated treatment and 65% of these were retained in care after three years (226). Similar retention rates have been found in the United States (227) and India (228).

POCT may improve loss to follow-up along this cascade: providing a CD4 test at HCT improves initiation rates (172, 229, 230) and ART eligibility assessment and allows immediate treatment initiation in HIV-positive pregnant women and youths (230-233). Placing a Pima™ CD4 analyser in a mobile van providing HCT demonstrated that patients who received an on-site CD4 test were more likely to complete their referral visits for HIV care, compared with those who were not offered a POC CD4 (234). Other studies however, have found little or no impact, with on-site CD4 testing not impacting on the uptake in HIV care and treatment or on retention in care (172, 229, 235).

1.5 THE ROLE OF POINT-OF-CARE TESTING IN SCALING UP TESTING SERVICES

By the end of 2014, ART programs in low- and middle-income countries had placed a staggering 13.5 million people on life saving ARVs, although this represented only 37-45% of those globally in need at the time (236). Scaling up of ART programs will necessitate a similar scale up in laboratory testing capacity to meet demands (1). With talks around a 'test-and-treat' strategy on the way, the numbers of persons requiring treatment will significantly increase, especially in low resource, high burden settings. This will require substantial speed in resource scale-up and health systems strengthening to meet increased testing demands (Figure 1.7). Many countries however, still cannot afford routine VL monitoring.

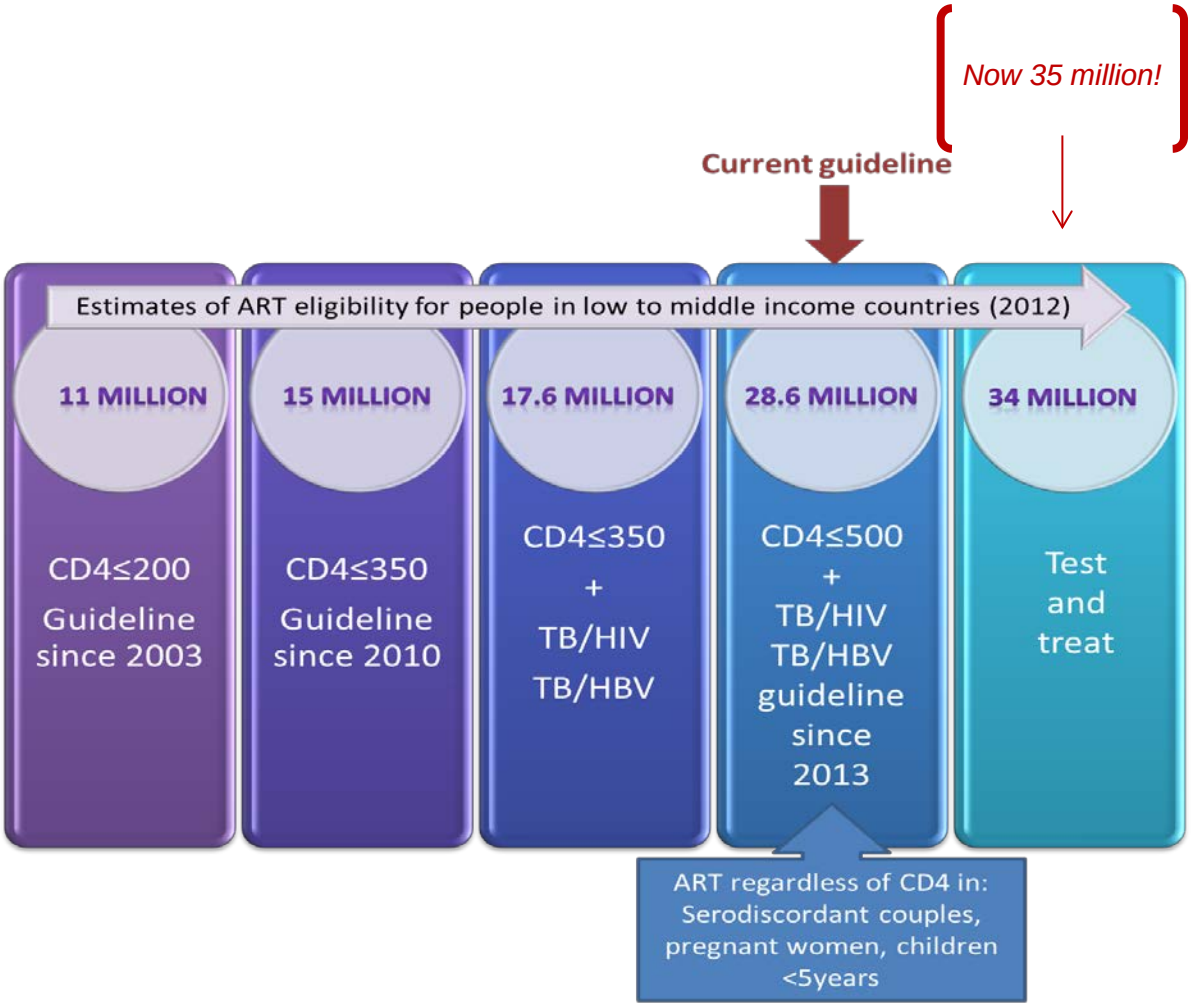

Figure 1.7: The WHO scenarios of treatment eligibility estimated in 2012. Even though numbers have increased, this diagram illustrates the sheer numbers of people requiring ART as guidelines have changed and potentially move to 'test and treat' strategy. Adapted from UNAIDS '90-90-90' document (2).

In South Africa, to cope with the increased testing burden needed to meet the NSP and '90-90-90' goals, health system strengthening will be needed. To achieve this, both decentralisation (moving laboratory services closer to the patient) of ART services to PHC clinics and integration with TB services (237) in a triaged fashion (bearing in mind infection control practices), together with innovation around linkage to care, will be needed. Integration of laboratory systems and services can be defined as a laboratory network, which

can provide all the primary diagnostic services needed by a patient for their appropriate care and treatment, without requiring them to go to different laboratories for specific tests (238).

South Africa has 3,991 public health facilities delivering HIV care (Figure 1.8) and in order to integrate HIV and TB services into a single one-stop facility (supermarket approach), significant restructuring of PHC facilities will be required (239). To this end, the South African NDoH has recently embarked on a new project called the 'Ideal Clinics Initiative', which aims to strengthen and improve service delivery at PHC clinics (240). The project will focus on certain key challenges faced by PHC clinics, one of which deals with laboratory service delivery. The vision is to integrate various new innovations such as extension of Short Message Service (SMS) printers and electronic gatekeeping, extending the use of phlebotomists, community based access to results and implementation of POCT to achieve health system strengthening (241).

The latter, despite much interest and rapid growth globally, remains controversial in part because POCT challenges the conventional approach to laboratory testing, and specifically for South Africa, the prevailing paradigm.

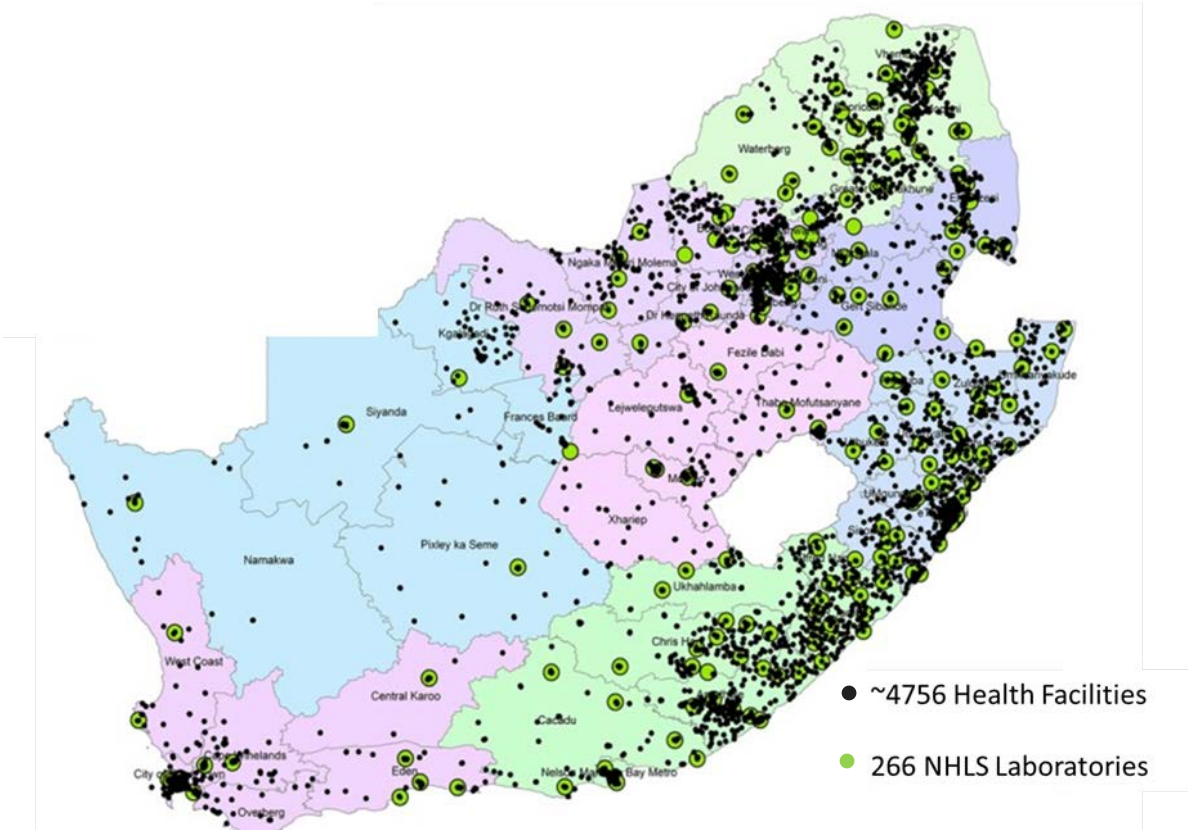

Figure 1.8: Health care facilities and NHLS laboratory coverage throughout South Africa. There are 4576 health facilities serviced by the NHLS, of which 3991 provide ART. Within this network, there are 60 CD4 laboratories, 17 HIV viral load laboratories and 216 GeneXpert® testing sites. In 2014, 4 million CD4 tests, 2.8 million viral loads were performed. Since implementation of the GeneXpert® in March 2011, >5.7million Xpert® MTB/RIF tests have been conducted nationally (84, 242).

1.5.1 POSSIBLE IMPLEMENTATION MODELS: THE TIERED HYBRID (LABORATORY/POCT) MODEL

There are differing views on how POCT should be implemented on a national scale in South Africa. The NHLS laboratory testing network currently servicing 80% of the population with 266 laboratories throughout the nine provinces of South Africa (1) (Figure 1.10). Despite the

advantages of centralised laboratory testing (high throughput, automation), the need for high level technical skill, infrastructure, space, cold chain requirements and instrument maintenance are limiting full implementation and scale up of this type of testing (60, 243). As mentioned previously, logistical issues also exist such as need for venous blood draw and proper transport of venous specimens to centralized facilities. Many patients are also lost to follow up due to increased result turnaround times and great distances between testing centres and patients' homes (244). Services need to be brought closer to the patient or logistics for sample collection and transport to centralised laboratories (such as the use of DBS) need significant improvement (2). On the other end of the spectrum, a total decentralization model through POCT will not be possible due to the sheer volumes of testing required. A hybrid model that includes both POCT and different tiers of laboratory testing may provide the best fit.

The NHLS's intent is to expand the three-tiered model to a hybrid model for CD4 testing which encompasses a six-tiered network in order to provide total coverage of services, even to areas where there is no access to laboratories (1) (Figure 1.9). Through this hybrid model which integrates both laboratory and POC-based testing, testing coverage is not only extended, but result turnaround times are decreased and programmatic costs are better contained (1).

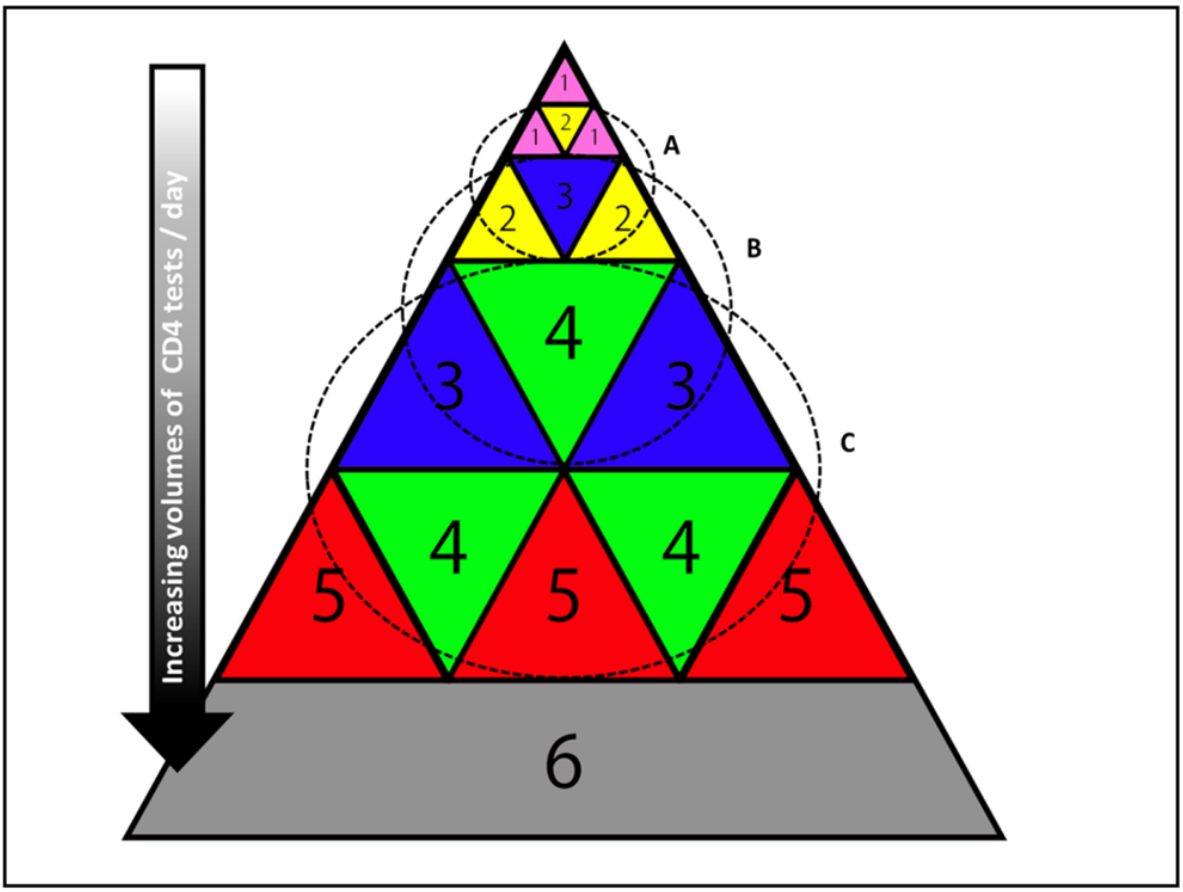

Figure 1.9: The current NHLS CD4 tiered laboratory network. Tier 1 represents 'true' POC in remote areas; Tier 2 is a 'POC Hub' which consolidates POCT across clinics and provides low throughput laboratory testing; Tier 3 represents a community laboratory which services health clinics; Tier 4 is a district laboratory; Tier 5 is a centralized laboratory with high volume testing; Tier 6 represents coordinated national support from an expert team (1).

1.5.2 CHALLENGES FACING MULTIPLE POINT-OF-CARE TESTING FOR SOUTH AFRICA

For implementation of POCT, South Africa will be faced with unique operational challenges due to the need for multi-disciplinary POCT that will require careful consideration.

With the surplus of POC diagnostics in the pipeline for both HIV and TB, how will we go about ensuring that upcoming technologies are appropriate for our clinical setting? Prior to implementation of a POC test, evaluations need to be conducted in the population for which the test is intended. Many of the new POC technologies have limited evaluation data or are in the early stages of development. CHAI, UNITAID and especially the London School of Hygiene and Tropical Medicine (LSHTM) have been given funding to develop standardised protocols and conduct validations on new POC assays. With multi-disciplinary testing requirements for South Africa, the question of how multiple tests will be performed in the field by nurses on a single patient within the current clinic workflow is also pertinent and may require reengineering. In remote settings, phlebotomy skills are a limiting factor to increasing access to testing. In this context, the use of finger or heel sticks would be ideal, however, for an HIV-positive individual, this would translate to up to four finger sticks per single clinic visit as per national in-country guidelines (47), not to mention the two finger stick performed at HCT, potentially on the same day for the coming “test and treat” option.

Infrastructure and operational requirements are also likely to be major barriers to scaling up POCT in South Africa (105) as it is unknown whether nurses at clinic sites have the available time or skills to perform multiple POCT appropriately or if clinics have the available resources. Studies have shown that certain POC tests may require additional human resource requirements. For the GeneXpert® MTB/RIF implementation in a PHC clinic in Johannesburg, 2.5 staff members were required for every 15 patients receiving same day treatment (245). Implementation of POCT may also require additional infrastructure, often

lacking from PHC clinics such as stable electricity, waste disposal and temperature control (112).

Although POC tests are designed to be simple and easy to use, the need for training should not be underestimated. The skill set of the local workforce to operate the tests also needs to be taken into account (207). In an ideal world, POC systems should be easy to operate by non-laboratory staff with minimal training and require very little sample manipulation. However, besides operation of the test device, training also needs to include other aspects such as good laboratory practice, quality control, safety and maintenance as well as how the test fits into the prevailing clinical algorithm (207).

1.6 RESEARCH OBJECTIVES

The South African NDoH requires a concrete recommendation for the country on laboratory and POCT for HIV and TB, incorporating either a centralised, decentralised or hybrid model. During 2011, a Grand Challenges Canada (GCC) Grant (grant # 0007-02-01-01-01) (246) was awarded to Professor Wendy Stevens with the aim of assessing the feasibility of implementing multidisciplinary POCT for HIV and TB service integration in South Africa. The main objective of this project was to determine whether multidisciplinary POCT for HIV and TB diagnosis and monitoring could be performed in remote settings, by non-laboratory personnel; that it was cost effective and could impact positively on patient treatment outcomes. This was an ambitious, global project requiring a multidisciplinary team to execute; scientists, bioengineers, nurses, health economists and various clinical partners.

The current PhD candidate's work is embedded within the objectives of the GCC project (Figure 1.10) and focused on investigating operational requirements for POC implementation and ensuring best practice guidelines to ensure POCT not only integrates with the existing laboratory testing network, but also complements it without disrupting patient care.

This was achieved by taking the core requirements for a quality diagnostic laboratory testing framework (247) and adapting and developing it specifically for POCT, as detailed in the objectives below.

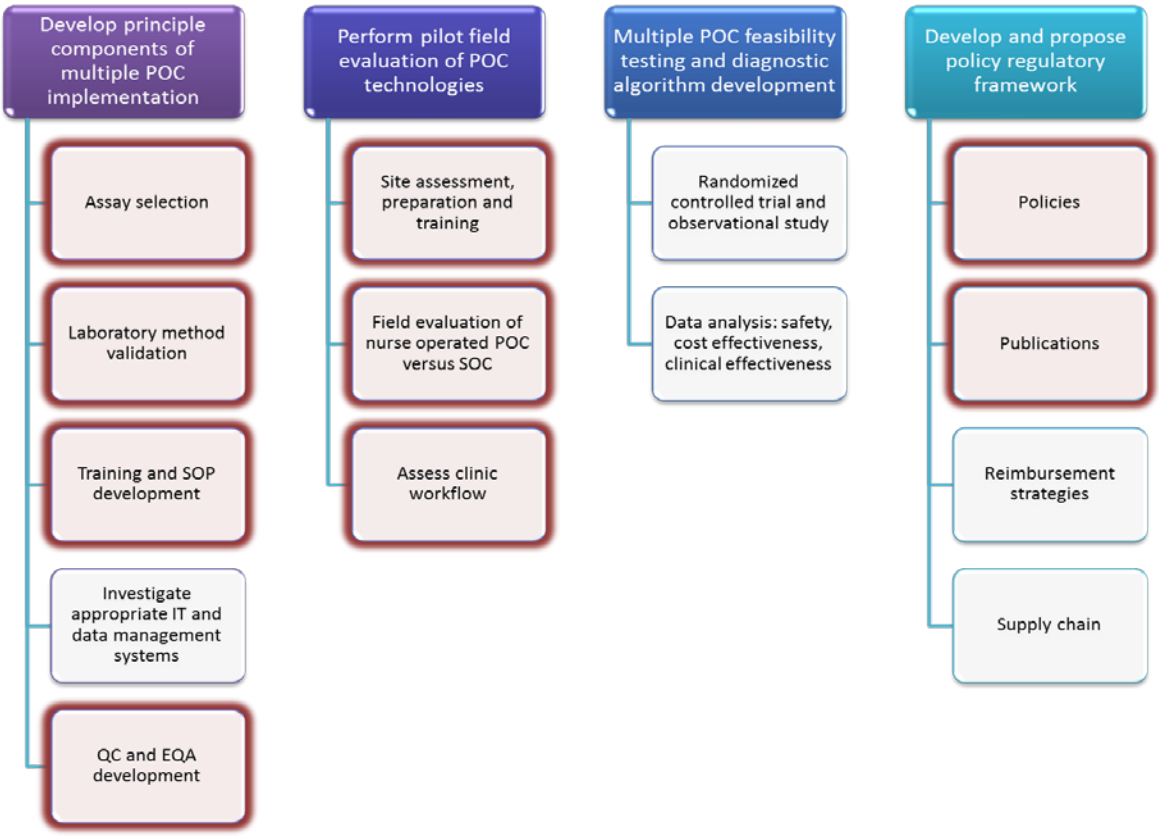

Figure 1.10: Outline showing the main objectives of the GCC project, with red highlighted boxes indicating the contribution of work from the PhD candidate.

Objectives:

1. To identify the key challenges and potential solutions for multiple POCT in South Africa through
    a. assessment of clinical needs for POCT, and
    b. engaging with government to develop the basis for standards and guideline documents to inform the use of POCT in future.
2. To determine standard acceptance criteria for evaluating new HIV and TB POC technologies:
    a. Establish analytical acceptance of POC technologies through laboratory evaluations;

b. Establish field acceptance for POC technology performance through clinical evaluations.

In parallel with objective 2:

3. To develop best practice guidelines for clinical implementation of multi-disciplinary POCT in terms of:
   a. Site, training and quality requirements.
4. To determine the performance and operational requirements of multiple POCT for HIV and TB service integration in South Africa.

The abovementioned objectives are addressed by subsequent chapters.

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CHAPTER 2: ASSESSING CLINICAL NEEDS FOR POCT AND ENGAGING GOVERNMENT

The clinical needs for POCT and potential approaches to implementing POCT were highlighted in the following manuscript and a summary presented as an oral abstract at the following conference:

a) Natasha Gous on behalf of Wendy Stevens. 2013. Point of Care diagnostics: South Africa's experience. In: Laboratory Medicine Congress, Cape Town, South Africa, 28th-31st July.

2.1 FEASIBILITY OF HIV POINT-OF-CARE TESTS FOR RESOURCE-LIMITED SETTINGS: CHALLENGES AND SOLUTIONS

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REVIEW

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Feasibility of HIV point-of-care tests for resource-limited settings: challenges and solutions

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Abstract

Improved access to anti-retroviral therapy increases the need for affordable monitoring using assays such as CD4 and/or viral load in resource-limited settings. Barriers to accessing treatment, high rates of loss to initiation and poor retention in care are prompting the need to find alternatives to conventional centralized laboratory testing in certain countries. Strong advocacy has led to a rapidly expanding repertoire of point-of-care tests for HIV. point-of-care testing is not without its challenges: poor regulatory control, lack of guidelines, absence of quality monitoring and lack of industry standards for connectivity, to name a few. The management of HIV increasingly requires a multidisciplinary testing approach involving hematology, chemistry, and tests associated with the management of non-communicable diseases, thus added expertise is needed. This is further complicated by additional human resource requirements and the need for continuous training, a sustainable supply chain, and reimbursement strategies. It is clear that to ensure appropriate national implementation either in a tiered laboratory model or a total decentralized model, clear country-specific assessments need to be conducted.

Keywords: Anti-retroviral therapy, CD4, Challenges, HIV, Implementation, Point-of-care, Viral load

Introduction

Globally, the number of persons living with HIV has increased from 34 million (31.4 to 35.9 million) in 2011 to an estimated 35.3 million (32.2 to 38.8 million) in 2012; approximately 69% of the global HIV burden resides in sub-Saharan Africa [1]. In response to anti-retroviral therapy (ART) programs, a concurrent drop in AIDS-related deaths from 2.3 million (2.1 to 2.6 million) in 2005 to 1.6 million (1.4 to 1.9 million) in 2012 has been recorded [1]. In order to reach the expected 2020 goals, a massive increase in HIV testing capacity will be required.

The expansion of ART programs can only be described as a huge success in low- and middle- income countries. Estimates reached 9.7 million on ART at the end of 2012, representing some 60% of those in need at that time [2]. With the new World Health Organization (WHO) guidelines changing the CD4 test threshold for treatment initiation from mid-2013, the number of individuals infected with HIV potentially requiring access to treatment has increased to an estimated 28.6 million [1]. Challenges to continued ART scale-up remain, and include improving access to HIV testing, ensuring universal access to testing, earlier initiation of treatment by improved access to HIV testing, ensuring subsequent linkage to care, and finally long-term retention in care. Each phase of HIV diagnosis and monitoring is supported by a number of tests conducted according to different algorithms in many high-burden countries, each with human and technical resource requirements. HIV rapid tests, used in adults in serial or parallel algorithms using one to three different assays, have been instrumental in ensuring wide-scale diagnosis and access to care, albeit with ongoing challenges to ensure quality. A recent estimate from President's Emergency Plan for AIDS Relief (PEPFAR) countries suggests over 80 million HIV rapid assays were performed in 2013 and that 11% of all assays were conducted as point-of-care tests (POCTs) (Jason Williams, personal communication).

CD4 testing has been the gatekeeper for assessing immune status and establishing eligibility for treatment and care. Treatment eligibility threshold levels have changed

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over time from 200 cells/μl in 2002 [3] to 350 cells/μl in 2010 [4]. More recently, the new consolidated WHO recommendations suggest initiation at CD4 counts of <500 cells/μl [5]. Further suggestions of universal access and test and treat strategies are also being evaluated and hotly debated [6]. The latter approach is already occurring for certain high-risk population groups such as those co-infected with tuberculosis (TB), pregnant women, and children under 5 years of age. CD4 count has also been used for regular monitoring of immunological recovery on treatment, generally at six-monthly intervals. CD4 testing can be done at different tiers of the laboratory service [7] and the frequent delay in linking this assay to the initiation of patient care can result in significant loss to follow-up [8]. CD4 testing is also recommended by WHO and used in South Africa as a benchmark for establishing the risk of cryptococcal infection, where testing for cryptococcal antigen can now be done at point-of-care (POC) and the onset of meningitis can be prevented if treated with fluconazole [9].

The HIV viral load (VL) assay, a nucleic-acid-based test, is used to monitor response to treatment; an undetectable viral load defines treatment success. VL testing is frequently done in centralized facilities and currently requires expensive instrumentation, technical skill, and has relatively high costs per assay. Despite these challenges, this assay has gained its rightful place in guidelines and clinical practice and is thought to be the most reliable marker for treatment success [10,11]. The development pipeline of POC VL assays promises to deliver a number of options to improve access and facilitate earlier identification of treatment failure. This will allow clinicians to avoid premature switching of regimens, particularly in regions with limited drug availability, potentially improving patient adherence and reducing the development of drug resistance [12]. Also, the percentage of failures using this assay can provide a monitor of both individual and program success [13]. As access to VL testing is improving, the role of CD4 measurements is being reassessed. Numerous studies have demonstrated that for the vast majority of people living with HIV who are receiving ART and are virally suppressed, CD4 cell count does not decline over time [14]. Other studies have shown that one third of individuals whose CD4 count was greater than 350 cells/μl had viral loads greater than 100,000 HIV RNA copies/ml [15]. A meta-analysis of seven studies assessing the accuracy of clinical or CD4 tests in predicting virological failure found a poor sensitivity of 26.6% and a positive predictive value of 49.4% [11]. This suggests that in situations where viral load is available routinely, CD4 monitoring can be reduced in frequency or stopped altogether. Recognizing this opportunity to save resources, the South African ART guidelines in 2013 recommended stopping routine CD4 monitoring in people who are stable on ART and a number of other countries are considering moving in this direction [16].

In addition to the core assays described in individuals with HIV, there are also hematology and biochemistry assays that remain important, including hemoglobin, creatinine (especially for tenofovir initiation) and liver transaminase tests as well as assays for the diagnosis of opportunistic infections such as TB and cryptococcal infection. The diagnosis and treatment of TB is critical in low- and middle- income countries where a significant proportion of individuals with HIV infection are co-infected with TB. In South Africa as an example, co-infection rates are as high as 65% to 70% [17].

To address all the needs described above and in the face of the successes of rapid tests such as those for HIV, malaria and, more recently, cryptococcal antigen, there is a drive now towards using POCT for the non-communicable diseases such as diabetes, cardiovascular disease and cancer, many of which are associated with long-term management of people living with HIV. Thus, there is an expanded list of multidisciplinary testing needs at primary health clinics (PHC). Performing and interpretation of these tests will potentially require significantly more expertise than a single rapid HIV antibody test.

History of point-of-care testing

POCT is an old approach to testing that has been around for decades and remains as controversial today as it was when first introduced. POCT refers to testing that is performed near to or at the site of patient care, with the result leading to a possible or immediate change to patient care [18]. The rationale is largely based on a need for shortening the time to decision making. The literature provides a myriad of different definitions such as the Clinical Laboratory Standards Institute in the USA, which defines the purpose of POCT being the provision of timely results that clinically and cost-effectively contribute to management decisions [19]. The first references to POCT date back to the early 1990s and focused largely on glucose testing for diabetes and blood gas analyzers in ICUs and operating theaters [20]. The controversy around managerial, quality and regulatory ownership remains a problem and it has been suggested that this is still a 'work in progress' [21]. Despite this, POCT is the fastest growing segment of the diagnostic industry (10% to 14% annually), accounting for one in four tests within the developing world [22,23]. A recent review reported that POCT accounts for 25% of total laboratory revenue [24]. New diagnostics into which POCT has expanded include cardiac markers, coagulation assays, substance abuse and home-based HIV testing, to name a few [25]. Interestingly, POC devices include not only *ex vitro* but also *in vitro* and

in vivo methodologies (continuous monitoring devices) [26]. Technological advances such as microfluidics [27], miniaturization [28], microfabrication, simple power and affordable light sources, electromagnetic actuation of fluids using microelectronics and, more recently, nano-diagnostics [29,30] have facilitated the development of more complex assays capable of placement at the POC [28]. Thus, rapid tests described for HIV diagnosis have been described as first-generation POC assays, involving antigens and antibodies and simple biochemistry and hematology; the second generation is infinitely more complex and based on cell detection or nucleic acid amplification and detection; the third generation will involve complex analyzers that could have multiplexing capabilities [31].

Global perspective on point-of-care testing

The unmet laboratory needs for assays to address communicable diseases such as HIV, TB and malaria appear to have assisted in catalyzing the POC diagnostics industry as a whole. Both communicable and non-communicable diseases will in future reap the benefits as appropriate implementation strategies are developed [31]. This is particularly important when predictions for the future suggest that diabetes may well be a more important risk factor for TB than HIV. Global market assessments have suggested that the increase in diabetes and thus glucose testing comprises at least 10% of the global POCT market [32]. The growth in POC HIV testing has been further reinforced by strong advocacy from groups such as the WHO (One pillar of Treatment v2.0 {WHO Department of HIV/AIDS, 2011 #99}guideline [33], WHO 2013 treatment guidelines [34]), UNITAID (market catalysts; Geneva), the Bill and Melinda Gates Foundation, the Clinton Foundation, PEPFAR and the African Society of Laboratory Medicine, who have been tasked with promoting guidance and implementation in field sites. This drive has begun to address many of the factors mentioned above, such as the absence of laboratories or access to assays such as CD4 and VL testing for the diagnosis and monitoring of HIV in remote sites. Alternatives to conventional centralized testing are being driven by the high rates of loss to initiation for both HIV and TB, as well poor retention in care [35]. These activities have catalyzed funders, suppliers, users and patients in galvanizing the POC diagnostics industry into action. In addition, POCT has been incorporated into the Global Health Strategy on HIV/AIDS [36]. Both the WHO and the London School of Tropical Medicine and Hygiene have been tasked with bringing forward multi-center laboratory-based validations of POC assays followed by an evaluation of their implementation in the field [37]. Strong emphasis has also been placed on the need for monitoring the impact and cost of the interventions across the entire continuum of care. By nature of the low throughput of these technologies and the additional human resources required in the field for testing and maintenance, the total assay costs can be as expensive, or more expensive, than laboratory testing. A strong emphasis needs to be placed on innovative strategies to ensure quality for tests that are being conducted in volumes far beyond that covered by conventional laboratory quality assurance plans and accreditation status. In South Africa, there is an ISO standard (ISO22789) that has been implemented for accredited laboratories to follow if they are conducting and supporting POC testing [38]. Perhaps a similar approach to accreditation of clinic sites conducting POC testing with a simpler standard and checklist could be used to ensure quality is maintained in field-testing sites.

The pipeline for HIV diagnosis and monitoring

There is an ever-expanding pipeline associated with the strong advocacy for POCT from global players, who maintain that universal access for HIV and TB care requires the use of POCT for earlier testing and improved retention in care. Cited advantages of POCT include improved turnaround time, greater accessibility, potentially improved patient retention and possible reduction in overall healthcare costs. However, despite the rapid growth and interest in POCT, many aspects remain controversial, in part because this approach challenges the conventional approach to laboratory testing, which remains the prevailing paradigm in many countries. In addition, while numerous early or near market entry products are available, at the time of writing few could be purchased on a large scale, outside of rapid HIV and malaria strip-based tests, and a monopoly exists of one or two suppliers with a proven track record for CD4 testing, such as the PIMA assay (Alere Inc., Waltham, MA, USA). In the VL arena, many early market entry products are available and development has been heavily funded, yet only three were available for clinical validation as of April 2014 - the LIAT™ Analyzer (IQuum, Inc., Roche) [39], Alere™ q HIV-1/2 Detect (Alere) [40] and Samba (Diagnostics for the Real-World, Ltd.) [41] - and manufacturing track records for scale-up were not available. The upcoming pipeline for HIV CD4 and VL testing with their performance characteristics are summarized in the landscape document produced annually by UNITAID [12]. A plethora of fast followers are in various stages of research, development and evaluation and include the MBio POC CD4 (MBio Diagnostics, Inc)(Co,USA) [42], Daktari CD4 Counter (Daktari Diagnostics, Inc.)(MA, USA), FACSPresto™ (BD Biosciences)(NJ, USA) [43], Visitect® (Omega Diagnostics), Zyomyx CD4 (Zyomyx, Inc.) and EMD Millipore® Muse™ (Merck)(Darmstadt,Germany) [12]. For VL testing, these include the GeneXpert® Viral Load system (Cepheid, Sweden), the EOSCAPE-HIV™ Rapid RNA Assay system

(Wave 80 Biosciences)(CA,USA) [44], TrueLab™ Real Time micro PCR system (MolBio Diagnostics, Ltd.), Goa, India Savanna VL test and platform (Northwestern Global Health Foundation in collaboration with Quidel Corporation) and Bioluminescent Assay in Real Time technology (Lumora, Ltd.)(cambridgeshire,UK) [45], amongst others [12].

In countries where significant laboratory infrastructure currently exists in both the public and private sectors, the sheer volumes of testing may make total decentralization prohibitive in terms of instrumentation and human resource requirements. In these instances, POC assays may and do have a role to play where gaps in service are noted, which can be identified by approaches such as geographic information systems mapping to ensure a national 'total coverage model'. The total coverage model is a new term being used in laboratory testing circles which refers to a tiered implementation model that includes both POC testing and different tiers of laboratory testing to ensure access for the entire national population. POCTs are also used heavily in specific niche areas such as hemoglobin in emergency rooms or renal clinics. A particular niche for the VL assay could, for example, be in maternity wards and antenatal care clinics where pregnant women infected with HIV could be monitored for risk of transmission and success of treatment strategies, and exposed infants could be tested at birth for HIV and then treatment initiated as soon as possible.

Major issues surrounding the implementation of POCT exist and include poor regulatory control, difficulties in ongoing monitoring of quality, and limited availability of guideline documents for the safe implementation of POC devices. In addition, there are few studies that report data on full economic costing for POC [46], which is likely to vary depending on tests used, diseases investigated and model input parameters.

There is a dearth of well-designed randomized controlled clinical trials (RCTs) to evaluate the outcomes and impact of the implementation of POCT. Most notable for their contributions to the POC literature are a group led by Shephard in Australia [47,48]. Although evaluating other assays in a general practitioner setting in Australia, the final study conclusions were that POCT was not inferior to laboratory-based testing, but came at a substantially higher cost that needs to be weighed against overall health benefits. Various clinical experiences were presented at a recent forum held in South Africa, with a number of studies reporting progress in RCT studies such as the Home-based Care Plus trial in Kwazulu-Natal, Rapid Initiation of Anti-retrovirals in Pregnancy (RAP) study in Cape Town, the Grand Challenges Canada RCT, and RapIT (Midrand PHC, South Africa). Results are still awaited eagerly and will help form policy but have shown clearly that POCT is just one step in a multi-step process along the continuum of care [49]. Other experiences show that POCT has great potential for certain high-risk populations such as migrants or adolescents where loss to follow-up is high and where immediate results would add value [49].

Pilot studies on the implementation of PIMA CD4 POC testing in South Africa and Mozambique have demonstrated that time to initiation is reduced; however, challenges were identified in that nurses perceived POC implementation as additional workload, and patients migrated from facilities before staff were able to track, record and file the results in patients' folders [49]. Experiences from Mozambique showed that after the introduction of POC CD4, the loss to follow-up before CD4 staging dropped, ART initiation rate increased, and time to ART initiation was reduced from 48 days to 20 days [50]. Retention rates in care, however, remained the same. It was recommended by this group that deploying POC should be done in in conjunction with conventional testing as part of a total laboratory network and there was acknowledgement that POC testing is far from error-proof. Only 20% of Mozambique's CD4 counts are conducted at POC. High invalid rates were noted using POC CD4 tests in this study. The authors warned that simple implementation is not always efficient - access does not necessarily mean that the patient gets care (approximately 25% of patients did not get CD4 testing even with POCT on site). They also highlighted that significant health systems strengthening is needed and clinic workflow re-engineering. A meta-analysis of the performance of PIMA is underway and preliminary analysis have revealed that the performance of the instrument on venous specimens is as good as current gold standard technology. However, the performance on capillary-derived specimens showed increased variability at the 350 cells/μl threshold, resulting in higher false-positive rates that would lead to more patients being placed on ART (unpublished results, Lesley Scott personal communication).

Approaches to ensuring quality testing

The US Food and Drug Administration requirements for defining a simple test are that it should be rapid, easy to perform, require minimal training and no specialized laboratory setup, and reagents should be stable and temperature independent. However, few assays actually meet these requirements. It should be noted that assay transfer from the laboratory to POC is not synonymous with improved quality of care. Implementation at the POC will require facilitation in a step-wise fashion with careful monitoring and evaluation at each step. The approach to quality of rapid lateral flow-based assays will be different to those that are device based. Several guidelines for

HIV rapid testing have been written over the years, but uptake of these recommendations has been poor in most resource-limited settings. In fact, many of these assays are considered Clinical Laboratory Improvement Amendment-waived because they are simple tests with a low risk for an incorrect result and are thus not quality assured in developed countries such as the USA.

While programs such as the WHO pre-qualification process [51] have provided guidance by conducting product and supplier evaluations and validations, and the Centers for Disease Control and Prevention (CDC) has done similar work for PEPFAR-related programs, there is a need for harmonization of approaches and standardization of protocols with greater co-operation between stakeholders. There needs to be co-ordination between and a review of all strategies and guidelines so that a simple, single guidance can be provided for countries. Quality needs to be addressed, within the laboratory and at the pre-analytic, analytical and post-analytical phases [22]. For rapid assays, the sheer volumes of assays conducted make conventional internal and external quality approaches extremely difficult to implement. Strategies employed have included the use of external quality assurance (EQA) material using dried tube spots for various HIV rapid assays [52] or dried culture spots for near POCT for TB [53,54]. Innovative strategies are required for material distribution and data collection across large programs. Regular training and re-training, competency assessments, and ongoing supervision and mentoring of staff conducting assays are all critical to ensuring continuous maintenance of quality.

For device-based assays, an approach that is under scrutiny is the use of real-time continuous monitoring using various connectivity systems linked to analyzers in the field [55,56]. Connectivity provides a means not only to ensure analyzer performance meets requirements, but also of collecting programmatic data, distribution of results and identifying the need to intervene should problems arise. Data ownership and data security are issues that need to be addressed. Each analyzer, however, frequently connects to the middleware or software solution via a different mechanism and there is thus a need for industry standards for POCT connectivity [57]. Several middleware programs have been evaluated that link to laboratory information systems in South Africa with success, although approaches differ in different regions depending on wireless availability, internet access and computer literacy. Thus solutions may need to be contextualized within different geographic regions. Simpler approaches may include the use of bi-directional short message service printers with additional capabilities for data collection and acknowledgement of receipt of results [58]. To improve retention in care, patients can be recalled for results, and this makes for a reasonably successful means of improving adherence [59]. The role of secondary and tertiary laboratories in the management of quality in PHCs is essential and many believe POCT should be a natural extension of the laboratory [60].

Supply chain management and procurement strategies need to be well planned. Global procurement and global forecasting may play a larger role than for other assays because the production lines for new assays entering the market are frequently unable to meet the demand of rapid recommendations that lead to rapid global uptake. Engagement with industry in the pre-market phase may help to ensure quality features are built into the system, connectivity is considered, and production meets the needs based on information provided on disease prevalence and likely test numbers. UNITAID, as an organization that funds approaches to catalyze and effect market changes, can stimulate additional approaches improving access. Advocacy for quality assured, appropriately selected assays used in settings where impact can be demonstrated is strongly needed.

Ownership and accountability

There is a general consensus that ownership should be at the level of in-country ministries of health. A POCT policy needs to be embedded within national strategic laboratory plans, the development of which was strongly advocated for by the Maputo declaration [61]. A single strategic national plan for the introduction of POCT in a country is likely to solicit donor funding or that of local treasuries in a far more effective manner. It is imperative that technical task teams are established to support decision making. The composition of the team should include clinicians; laboratorians; health economists; procurement, supply and distribution workers; and funders. Strong partnerships with industry need to be facilitated because the ongoing procurement, maintenance of analyzers and product failures need to be addressed. As a result of recent product failures in the HIV arena impacting many countries, a task team was established with expertise from organizations such as the WHO, CDC and other partners. This may be useful going forward to urgently address product failures as this body is formalized. This brings in the concept of a far more active reporting to support post-market surveillance, currently poorly coordinated the world over. Ownership of the POCT process, however, needs to extend to users of the assays and the communities that are tested, with creative ways developed for incentivizing healthcare workers conducting the tests to maintain high quality standards.

Conclusions

POCT will improve access to needed HIV and associated diagnostics, but these assays are not without limitations

that should be noted and reported. There is a need to integrate these technologies cost-effectively and efficiently into clinical algorithms and existing laboratory networks. In costing, it should be emphasized that context matters, particularly human resources and test volumes. There is much to be done in this field. Notably, large randomized studies measuring the impact of a diagnostic intervention along the entire continuum of care are currently an exception and need to be encouraged and supported. Standardization of assay evaluation and development of appropriate internal and external quality control are important activities that need support. Regulatory hurdles need to be overcome and developed in many countries. Global harmonization of all stakeholder activities is essential to get the product from an idea to the bench and ultimately to the patient bedside. The likelihood is that in many countries POCT will be strategically deployed in a hybrid model with support from the conventional tiers of in-country laboratories.

Abbreviations

ART: antiretroviral therapy; CDC: Centers for Disease Control and Prevention; EQA: external quality assurance; PEPFAR: President's Emergency Plan for AIDS Relief; PHC: primary health-care clinic; POC: point-of-care; POCT: point-of-care testing; RCT: randomized control trial; TB: tuberculosis; VL: viral load; WHO: World Health Oganization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

WS wrote the first draft of the manuscript. LS and NG did a review of the manuscript; both are involved in POC projects in the National HIV/TB program in South Africa and provided information. NG assisted with sourcing references. NF reviewed the document. All authors have read and approved the final manuscript.

Authors' information

WS is currently Professor and Head of the Department of Molecular Medicine and Haematology at the University of the Witwatersrand; and the National Health Laboratory Service (NHLS) from 2003 to current. Her research efforts have been largely focused in HIV for the past 12 years and this can be supported by over 170 peer-reviewed publications and 140 conference presentations. She has contributed significantly to the development of capacity for affordable, accessible HIV diagnosis and monitoring in South Africa and over 60 centers in sub-Saharan Africa. Research activities have included the expansion of early infant diagnosis of HIV, affordable viral load, CD4 and investigation of HIV drug resistance. Since November 2010, she has been appointed head of National Priority Programs at the NHLS focusing on laboratory efforts related to HIV and TB. Her current roles include National Rollout of GeneXpert technology across microscopy centers in South Africa. Her current work is the evaluation and validation of POCT, both instruments and positioning thereof within the healthcare system in South Africa.
NG is a PhD student, currently holding the position of Medical Scientist in the Research and Development Unit in the Department of Molecular Medicine and Haematology at the University of the Witwatersrand. Her main areas of research include the development and evaluation of novel, rapid and affordable HIV and TB diagnostic assays, particularly for use in low-resource settings. NG is part of the POC Research Group established by the NHLS National Priority Program to investigate integration of HIV/TB services at POC and was the R&D scientist involved in the development and production of an EQA and verification program for the NHLS National GeneXpert rollout program (under LS).
NF has worked on improving access to HIV/AIDS treatment and care in resource-limited settings for the last 15 years, with a particular focus on sub-Saharan Africa.
LS is an applied scientist in the Department of Molecular Medicine and Haematology, and has for the past 15 years focused on designing, developing, evaluating and implementing laboratory diagnostic technologies for individuals infected with HIV and TB. She has over 50 publications, more than 100 abstracts at local and international conferences, and is a reviewer for several journals and part of editorial boards within her field. Her more recent innovation is the development of a novel quality monitoring system for the Gene Xpert MTB/RIF test using dried culture spots of inactivated *Mycobacterium tuberculosis*. These developments together with three other WITS patents and one trademark emphasize the contribution of LS's research to improving healthcare in South Africa.

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CHAPTER 3: THE EVALUATION OF NEW TECHNOLOGIES FOR THE DIAGNOSIS AND/OR MONITORING OF HIV AND TB

Several new technology evaluations for HIV and TB were performed, with a particular focus on potential POC technologies, two of which are featured in subsequent publications.

Further HIV and TB technology evaluations were also presented in the following list of conference abstracts:

HIV technology evaluations:

a) Gous N. 2015. Experience with a polyvalent molecular platform: The Xpert® MTB/RIF assay and the Xpert® HIV-1 Quant assay. In: Cepheid Satellite Session on Your GeneXpert® Can Do So Much More!! 7th SA AIDS Conference, Durban, SA, 9-12 June.

b) Scott LE, Gous N, Carmona S, Stevens WS. 2014. HIV viral Load Monitoring: Introducing New Technologies. In: Cepheid Lunch and learn. ASLM, Cape Town, South Africa, 30th November – 4th December.

c) Scott LE, Gous N. 2014. Alere Q whole blood viral load assay (prototype) evaluation for ART monitoring. Symposium. In: ASLM, Cape Town, South Africa, 30th November - 4th December.

d) Gous N, Scott LE. 2015. Longitudinal cohort analysis of dried blood spots for viral load monitoring. In: 7th SA AIDS Conference, Durban, SA, 9th-12th June.

TB technology evaluations

e) Stevens W, Gous N, Erasmus L, Coetzee G, Cunningham B, Scott L. 2011.Taking the first steps in national GeneXpert® implementation: lessons learned from South Africa. In: IAS, Italy, 17th – 20th July.

f) Gous N, Scott LE, Khan S, Reubenson G, Coovadia A, Cunningham B, Stevens W. 2012. The Reality of Xpert® MTB/RIF at Point of Care (POC) for the Diagnosis of Childhood TB using Raw Sputum. In: CROI, Seattle, USA, 5th – 8th March.

g) David A, Gous N, Stevens W, Scott LE. 2014. Laboratory validation of Ustar EasyNAT™ Diagnostic test compared to GeneXpert® MTB/RIF for qualitative detection of Mycobacterium tuberculosis using Dried Culture Spots. In: ASLM, Cape Town, South Africa, 30th November - 4th December.

3.1 LABORATORY EVALUATION OF THE LIAT HIV QUANT (IQUUM) WHOLE-BLOOD AND PLASMA HIV-1 VIRAL LOAD ASSAYS FOR POINT-OF-CARE TESTING IN SOUTH AFRICA

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Laboratory Evaluation of the Liat HIV Quant (IQuum) Whole-Blood and Plasma HIV-1 Viral Load Assays for Point-of-Care Testing in South Africa

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Point-of-care (POC) HIV viral load (VL) testing offers the potential to reduce turnaround times for antiretroviral therapy monitoring, offer near-patient acute HIV diagnosis in adults, extend existing centralized VL services, screen women in labor, and prompt pediatrics to early treatment. The Liat HIV Quant plasma and whole-blood assays, prerelease version, were evaluated in South Africa. The precision, accuracy, linearity, and agreement of the Liat HIV Quant whole-blood and plasma assays were compared to those of reference technologies (Roche CAP CTMv2.0 and Abbott RealTime HIV-1) on an HIV verification plasma panel ($n = 42$) and HIV clinical specimens ($n = 163$). HIV Quant plasma assay showed good performance, with a 2.7% similarity coefficient of variation (CV) compared to the Abbott assay and a 1.8% similarity CV compared to the Roche test on the verification panel, and 100% specificity. HIV Quant plasma had substantial agreement (p_c [concordance correlation] $= 0.96$) with Roche on clinical specimens and increased variability ($p_c = 0.73$) in the range of <3.0 log copies/ml range with the HIV Quant whole-blood assay. HIV Quant plasma assay had good linearity (2.0 to 5.0 log copies/ml; $R^2 = 0.99$). Clinical sensitivity at a viral load of 1,000 copies/ml of the HIV Quant plasma and whole-blood assays compared to that of the Roche assay ($n = 94$) was 100% (confidence interval [CI], 95.3% to 100%). The specificity of HIV Quant plasma was 88.2% (CI, 63.6% to 98.5%), and that for whole blood was 41.2% (CI, 18.4% to 67.1%). No virological failure (downward misclassification) was missed. Liat HIV Quant plasma assay can be interchanged with existing VL technology in South Africa. Liat HIV Quant whole-blood assay would be advantageous for POC early infant diagnosis at birth and adult adherence monitoring and needs to be evaluated further in this clinical context. LIAT cartridges currently require cold storage, but the technology is user-friendly and robust. Clinical cost and implementation modeling is required.

HIV viral load (VL) testing is used to monitor the effectiveness of antiretroviral therapy (ART) after initiation, identify early virological failure and targeted adherence, and finally to provide guidance on when to switch therapy in late failures (1). VL testing has been used in the diagnosis of HIV acute infection (2) and early infant diagnosis (EID; <18 months of age) (3, 4); however,VL testing may be limited in the developing world, and EID still is widely performed with a qualitative test and VL is used as a secondary test (5). Several well-established VL technologies are available (6) in high-throughput and, more recently, ultrahigh-throughput platforms designed for centralized laboratories with good quality control. There is a dire need to increase access to VL testing in resource-limited settings (7) and to simplify and improve efficiency in diagnostics to ensure patient care is not compromised. Complexities related to logistics and sample transport to ensure RNA integrity in blood specimens is one limitation to providing full access to VL testing (8). Solutions to increasing access to VL testing being investigated are the use of dried blood spots (9–11), which are easy to transport and appear to extend sample RNA integrity (12), and on-site (point-of-care [POC]) VL testing (without requiring specimen transport and potential loss of specimen integrity) (13). POC VL testing, however, has been slow in commercialization, especially for large-scale implementation programs, such as that in South Africa. In spite of a rich diagnostic development pipeline and ample global support, including the Liat (laboratory-in-a-tube) analyzer (Roche Molecular Systems, NJ, USA), Alere q HIV-1/2 (Alere, Jena, Germany), SAMBA (Diagnostics for the Real World, Ltd., Cambridge, United Kingdom), GeneXpert viral load system (Cepheid, Sunnyvale, USA), and the EOSCAPE-HIV rapid RNA assay system (Wave 80 Biosciences, San Francisco, CA), among others (14, 15), few HIV VL POC tests have been evaluated and are commercially available (16, 17).

One such promising technology is the Liat HIV Quant POC VL assay (IQuum, Inc., Marlborough, MA, which was recently acquired by Roche Molecular Systems, Inc., Branchburg, NJ, USA) (18 and http://www.roche.com/media/media_releases/med-cor-2014-04-07.htm). A validation of this technology's initial plasma assay, requiring 200 μl plasma, showed good performance against the HIV-1 real-time test (Abbott Molecular, Des Plaines, IL, USA) (17). The current Liat HIV Quant molecular assay is a fully automated POC test that generates a quantitative HIV VL within 30 to

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TABLE 1 Evaluation matrix of the specimen numbers, specimen type, and comparator VL tests performed

Purpose	Material	No. and type of specimens	Comparator assay
Platform verification	Assessment-quality frozen plasma from 42-member panel[a]	10 HIV^-, 25 HIV^+	Roche CAP/CTMv2; Abbott RealTime HIV-1
Precision (intra- and intervariability)	4 ml whole blood (and plasma)	3 patient specimens (3, 4, and 5 log copies/ml) repeated 6 times; all tested on 3 instruments	Not applicable
Linearity and variability of LDL	Clinical specimen plasma diluted into HIV-negative plasma	3 patient specimens (>5.0 log) serially diluted 1:10 down to 3.0 log copies/ml and then diluted 1:2 to 2.0 log copies/ml (repeated 9 times); all tested on 3 Liat HIV Quant assay instruments	Roche CAP/CTMv2
Accuracy and misclassification	HIV^+ clinical specimens	157 clinical specimens tested on the CAP/CTMv2 and Liat HIV Quant assay plasma assays; 94 (of 157) tested by Liat HIV Quant assay whole blood and 63 (of 157) tested by RealTime HIV-1	Roche CAP/CTMv2

[a] Ten of 17 HIV-negative panel members were tested, and all ($n = 25$) of the quantifiable panel members were tested on a Liat analyzer due to limited numbers of cartridges being available at the time of study.

35 min using either 150 μl plasma (Liat HIV plasma Quant assay) or 75 μl whole blood (Liat HIV blood Quant assay). The ability to rapidly perform VL testing on both plasma and whole-blood specimens using the same instrument is an attractive feature, particularly where specialized skill is required for performing phlebotomy on infants, and it is not available for POC HIV VL testing in South Africa.

MATERIALS AND METHODS

Specimen collection and study site description. Ethics approval was obtained from the University of the Witwatersrand, Johannesburg (M110139), for an additional 4 ml to 16 ml anticoagulated ($EDTAK_3$) venous derived blood from adult patients attending two primary health clinics in Johannesburg for their routine antiretroviral therapy (ART) and tuberculosis (TB) monitoring between June 2012 and September 2012. The two clinics were Hillbrow Community Health Centre, Johannesburg (approval for 4 ml [1 tube] blood collection), and Themba Lethu HIV Clinic, Helen Joseph Hospital, Johannesburg (approval for 16 ml [4 tubes] blood collection). The blood specimens were couriered (~30 min transport time) to the University of the Witwatersrand Research Diagnostics testing laboratory on the same day that venesection was performed. Whole-blood testing on the Liat HIV Quant assay was performed first, and then residual whole blood was centrifuged at 3,000 × *g* for 15 min using a Hettich EBA-20 centrifuge (Hettich AG, Germany), followed by plasma Liat HIV Quant assay testing. Residual plasma was tested on the Roche COBAS AmpliPrep/COBAS TaqMan (CAP/CTMv2) (Roche Molecular Systems, Inc., Branchburg, NJ, USA), and residual plasma with sufficient volume was tested with the Abbott RealTime HIV-1 assay (Abbott Diagnostics Abbott Molecular Inc., Des Plaines, IL, USA) using m2000sp and m2000RT. The Liat HIV whole-blood Quant assay was tested on the same day as blood draw, followed by plasma testing either the same or the next day (overnight storage at 4°C). Residual plasma was stored at −70°C, and batch testing on the comparator assays was performed in the accredited National Health Laboratory Service HIV PCR laboratory of the same department.

Liat HIV Quant assay VL testing. Three Liat analyzers were couriered from the supplier (in Massachusetts, USA) to the University of the Witwatersrand in Johannesburg, where they were self-installed by the laboratory scientist using the manufacturer's package insert instructions. The Liat analyzer is a quantitative, fully automated instrument that performs silica magnetic bead sample extraction, multiplex real-time PCR amplification, and detection of HIV in a single assay tube and has a barcode reader and digital screen display with integrated keypad. The lower limit of detection is reported as 81 copies/ml in 150 μl plasma, and the dynamic range is 10^2 to 1.5×10^6 copies/ml. Both Liat HIV Quant assays (one assay tube for plasma and one assay tube for whole blood) have an internal control and require 75 μl whole blood or 150 μl plasma to generate results within 30 to 35 min. The testing cartridges require a cold chain (4°C).

Evaluation protocol and statistical methodology. The blood specimens (whole blood and plasma) were tested on the Liat HIV Quant plasma and the Liat HIV Quant whole-blood assays to determine quantitative precision, accuracy (including misclassification), and linearity as well as the qualitative performance (ease of use, number of invalids and errors, and number of tests per day). An evaluation matrix is presented in Table 1 outlining the various evaluation components. A 42-member frozen plasma verification panel (termed the South African viral quality assessment [SAVQA], developed by the South African National Priority Program in Johannesburg using HIV subtype C plasma for verifying newly installed Roche CAP/CTMv2 and Abbott RealTime HIV-1 platforms), which included several confirmed HIV-negative blood specimens obtained from SANBS (South African Blood Transfusion Services) (19), also was included in the evaluation matrix. The panel is designed to measure precision (intra- and intervariability) as well as carryover (instrument contamination) and the limit of the blank (i.e., to correctly identify all HIV-negative specimens). Due to a limited number of Liat plasma cartridges available, 10 of 17 HIV-negative panel members were tested, and all ($n = 25$) of the quantifiable panel members were tested on a Liat analyzer. The results generated by the Liat HIV Quant plasma assay were compared to published criteria (<35% coefficient of variation on untransformed data; <0.19 log copies/ml standard deviations [SD]; <0.3 log copies/ml bias; <2.9% similarity coefficient of variation [CV] [21]) determined previously for the Roche CAP/CTMv2 and Abbott HIV-1 RealTime HIV-1 platforms on this panel (19).

Liat HIV Quant plasma assay precision was determined for all three Liat analyzers and encompassed intra- and interinstrument precision and total precision [the square root of (intrainstrument SD^2 + interinstrument SD^2)]. The latter was established for both the whole blood and plasma Liat HIV Quant test cartridges for three VL log ranges (3.0 log copies/ml, 4.0 log copies/ml, and 5.0 log copies/ml). Intravariability was calculated using the means and SD across six replicate specimens per Liat analyzer. The intervariability was calculated from one (the same) specimen tested on each Liat analyzer.

To determine the Liat HIV Quant plasma assay linearity, three clinical plasma specimens with VL values of 5.0 log copies/ml were selected and serially diluted (1:10) in HIV-negative plasma down to 3.0 log copies/ml (Table 1). A further dilution of 1:2 was performed to obtain samples of 2.0 log copies/ml, and 9 repeats were prepared. Liat HIV Quant plasma assay and the reference testing (Roche CAP/CTMv2) were performed over 1 day on all instruments. Linearity was determined by linear regression (including R^2).

Agreement (accuracy) was determined between the Liat HIV Quant plasma and the Roche CAP/CTMv2 and Abbott RealTime HIV-1 plat-

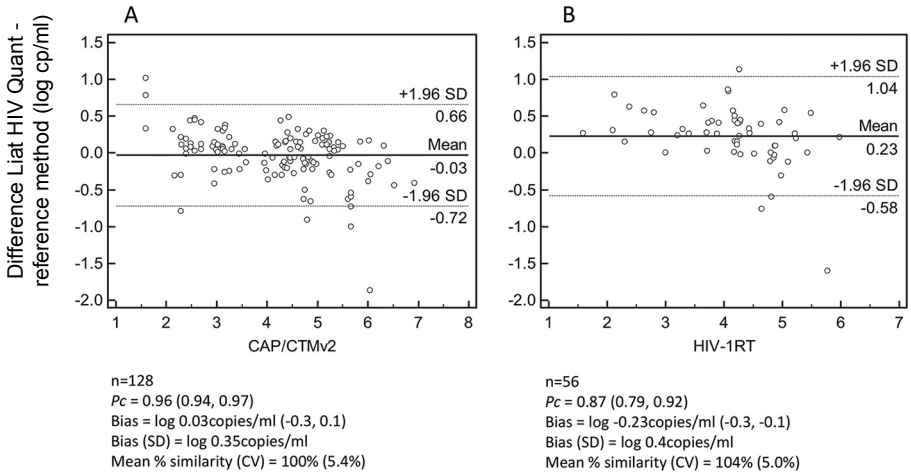

FIG 1 Modified Bland-Altman difference plots comparing the Liat HIV Quant plasma to the Roche CAP/CTM v2 reference comparator method (A) and the Abbott RealTime HIV-1 (HIV-1RT) reference comparator assay (B). Mean difference and SD lines are present, as well as an additional legend containing the concordance correlation, absolute bias, and percent similarity values for each comparison. The vertical axes are the paired differences, and the horizontal axes are the log copies/ml VL values for the reference technology.

forms, as well as between the Liat HIV Quant whole-blood assay and the Liat HIV Quant plasma assay on paired specimens. This was measured by concordance correlation (p_c) (22), Bland-Altman difference plots (23), and percent similarity (24). Concordance correlation was calculated as a measure of agreement strength (accuracy and precision) between a new assay and a reference method (22, 25). The formula applied is $p_c = p \times C_b$ (22, 25), where p is the Pearson correlation (a measure of precision) and C_b is the bias correction factor (a measure of accuracy). The value of p_c (strength of agreement) is suggested to be <0.9 (poor), 0.90 to 0.95 (moderate), 0.95 to 0.99 (substantial), and >0.99 (almost perfect) (22). Any paired Liat HIV Quant whole-blood or plasma assay value of >1.0 log copies/ml difference from the reference was considered clinically relevant (i.e., the point at which patient management may change).

Clinical sensitivity, specificity, and misclassification. The sensitivity and specificity (including the 95% confidence interval [CI]) at a clinical threshold of 1,000 copies/ml of the Liat HIV Quant plasma and whole-blood assays was determined using Roche CAP/CTMv2 as the reference. Detection by the Liat HIV Quant whole-blood and plasma assay quantifiable test results was compared to that of the Roche CAP/CTMv2 plasma VL as the reference method. The Roche CAP/CTMv2 results were sorted into 4 categories (1, TND [target not detected]; 2, lower detection limit [LDL; <1.59 log copies/ml]; 3, 2.0 to 2.99 log copies/ml; 4, 3.0 to >6.99 log copies/ml). Any paired Liat HIV Quant whole-blood or plasma assay value outside these reference categories were considered misclassified and were represented as a percentage (rate) of the number of reference test results in each category. Statistical analyses were performed using STATA version 12 (StataCorp, College Station, TX).

RESULTS

Performance of the Liat HIV Quant plasma assay using a standard verification panel. The Liat HIV Quant plasma assay reported all HIV-negative and HIV-positive specimens correctly (100% concordance), with no carryover between specimen tests on the SAVQA (19) plasma panel. The Liat HIV Quant plasma assay had increased assay variability (reduced precision) in the low-VL ranges (≤3.0 log copies/ml). However, this increased variability was within the acceptable clinically relevant difference (bias) of 1.0 log copies/ml, and patient management would not have been changed. The Liat HIV Quant plasma assay demonstrated acceptable bias of <0.3 log copies/ml for the majority of VL values of >3.0 log copies/ml, with an overall acceptable percentage similarity CV of 2.7% compared to that of the Abbott RealTime HIV-1 assay and even better performance of 1.8% compared to that of Roche CAP/CTMv2. These are below the expected limit of 2.9% CV reported for the comparison between Roche CAP/CTMv2 and Abbott RealTime HIV-1 on the SAVQA panel (19). Twenty confirmed HIV-negative specimens all were reported as undetectable on both the Liat HIV Quant whole-blood and plasma assays.

Performance of the Liat HIV Quant plasma and whole-blood assay precision on clinical HIV-positive specimens. The Liat HIV Quant whole-blood and plasma assay precision (variability) on clinical specimens shows overall good intravariability and intervariability, with median SD of <0.19 log copies/ml for whole blood and median SD of <0.22 log copies/ml for plasma across values of 3.0 log copies/ml to 5.0 log copies/ml. Increased variability, however, was noted with the Liat HIV Quant whole-blood assay in the category of 3.0 log copies/ml. The Liat HIV Quant plasma assay generated acceptable linearity ($R^2 = 0.99$) for three analyzers across the range (5.0 log copies/ml to 2.0 log copies/ml). The variability at 2.0 log copies/ml for two analyzers (11% CV and 12% CV) was similar to that of the CAP/CTMv2 assay (12% CV), with one analyzer showing somewhat increased variability to 23% CV. The quantifiable results generated by the assays were the following: 94% (59/63) quantified by Abbott RealTime HIV-1, with a median of 4.2 log copies/ml; 93% (88/94) quantified by the Liat HIV Quant whole-blood assay, with a median of 4.5 log copies/ml; 88% (138/157) quantified by Roche CAP/CTMv2, with a median of 4.3 log copies/ml; and 82% (128/157) quantified by the Liat HIV Quant plasma assay, with a median of 4.3 log copies/ml. Of all HIV-positive clinical specimens analyzed by the Liat HIV Quant whole-blood, plasma, and comparator assays, the Liat HIV Quant plasma assay values cluster with those of Roche CAP/CTMv2, whereas the Abbott RealTime HIV-1 assay generates lower viral load values than both the Liat HIV Quant plasma assay and Roche CAP/CTMv2. Overall, the Liat HIV Quant assays align more with the Roche CAP/CTMv2 than Abbott RealTime HIV-1 assay (Fig. 1A and B).

The Liat HIV Quant whole-blood assay has more variability

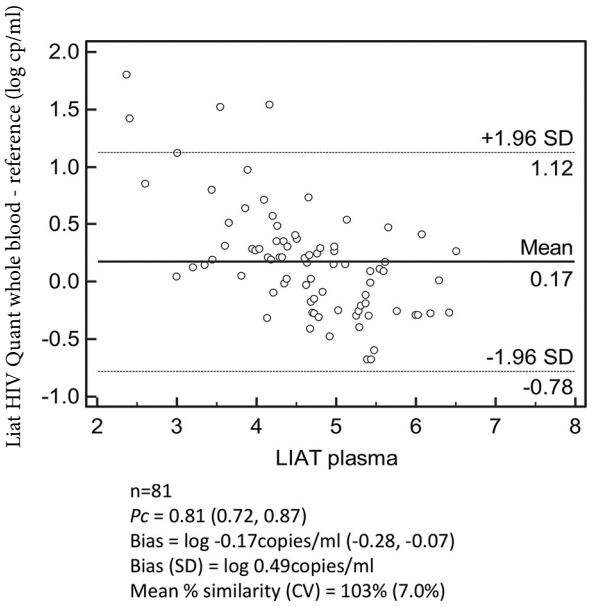

FIG 2 Modified Bland-Altman difference plot of the Liat HIV Quant plasma assay (considered the reference in this comparison) compared to the Liat HIV Quant whole-blood assay. The mean difference and SD lines are present, as well as an additional legend containing the concordance correlation, absolute bias, and percent similarity values for each comparison. The vertical axis is the paired difference, and the horizontal axis is the log copies/ml VL values for the reference technology.

than the Liat HIV Quant plasma assay (Fig. 2), because it generates overall higher viral load values and has several outliers in the viral load range of <4.0 log copies/ml. Overall, the Liat HIV Quant whole-blood assay misclassified 16% and the Liat HIV Quant plasma assay misclassified 5% of samples compared to the reference Roche CAP/CTMv2 (Table 2). The misclassified specimens tested by the Liat HIV Quant plasma assay all had values of <3.0 log copies/ml (<1,000 copies/ml) and would not result in treatment switching. Testing on the Liat HIV Quant whole-blood assay, however, did misclassify 10 of 94 (10.6%) specimens that would have resulted in a change in patient management through upward misclassification. Neither the Liat HIV Quant plasma nor the Liat HIV Quant whole-blood assay generated any downward misclassification (i.e., virological failure [>1,000 copies/ml] classified by the reference) that would have been missed by either Liat HIV Quant assay. The sensitivity and specificity of the Liat HIV Quant plasma and Roche CAP/CTMv2 assays ($n = 94$) were 100% (CI, 95.3%, 100%) and 88.2% (CI, 63.6%, 98.5%), respectively. The sensitivity and specificity of the Liat HIV Quant whole-blood and Roche CAP/CTMv2 assays ($n = 94$) were 100% (CI, 95.3%, 100%) and 41.2% (CI, 18.4%, 67.1%), respectively.

Figure 1A and B further show that the assays with the most agreement between their paired viral load results are the Liat HIV Quant plasma assay and the Roche CAP/CTMv2. Their paired viral load values have substantial strength of agreement ($p_c = 0.96$) with overall good accuracy and precision (5.4% CV) and acceptable variability in their bias (0.35 log copies/ml). The agreement between the Liat HIV Quant plasma and the Abbott RealTime HIV-1 is less ($p_c = 0.87$) due to an increased variability in their bias (0.41 log copies/ml). The agreement between the Liat HIV Quant whole-blood assay and the Liat HIV Quant plasma assay (Fig. 2) shows all outliers occur in the plasma viral load range of <3.0 log copies/ml, with the Liat HIV Quant whole-blood assay generating higher viral load values with a concordance correlation of 0.81.

Qualitative analysis of the Liat HIV Quant assays. The staff who performed this evaluation reported that the Liat analyzer installation was easy to perform, and no supplier support was necessary. This installation and self-training (familiarity with the assay features and testing procedures) were performed within 2 h, and no adverse events were experienced by the operators. The Liat analyzer had a small footprint (approximately 11.4 cm by 19 cm by 24.1 cm) with a touchscreen user interface. Throughout the testing period ($n = \sim$437 Liat HIV Quant assay tubes tested), seven errors were reported (1.6% error rate). These all were due to a scanning error; after rescanning, no specimen results were lost. A total of 12 to 14 specimens could be tested on a single Liat analyzer using either the HIV Quant whole-blood or plasma assay within a single 8-h day. As the assay cartridge is a closed system, no special safety precautions were required, and standard biohazardous waste disposal was needed. Data could be exported by USB port or Ethernet cable (however, no connectivity was used for this evaluation).

DISCUSSION

POC VL testing offers a solution to improved access, decreased result turnaround time, reduced patient return visits for results, immediate adherence counseling, and reduced concerns around

TABLE 2 Liat HIV Quant plasma and whole-blood assay detection of HIV compared to detection by Roche CAP/CTMv2 on HIV-positive clinical specimens

Category of plasma VL ($n = 94$)	Result by[a]: CAP/CTMv2	Liat HIV Quant plasma assay	Liat HIV Quant whole-blood assay
TND (log copies/ml)	TND	TND	2.77
	TND	TND	4.10
LDL (<1.60) (log copies/ml)	TND	TND	4.83
	TND	TND	3.29
	1.30	TND	3.70
	1.30	TND	4.08
	1.38	TND	3.38
	1.60	2.61	3.46
	1.60	2.37	4.17
2.0–2.99 log (log copies/ml)	2.17	1.86	TND
	2.19	TND	TND
	2.30	2.41	3.83
	2.48	2.92	TND
	2.92	3.01	4.13
	2.99	3.03	TND
3.0–6.99 log (n)	79	79	79
Overall misclassification (%)	NA	5.3 (5/94)	15.9 (15/94)
Misclassification resulting in a change in patient management (%)	NA	0	10.6 (10/94)

[a] Underlined results indicate clinical relevance for ART monitoring (>1.0 log copies/ml difference at values of >1,000 copies/ml) compared to the reported comparator results (≤1,000 copies/ml).

sample integrity. The Liat HIV Quant assay has several features that are attractive to POC testing, such as a 30- to 35-min sample input to result time, ease of use by nonlaboratory personnel, and a small footprint. However, plasma testing would require the added step of centrifugation, and the current reagent has a short shelf life of 6 months and requires cold storage. The latter requirement, however, is not unachievable in areas where both assays and drugs use similar conditions in the clinic pharmacy. If used at the POC, however, viral load testing will require ongoing quality monitoring, which currently is accessible only to high-throughput laboratories and will require development for POC.

Overall, the Liat HIV Quant whole-blood and plasma assays show good precision and accuracy compared to existing technologies, such as the Roche CAP/CTMv2 and Abbott RealTime HIV-1. This was evident on the frozen plasma verification panel and fresh clinical specimens and also shows that the Liat HIV Quant whole-blood and plasma assays are well suited to HIV subtype C specimens representative of the South African HIV-infected population (although no sequencing was performed) (26). Overall, the agreement of the Liat HIV Quant assays are aligned more with that of Roche CAP/CTMv2 than that of Abbott RealTime HIV-1. The Liat HIV Quant assays and analyzer appear to have good reproducibility between and within repeat specimen testing for both whole blood and plasma and good linearity between 2.0 log copies/ml and 5.0 log copies/ml. However, increased variability was found in the viral load range of 2.0 log to 3.0 log copies/ml, which is similar to that for testing dried blood spots.

Compared to the Roche CAP/CTMv2, Liat HIV Quant plasma assay testing did not generate any clinically misclassified results that would have resulted in a change in patient management at the 1,000-copies/ml threshold. The Liat HIV Quant whole-blood assay testing, however, did yield 10.6% clinical misclassification on these same specimens, but these all were upward misclassified (i.e., identified higher viral load values than reference testing), and no specimens identified by reference testing as virological failure were missed. This is due to the assay's total nucleic acid extraction protocol, with amplification from whole-blood specimens of cell-associated HIV-1 DNA and RNA. This indicates repeat testing on plasma of all whole-blood specimens with Liat HIV Quant whole-blood values of ~4.5 log copies/ml but is advantageous in assessing HIV-1 infection among newly infected persons receiving pre- or postexposure prophylaxis, where plasma HIV-1 RNA levels may be suppressed, and for newly infected neonates whose mothers had received ART at parturition. The sensitivity and specificity of the Liat HIV Quant whole-blood assay (100% [CI, 95.3%, 100%] and 41.2% [CI, 18.4%, 67.1%], respectively) compared to those of the Roche CAP/CTMv2 platform ($n = 94$) is not too dissimilar from values reported for dried-blood-spot VL monitoring (11); however, this study is limited by a small sample size.

Analysis of a national program's patient's HIV VL data has value in measuring a program's treatment performance (community VL) (27). However, this is possible only with central collection of VL results, such as through a laboratory information system (LIS). In South Africa, this is very well managed through the use of Abbott link, Roche Ampli-link (28), and Axeda systems; therefore, the introduction of new HIV VL technology must ensure connectivity in this context. The Liat analyzer does have full connectivity with HL7 communication protocols and wireless and Ethernet capabilities.

Positioning such an assay at POC in South Africa (currently performing ~2 million VL analyses through centralized testing laboratories) would be best in underserviced areas and niche settings to extend access to VL testing. The goal of the South African national HIV treatment program was to add 500,000 new people to ART per year in their 3,540 clinics, which are staffed by ~23,000 trained nurses (29). Areas where sample integrity may be compromised (>4-h specimen transport times), and where rapid turnaround of VL results is required (maternity wards to identify HIV-positive mothers and babies at birth), would benefit from using the Liat HIV Quant assays and ensure equity of access. The Liat HIV Quant plasma assay, as shown in this study, performs well against the two existing in-country VL plasma technologies, making it an ideal candidate for implementing in this existing program. Concerns would be minimal for cross-platform VL plasma test reporting, leading to clinical differences and unnecessary (or undetected) changes in patient management. Although the Liat HIV Quant assay was not evaluated on infant specimens in this study, whole-blood testing in the maternity setting would have the benefit of its total nucleic acid extraction protocol. However, both scenarios require further clinical evaluation (especially finger stick-derived whole-blood specimen testing), cost analyses (single-use specimens versus high throughout), procurement, and implementation modeling.

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3.2 COMPARISON OF XPERT MTB/RIF WITH OTHER NUCLEIC ACID TECHNOLOGIES FOR DIAGNOSING PULMONARY TUBERCULOSIS IN A HIGH HIV PREVALENCE SETTING: A PROSPECTIVE STUDY

Comparison of Xpert MTB/RIF with Other Nucleic Acid Technologies for Diagnosing Pulmonary Tuberculosis in a High HIV Prevalence Setting: A Prospective Study

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Abstract

Background: The Xpert MTB/RIF (Cepheid) non-laboratory-based molecular assay has potential to improve the diagnosis of tuberculosis (TB), especially in HIV-infected populations, through increased sensitivity, reduced turnaround time (2 h), and immediate identification of rifampicin (RIF) resistance. In a prospective clinical validation study we compared the performance of Xpert MTB/RIF, MTBDRplus (Hain Lifescience), LightCycler Mycobacterium Detection (LCTB) (Roche), with acid fast bacilli (AFB) smear microscopy and liquid culture on a single sputum specimen.

Methods and Findings: Consecutive adults with suspected TB attending a primary health care clinic in Johannesburg, South Africa, were prospectively enrolled and evaluated for TB according to the guidelines of the National TB Control Programme, including assessment for smear-negative TB by chest X-ray, clinical evaluation, and HIV testing. A single sputum sample underwent routine decontamination, AFB smear microscopy, liquid culture, and phenotypic drug susceptibility testing. Residual sample was batched for molecular testing. For the 311 participants, the HIV prevalence was 70% ($n = 215$), with 120 (38.5%) culture-positive TB cases. Compared to liquid culture, the sensitivities of all the test methodologies, determined with a limited and potentially underpowered sample size ($n = 177$), were 59% (47%–71%) for smear microscopy, 76% (64%–85%) for MTBDRplus, 76% (64%–85%) for LCTB, and 86% (76%–93%) for Xpert MTB/RIF, with specificities all >97%. Among HIV+ individuals, the sensitivity of the Xpert MTB/RIF test was 84% (69%–93%), while the other molecular tests had sensitivities reduced by 6%. TB detection among smear-negative, culture-positive samples was 28% (5/18) for MTBDRplus, 22% (4/18) for LCTB, and 61% (11/18) for Xpert MTB/RIF. A few ($n = 5$) RIF-resistant cases were detected using the phenotypic drug susceptibility testing methodology. Xpert MTB/RIF detected four of these five cases (fifth case not tested) and two additional phenotypically sensitive cases.

Conclusions: The Xpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis* over existing AFB smear microscopy and other molecular methodologies in an HIV- and TB-endemic region. Its place in the clinical diagnostic algorithm in national health programs needs exploration.

Please see later in the article for the Editors' Summary.

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Abbreviations: AFB, acid fast bacilli; DST, drug sensitivity testing; INH, isoniazid; LCTB, LightCycler Mycobacterium Detection; MDR, multidrug-resistant; MGIT, Mycobacteria Growth Indicator Tube; *M.tb*, *Mycobacterium tuberculosis*; NAAT, nucleic acid amplification technology; NALC, N-acetyl-L-cysteine-sodium hydroxide; NTM, non-tuberculous mycobacteria; RIF, rifampicin; TB, tuberculosis; WHO, World Health Organization

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Introduction

The tuberculosis (TB) and HIV epidemic in sub-Saharan Africa continues to pose enormous challenges to public health. South Africa alone has 1 million people currently receiving HIV antiretroviral treatment [1,2], the TB incidence is 941 per 100,000 individuals [3], and 9,070 cases of multidrug-resistant (MDR) TB were reported in 2009 [4]. A recent post-mortem study from KwaZulu-Natal observed that TB is still the leading cause of death in HIV+ individuals [5], suggesting that the diagnosis of TB is made too late to avert mortality. Early diagnosis and management of TB is also critical to reduce TB transmission in communities and health care facilities. In 2009, 3 million smears and 740,000 cultures were performed in South Africa public sector health care facilities (excluding the KwaZulu Natal province) [6,7]. In recent years, the South African National Health Laboratory Service scaled up its infrastructure to 249 sites for smear microscopy and 16 laboratories for Mycobacteria Growth Indicator Tube (MGIT) culture. While culture remains the most sensitive method for confirmation of TB, the prolonged turn-around time, biosafety requirements, and laboratory operational requirements [8] limit its contribution to clinical decision making [9]. In 2009, the World Health Organization (WHO) approved the MTBDRplus assay (Hain Lifescience) for use in smear-positive specimens and *Mycobacterium tuberculosis* (*M.tb*) isolates [10]. To hasten diagnosis of drug-resistant TB, 20 National Health Laboratory Service laboratories were earmarked for implementation of the MTBDRplus assay in South Africa in 2010. The assay is limited in its application because laboratory infrastructure must accommodate PCR technology, the assay is not approved for use in smear-negative cases, and in high-throughput laboratories, it can take up to 7 d from sample receipt to result reporting [6]. All these factors limit its potential to contribute to the control of drug-resistant TB.

The diagnostic development pipeline for both high-throughput and point-of-care laboratories has seen rapid innovations in the last decade [11,12] through WHO, Stop TB Partnership, and Foundation for Innovative New Diagnostics partnerships [13,14]. The most recently WHO-endorsed [15] diagnostic tool, the Xpert MTB/RIF (Cepheid) assay, has been reported in a multi-country study to have sensitivities of 98.2% among smear-positive, culture-positive patients and 72.5% among smear-negative, culture-positive patients on a single direct Xpert MTB/RIF test compared to three smears and four culture results [16]. Two sites from South Africa (Durban and Cape Town) with average HIV infection rates of 73% were included in this multi-center study. The sensitivity of the Xpert MTB/RIF assay (three tests performed per participant) among smear-negative, culture-positive individuals compared to standard testing (three smears and four culture results per participant) was 87% (95% CI 62%–96%) for samples from Durban and 90% (95% CI 79%–96%) for samples from Cape Town. Other studies have also recently reported the performance and clinical role of the Xpert MTB/RIF test for detecting TB in extrapulmonary specimens, with sensitivities of 69% to 85.7% for tissue specimens and up to 100% in urine and stool specimens [17,18].

Our study aims to (1) further assess the performance of a single-sputum Xpert MTB/RIF test against culture-confirmed and clinically defined cases of TB in a cohort of adults being investigated for TB with high prevalence of HIV infection from South Africa (Johannesburg region) and (2) to compare this nucleic acid amplification technology (NAAT) to two existing molecular TB assays, the LightCycler Mycobacterium Detection (LCTB) assay (Roche) and the MTBDRplus assay (Hain Lifescience), for use directly on sputum.

Methods

Ethics Approval

The study was approved by the University of the Witwatersrand Human Ethics Review Committee (M070826).

Study Design for Investigation of Potential TB Patients and Data Management

This prospective study investigated consecutive adults presenting with suspected pulmonary TB to a primary health care clinic in Johannesburg, South Africa, over a 9-mo period between 3 August 2009 and 28 May 2010. Individuals were eligible if they were ≥18 y of age and presented with a cough of ≥2 wk duration, with or without fever, night sweats, loss of weight, chest pain, and signs of extrapulmonary involvement (such as lymph nodes, pleural effusions, or abdominal TB), independent of a history of TB treatment and acceptance of HIV testing. Persons were excluded if they were not able to produce sputum, had symptoms only of extrapulmonary TB, were already on TB treatment, or required hospital admission.

TB and HIV diagnosis and management were performed according to South African guidelines [1,19]. As part of routine care, participants on first presentation were asked to provide two sputum specimens for smear microscopy. On return (within 1 wk) for results, participants were invited to participate, and written informed consent was obtained by the study nurse. At this visit, all participants were asked to provide a third sputum specimen for routine smear and culture, and investigational tests. Smear-positive patients were started on TB treatment. AFB-smear-negative patients underwent chest radiography and were prescribed amoxicillin. One week later, response to antibiotic therapy was evaluated, the chest X-ray was read, and the case was assessed by the study physician. Smear-negative participants with no response to antibiotics and chest X-ray findings compatible with TB were initiated on TB treatment. Participants without these criteria were deemed not to have TB. When in doubt, participants were referred to a tertiary center for further investigation. For all participants, data were collected on history of TB, HIV status, most recent CD4 count, antiretroviral therapy, weight, and oral temperature at baseline, and a follow-up visit was conducted approximately 60 d after enrolment. The third sputum sample underwent routine and immediate *N*-acetyl-L-cysteine-sodium hydroxide (NALC)–NaOH decontamination for AFB smear and MGIT culturing. Residual processed specimen was stored at −70°C, batched, and later used for the three NAAT tests. The NAAT tests were performed by a scientist, blinded to smear, culture, and clinical evaluations in an off-site laboratory. All culture-positive specimens underwent routine MTBDRplus testing for rifampicin (RIF) and isoniazid (INH) resistance, and where one or other of these was found present, phenotypic drug sensitivity testing (DST) was performed. These routine smear and culture DST results were reported to clinicians and used for patient management and clinical decision making. The sensitivity, specificity, and positive and negative predictive values for the NAAT tests compared to MGIT culture and clinical case definition were calculated from the results generated from this single processed sputum sample.

Patient data were recorded using a standardized case report form, entered periodically into MS Access and exported into STATA 10 (StataCorp) for analysis. Characteristics between

groups were compared using chi-square and *t*-tests as appropriate. Sensitivity, specificity, and positive and negative predictive values were calculated using either MGIT culture (excluding contaminated cultures and non-tuberculous mycobacteria [NTM]) or any TB (definite, probable, and possible TB) as a gold standard. NAAT test performance was established for those specimens where sufficient sample allowed all tests to be done on each specimen. Analysis was stratified by HIV status and smear microscopy.

Definitions for TB Case Classification

Participating individuals were classified as "definite TB" if sputum culture yielded *M.tb* (with or without positive smears); "probable TB" if *M.tb* culture was negative/contaminated and at least one smear was positive for AFB; "possible TB" if smear was negative for AFB, *M.tb* culture was negative or contaminated, but the patient had TB-compatible chest X-ray and any documented weight gain in response to TB treatment; and "no TB" if smear was negative for AFB, *M.tb* culture was negative or contaminated, symptoms resolved without TB treatment, or if the culture grew NTM. Individuals who were smear-negative, had a culture that was negative or contaminated, had a chest X-ray suggestive of TB, and were initiated on TB treatment, but in whom weight gain was not documented, were classified as "indeterminate TB." Participants who were not started on TB treatment and were lost to follow-up or died were also classified as indeterminate TB. The clinical classification of TB status was performed blinded to the NAAT results.

Laboratory Methods

The single sputum sample was processed and analyzed using standard operating procedures in an accredited biosafety level 3 laboratory. Following decontamination using NaOH (1%)–NALC [20], the specimen was centrifuged and resuspended in approximately 2 ml of phosphate buffer (pH 6.8) to ensure maximum recovery of bacteria and easy homogenization before aliquots were removed for testing methodologies. The reconstituted pellet was used fresh for smear microscopy (~50 µl) and culture (0.5 ml), and the residual sample was stored at −70°C for NAAT processing. The MTBDRplus and the LCTB assays were the first NAATs to be performed in batches of 12 per day (extraction protocols performed on day 1 followed by amplification the following day). Once the Xpert MTB/RIF became available (June 2009), 4–5 residual frozen (−70°C) specimens stored after completion of the MTBDRplus and LCTB assays were tested daily. Use of residual pellet for Xpert MTB/RIF (0.5 ml), MTBDRplus (0.5 ml), and LCTB (0.1 ml) depended on availability of residual sample after smear and culture. Any specimen yielding an invalid NAAT result was re-tested if there was sufficient residual material. This latter result was used in the sensitivity and specificity calculations.

The sputum smear was stained using standard auramine reagent and 100 high-power fields examined using a fluorescent microscope (Olympus CX31 with LED attachment, Wirsam). Culture was performed using MGIT containing modified Middlebrook 7H9 broth base, supplemented with MGIT Growth Supplement and PANTA (BD) and incubated at 37°C up to 42 d in a BACTEC cabinet (Becton Dickinson). Positive cultures were subjected to Ziehl-Neelsen staining to confirm the presence of AFB, and to routine MTBDRplus assay to confirm identity as *M.tb* and establish INH and RIF susceptibility profiles. Routine phenotypic MGIT DST was performed when MTBDRplus assay detected genotypic resistance. All cultures were preserved and stored. At completion of the study MGIT DST was performed as per manufacturer's instructions on additional isolates when this had not been done before.

All NAAT methods were performed according to the manufacturer's instructions and are detailed below. The LCTB assay is a real-time PCR assay, with bacterial nucleic acid extracted using the COBAS Amplicor Respiratory Specimen Preparation kit (Roche Diagnostics) by adding a wash and lysis solution to the pellet followed by 45 min incubation at 60°C and addition of a neutralization buffer before the PCR step. PCR is performed using the LCTB amplification kit (Roche Diagnostics) designed to amplify a 200-bp fragment of the 16s rRNA gene containing the hypervariable region A using fluorescent resonance energy transfer hybridization probes designed for the LightCycler instrument (Roche Diagnostics). Melting curve analysis is performed for species differentiation (positive control, 59±1.5°C; negative control not defined; *M.tb*, 55.9±1.5°C; *M. kansasii*, 59±1.5°C; *M. avium*, 47.5±1.5°C).

The MTBDRplus assay in this study was performed directly on sputum (irrespective of smear result) and routinely on positive cultures. In this assay, bacterial nucleic acid extraction is performed by heat followed by sonication. The PCR is a multiplex amplification using biotinylated primers, followed by reverse hybridization onto nitrocellulose strips. A strip contains 17 probes, including five sample and hybridization controls [21]. The targets amplified are (1) the core region of the *rpoB* gene, positions 505–533, analyzed for RIF resistance based on eight wild-type probes and four mutant probes (D516V, H526Y, H526D, and S531L), (2) the *katG* gene, analyzed for high-level INH resistance based on the wild-type S315 and two mutants (AGC to ACC and AGC to ACA, both producing S315T mutations), and (3) the *inhA* gene, analyzed for low-level INH resistance based on the wild-type 1 probe spanning positions 9–22 and wild-type 2 probe spanning positions 1–12, as well as four mutation probes (C15T,1A6G, T8C, and T8A) [21]. After several washes and chromogenic substrate reaction, the bound probes are visually inspected for the presence or absence of control, wild-type, and mutant bands. Omission of a wild-type band or the appearance of a mutant band in the resistance-determining region of a gene indicates the existence of a resistant strain.

The Xpert MTB/RIF assay is a hemi-nested real-time PCR method that amplifies the 81-bp region of the RIF-resistance-determining region of the *rpoB* gene, positions 507–533. A sample reagent buffer containing NaOH and isopropanol is added in a 2:1 ratio to the processed sputum ensuring a final volume of at least 2 ml. After 15 min of incubation with intermittent hand mixing, 2 ml of the liquefied inactivated sample is added to the cartridge that contains the wash buffer, reagents for lyophilized DNA extraction and PCR amplification, and fluorescent detection probes (five for the *rpoB* gene and one for an internal control, *Bacillus globigii* spores). After the cartridge is placed in the instrument module, the automated processes include the following: specimen filtering, sonication to lyse the bacilli and internal control spores, released DNA collection and combination with the PCR reagents, amplification, target detection by five-color fluorescence of overlapping molecular beacon probes, and one-color fluorescence for the internal control. Results are automatically generated within 2 h and reported as *M.tb*-negative or -positive (with semi-quantification) and RIF sensitive or resistant. The former determination is based on the amplification of any two *rpo* gene regions, and the latter determination is based on a difference of >3.5 amplification cycles of any probe. The Xpert MTB/RIF assay definition files versions 1.0 and 2.0 were used in this study. Data analysis for RIF resistance detection, however, reports results with both the 3.5 and 5.0 cycle threshold differences as per the manufacturer's suggestion.

Results

Patient Population and TB Case Classification

During the study period, 402 potential adults with suspected TB presented to the clinic, and 319 agreed to participate (Figure 1). Participants' mean age was 32.4 y (range 19–75 y); 188 (59%) were male (Table 1). Most participants (274, 86%) accepted HIV counseling and testing, among whom 220 (70%) were HIV positive. Eight patients did not provide a sputum sample for study procedures and were excluded from the analysis. Among the 311 patients included in the analysis (Figure 1), 88 (28.2%) were smear- and culture-positive TB cases, 32 (10.2%) had smear-negative, culture-positive TB, and three (0.9%) had smear-positive, culture-negative TB. Culture was contaminated for 19 (6.1%) participants. Among the 188 (60.4%) participants without bacterial confirmation, 50 (26.5%) had possible TB, 58 (30.9%) were classified as not TB (including five patients with NTM), 31 (16.4%) who started TB treatment were classified as indeterminate TB because of failure to gain weight on treatment or because weight at follow-up was not documented, and 50 (26.6%) were classified as indeterminate TB because they were not started on treatment and were lost to follow-up or died.

Case Detection by NAAT Assay

Sufficient sputum sample was available to perform NAAT analysis using Xpert MTB/RIF in 205 (64%) participants, MTBDRplus in 283 (89%) participants, and LCTB assay in 280 (88%) participants. There was no significant difference in mean age, gender, smear microscopy, culture, and HIV status between patients in whom the different NAAT assays were performed (all comparative p-values>0.05). Overall, NAAT analysis yielded a positive result for *M.tb* in 33% (67/205) by Xpert MTB/RIF, 29% (83/283) by MTBDRplus, and 31% (88/280) by the LCTB assay. Among smear-negative participants ($n = 227$), the proportion of NAAT tests yielding a positive result for *M.tb* was 11.8% (17/143), 6.7% (13/194), and 6.1% (12/199) for Xpert MTB/RIF, MTBDRplus, and LCTB, respectively.

Amongst the NAAT tests, the highest rate of indeterminate or invalid test results was observed for Xpert MTB/RIF (12/205, 5.9%) due to power failures during instrument performance before an uninterrupted power supply was installed ($n = 2$), inability to determine presence or absence of *M.tb* due to improper sample processing (cartridge error) or PCR inhibition (reported as "invalid results") ($n = 5$), probe check failure (reported as "error") ($n = 4$), and operator error ($n = 1$). Of these invalid results there was sufficient residual material to re-analyze seven samples, which were then included in the sensitivity and specificity calculations. Only 2.3% of MTBDRplus assays were indeterminate (due to positive *M.tb* control [TUB] band detection issues). None of the LCTB tests results were indeterminate.

NAAT Sensitivity and Specificity

As detailed in Table 2, compared to MGIT culture, the lowest sensitivity was observed for smear microscopy (59%, 95% CI 47%–71%), followed by MTBDRplus and LCTB with identical performance (76%, 95% CI 64%–85%), and Xpert MTB/RIF (86%, 95% CI 76%–93%), with the highest sensitivity. Sensitivity estimates did not differ for each NAAT when test results were included for specimens not having been tested on all NAAT formats. These results were as follows: smear microscopy, $n = 289$, sensitivity 59% (95% CI 49%–68%); MTBDRplus, $n = 254$, sensitivity 74% (95% CI 64%–81%); LCTB, $n = 236$, sensitivity 75% (95% CI 67%–84%); Xpert MTB/RIF, $n = 182$, sensitivity 86% (95% CI 76%–93%). Specificity was 100% for smear microscopy and >96% for all three NAAT assays. Among culture-negative TB cases, clinical classifications for participants with positive NAAT results were as follows: Xpert MTB/RIF, possible TB ($n = 1$), not TB ($n = 1$), and indeterminate TB status ($n = 1$); MTBDRplus, indeterminate TB status ($n = 3$); and LCTB, indeterminate TB status ($n = 2$).

NAAT test performance amongst the cohort of HIV-uninfected participants had similar sensitivities to test performance on the entire cohort, although the confidence intervals were wide on account of the small numbers. However, amongst HIV-infected participants MTBDRplus and LCTB sensitivities dropped, while that of Xpert MTB/RIF assay remained similar to that of test performance in the entire cohort. As expected for all three NAAT assays, sensitivity was higher among smear-positive than among smear-negative patients (Table 2). Amongst smear-negative, culture-positive cases, Xpert MTB/RIF had the highest sensitivity, 61%, detecting 11/18 cases.

The sensitivity for diagnosis of any TB (smear- and/or culture-positive TB plus possible TB), was 40%, 66%, 51%, 51%, and 58% for smear, culture, MTBDRplus, LCTB, and Xpert MTB/RIF, respectively.

Detection of Drug Resistance by NAAT

Phenotypic DST results were available for 89 participants, and identified two MDR strains, five INH mono-resistant strains, and three RIF mono-resistant strains. Resistance was detected by MTBDRplus (on sputum or culture) and/or Xpert MTB/RIF in 23 patients (Figure 2). Xpert MTB/RIF identified RIF resistance in nine patients (using the amplification cycle threshold maximum 3.5 of Xpert MTB/RIF software version 1), of which three were not reported as RIF-resistant by other DST methods. These are likely false-positive RIF resistance results, as these samples were reported as RIF-sensitive by Xpert MTB/RIF when using a maximum 5.0 amplification cycle threshold (as per Xpert MTB/RIF software versions 2 and 3). The MTBDRplus test directly on sputum identified eight patients with RIF-resistant TB, seven of these had AFB-smear-positive TB. Two were confirmed by MGIT DST, three were sensitive by MGIT DST, one was culture-negative, one culture was not done, and one culture was contaminated. MTBDRplus directly on sputum did not identify three smear-negative isolates with RIF resistance on phenotypic MGIT DST. MTBDRplus performed on culture isolates identified six patients with RIF resistance, of which five were confirmed by MGIT DST.

INH resistance was detected in ten patients using the MTBDRplus test directly on sputum. Of these, four were also resistant on MGIT DST and MTBDRplus on cultured isolates, four were INH-sensitive by MGIT DST and MTBDRplus done on cultured isolates, while one was negative for *M.tb* on MGIT. MTBDRplus directly on sputum missed INH resistance identified by MGIT DST in two cases, one of which was AFB-smear-negative.

Discussion

This is a real-world comparison of different TB sputum detection technologies, integrated within a national TB screening guideline. The sensitivity of a single NAAT test compared to a single MGIT culture in our cohort of South African outpatients with suspected pulmonary TB (70% HIV-co-infected) was higher for Xpert MTB/RIF 86% (76%–93%) than MTBDRplus 76% (64%–85%) and LCTB 76% (64%–85%). This difference in sensitivities was especially prominent for the diagnosis of pulmonary TB in HIV-infected individuals (84% versus 70%

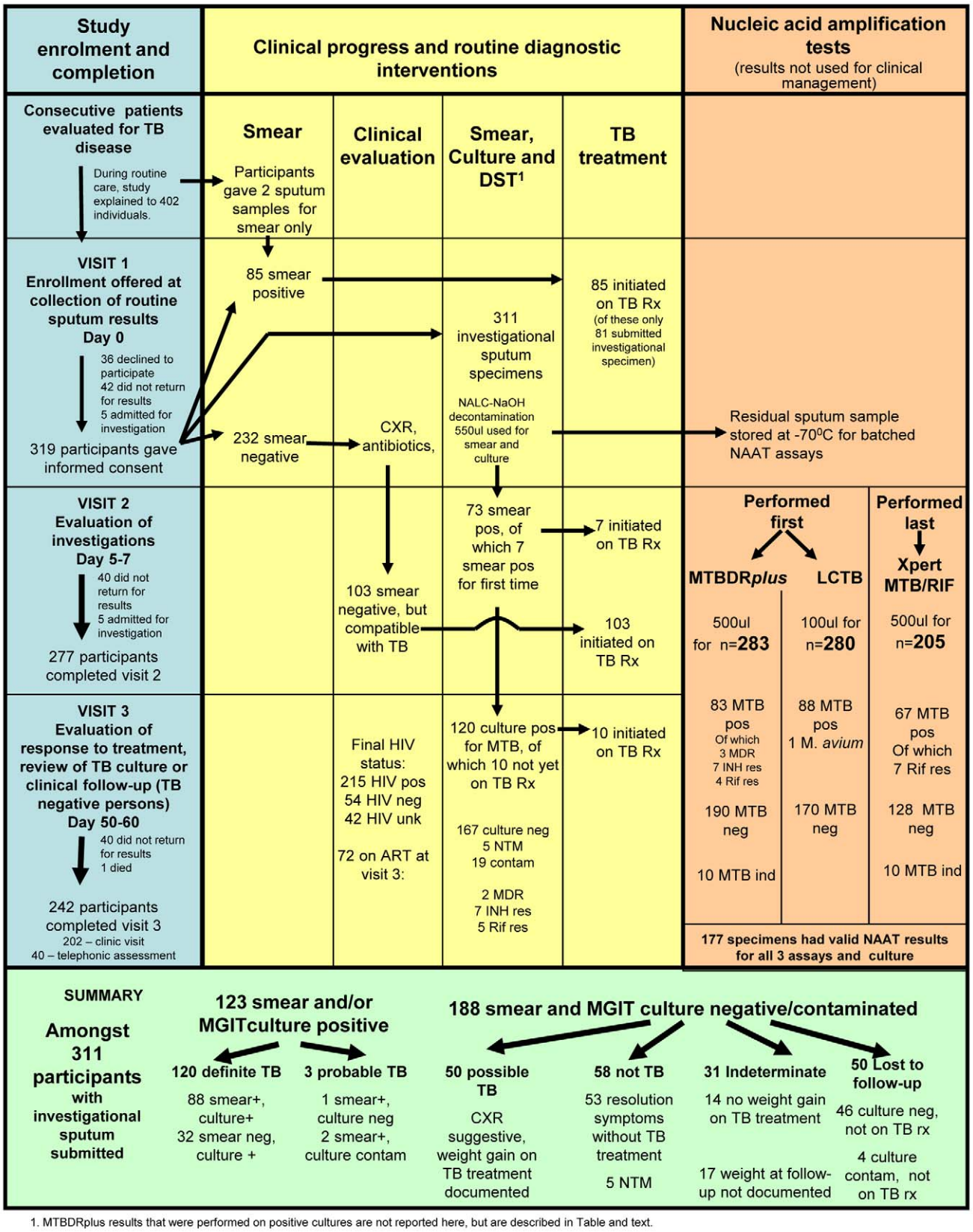

Figure 1. Study algorithm. ART, antiretroviral therapy; contam, contaminated; CXR, chest X-ray; ind, indeterminate; neg, negative; pos, positive; res, resistant; Rx, drug treatment.
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Table 1. Demographics, clinical characteristics, and results of TB diagnostics in 311 adults with suspected pulmonary TB.

Characteristic[a]	All Participants ($n=319$)	Smear Microscopy, MGIT Culture, and Susceptibility ($n=311$)	NAAT Performed Directly on Sputum		
			MTBDRplus ($n=283$)	LCTB ($n=280$)	Xpert MTB/RIF ($n=205$)
Demographics					
Age in years, mean (range)	32 (19–75)	32 (19–75)	32 (19–57)	32 (19–57)	32 (19–56)
Male gender, number (%)	188 (59)	185 (59%)	165 (58)	161 (58)	115 (56)
Clinical signs and symptoms at presentation					
Duration of cough in weeks, mean (range)	4.1 (0–12)	4 (0–12)	3.9 (0–12)	4.0 (0–12)	3.9 (0–12)
Night sweats	287 (90)	281 (90)	256 (90)	253 (90)	186 (90)
Loss of weight	314 (98)	307 (98)	278 (98)	275 (98)	202 (99)
Chest pain	314 (98)	307 (98)	278 (98)	275 (98)	201 (98)
Concurrent extrapulmonary TB symptoms	31 (10)	30 (10)	26 (9)	26 (9)	25 (12)
Pyrexial at presentation	53 (17)	52 (17)	45 (16)	47 (17)	37 (18)
HIV-related information					
Agreed to HIV testing	274 (86)	269 (86)	244 (86)	243 (86)	175 (85)
Tested positive	220 (70)	215 (69)	197 (70)	195 (70)	143 (70)
Tested positive: on ART at presentation	17 (5)	17 (5)	12 (4)	11 (4)	8 (4)
Tested positive: mean CD4 count, cells/μl (*n*, range)	214 (166, 0–818)	217 (162, 0–818)	215 (151, 0–818)	214 (149, 0–818)	221 (109, 0–818)
Refused testing	43 (14)	42 (13)	38 (14)	36 (13)	30 (15)
Tested negative	54 (17)	54 (17)	47 (17)	48 (17)	32 (16)
Bacteriological classification[b]					
Smear- and culture-positive	88 (28)	88 (28)	82 (29)	81 (29)	54 (26)
Smear-negative, culture-positive	32 (10)	32 (10)	28 (10)	28 (10)	19 (9)
Smear-negative, culture-negative	166 (52)	166 (53)	150 (53)	148 (53)	115 (56)
Smear-negative, culture contaminated	17 (5)	17 (5)	16 (6)	16 (6)	11 (5)
Clinical classification					
Definite TB	120 (38)	120 (39)	110 (39)	109 (39)	73 (36)
Probable TB	4 (1)	3 (1)	2 (1)	2 (1)	1 (1)
Possible TB	51 (16)	50 (16)	45 (16)	44 (16)	40 (20)
No TB	57 (17)	58 (19)	50 (18)	48 (17)	37 (18)
Indeterminate TB status (on TB drugs)	31 (10)	30 (10)	29 (10)	29 (10)	22 (11)
Lost to follow-up, not on TB drugs	56 (18)	50 (16)	47 (17)	48 (17)	32 (15)
***M.tb* case detection**					
Percent with indeterminate results[c], number/total (percent)	NA	19/311 (6.1)	10/283 (3.5)	0/280 (0)	12/205 (5.9)
Percent positive among those with valid results, number/total (percent)	NA	120/292 (41)	83/273 (30)	88/280 (31)	67/195 (34)
Detection of RIF and/or INH resistance[d]					
RIF resistance, number/total done (percent)	NA	5/89 (6)	8/273 (3)	NA	7/195 (4)
INH resistance, number/total done (percent)	NA	7/89 (8)	10/273 (4)	NA	NA
MDR (INH+RIF resistance), number/total done (percent)	NA	2/89 (1.0)	3/273 (1)[e]	NA	NA

[a]Values are number (percent) unless otherwise indicated.
[b]One case was smear-positive, culture-negative, and two cases were smear-positive, culture contaminated.
[c]No indeterminate smear results; for MGIT culture, indeterminate = contaminated; for Xpert MTB/RIF, indeterminate = error or other result.
[d]MGIT susceptibility testing done on selected isolates including all cultures where NAAT tests detected resistance.
[e]Two cases were culture-positive with phenotypic-confirmed MDR; a third case was culture-negative.
NA, not applicable.
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and 70%, respectively) and among smear-negative, culture-positive patients (61% versus 28% and 22%, respectively). The potential underpowering of this limited sample size should be noted, and it should be clarified that the confidence intervals for all three NAATs do overlap (even with the sample size increased to 289 by including samples not tested by all assays); however, there is

Table 2. Test performance (including comparison to clinical case definitions) for smear microscopy, MGIT culture, MTBDRplus directly on sputum, LCTB, and Xpert MTB/RIF assays stratified by smear microscopy and HIV status.

Test Performance Measure[a]	Smear Microscopy	MGIT Culture	NAAT Performed Directly on Sputum		
			MDRTBplus	LCTB	Xpert MTB/RIF
Comparison to MGIT culture ($n=177$)					
Sensitivity	59 (47–71)	NA	76 (64–85)	76 (64–85)	86 (76–93)
Specificity	100 (96–100)		97 (92–99)	98 (93–99)	97 (92–99)
PPV	100 (91–100)		94 (84–98)	92 (87–99)	95 (86–99)
NPV	80 (72–86)		87 (79–92)	87 (79–92)	92 (85–96)
Comparison to MGIT culture (HIV-positive cohort only, $n=124$)					
Sensitivity	54 (38–69)	NA	70 (54–83)	70 (54–83)	84 (69–93)
Specificity	100 (95–100)		96 (89–99)	98 (93–100)	96 (89–99)
PPV	100 (85–100)		91 (76–98)	97 (83–100)	92 (79–98)
NPV	80 (70–87)		85 (76–92)	86 (77–92)	92 (84–97)
Comparison to MGIT culture (HIV-negative cohort only, $n=26$)					
Sensitivity	66 (35–90)		75 (43–95)	75 (42–94)	83 (52–98)
Specificity	100 (70–100)		100 (76–100)	100 (76–100)	100 (76–100)
PPV	100 (63–100)		100 (66–100)	100 (66–100)	100 (69–100)
NPV	79 (52–93)		82 (56–96)	82 (56–96)	88 (62–98)
Comparison to clinical case definition "Any TB including definite, probable, and possible TB" ($n=177$)					
Sensitivity	40 (30–50)	66 (56–75)	51 (40–60)	51 (40–60)	58 (48–68)
Specificity	100 (95–100)	100 (95–100)	96 (88–99)	97 (91–99)	97 (91–99)
PPV	100 (91–100)	100 (94–100)	94 (84–98)	96 (87–99)	97 (88–99)
NPV	56 (47–64)	69 (59–77)	59 (50–68)	60 (51–68)	63 (54–72)
Percent detection[b]					
Smear-positive, culture-positive[c], number/total (percent)	40/49 (81)	49/49 (100)	46/49 (94)	47/49 (96)	47/49 (96)
Smear-negative, culture-positive[d], number/total (percent)	0/18 (0)	18/18 (100)	5/18 (28)	4/18 (22)	11/18 (61)
Smear-negative, culture-negative, number/total (percent)	0/107 (0)	0/107 (0)	3/107 (3)	1/107 (1)	3/107 (3)

All tests performed on the same 177 sputum specimens. Confidence intervals 95%.

[a]All values are percent (95% CI) unless otherwise indicated.

HIV status distribution was as follows: HIV-positive, 124; HIV-negative 26; HIV status unknown, 27.

[b]Amongst 177 cases where all tests were done, 49 were smear-positive, culture-positive; 18 were smear-negative, culture-positive; 107 were smear-negative, culture-negative; in three cases NTM was isolated.

[c]Where any of the three smears taken during the study period were positive.

[d]Where all of the three smears taken during the study period were negative.

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no confidence interval overlap between the Xpert MTB/RIF assay (76%–93%) and smear microscopy (47%–71%). This therefore supports the WHO policy that the Xpert MTB/RIF should be the initial test in adults with HIV infection suspected of having TB and can replace smear microscopy. The sensitivity of a single Xpert MTB/RIF assay in our Johannesburg cohort was slightly lower than in the landmark multi-country study (86% versus 92.2% overall and 61% versus 72% for smear-negative, culture-positive specimens) by Boehme et al. [16]. This may be due to differences in study population, as HIV infection rates reported in the multi-country study ranged from 1.7% to 76% across sites [16]. Although the two South African sites (Durban and Cape Town) involved in the multi-center Xpert MTB/RIF study [16] reported HIV infection rates (71.4% and 76.1%, respectively) similar to that found in our Johannesburg population (70% HIV-infected), breakdown of a single Xpert MTB/RIF test compared to a single culture result from these sites was not provided. A recent study [22] performed in a East African (Tanzania) population using a single Xpert MTB/RIF test reported sensitivities of 84.1% overall and 61% for smear-negative, culture-positive isolates, similar to the results in our study. A more recent study also from South Africa in the Cape Town population reports even lower sensitivities of 78.1% overall (performed on raw or processed sputum stored at $-20^{\circ}C$) and 55% for smear-negative, culture-positive samples (1 ml unprocessed archived sputum) [23]. This latter study further reported a sensitivity of the Xpert MTB/RIF assay among HIV-infected individuals of 69.6% ($n=46$), which, although lower than our study findings (84%, $n=124$), was not significantly different ($p=0.09$) from the sensitivity reported for the HIV-uninfected group in their study (82.9%, $n=82$) [23]. All together, these studies and our findings provide evidence of the much improved

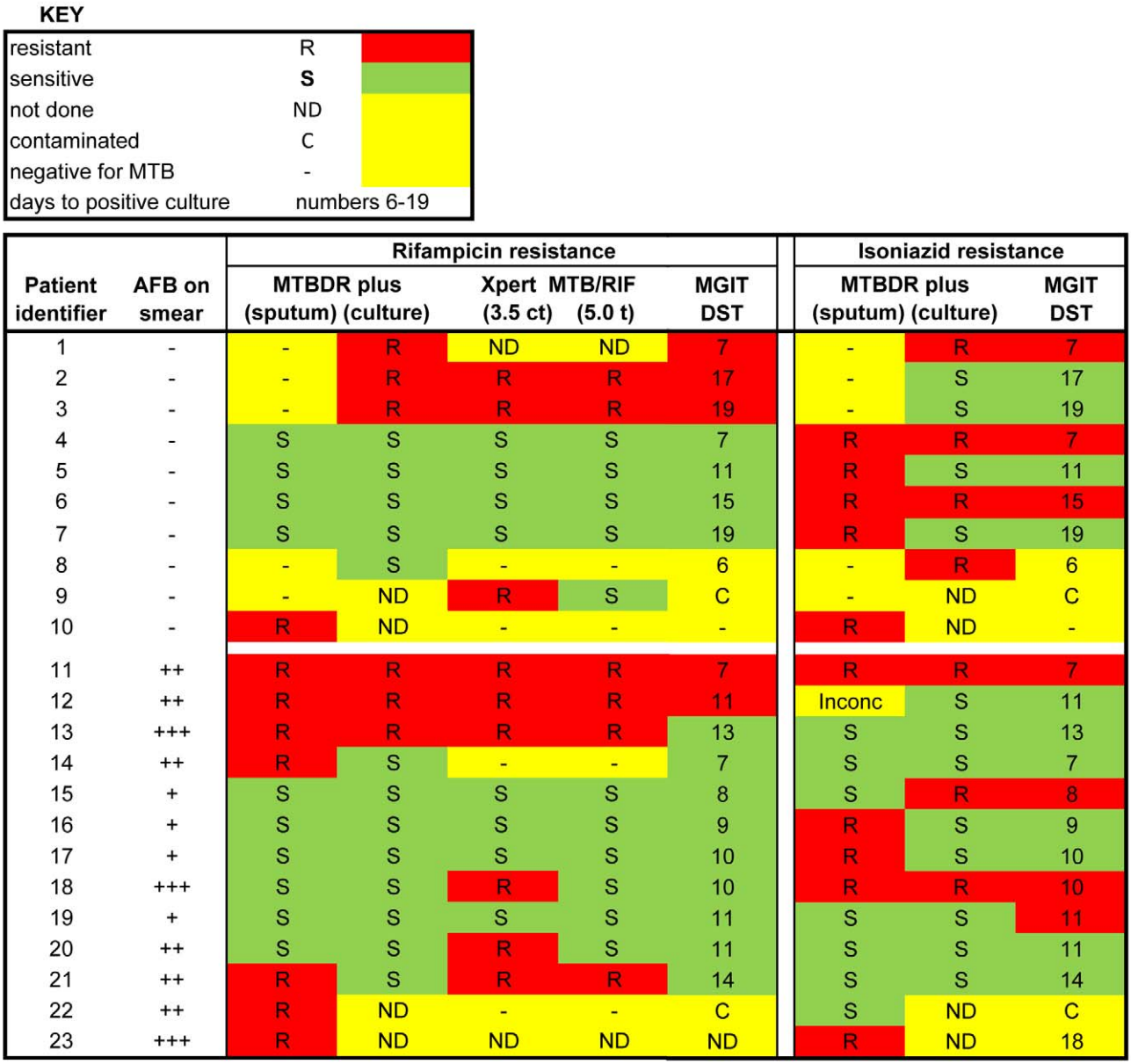

Figure 2. Heat map showing drug susceptibility profiles from 23 samples based on Xpert MTB/RIF, MTBDRplus on sputum, MTBDRplus on cultured isolates, and phenotypic culture (MGIT DST). The 23 samples were from a cohort of 311 participants. The heat map shows samples represented in rows and assigned numerical patient identifiers and testing methodologies in columns. Three colors are used to indicate the results: red, resistant; green, sensitive; yellow, not done, negative for *M.tb*, contaminated, or inconclusive. The samples are sorted into AFB-negative or -positive, with RIF and INH profiles in blocks side by side. Two columns are shown for the RIF results generated from the Xpert MTB/RIF using the amplification cycle threshold maximums 3.5 and 5.0. MDR TB was identified in two patients.
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performance of the Xpert MTB/RIF test compared to smear microscopy. Our findings further show the superior sensitivity of the Xpert MTB/RIF compared to the MTBDRplus and the LCTB assays, especially in the context of HIV co-infection. Some studies have reported Xpert MTB/RIF performance compared to other NAATs not evaluated in our study: the sensitivity of the Xpert MTB/RIF is reported to be higher than that of COBAS Amplicor MTB (Roche) (94% versus 86.8%) and similar to that of ProbeTec ET MTB Complex Direct Detection Assay (BD) (83.7% versus 83.9%) [16]; the sensitivity of the Xpert MTB/RIF assay is reported to be 79%, compared to an in-house IS*6110*-TaqMan real-time PCR assay with 84% sensitivity [24]; the Xpert MTB/RIF is suggested to be as good as the Gen-Probe MTB (Gen-Probe), but no data are available [25].

In our study, the decreased sensitivities of all tests (smear, culture, and NAATs) when using "clinical TB" as a gold standard instead of MGIT culture reflect the paucibacillary nature of pulmonary TB in a community of high HIV seroprevalence and the preference of clinicians to potentially overtreat than undertreat TB in HIV-infected individuals. Amongst these cases, confirmation of TB could be improved through additional MGIT cultures or additional Xpert MTB/RIF assays [16]. However, we elected to remain with this study design (one specimen sample for all investigational NAATs and MGIT culture) as it more closely

resembles current South African National TB Control Programme guidelines, and may remain practicable should Xpert MTB/RIF be implemented into routine diagnostic algorithms.

We further compared the assays' performances for the diagnosis of drug-resistant TB. Xpert MTB/RIF can detect mutations in the *rpoB* gene which occur in 95%–99% of RIF-resistant isolates [26–29] and are considered a good indicator for MDR TB [30]. The MTBDRplus assay is able to detect *katG* and *inhA* gene mutations that confer INH resistance in phenotypically resistant INH isolates, in addition to *rpoB* gene mutations. The LCTB assay does not detect mutations in resistance-determining regions of *M.tb*. Regarding RIF resistance, over-reporting has previously been described for the Xpert MTB/RIF assay compared with phenotypic DST [16]. Boehme et al. [16] further investigated isolates reported by Xpert MTB/RIF as RIF-resistant, and established by gene sequencing the presence of resistance-associated *rpoB* mutations or mixed infection with wild-type and mutant strains in the same culture. We did not genotype our resistant isolates further but initially observed a higher yield of the Xpert MTB/RIF assay for diagnosis of RIF resistance compared to MTBDRplus or MGIT DST. On re-evaluation using the new recommended software amplification cycle threshold of maximum 5.0, no discrepancies with MTBDRplus were found. We also observed a loss of detection of RIF and INH resistance between MTBDRplus directly on sputum and MTBDRplus on culture isolate. This difference could be due to the presence of a mixed-drug-susceptible and drug-resistant population with different growth potentials [31].

Overall, of 23 resistant samples detected by any methodology amongst 311 patients, we found nine discrepancies between phenotypic and genotypic results. In practice, discrepancies may lead to inappropriate management of TB, with unnecessary exposure to potentially toxic drugs or suboptimal treatment; however, the small sample size limits the full powering for DST accuracy testing.

In addition to the investigation of the Xpert MTB/RIF and MTBDRplus NAAT tests, this study also investigates the new LCTB NAAT assay, which may find place in laboratory settings for cost-effective high-throughput rapid screening (76% sensitive) in place of smear microscopy (59% sensitivity), with similar turnaround times.

A limitation of our study is that NAAT assays were performed on frozen aliquots, while smear microscopy and MGIT culture were performed on fresh samples. This may have impaired *M.tb* detection, and reduced the sensitivity of the NAATs in comparison to culture. In addition, the resuspension of the single processed sputum in ~2 ml of buffer, as opposed to the recommended 1.5 ml, increased the sample volume, resulting in a dilution and possibly reduced NAAT sensitivities. Freezing of sample aliquots may have caused bacterial disintegration, and consequent suboptimal performance of Xpert MTB/RIF, which relies on capturing whole (intact) bacteria. Several other studies too have recently reported Xpert MTB/RIF assay performance using stored samples: 217 samples from three sites within the western United States processed by NALC-NaOH and then stored at −80°C showed sensitivities of 98% for smear-positive and 72% for smear-negative samples [25]; 125 smear-negative clinical specimens processed by NALC-NaOH and then stored at −80°C for up to 10 y had reported sensitivities (on 1 ml) of 75.3% on the Xpert MTB/RIF assay [32]; 97 clinical specimens processed by NALC-NaOH and then stored at −80°C before Xpert MTB/RIF testing had reported sensitivities of 79% [24]; and the Cape Town study also tested the Xpert MTB/RIF assay using archived specimens, as mentioned above [23]. Despite these limitations, Xpert MTB/RIF still showed superior performance among all NAATs. In favor of the Xpert MTB/RIF assay design is the sample input volume of processed sputum of 500 µl, compared to 100 µl used for the LCTB assay, and product detection using automated, more sensitive fluorescence, not visual detection as with the MTBDRplus assay. Although the Xpert MTB/RIF assay invalid rate appeared higher than previously documented, the use of an uninterrupted power supply did improve result reporting, and should therefore be considered during field implementation.

It has been estimated that the diagnosis of active TB with a sputum-based assay with a sensitivity of 85% and specificity of 97% has the potential to save more than 400,000 lives per year [33]. The only NAAT assay that achieved these targets in our study was Xpert MTB/RIF. Combined with the fast turnaround time and the potential for point-of-care implementation (latter not evaluated in this study), the assay could revolutionize TB diagnosis. Already in a first implementation study of the Xpert MTB/RIF assay [34] in sites in South Africa, Peru, and India, and totaling 6,648 participants, use of the Xpert MTB/RIF assay reduced the median treatment duration for smear-negative TB from 56 d to 5 d. Further research is needed to determine how best to integrate this assay into current TB diagnostic algorithms and to improve our understanding of the prevalence and causes of discrepant drug resistance profiles.

The implementation of point-of-care testing including NAATs such as the Xpert MTB/RIF will need to be assessed for appropriate management of quality assurance, the adequacy of clinic resources (infrastructural and human), data collection, acceptance by patients and health care providers, and affordability, especially in resource-constrained settings.

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Author Contributions

Conceived and designed the experiments: LS KM WS IS FV AD. Performed the experiments: NG LS. Analyzed the data: LS KM AVR NG. Contributed reagents/materials/analysis tools: WS IS FV. Wrote the paper: LS KM AVR NG WS. ICMJE criteria for authorship read and met: LS KM NG MN AVR IS FV AD WS. Agree with the manuscript's results and conclusions: LS KM NG MN AVR IS FV AD WS. Enrolled patients: MN. Wrote the first draft of the paper: LS KM NG WS. Clinic management where study conducted: FV IS. Managed infection control necessary for study to be performed and assistance with RIF resistance data interpretation: AD.

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Editors' Summary

Background. Tuberculosis (TB)—a contagious bacterial infection that mainly affects the lungs—is a global public health problem. In 2009, 9.4 million people developed TB, and 1.7 million people died from the disease; a quarter of these deaths were in HIV-positive individuals. People who are infected with HIV, the virus that causes AIDS, are particularly susceptible to TB because of their weakened immune system. Consequently, TB is a leading cause of illness and death among people living with HIV. TB is caused by *Mycobacterium tuberculosis*, which is spread in airborne droplets when people with the disease cough or sneeze. Its characteristic symptoms are a persistent cough, night sweats, and weight loss. Diagnostic tests for TB include sputum smear analysis (the microscopic examination of mucus brought up from the lungs by coughing for the presence of *M. tuberculosis*) and mycobacterial liquid culture (in which bacteriologists try to grow *M. tuberculosis* from sputum samples and test its drug sensitivity). TB can usually be cured by taking several powerful drugs daily for at least six months.

Why Was This Study Done? Mycobacterial culture is a sensitive but slow way to diagnose TB. To halt the disease's spread, it is essential that TB—particularly TB that is resistant to several treatment drugs (multidrug-resistant, or MDR, TB)—is diagnosed quickly. Recently, several nucleic acid amplification technology (NAAT) tests have been developed that rapidly detect *M. tuberculosis* DNA in patient samples and look for DNA changes that make *M. tuberculosis* drug-resistant. In December 2010, the World Health Organization (WHO) endorsed Xpert MTB/RIF—an automated DNA test that detects *M. tuberculosis* and rifampicin resistance (an indicator of MDR TB) within two hours—for the investigation of patients who might have TB, especially in regions where MDR TB and HIV infection are common. TB diagnosis in HIV-positive people can be difficult because they are more likely to have smear-negative TB than HIV-negative individuals. In this prospective study, the researchers compare the performance of Xpert MTB/RIF on a single sputum sample with that of smear microscopy, liquid culture, and two other NAAT tests (MTBDRplus and LightCycler Mycobacterium Detection) in adults who might have TB in Johannesburg (South Africa), a region where many adults are HIV-positive.

What Did the Researchers Do and Find? The researchers evaluated adults with potential TB attending a primary health care clinic for TB according to national guidelines and determined their HIV status. A sputum sample from 311 participants underwent smear microscopy, liquid culture, and drug susceptibility testing; 177 samples were also tested for TB using NAAT tests. They found that 70% of the participants were HIV-positive and 38.5% had culture-positive TB. Compared to liquid culture, smear microscopy, MTBDRplus, LightCycler Mycobacterium Detection, and Xpert MTB/RIF had sensitivities of 59%, 76%, 76%, and 86%, respectively. That is, assuming that liquid culture detected everyone with TB, Xpert MTB/RIF detected 86% of the cases. The specificity of all the tests compared to liquid culture was greater than 97%. That is, they all had a low false-positive rate. Among people who were HIV-positive, the sensitivity of Xpert MTB/RIF was 84%; the sensitivities of the other NAAT tests were 70%. Moreover, Xpert MTB/RIF detected TB in 61% of smear-negative, culture-positive samples, whereas the other NAATs detected TB in only about a quarter of these samples. Finally, although some TB cases were identified as drug-resistant by one test but drug-sensitive by another, the small number of drug-resistant cases means no firm conclusions can be made about the accuracy of drug resistance determination by the various tests.

What Do These Findings Mean? Although these findings are likely to be affected by the study's small size, they suggest that Xpert MTB/RIF may provide a more accurate rapid diagnosis of TB than smear microscopy and other currently available NAAT tests in regions where HIV and TB are endemic (i.e., always present). Indeed, the reported accuracy of Xpert MTB/RIF for TB diagnosis—85% sensitivity and 97% specificity—has the potential to save more than 400,000 lives per year. Taken together with the results of other recent studies (including an accompanying article by Lawn et al. that investigates the use of Xpert MTB/RIF for screening for HIV-associated TB and rifampicin resistance), these findings support the WHO recommendation that Xpert MTB/RIF, rather than smear microscopy, should be the initial test in HIV-infected individuals who might have TB.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.1001061.

- This study is further discussed in a *PLoS Medicine* Perspective by Carlton Evans; a related *PLoS Medicine* Research Article by Lawn et al. is also available
- WHO provides information (in several languages) on all aspects of tuberculosis, including general information on tuberculosis diagnostics and specific information on the Xpert MTB/RIF test; further information about WHO's endorsement of Xpert MTB/RIF is included in a recent Strategic and Technical Advisory Group for Tuberculosis report
- WHO also provides information about tuberculosis and HIV
- The US National Institute of Allergy and Infectious Diseases has detailed information on tuberculosis and HIV/AIDS
- The US Centers for Disease Control and Prevention also has information about tuberculosis, including information on the diagnosis of and on tuberculosis and HIV co-infection
- Information is available from Avert, an international AIDS charity on many aspects of HIV/AIDS, including information on HIV-related tuberculosis (in English and Spanish)

CHAPTER 4: DEVELOPING THE PRINCIPLE COMPONENTS TO ENSURE BEST PRACTISE FOR MULTI-DISCIPLINARY POCT

Several components of a quality assurance system were addressed and presented in Chapter 4.1 to 4.4, which detail the stringent process involved in verification and EQA quality material development for HIV and TB. For molecular HIV plasma-based assays, the South African Viral Quality Assurance (SAVQA) panel was developed and is now being used to assess newly developed POC technologies. The Dried Culture Spot (DCS) program was developed for molecular TB assays and also trialled at POC.

In addition, further components to ensuring the quality of POCT results in the field, in terms of ensuring clinic site readiness and appropriate POC operator training, were presented in the following conference presentations:

a). Scott LE, Gous N, Carmona S, Stevens W. 2014. Performance of Xpert® HIV-1 Quant compared to Roche CAP/CTM v2 and Abbott RealTi*m*e HIV-1 on a prequalification plasma validation panel. In: ASLM, Cape Town, South Africa, 30th November - 4th December.

b). Gous N, Scott LE, Stevens WS. 2012. Requirements for Point of Care testing: A checklist for implementation. In: Grand Challenges Diagnostic Meeting, Seattle, Canada, September.

c). Gous N, Scott L, Cunningham B, Stevens W. 2012. Site and training requirements for Xpert® MTB/RIF assay implementation in remote settings: Prepare for the unexpected. In: 3rd SA TB Conference, South Africa, 12 – 15 June.

4.1 USE OF A PREQUALIFICATION PANEL FOR RAPID SCALE-UP OF HIGH-THROUGHPUT HIV VIRAL LOAD TESTING

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Use of a Prequalification Panel for Rapid Scale-Up of High-Throughput HIV Viral Load Testing

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Increased access to antiretroviral drugs expands needs for viral load (VL) testing. South Africa's National Health Laboratory Service responded to demands by implementing two testing platforms in 17 laboratories within 8 months. An industry partner's collaboration, training programs, and method verification with a VL prequalification panel ensured testing quality and rapid implementation.

More than 1.4 million patients receive antiretroviral (ARV) treatment in South Africa through the public health services (1, 8). In response to such demand, 17 HIV viral load (VL) testing laboratories were identified for the national HIV program between May 2010 and January 2011. These were distributed throughout the 9 provinces at both rural and urban laboratory centers. Several sites were newly implemented ($n = 6$), and the remaining required minimal renovation to accommodate the new VL testing platform footprints. A total of 75 technical staff members were trained. Two viral load testing platforms were selected for implementation using a stringent tender-based procurement process. The first platform was the COBAS TaqMan HIV-1(CAP/CTM) version 2.0 docked system (Roche Molecular Systems, Branchburg, NJ) ($n = 20$), which combines the extraction of total nucleic acids on the COBAS AmpliPrep (CAP) with real-time PCR on the COBAS TaqMan analyzer (CTM). Each site with this platform also installed a preanalytical sample-handling P630 device (Roche), ensuring further automation. The second platform was the Abbott *m*2000sp ($n = 23$) for nucleic acid extraction and the *m*2000*rt* for amplification and detection to perform the Abbott RealTime HIV-1 assay (Abbott Molecular Inc., Des Plaines, Illinois). Both automated systems are based on real-time PCR and were connected to the National Health Laboratory Service's (NHLS) laboratory information system (LIS). Both assays have been validated (4), including in-country validation (5, 7), and therefore did not require further validation but, rather, verification after platform placement to ensure adequate site performance. The verification was required within 1 week of installation to ensure each instrument was "fit for purpose" for clinical sample testing and result reporting.

The material used to prepare the verification panels was a combination of known HIV-positive and -negative plasma packs (~200 ml) obtained from the South African National Blood Services (SANBS). SANBS tests all plasma using the Procleix Ultrio blood donor screening test (Gen-Probe and Novartis Diagnostics, Emeryville, CA) to confirm positive or negative HIV status. Each pack was quantitated (using both assays at the NHLS Charlotte Maxeke Academic Hospital PCR reference laboratory in Johannesburg) and kept frozen (−70°C) until results were obtained by testing on both VL testing platforms in the reference laboratory in Johannesburg. The bulk plasma packs were then thawed in a 37°C water bath and diluted (using negative plasma) or pooled and aliquoted into 6 bulk lots calculated to produce a range of VL. Once manufactured, these bulk lots were mixed thoroughly at room temperature on an orbital shaker (Labotec, SA) and then retested on the Abbott RealTime HIV-1 assay to confirm the correct dilutions/pooling of VL. The 6 bulk lots, each a maximum volume of ~120 ml, consisted of one negative and five quantifiable bulk lots in the following viral load ranges (500 copies/ml, 2.7 log copies/ml; 1,000 copies/ml, 3.0 log copies/ml; 5,000 copies/ml, 3.7 log copies/ml; 50,000 copies/ml, 4.7 log copies/ml; and 100,000 copies/ml, 5.0 log copies/ml). These were then assigned into a 42-member verification panel (Table 1) to be processed in the order stated. Each range was repeated five times and interspersed between 17 negative samples. The choice of 42 tubes was to ensure coverage of two racks in the CAP/CTM v2 assay.

The verification panel was shipped using couriers with dry ice packaging to each site. Testing was performed directly from the dry-ice-transported panel, or panels were stored at −70°C until testing. Testing at each site was performed over 1 day with the same lot numbers of reagents and controls per instrument. Once the results were obtained, they were entered by the site personnel into a template MS Excel spreadsheet and emailed to the Department of Molecular Medicine and Hematology, Research Diagnostic Laboratory, NHLS, in Johannesburg. Statistical parameters measured were accuracy, precision, carryover, and limit of blank. The mean (average), standard deviation (SD), and coefficient of variation (CV) were calculated in each category for both the untransformed value (copies/ml) and the log-transformed value (log copies/ml). Levels of acceptable variability (within-run precision) were determined as previously reported (2) and according to the international Viral Quality Assurance (VQA) program (Rush Presbyterian-St. Luke's Medical Center, Chicago, IL). These values were ≤35% CV on the untransformed copies/ml values and ≤0.19 SD on the log transformed copies/ml values. The log difference (reference − new site) or bias was calculated using the log-transformed values. An acceptable bias was considered ≤0.3

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TABLE 1 Panel constitution

Tube no.	Panel member VL
1	Negative
2	500 copies/ml
3	1,000 copies/ml
4	5,000 copies/ml
5	Negative
6	50,000 copies/ml
7	100,000 copies/ml
8	Negative
9	Negative
10	500 copies/ml
11	1,000 copies/ml
12	5,000 copies/ml
13	Negative
14	50,000 copies/ml
15	100,000 copies/ml
16	Negative
17	Negative
18	500 copies/ml
19	1,000 copies/ml
20	5,000 copies/ml
21	Negative
22	50,000 copies/ml
23	100,000 copies/ml
24	Negative
25	Negative
26	500 copies/ml
27	1,000 copies/ml
28	5,000 copies/ml
29	Negative
30	50,000 copies/ml
31	100,000 copies/ml
32	Negative
33	Negative
34	500 copies/ml
35	1,000 copies/ml
36	5,000 copies/ml
37	Negative
38	50,000 copies/ml
39	100,000 copies/ml
40	Negative
41	Negative
42	Negative

log copies/ml across all categories. In addition, the percentage similarity was calculated (6) across all log-transformed quantified values irrespective of categories, and the percentage similarity SD and percentage similarity CV were calculated. All Abbott RealTime HIV-1 results were compared to one Abbott RealTime HIV-1 panel, and all CAP/CTM v2 results were compared to one CAP/CTM v2 panel tested on both platforms at the central reference laboratory in Johannesburg and considered the reference standard for statistical analysis. Outcomes were reported back to the sites via email in a standard report document. Carryover and limit of the blank were reported if any negative sample directly after a high-VL sample was reported as positive or if any negative sample irrespective of position was reported as positive. The reports distributed to the sites recorded the maximum SD and CV.

TABLE 2 Problems identified through the program between May 2010 and January 2011 from 45 instruments placed in the field and enrolled in the prequalification program

Problem reported	No. of instruments
Results flagged outside the acceptable statistical criteria	4
Failed the limit of the blank	2
Failed the bias	1
Target not detected in a positive sample	1
Instruments moved after initial verification due to laboratory renovations and were verified again before clinical sample testing	4
Instruments did not generate verification panel results due to run losses	12
Power outage	2
Instrument error	1
Transcription error	2
Incorrect carriers	1
Instrument alignment	1
Incorrect sample storage	1
Failed controls	1
Faulty thermocycler	1
Instrument replaced	2

Forty-five instruments were initially enrolled in this verification program across the 17 laboratories: 2 instruments did not pass verification and were removed, and 43 passed verification and were able to be used for clinical testing. One panel was tested on each instrument, and where problems were identified, additional panels were prepared for testing. Four instrument verifications flagged above the acceptable statistical criteria (2 failed the limit of the blank, 1 failed the bias, and 1 target was not detected in a positive sample). Four instruments were moved after initial verification due to laboratory renovations and were verified again before clinical sample testing. Twelve instruments did not generate verification panel results due to run losses for reasons listed in Table 2. A total of 65 panel units were tested, with 59 units from the same bulk manufacturing lot (panel 1). A second bulk plasma batch (panel 2) needed to be manufactured to ship six additional units to the sites. This second bulk lot was manufactured from different plasma packs but followed the same manufacturer's protocol as the first bulk.

Table 3 lists the summary statistics of the final verification values for 43 instruments, excluding two instruments that were replaced. Panel 1 and panel 2 results are also shown separately, as the reference comparators were different. In addition to the within-platform comparison, a section is also included for the across-platform comparison, in which the Roche (1 instrument) and Abbott (1 instrument) assays are compared to each other using the reference panel results for panel 1 and panel 2. This comparison shows the maximum percentage similarity CV obtained between the two assays and, therefore, used as the maximum limit for within-platform precision acceptability. Any percentage similarity CV value above this level (maximum, 2.9%) was flagged for further investigation.

The average maximum CV and bias for both panels on both platforms were similar, showing that both platforms are suitable for HIV VL testing on clinical samples from the region. Two in-

TABLE 3 Summary statistics for two manufactured panels showing the performance across and within platforms for their final verification values

Comparison and laboratory no.[a]	Instrument no.	Maximum CV	Maximum SD	Maximum bias	SD bias	% similarity CV
Reference panel comparison across platforms[b]						
Reference panel 1		22.5	0.1	0.32	0.1	2.9
Reference panel 2		34.5	0.2	0.25	0.2	2.5
Panel 1 comparison within Roche instruments						
1	1	15.4	0.07	−0.21	0.11	1.1
	2	23.2	0.1	−0.26	0.13	1.6
2	3	26.1	0.11	0.09	0.08	1.1
	4	30.2	0.14	−0.08	0.12	1.5
	5	13.2	0.06	−0.14	0.07	0.9
	6	30.1	0.15	−0.32	0.14	2.3
	7	18	0.08	−0.12	0.13	1.5
	8	17.8	0.08	−0.16	0.08	1.4
3	9	36.3	0.16	−0.19	0.18	2.3
	10	17.7	0.08	−0.16	0.09	1
4	11	20.1	0.09	−0.14	0.1	1.7
	12	25.9	0.1	−0.26	0.16	2
5	13	25.9	0.1	−0.18	0.18	2
	14	25.5	0.12	−0.11	0.12	1.4
6	15	22.6	0.09	−0.17	0.12	1.4
	16	22.2	0.1	0.12	0.1	1.6
7	17	23.3	0.1	−0.15	0.1	1
8	18	28.4	0.15	−0.13	0.13	0.9
	19	19.4	0.09	−0.11	0.1	1.1
Avg		23.2	0.1	−0.1	0.1	1.5
Panel 1 comparison within Abbott instruments						
2	20	18.1	0.08	−0.22	0.12	1.3
9	21	13.3	0.06	0.15	0.08	1.2
	22	24.1	0.1	−0.08	0.14	1.5
	23	18.1	0.08	−0.1	0.07	1.1
	24	36.4	0.17	0.09	0.15	1.6
10	25	11.6	0.05	0.16	0.09	1.6
	26	16.5	0.07	0.06	0.08	1
11	27	23.8	0.12	0.12	0.12	1.6
	28	19.1	0.08	0.1	0.09	1.2
12	29	12.8	0.06	0.17	0.11	1.6
	30	19	0.09	0.15	0.13	1.4
13	31	26.6	0.1	−0.05	0.12	1.3
14	32	20.5	0.09	0.06	0.11	1.2
15	33	26.8	0.14	0.03	0.11	1.1
	34	29.1	0.12	−0.06	0.17	1.5
16	35	27.4	0.12	0.1	0.16	1.7
	36	16.9	0.08	−0.11	0.09	1.1
17	37	15.1	0.06	−0.1	0.08	0.9
	38	33.2	0.13	0.11	0.15	1.7
Avg		21.5	0.1	0.0	0.1	1.3
Panel 2 comparison within Roche instruments						
2	39	18.2	0.1	−0.25	0.19	2.4
7	40	17	0.08	−0.19	0.13	1.8
	41	15.7	0.1	−0.23	0.12	2
Avg		17.0	0.1	−0.2	0.1	2.1
Panel 2 comparison within Abbott instruments						
17	42	23.4	0.11	0.12	0.14	1.7
	43	16.1	0.07	0.11	0.14	1.6
Avg		19.8	0.1	0.1	0.1	1.7

[a] Numbers in the stub are laboratory numbers unless otherwise indicated (i.e., reference panel numbers).
[b] Roche ($n = 1$) versus Abbott ($n = 1$).

strument (numbers 9 and 24) maximum CVs on the untransformed values were >35% but were considered borderline acceptability, as their biases were within acceptable limits. Apart from the instrument errors identified through this program and listed in Table 2, a further 11 individual sample tubes (0.4%; 11/2,730) generated errors (1 internal control failed and 10 were invalid due to a clot being detected).

Statistical analysis for verification may be daunting, especially when implementing different platforms, different samples, and different scoring parameters; however, the design of this panel of 42 samples was well suited to both platforms testing run sizes, and the selection of panel members well represented the assays' dynamic range and clinically relevant treatment switch range (500 to 1,000 copies/ml). The five replicates in each range also appeared suitable to identify any issues of precision within these clinically important ranges, and the 17 negative samples appeared adequate to investigate carryover and limit of the blank. The percentage similarity CV was useful as an overall measure of variability to highlight instrument problems. If the within-assay percentage similarity CV is greater than the between-assay percentage similarity CV (>2.9%), then further investigation is needed within each category using the bias, SD, and CV.

This prequalification program design, its central location, and its rapid deployment (a not-scheduled scheme) with local resources proved suitable for both VL testing platforms. The process identified errors related to both the instrument and the laboratory operator and proved useful in training and managing new sites (installation, on-site training, and verification within 1 week). It identified the need to manufacture larger bulk batch sizes but also that standard laboratory equipment is suitable for such bulk manufacture. Plasma packs were selected as the choice of testing material for instrument verification because they were relatively easy to source (local blood bank material), truly represented clinical testing material (predominately subtype C [3]), and showed few sample errors due to clots detected. However, other testing material, such as viral cultures in synthetic matrix, spiked negative plasma, and plasmid preparations, may be investigated. The potential future use of dried blood spots (DBS) for HIV VL testing will also require instrument verification and thereby also require a specialized DBS verification panel, which is being investigated. An ongoing VL assessment program is now being developed to continue quality VL testing.

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4.2 DRIED CULTURE SPOTS FOR XPERT MTB/RIF EXTERNAL QUALITY ASSESSMENT: RESULTS OF A PHASE 1 PILOT STUDY IN SOUTH AFRICA

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Dried Culture Spots for Xpert MTB/RIF External Quality Assessment: Results of a Phase 1 Pilot Study in South Africa▽

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Implementation of Xpert MTB/RIF requires quality assessment. A pilot program using dried culture spots (DCSs) of inactivated *Mycobacterium tuberculosis* is described. Of 274 DCS results received, 2.19% generated errors; the remainder yielded 100% correct *Mycobacterium tuberculosis* detection. The probe A cycle threshold (C_T) variability of three DCS batches was ≤3.47. The study of longer-term DCS stability is ongoing.

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) (1, 3–5, 9, 12, 13, 15, 19, 25) for the diagnosis of *Mycobacterium tuberculosis* has recently been endorsed by the WHO (28), and recommendations for data collection to quantify the impact of this GeneXpert (GX) technology are provided (26). Guidance, however, with respect to appropriate external quality assessment (EQA) programs is lacking (17). Current international tuberculosis (TB) EQA programs focus on microscopy, culture, and susceptibility testing laboratories (24) and highlight the difficulties in expansion due to labor-intensive preparatory work and the high cost and regulations associated with shipping drug-resistant isolates (27).

Criteria for a verification ("fit for purpose") and EQA program suited to the characteristics of the Xpert MTB/RIF assay (3, 8) will require the following elements. (i) The testing material must contain whole *M. tuberculosis* (8). (ii) Transportation of EQA material needs to be safe. (iii) The testing procedure needs to be safe and compatible with the Xpert MTB/RIF current testing protocol. (iv) Health care workers who do not have laboratory skills must be able to perform the testing in nonlaboratory settings. (v) Finally, the programs will need to be cost-effective and sustainable. Such a program using whole inactivated *M. tuberculosis* spotted onto filter paper was developed and piloted in South Africa as part of the National Health Laboratory Service (NHLS) GX rollout.

M. tuberculosis was obtained from (i) pooled samples from 20 microbial growth incubation tubes (MGIT) of rifampin (RIF)-susceptible clinical isolates and tested with the MTBDR*plus* (Hain Life Sciences), (ii) 20 pooled MGIT cultures comprising American Type Culture Collection (ATCC) strain S-MYCTU-02-P2 (ATCC 25177 [H37Ra]) and well-characterized local clinical strain MYCTU 15, and (iii) the ATCC 25618 (H37Rv) laboratory strain grown for single-cell-organism suspensions (11). The MGIT cultures S-MYCTU-02-P2 and MYCTU 15 and clinical isolates were pooled in their respective batches (with strains kept separate and not mixed), centrifuged (3,000 × *g* for 15 min at 4°C) to pellet cells, and resuspended in 40 ml phosphate-buffered saline (PBS) followed by addition of 80 ml (2:1 ratio of buffer to culture) of the Xpert sample reagent (SR) buffer. For the H37Rv strain, 200 ml of culture was harvested (by centrifugation at 3,500 × *g*) at room temperature for 10 min, and cells were resuspended in PBS to 40 ml followed by addition of 80 ml SR buffer (2:1 ratio of buffer to cells). Both MGIT-grown and H37Rv strain cultures were inactivated in SR buffer for 2 h at room temperature, with intermittent mixing. The inactivated material was washed twice with sterile PBS and resuspended in final volumes of 10 ml (S-MYCTU-02-P2 and MYCTU 15) and 40 ml (H37Rv) PBS. For confirmation of inactivation, washed cultures (0.5 ml) were reinoculated into new MGIT tubes in Bactec cabinets for 42 days. These inactivated bulk stocks were enumerated by flow cytometry (FC500 using Flow count microspheres; Beckman Coulter) and tested with the Xpert MTB/RIF assay. The cycle threshold (C_T) values of the semiquantitative categories (high, C_T of <16; medium, C_T of 16 to 22; low, C_T of 22 to 28; and very low, C_T of >28) were recorded for probe A and were compared to the flow cytometry enumeration score. Dilutions that generated a medium (C_T of 16 to 22) qualitative Xpert MTB/RIF result were used to prepare the dried culture spots (DCSs).

DCSs were prepared by spotting 25-μl amounts of inactivated culture material onto Whatman 903 filter cards (Merck) together with 2 μl of DNA loading dye (Sigma-Aldrich) per spot for visualization purposes, as illustrated in Fig. 1, and dried for 1 h at room temperature before being placed in sealed plastic bags with a desiccant sachet (Sigma-Aldrich).

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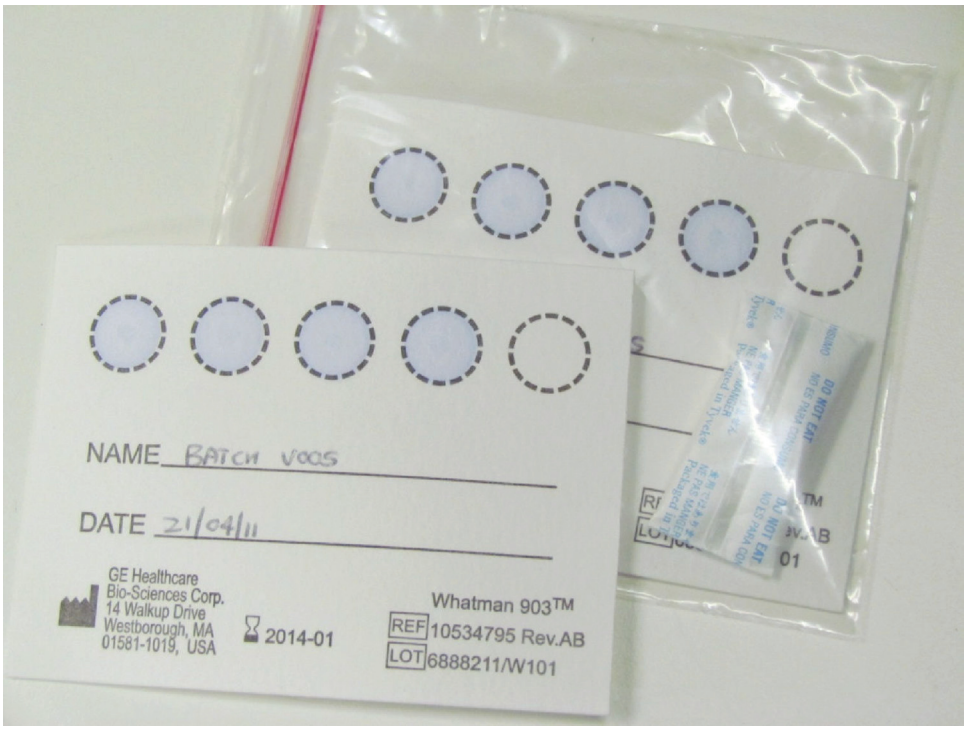

FIG. 1. A sample of the DCSs on filter cards and in plastic transport bags with dessicant sachets. Four DCSs on a card containing inactivated *M. tuberculosis* culture are visualized by the blue dye.

These were couriered ($n = 16$), hand delivered ($n = 10$), or surface mailed (repeat DCSs to 4 sites) to various participating sites, where each spot was cut (using a sterile pair of scissors) into a 50-ml standard laboratory Nunc centrifuge tube (AEC Amersham), and 2.8 ml SR buffer (to ensure there was a sufficient 2-ml concentration to pipette into the Xpert MTB/RIF cartridge after the DCS incubation) was added to the tube. The tubes were vortexed (or hand shaken by swirling

TABLE 1. Performance of the three DCS batches on 286 GX modules

Parameter	Result for DCS batch no.:		
	V002	V004	V005
M. tuberculosis bulk culture material	MGIT clinical controls (RIF-sensitive *M. tuberculosis*)	MGIT ATCC strain (RIF-sensitive *M. tuberculosis*)	H37 laboratory strain (RIF-sensitive *M. tuberculosis*)
No. of GX modules tested by DCS	49 (all RIF-sensitive *M. tuberculosis*)	173 (all RIF-sensitive *M. tuberculosis*)[a]	64 (all RIF-sensitive *M. tuberculosis*)
No. of errors[b]			
Error 5007		1	
Error 5011	1	3	1
No. of DCSs for statistical analysis	48	157	63
% of testing in qualitative category:			
Very low	0	5.1	6.25
Low	26.53	47.13	42.19
Medium	69.39	47.77	48.44
High	2.04	0	1.56
C_T for probe A			
Mean	20.75	22.58	21.89
SD	2.20	2.76	3.47
CV (%)	10.6	12.22	15.86

[a] A total of 161 modules returned results.
[b] Error 5011 refers to signal loss detected in an amplification curve, and error 5007 refers to a probe check failure.

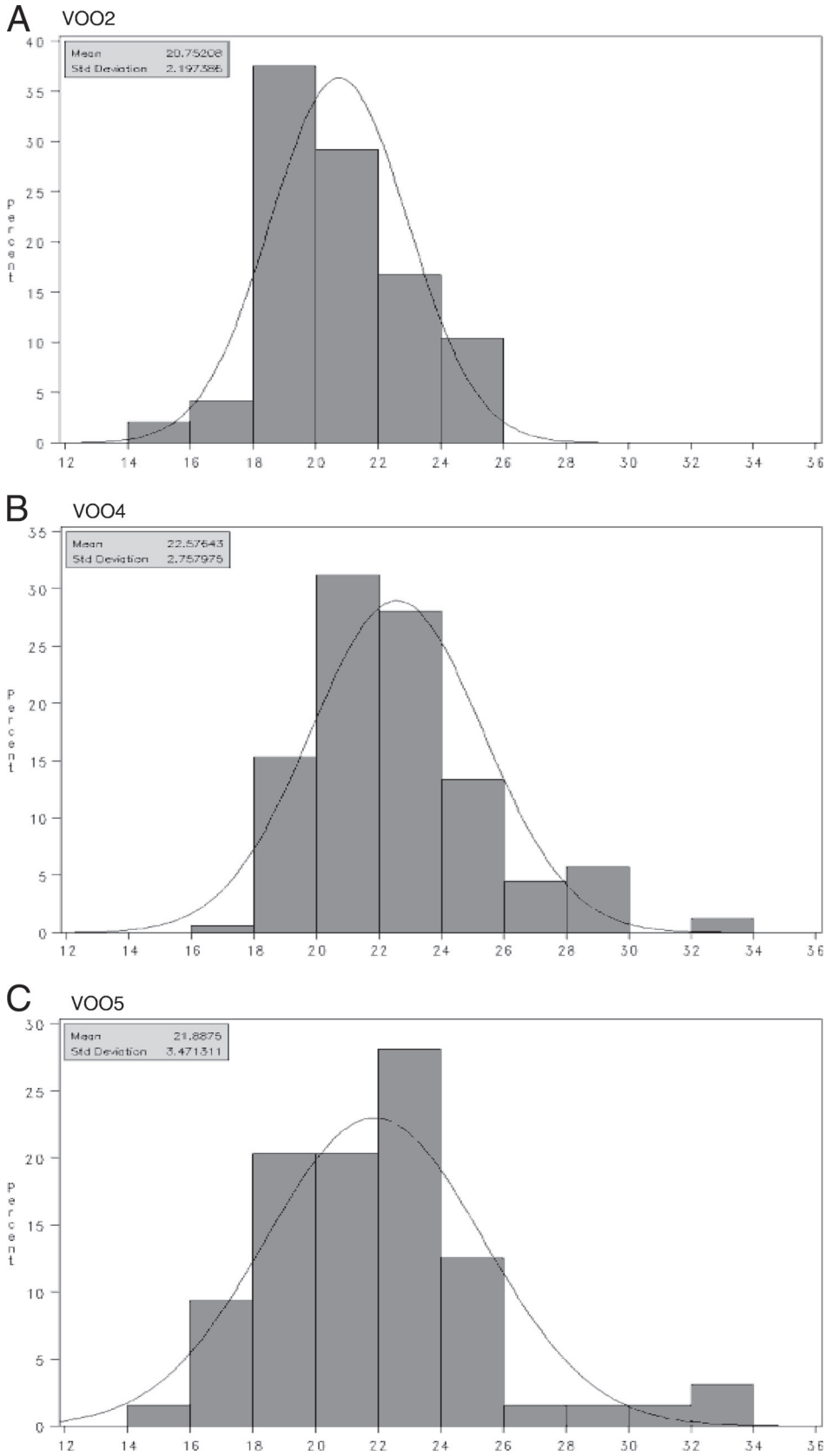

FIG. 2. Frequency distributions overlaid with normal curves of the C_T values for probe A from the three DCS batches. (A) Batch V002; (B) batch V004; (C) batch V005. The standard deviation and mean C_T values are represented in insets in each of the panels.

vigorously if no vortexer was available) and left at room temperature for 15 min with intermittent mixing. One DCS was then tested on each Xpert MTB/RIF module. The C_T mean, standard deviation, and coefficient of variation (CV) were calculated for probe A.

Three DCS batches were manufactured for 31 GXs: GX Infinity-48 ($n = 1$), GX16 ($n = 9$), and GX4 ($n = 21$). A total of 286 DCSs were distributed to the 26 participating sites, and results were received for 274 DCSs, thereby identifying sites with nonconformities. Six testing errors (error no. 5011 [$n = 5$] and 5007 [$n = 1$]) were reported, and the remaining 268 DCSs generated results with 100% *M. tuberculosis* positivity and RIF sensitivity (Table 1). Probe A was the first probe to reach the amplification C_T, with similar standard deviations across three DCS batches with a C_T of ≤3.47. Frequency distributions in Fig. 2 illustrate the greatest variability in batch V005 (CV of 15.86%) from the single-cell-generated culture.

National Xpert MTB/RIF implementation programs are challenged by determining the scope and composition of EQA panels and the infectious nature of *M. tuberculosis* material. This study provides a preliminary demonstration through the use of inactivated *M. tuberculosis* coupled with easier transportation of DCS material that an EQA program can be safely provided. The DCS material proved successful for verification of GX instruments and highlighted expected error code frequencies (2.1%) and site nonconformities.

Although this is a uniquely designed EQA program that appears so far suitable for Xpert MTB/RIF verification using different strains from different culture methods, the individual components are not unfamiliar to the field: filter paper has been used for the transportation and molecular testing of *M. tuberculosis* DNA (7, 14), and flow cytometry has been used for the analysis of *M. tuberculosis* (2, 10, 16, 18, 20–23). Flow cytometry has the advantage of rapidly and accurately identifying inactivated single whole bacterial cells, which circumvents conventional, time-consuming CFU enumeration methodologies. Enumeration of flow cytometric events can also be performed below the minimum McFarlane concentrations (1×10^7 CFU/ml) and could more accurately be used in strain mixing to test "dropout" or "delayed" C_Ts (3). Flow cytometry is also available in settings that currently perform CD4 counting of HIV patients for treatment initiation and monitoring and therefore represent a platform and infrastructure already in place (6).

The variability in C_T values may result from the spotting technique, different DCS reconstitution techniques (including vortexing/hand shaking), and variability in the amount of SR buffer added to each DCS. Other sources of variability may be explained by *M. tuberculosis* clumping from the MGIT-grown cultures being better trapped by the Xpert MTB/RIF filter membrane, whereas an *M. tuberculosis* single cell (~0.4 μm wide by 1.0 μm long) may pass through the 0.8-μm membrane pore. The advantage of single-cell-cultured material is that no sonication or declumping methods are required before flow cytometry enumeration and spotting.

Future design of an Xpert MTB/RIF EQA program could be similarly based on line probe assay programs using one pansusceptible strain, one RIF-monoresistant strain with a common *rpoB* mutation, one multidrug-resistant (MDR) strain, one nontuberculous mycobacterium (NTM) strain, and a negative control (17), each placed on a DCS card and distributed 3 times per year.

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4.3 PERFORMANCE MONITORING OF MYCOBACTERIUM TUBERCULOSIS DRIED CULTURE SPOTS FOR USE WITH THE GENEXPERT SYSTEM WITHIN A NATIONAL PROGRAM IN SOUTH AFRICA

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Performance Monitoring of *Mycobacterium tuberculosis* Dried Culture Spots for Use with the GeneXpert System within a National Program in South Africa

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The use of dried culture spots (DCSs) has been reported in the verification of GeneXpert instruments as being "fit for purpose" for the South African National implementation program. We investigated and compared the performance of the DCSs for verification across different bulk batches, testing the settings and cadre of staff, and the Xpert MTB/RIF assay version. Four bulk batches (V005 to V008) were used to prepare (i) 619 DCS panels for laboratory testing on G3 or G4 cartridges by a technologist, (ii) 13 DCS panels (batch V005) used for clinic verification on G3 cartridges by a nurse or lay counselor, and (iii) 20 DCS panels (batch V005) used for the verification of 10 GeneXpert 16 module instruments in mobile vehicles on the G3 cartridge performed by a scientist. The stabilities of the DCSs over 6 months at 4°C, room temperature, and 37°C were investigated. The mean cycle threshold (C_T) and standard deviation (SD) for probe A were calculated. The proportions of variability in the C_T values across bulk batches, assay versions, and settings and cadre of staff were determined using regression analysis. Overall, the DCSs demonstrated SDs of 3.3 ($n = 660$) for the G3 cartridges and 3.8 ($n = 1,888$) for the G4 cartridges, with an overall error rate of 1.5% and false rifampin resistance rate of 0.1%. The proportions of variability (R^2) in the C_T values explained by batch were 14%, by setting and cadre of staff, 5.6%, and by assay version, 4.2%. The most stable temperature in a period of up to 6 months was 37°C (SD, 2.7). The DCS is a robust product suitable for storage, transport, and use at room temperature for the verification of the GeneXpert instrument, and the testing can be performed by non-laboratory-trained personnel in nonlaboratory settings.

Following the endorsement of the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) by the World Health Organization (1), the South African National Department of Health (NDoH) and the National Health Laboratory Service (NHLS) undertook national implementation of the GeneXpert MTB/RIF assay in March 2011. The implementation involved rapid successive placement of GeneXpert instruments in a phased approach into smear microscopy centers across high-burden tuberculosis (TB) districts, encompassing all 9 provinces in South Africa. By 31 March 2013, approximately 2,315,380 Xpert MTB/RIF cartridges had been sold globally, over half of which had been procured for use in South Africa alone (2). In parallel to this implementation, a GeneXpert instrument verification program consisting of inactivated *Mycobacterium tuberculosis* organisms spotted onto filter cards, termed dried culture spots (DCSs), was developed and successfully used to verify and ensure that newly placed instruments during phase I of the implementation ($n = 26$ sites) were "fit for purpose" before clinical specimen testing (3). A website (www.tbgxmonitor.com) was developed to automatically perform statistical analyses and to generate verification reports in real time.

With the ongoing South African national GeneXpert implementation program (4), continuous monitoring and field testing of the DCS program need to be investigated to ensure that the material is not only suitable for the verification of instruments in laboratory settings by skilled personnel but that it is also appropriate for instrument verification in remote nonlaboratory settings, such as clinics, by non-laboratory-trained personnel.

We report here the GeneXpert assay verification results for DCSs in various settings, namely: (i) laboratory instrument verification from the NHLS national implementation program, (ii) clinic instrument verification by non-laboratory-trained personnel, and (iii) instrument verification of GeneXpert assays situated in mobile vehicles for an intensified case finding event for World TB Day 2012 at KDC gold mine in Carltonville, South Africa (see http://www.nhls.ac.za/?page=world_tb_day_2012&id=77). The performance of the DCSs under the most common transport and storage conditions was also evaluated to demonstrate the stability of the material.

MATERIALS AND METHODS

Preparation of DCSs and testing in different settings. The manufacture of the DCSs has been reported previously (3); briefly, it involves growing the culture strain *M. tuberculosis* ATCC 25618 (H37Rv) in a single-cell suspension (5) in bulk, followed by inactivation. Although not reported, prior to DCS panel preparation, all bulk manufactured stock was quantified using flow cytometry, which included quality control parameters for single-cell counting, such as measurement of the percentage of doublets. The material was then spotted with a blue dye (Sigma-Aldrich) onto perforated Munktell specimen collection cards (Lasec, South Africa) as previously described, dried, packed, and sent to the sites (3). Four bulk batches (V005 to V008) of this inactivated single-cell stock were used to prepare DCS panels for the program.

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TABLE 1 Performance of DCSs by bulk stock, assay version, and cadre of staff or testing setting[a]

Performance variable and result	Performance by bulk batch and cartridge type								
	V005				V006		V007		V008
	G3			G4	G3	G4	G3	G4	G4
Setting (operator)	Clinic (nurse)	Mobile (scientist)	Laboratory (technologist)	Laboratory (technologist)	Laboratory (technologist)		Laboratory (technologist)		Laboratory (technologist)
Incubation time for DCSs	15 min	1 h	15 min	15 min	15 min	15 min	15 min	15 min	15 min
Method of DCS resuspension	Hand shaking	Hand shaking	Vortex	Vortex	Vortex	Vortex	Vortex	Vortex	Vortex
Results (no.)	48	79	520	444	24	548	4	504	432
Errors (no. [%])	1 (2.1)	3 (3.8)	8 (1.5)	6 (1.4)	None	9 (1.6)	None	7 (1.4)	7 (1.6)
"No result" (no. [%])			1 (0.4)					2 (0.4)	
Invalid results (no. [%])									2 (0.5)
Rifampin sensitivity results (no. [%])									
RIF sensitive	47 (100)	76 (100)	509 (99.6)	437 (99.8)	24 (100)	537 (99.6)	4 (100)	491 (99.2)	423 (100)
RIF indeterminate						1 (0.2)		3 (0.6)	
RIF resistant			2 (0.4)			1 (0.2)			
M. tuberculosis not detected				1 (0.2)				1 (0.2)	
C_T for probe A (mean [SD])	24.1 (3.3)	16.2 (1.9)	21.6 (3.3)	17.7 (3.5)	22.8 (3.4)	17.3 (3.0)	17.8 (2.1)	21.5 (3.0)	21.4 (3.5)
%CV of the C_T for probe A	13.8	11.8	15.3	17.7	14.8	17.5	11.6	13.8	16.2

[a] The overall SD and error rate for G3 ($n = 660$) were 3.3 and 1.8%, respectively, and for G4 ($n = 1{,}888$) were 3.8 and 1.5%.

At the testing sites, a single-use DCS was tested for each GeneXpert module by pushing the perforated spot into a sterile 50-ml Nunc tube using an additional pipette. A volume of 2.8 ml of sample reagent buffer (SR; Cepheid, Sunnyvale, CA) was added to the spot to resuspend the bacteria. This was incubated for 15 min, unless otherwise stated. During the incubation period, the DCS was mixed by either hand shaking (field test setting) or vortex (laboratory setting). Following incubation, all the SR mixture (>2 ml) was added to the cartridge and tested as per the manufacturer's instructions (6). In January 2012, a new cartridge version, G4, was released for use in the national program. The most prominent modifications to the G3 cartridge included sequence changes to probe B, a new quencher, and minor PCR cycle time reductions.

At the completion of testing, all comma-separated value (CSV) files were uploaded onto the Web-based platform (www.tbgxmonitor.com) by staff performing the verification, and all data were downloaded from the website into MS Excel format. These included the semiquantitative ranges and the cycle threshold (C_T) values, which is the point at which fluorescence from the hybridized probes increases with product amplification and is used as an indication of the bacterial load (7). A C_T of <16 indicates a high bacterial load, 16 to 22 indicates a medium load, 22 to 28 indicates a low load, and >28 indicates a very low load.

DCS batch usage. (i) NHLS laboratories. Four bulk batches, V005 to V008, of the *M. tuberculosis*-positive rifampin (RIF)-sensitive stock cultures were used to prepare DCSs for the verification of GeneXpert modules in NHLS laboratory sites performing the Xpert MTB/RIF assay. A total of 619 DCS panels were sent out to verify 2,476 GeneXpert modules, some of which had already been switched to the newer G4 cartridge. All verification was performed by the technologists who routinely performed GeneXpert testing.

(ii) Primary health care clinics. Batch V005 was used to prepare DCSs for 13 nongovernmental organization (NGO)-funded primary health care clinics using the GeneXpert assay for research purposes. All these clinics are primarily HIV counseling and testing (HCT) sites that provide antiretroviral and TB treatment to patients. Thirteen DCS panels were sent out as follows: 7 sites in region F, Johannesburg, 4 sites in Motlasana District, North West Province, one site at Witkoppen Clinic, and one at Themba Lethu Clinic, both in Johannesburg. Verification at the clinical sites was performed on G3 cartridges by the nurse or lay counselor doing the GeneXpert testing for the study.

(iii) Mobile vehicles. Twenty DCS panels were prepared from batch V005 for verification of 10 GeneXpert 16 module instruments situated in mobile vehicles at the KDC gold mine in Carltonville, South Africa, for an NDoH health and wellness campaign held on National TB Day 2012. Due to electrical power failures on the day of testing, DCSs were incubated in SR buffer for up to 1 h. All verification was performed on G3 cartridges by a scientist. Time constraints on the day caused by the power failures led to only 79 modules being randomly chosen for verification (in an even distribution across the four frames of the instrument).

DCS performance testing: stability over time and temperature. Twenty-seven panels (3 DCSs/card) were prepared from batch V005 for DCS performance testing. DCS panels were packaged in zip-locked plastic packets with a desiccant and stored in either a 4°C refrigerator (range, 4° to 8°C), at room temperature (RT; approximately 25°C), or at 37°C (IncoTherm digital incubator; Labotec, South Africa), with stability evaluations performed at 9 different time points. One entire card (containing 3 DCSs) per temperature was then tested in the Xpert MTB/RIF assay at each time point: 1, 2, 3, 4, 8, 12, 16, 20, and 24 weeks (6 months).

Statistical analysis. The mean cycle threshold (C_T) values, standard deviations (SD), and coefficients of variation (%CV) for probe A (the first probe to bind) (3) were calculated for all DCS Xpert verification and stability results. All errors/invalids/no results findings were described but excluded from the quantitative analysis. The proportions of variability in C_T values across (i) bulk batches (same assay version and operator or setting), (ii) operators and settings (same batch and assay version), and (iii) assay version (same batch and operator or setting) were determined using regression analysis and reported as the R^2 values (%) using Stata 12 software.

RESULTS

Performance of DCSs across bulk batches. The bulk batches V005, V006, V007, and V008, which were tested in similar laboratory settings on the same assay version (G4) by laboratory technologists, excluding errors, gave an overall mean C_T value of 19.4 ($n = 1{,}888$) and overall variability of 3.8 SD (Table 1). Batches V007 and V008 had greater overall mean C_T values (fewer bacteria on average). The overall error rate across all bulk batches (V005 to V008) was 1.5% (29/1,928), with an invalid rate of 0.1% (2/1,928).

Only one false-resistant result was reported for batch V006 (delayed hybridization on probe B), but the module passed verification on a repeat DCS. If batch V005 was used as the reference in regression analysis, the proportion of variability in C_T values explained by batch was approximately 14% (R^2).

Performance of DCS by operator/setting. A total of 647 DCSs (48 at the clinics, 79 at mobile vehicles, and 520 at the laboratory) from the same bulk batch (V005) were performed on the same cartridge assay version G3 but by a different cadre of staff (nurse or lay counselor, scientist, or laboratory technologist) in different test settings (Table 1). Both clinic and laboratory testing had similar variability (measured by the SD), while DCSs tested in the mobile vehicles had a lower SD of 1.9. The latter testing site was the only one where the incubation time in SR buffer increased beyond 15 min to approximately 1 h. In addition, the C_T values from the clinic (where no vortex was used) was greater than that from the laboratory. The error rate was highest for DCSs tested in the mobile vehicles, with only one of these being operator dependent (volume related). The proportion of variability in C_T values explained by setting (and, therefore, the cadre of staff) was 5.6% overall (R^2).

Performance of DCSs across different cartridge assay versions. In order to compare any differences in the assay versions, DCSs tested on the G3 and G4 cartridges from the same bulk batch V005 and in the same laboratory testing setting were compared. Both assay versions showed a similar SD for probe A and similar error rates (Table 1). G3 cartridges generated a higher false rifampin resistance result of 0.4% (1 dropout on probes D and E and 1 delayed hybridization on probe B), but one false *M. tuberculosis*-negative result was reported for the G4 cartridge, probably due to low bacterial load on the DCS. The proportion of variability in the C_T values (mean C_T values, 21.2 for G3 cartridges and 19.4 for G4 cartridges) explained by assay version was 4.2%.

Irrespective of the bulk batch or test setting, DCSs gave an overall SD of 3.3 ($n = 660$) on G3 cartridges and 3.8 ($n = 1{,}888$) on the G4 cartridges. DCSs tested on the older G3 cartridge had an overall higher mean C_T of 21.2 (medium semiquantitative category) versus 19.4 for G4 cartridges (also medium semiquantitative category) but lower overall %CV across the data set (17.5% versus 19.5%). The percent error rate on any G3 cartridge was 1.8% (12/675), whereas G4 cartridges had slightly fewer errors at a rate of 1.5% (29/1,928).

Long-term performance of DCS material: stability testing. A total of 81 DCSs were evaluated to determine the performance of the DCSs under various storage and temperature conditions (Table 2). Across all time points, three errors (signal loss failures) and 3 "no results" (2 due to on-site temporary power failures and 1 syringe motion error) were reported. Of the remaining results ($n = 75$) reported in Table 2, 2 RIF-indeterminate values were reported from two samples yielding very low semiquantitative results. The SD for probe A remained low for all temperatures tested, with the lowest SD (2.7) occurring for DCSs stored at 37°C. Overall, most of the DCS results were in the low semiquantitative category (mean C_T range, 22.6 to 24.7).

TABLE 2 Overall performance data for DCSs after 6 months at various temperatures in batch V005

Performance data	Results at temp of[a]: 4°C	RT	37°C
No. (%) of errors, invalids, or no results	1 (3.7) error	2 (7.4) errors, 2 (7.4) no results	1 (3.7) error
False RIF resistance calls	1 RIF indeterminate	1 RIF indeterminate	None
No. of analyzable results	25	22	26
Semiquantitative results (%)			
Very low	3.9	12.5	7.7
Low	61.5	62.5	76.9
Medium	34.6	25	15.4
High	0	0	0
C_T value for probe A (mean [SD])	22.6 (3.2)	24.5 (4.1)	24.7 (2.7)
%CV of the C_T for probe A	13.9	16.6	10.9

[a] $n = 27$ for each temperature.

DISCUSSION

DCS verification results from the National GeneXpert rollout showed comparable variation (660 G3 cartridges [SD, 3.3] and 1,888 G4 cartridges [SD, 3.8]) to previously reported findings on DCSs (268 G3 cartridges [SD, ≤3.8]) (3). The stability of intact mycobacterial cells on filter paper for up to 6 months at all temperatures (4°, RT, and 37°C) was well within the expected time limits for shipping to testing in a national program. Of the temperatures tested, DCSs were most stable at 37°C, which may be due to an increased number of bacteria being more easily able to resuspend from the paper. One potential limit would be that if GeneXpert users are found to prefer using a liquid external quality assessment (EQA) format instead of using DCSs, stability testing will need to be done on the bulk liquid batches.

The variability in the DCS product was minimal for the differences in assay version (4.2%) and operators or settings (5.6%) but higher between bulk batches (14%). This is to be expected due to the manufacturing process, which ensures single-cell format and spotting procedures. This did not, however, affect the overall mean semiquantitative results reported; all bulk batches, assay versions, and operator results were determined to be in the medium category.

The overall error rate across all batches, regardless of assay version, was 1.5%. This is currently below the average failure rate for the national program (~3%) (8), indicating that it is the minimum error rate to be expected in a national program.

The false rifampin resistance result generated from the DCS material was 0.1% (3/2,548), highlighting the overall good performance of the Xpert MTB/RIF assay. It is worth noting that two of these were due to the reduced probe B hybridization that is typical of the G3 cartridge. A total of four *M. tuberculosis*-positive/RIF-indeterminate values (stocks V006 and V007) and two *M. tuberculosis* negatives (stocks V005 and V007) were reported. These findings were most likely due to variable amounts of bacteria being spotted onto each DCS due to problems with retaining the cells in a homogenous suspension during the postmanufacture spotting process. Earlier bulk batches showed increased clumping during the inactivation process, which was subsequently corrected for all new batches.

The use of DCSs has also proven to be accurate (with accept-

able variability) and feasible for the verification of GeneXpert instruments in clinics, at the point of care (POC), by non-laboratory-trained personnel, although it highlights the need for the verification program to supply extra consumables (50-ml Nunc tubes and a pipette to push out the perforated DCSs) at an additional cost. Furthermore, a comparison between operators or settings with the newer G4 assay version cartridge would be beneficial.

The robustness of the DCS program was further demonstrated by its use at an outdoor NDoH campaign on World TB Day 2012, held at a mining community in Carltonville, South Africa. DCSs were used to verify 10 GeneXpert instruments situated in 5 mobile vehicles, but due to a power failure, the DCSs already prepared for use could not be tested following the 15-min incubation time. The lower mean C_T value observed was due to the increased number of bacteria resuspended off the filter paper after the longer incubation time. This effect was similarly shown in the lower C_T value that was observed for the laboratories where a vortex was used to resuspend the bacteria off the filter paper compared to the clinic, where hand shaking was used (C_T values of 24.1 versus 21.6, respectively).

The data presented in this study show the suitability of DCSs for GeneXpert instrument verification in all settings across a national program of broad geographic coverage and by all cadres of testing staff.

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4.4 A PILOT EVALUATION OF EXTERNAL QUALITY ASSESSMENT OF GENOTYPE MTBDRPLUS VERSIONS 1 AND 2 USING DRIED CULTURE SPOT MATERIAL

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A Pilot Evaluation of External Quality Assessment of GenoType MTBDR*plus* Versions 1 and 2 Using Dried Culture Spot Material

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Dried culture spots (DCS) of inactivated *Mycobacteria strains* designed as part of an external quality assessment (EQA) program for the GeneXpert system has applications to other molecular tuberculosis (TB) diagnostic platforms. DCS tested on the GenoType MTBDR*plus* and *Mycobacterium* CM assays performed well with MTBDR*plus* version 2 but require increased bacterial concentration for use with version 1.

Quality assurance (QA) refers to an umbrella of quality checks within a medical laboratory encompassing the entire testing process from specimen collection to result reporting (i.e., preanalytical, analytical, and postanalytical steps) to ensure high-quality testing (1). External quality assessment (EQA), sometimes referred to as proficiency testing, is just one component of a QA program and is defined as a system for objectively checking the performance of a laboratory using an external agency or facility (2). This is often challenging and costly for tuberculosis (TB) laboratories but is particularly exacerbated in low-resource settings (3), which may not always be equipped with an adequate level of biosafety to receive live *Mycobacterium tuberculosis* (MTB) cultures, and stringent requirements for transportation of such materials exist (4). Several TB EQA molecular schemes exist (http://www.cap.org; 5, 6), but some provide the EQA material in a format containing preextracted DNA, making them inappropriate for the monitoring of the entire testing process from extraction to detection.

To address the complexities of ongoing molecular diagnostic EQA systems in TB laboratories, a verification (fit-for-purpose) (7) and EQA program (pre-/postanalytics) (8) consisting of dried culture spot (DCS) material was developed for the molecular GeneXpert instrument performing the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) (9). The major advantage of this approach is that the DCS contain whole, inactivated, and quantitated mycobacterial strains that can be safely transported as one would transport documents by mail, without the requirement of cold-chain transport. The spots are easy to use and robust, with a minimum shelf life of 9 months confirmed to date (10).

The DCS EQA program has been successfully implemented in 207 National Health Laboratory Service (NHLS) GeneXpert testing laboratories in South Africa and 82 non-NHLS sites in 21 countries. The Global Laboratory Initiative (GLI) (advisors to the World Health Organization) has also endorsed the DCS for verification of the GeneXpert instrument. As the DCS material is not platform specific, we determined the potential application of the GeneXpert DCS program for the molecular line probe assay

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TABLE 1 Initial results on use of the DCS material on the GenoType MTBDR*plus* versions 1 and 2 tested in a research laboratory

No. of DCS tested by GenoType MTBDR*plus* version[a]	DCS strain	Observed result on GenoType MTBDR*plus* assay (no. observed/total no. [%])
Version 1		
8	*M. kansasii*	16/16 (100) negative
8	*M. intracellulare*	16/16 (100) negative
16	*M. tuberculosis* RIF^r, INH^s	15/16 (94) positive, RIF^r INH^s; 1/16 (6) negative
16	*M. tuberculosis* RIF^s, INH^s	8/16 (50) positive, RIF^s INH^s; 2/16 (12.5) positive, RIF and INH inconclusive; 6/16 (37.5) negative
Version 2		
8	*M. kansasii*	24/24 (100) negative
8	*M. intracellulare*	24/24 (100) negative
8	*M. fortuitum*	24/24 (100) negative
40	*M. tuberculosis* RIF^r, INH^s	40/40 (100) positive, RIF^r INH^s
16	*M. tuberculosis* RIF^s, INH^s	16/16 (100) positive, RIF^s INH^s

[a] Number of DCS tested per strain dependent on available stock.

TABLE 2 Pilot EQA GenoType MTBDR*plus* results for the DCS prepared and tested in four routine service laboratories

No. of sites by GenoType MTBDR*plus* version	No. of DCS tested	DCS strain	Observed result (no. observed/total no. [%])
Version 1			
2	6	*M. intracellulare*	No reportable result (blank strips)
	6	*M. tuberculosis* RIF^r, INH^s	No reportable result (blank strips)
	12	*M. tuberculosis* RIF^s, INH^s	No reportable result (blank strips)
Version 2			
2	6	*M. intracellulare*	6/6 (100) negative
	6	*M. tuberculosis* RIF^r, INH^s	5/6 (83) positive, RIF^r INH^s; 1/6 (17) positive, RIF^r INH^s (WT7 present, MUT2A present)
	12	*M. tuberculosis* RIF^s, INH^s	12/12 (100) positive, RIF^s INH^s

(LPA), Genotype MTBDR*plus* versions 1 and 2 (Hain Lifescience, GmbH, Nehren, Germany).

DCS panels (each comprising 4 spots) were initially tested in a research laboratory in Johannesburg and then piloted in four routine service TB laboratories in South Africa, namely, the Mycobacteriology Referral Laboratory, Johannesburg; Ampath, Pretoria; Lancet, Johannesburg; and Centre for Clinical Tuberculosis Research (CCTR) Laboratory, Task Applied Science, Cape Town.

The Xpert MTB/RIF DCS panels (7), comprising *M. tuberculosis* rifampin (RIF)-resistant (Xpert probe D mutant)/isoniazid (INH)-susceptible, *M. tuberculosis* RIF-susceptible/INH-susceptible, and nontuberculous mycobacteria (NTM) (namely *M. kansasii*, *M. intracellulare*, and *M. fortuitum*), were tested on the MTBDR*plus* versions 1 and 2 . This involved resuspending the DCS in a 50-ml Nunc tube containing 2 ml of phosphate-buffered saline (PBS) for 15 min, with intermittent vortexing. This was followed by manual extraction (for MTBDR*plus* version 1) or GenoLyse extraction (for MTBDR*plus* version 2) of the entire lysate, as per the manufacturer's instructions. The extracted material was then amplified and hybridized as per the standard manufacturer's protocol for versions 1 and 2. The species identifications of the negative results for the MTBDR*plus* version 2 (TUB band absent) were determined using the GenoType *Mycobacterium* CM assay (Hain Lifescience, Germany).

Table 1 lists the observed findings for the initial research laboratory testing and Table 2 for the pilot site testing.

The species of all DCS that tested negative on version 2 of the LPA in the research laboratory (Table 1) were further identified with the GenoType *Mycobacterium* CM assay. Of the eight *M. kansasii*, *M. intracellulare*, and *M. fortuitum* spots tested, the species of 6 from each were successfully identified (18/24 [75%]).

The *M. intracellulare* DCS tested on LPA version 2 at two of the pilot sites (Table 2; $n = 3$ per site) were also tested on the *Mycobacterium* CM assay, and the species of 5/6 (83%) were correctly identified. One DCS (17%) was incorrectly reported as *M. avium*.

Although partially successful in the initial laboratory testing phase, the two pilot sites performing version 1 failed to produce interpretable results. This was due to the low bacillary load on the DCS designed for detection at a minimum of 150 CFU/ml, which is the lower limit of detection of the Xpert MTB/RIF test (9). Since the LPA version 1 is validated only for use with smear-positive specimens, which has a limit of detection of 10,000 bacteria/ml[3] (11), it is feasible to accept that version 1 will not detect *M. tuberculosis* on the DCS below this lower limit.

The reverse is true for the use of DCS with the MTBDR*plus* version 2 assay, as all (80/80) DCS results from the research laboratory and 96% (23/24) of the pilot site results were correctly reported. Rifampin resistance was identified by an *rpoB* wild-type 7 (WT7) missing band and a mutant 2A (MUT2A) band present by the LPA, which corresponds to the Xpert MTB/RIF probe D mutant. One of the pilot sites detected an RIF-resistant DCS as having a WT7 band and MUT2A band present. This may have been due to incorrect interpretation or contamination, but the finding was not considered clinically relevant. Of the three sites (one research laboratory and 2 routine service laboratories) that performed *Mycobacterium* CM assay testing on the negative results by MTBDR*plus* version 2, only one incorrect species identification was reported, possibly due to a mislabeled laboratory specimen.

Overall, the DCS program appears to be suitable not only for the Xpert MTB/RIF assay but also for the MTBDR*plus* assay version 2, due to their similar sensitivities, and it provides measurements for the entire testing process (DNA extraction through result reporting), as opposed to just the amplification and hybridization steps on DNA-prepared EQA materials. The added advantage of the DCS technology is the ability to transport inactivated tuberculosis-positive specimens at room temperature, thereby reducing the overall program costs. Even though the GenoType MTBDR*plus* version 1 assay has been discontinued in several countries (Hain Lifescience, South Africa [Pty], Ltd., personal communication), if laboratories wish to use the DCS with this version, an increased bacterial concentration will need to be spotted onto the paper card.

Currently, the result reporting of the GeneXpert MTB/RIF EQA DCS program is automated and managed remotely through TBGxMonitor for real-time turnaround of the results, but it could be expanded to provide a more automated result reporting for the MTBDR*plus* assays. Other *M. tuberculosis* strains could also be included in the DCS panels that are suitable for second-line drug resistance testing, such as those with the MTBDR*sl* assay (Hain Lifescience, GmbH, Nehren, Germany).

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CHAPTER 5: DETERMINING THE FEASIBILITY OF MULTIPLE POCT FOR HIV AND TB SERVICE INTEGRATION IN THE FIELD

Once assay validations were complete and the various components of a quality management system were developed, the feasibility of a nurse performing multi-disciplinary POCT in a clinical setting was evaluated. This also included acceptance criteria by patients as well as the practical considerations for performing multiple POCT.

5.1 IMPLEMENTATION OF MULTIPLE POINT OF CARE TESTING IN TWO HIV ANTIRETROVIRAL TREATMENT CLINICS IN SOUTH AFRICA (SUBMITTED TO JAIDS)

Title page

Manuscript title: Implementation of multiple point-of-care testing in two HIV antiretroviral treatment clinics in South Africa.

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Conflict of interest and sources of funding

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Running title: Multidisciplinary POCT implementation

Abstract:

Background: A plethora of point-of-care (POC) tests exist in the HIV and TB diagnostic pipeline which require rigorous evaluation to ensure performance in the field. The accuracy and feasibility of nurse-operated multidisciplinary-POC testing for HIV antiretroviral therapy (ART) initiation/monitoring was evaluated.

Methods: Random HIV-positive adult patients presenting at two treatment clinics in South Africa for ART initiation/monitoring, were consented and enrolled. POCT was performed by a dedicated nurse on a venepuncture specimen; Pima (CD4), HemoCue (haemoglobin), Reflotron (alanine aminotransferase, creatinine), Accutrend (lactate) and compared to laboratory testing using the Bland-Altman and percentage similarity methods of agreement. External quality assessment (EQA), training, workflow and errors were assessed to determine feasibility of POC testing.

Results: n=324 enrolled at site1; n=469 enrolled at site2. Clinical data on n=305 participants: 65% (n=198) female with a mean age of 39.8[range 21-61] years; mean age of males 43.2[range 26-61] years; 70% of patients required 3 or more POC tests/visit. EQA material was suitable for POCT. CD4, haemoglobin and alanine aminotransferase testing showed good agreement with predicate methodology; creatinine and lactate had increased variability. Pima CD4 misclassified up to 15.6% of patients at 350cells/μl and reported 4.3-6% error rate. A dedicated nurse could perform POCT on 7 patients/day; inclusion of Pima CD4 increased time for testing from 6 to 110minutes. Transcription error rate was 1%.

Conclusions: Multi-disciplinary POCT is accurate and feasible for ART initiation/monitoring if performed by a dedicated nurse but will increase duties. Use of Pima CD4 will increase patients initiated on ART. Connectivity will be central to ensure quality management of results but overall impact will need to still be addressed.

Keywords

Multiple-disciplinary; point-of-care testing; HIV; antiretroviral; implementation

Introduction

Laboratory systems and services are critical in global health and point-of-care testing (POCT) may have a place within this framework to address unmet diagnostic needs, especially in resource-limited environments [1-3]. Appropriate clinical management of ill patients presenting at primary health care clinics (PHC) remains a global health challenge and lack of accessibility to an appropriate laboratory diagnosis is a major reason why health services are failing. A recent report in the New England Journal of Medicine describing 20 years of health care in South Africa, stated that improving access to health care requires restructuring and strengthening of existing district-based PHC facilities, with nurses and community healthcare workers (HCWs) playing an increasingly important role in remote areas [4].

POCT is currently the fastest growing market in medical diagnostics with many innovative technology developments [1, 5]. The purpose of POCT is to provide a test that has immediate impact on patient outcome [6] and that can be used in outpatient clinics, emergency rooms, theatres, mobile clinics, PHC clinics, or even small laboratories [7]. The potential benefits for POCT identified in low to middle income countries are to avoid patient hospitalisation (or reduce length of stay), help manage chronic conditions [3, 7, 8], improve accessibility of services, reduce turnaround times, potentially improve patient retention and improve staff convenience and satisfaction [9-13].

A number of disadvantages also exist, such as the poor regulatory control [14], lack of connectivity, inadequate quality control and assurance [15, 16], a potential increase in cost and over-use (duplication) of existing laboratory and POC services, as well the need for appropriately trained POC operators [17]. Several guideline documents detailing the requirements for POCT exist [18-20] and all emphasise the need for quality in POCT. The complexities of managing quality of the entire process (pre-analytical, analytical and post-analytical) however, are well described for glucose testing [21], but less so elsewhere.

POCT for infectious diseases in the developing world are largely in the form of strip-based lateral flow assays for the diagnosis of syphilis, malaria and HIV [14]. The massive expansion of anti-retroviral (ARV) therapy (ART) in lower and middle-income countries has relied entirely on the use of one or more rapid tests for diagnosing HIV, which are frequently performed by lay counsellors or lower-level HCWs. In many countries, this has facilitated expansion of services where laboratories were inadequately equipped to handle the volume of testing or where infrastructure hampered the safe and timeous transportation of biological samples [22, 23]. In South Africa, the track record for HIV rapid testing at POC has been challenged with studies showing poor compliance to standard operating procedures and poor quality management [24].

In addition to rapid strip-based testing for diagnosis of HIV, ART initiation has relied on CD4 count testing for treatment initiation. The value of the CD4 assay is under scrutiny with treatment thresholds increasing to 500cells/µl and more emphasis being placed on viral load testing for monitoring treatment success [25, 26]. ART treatment guidelines in South Africa [27] also include additional diagnostic assays prior to ART initiation: Alanine aminotransferase (ALT), creatinine (Cr) (for Tenofovir usage), haemoglobin (Hb) (particularly if Zidovudine is used) and hepatitis B antigen. This highlights the need for a multi-disciplinary array of testing requirements for ART initiation and subsequent monitoring.

Prior work has alluded to the fact that the expanded repertoire of assays could possibly be performed by nurses or other HCWs at POC [28]. A limitation to this process however, in many countries including South Africa, has been the need for phlebotomy skills for which the scope of work is defined for a certain cadre of nursing staff and is mandated by the Health Professions Council of South Africa (HPCSA) [29]. The use of finger stick testing can eliminate the need for skilled phlebotomists, but for HIV treatment could mean patients may require up to four finger sticks for POCT at each visit [30], over and above the initial two finger sticks required for HIV rapid testing.

While numerous reviews are available on the use of POCT and the need in resource limited settings [2, 31, 32] in the HIV testing arena, few have dealt with logistics around sample collection, connectivity, result reporting or whether improving logistics would be more cost-effective. In addition, most of the new POC technologies have limited field evaluation and few studies have evaluated clinical outcome, impact on overall health care, cost effectiveness and cost benefit of POC, especially in the developing world and specifically for multi-disciplinary POCT. To address some of these issues, we implemented nurse operated multiple POCT in two busy ART clinics in South Africa, to assess the feasibility and accuracy of the process.

Methods

This project formed part of a Grand Challenges Canada study (grant # 0007-02-01-01-01). Human Ethics was obtained and approved through the University of the Witwatersrand, (M10333) and the University of Pretoria, South Africa (M090688).

Clinic sites

All laboratory validations, training and research and development were performed at the National Health Laboratory Service (NHLS) Department of Molecular Medicine and Haematology in Johannesburg, South Africa. The clinic phase validation to determine nurse operated POCT versus laboratory testing was performed in two well managed urban ART PHC sites: a) non-governmental organisation (NGO) supported Themba Lethu Clinic, part of the Clinical HIV Research Unit (CHRU) located within the infrastructure of the Helen Joseph Hospital, Right to Care in Johannesburg, which manages 21 000 HIV infected patients on ARV treatment [33]; b) Comprehensive Care Management and Treatment (CCMT) Clinic, Tshwane District Hospital in Pretoria. Both clinics enrol more than 350 patients per month, 5 days a week and both are within walking distance from high throughput routine laboratories managed by the NHLS. A POCT laboratory was established in each clinic in a dedicated room adjacent to the consultation rooms. The POCT instruments were installed by suppliers

as per good clinical laboratory practice guidelines (GCLP) [34]. Reagent kits were procured and stored appropriately. Three dedicated research nurses (professional nurses experienced in phlebotomy and HIV/Tuberculosis (TB) treatment) were employed and trained on all POC instruments by the instrument suppliers and local laboratory staff. In all sites selected, provincial approval and support was obtained and dedicated staff and instrumentation were placed to support all the project activities.

Participant eligibility for POCT

Individuals (>18 years of age, with known HIV-positive status) were approached for enrolment in the study when they presented for routine phlebotomy related to their HIV ARV initiation and monitoring at the clinics. The nurses conducted informed consent, enrolled participants and performed the required phlebotomy. The selection of POCT was based on the SA HIV treatment guidelines at the time of the study [35], and included the following tests: CD4 for ART initiation, followed by Cr, ALT and Hb. Lactate was included but rarely requested (stavudine usage high at time of study). Each participant consented to providing an additional $EDTAk_3$ (for CD4, Hb, Cr and lactate) and/or a Heparin tube (for ALT), which was used for on-site POCT. The POC platforms were the PIMA (Alere, Inc., Waltham, MA, USA) for CD4; HemoCue DM201 (HemoCue AB, Ängelholm, Sweden) for Hb; Reflotron Plus (Roche Diagnostics, GmbH, Germany) for ALT and Cr; and COBAS Accutrend Plus (Roche Diagnostics, GmbH, Germany) for Lactate. The selection of these POCT platforms was based on the POC diagnostic pipeline document at the time[36], available literature [28, 37-41] and in-house validations.

Post-phlebotomy, the nurses took the additional blood tube to the POCT room in the clinic, opened the vacutainer tube and performed POCT. A fixed volume pipette supplied with the Reflotron (Roche Diagnostics) was used to dispense the required blood volume onto the rapid strips (ALT, Cr and lactate) or to dispense blood into the Hb microcuvette or PIMA cartridge. Results were manually recorded in a log book. At CHRU, the nurse also used a Vacudrop (Greiner Bio-One, Dublin, Ireland) device which allows the withdrawal of a single

drop of blood from a closed blood tube, similar to a finger stick. This method was investigated to determine the performance of the POC tests without the use of a pipette. POC results were not used for clinical management. The routine bloods were sent as per standard-of-care to the NHLS laboratories for routine laboratory testing. These reference result values were made available to the study through the NHLS laboratory information system and used for patient management.

POC instrument verification and quality management

After placing the POCT platforms in the clinic sites and prior to their use, each platform (Pima CD4, HemoCue and Reflotron) was verified using 25 specimens (ensuring they were "fit for purpose" as part of GCLP requirements)[42]. The verification specimens were randomly collected from routine, residual patient specimens in the adjoining NHLS reference laboratories. As lactate measurements need to be performed immediately upon blood collection, the first 25 patients recruited on the study requiring a lactate test, were used as verification material for the Accutrend Plus instrument.

For quality monitoring throughout the study, quality control (QC) material was tested on each of the instruments according to manufacturer's instructions by the POC nurses. A log sheet was used to record QC test results. In addition, one round of external quality assessment (EQA) was performed at the CHRU clinic on the PIMA CD4, HemoCue and Accutrend instruments using EQA material obtained from the NHLS EQA regional and national programs. Results were sent to the NHLS EQA Division for independent analysis and reporting (Z-scores of <2 were considered acceptable). No NHLS EQA material was available for the Reflotron instrument as this is a dry chemistry based system compared to the laboratory platforms which use wet chemistry.

Comparator laboratory testing platforms

NHLS derived results were considered the reference standards against which the POC results were compared, these included: CD4 single platform Pan*Leuco*gating method using

flow cytometry (Beckman Coulter, Miami, FL); Advia 120 and 2120 Heamatology system for Hb (Siemens, Diagnostic Solutions, Tarrytown, NY); Advia 1800 Chemistry analyser (Siemens Healthcare Diagnostics, Inc, Germany) and Synchron DXC 800 (Beckman Coulter, Miami, FL) for ALT and Creatinine; Advia 1800 for Lactate.

Statistical analysis

The performance of multidisciplinary POCT performed by nurses directly in clinics was compared to laboratory generated reference results using the Bland-Altman [43] and percentage similarity [44] methods of agreement. Accuracy was measured using the bias (laboratory reference - POCT) and this was reported in the context of the data sets summarised by their median values. Confidence intervals (CI) at 95% were included. Overall agreement between the laboratory reference and POC results was measured using the percentage similarity coefficient of variation (CV), which includes accuracy and precision. Total misclassification (false positive and false negative compared to predicate) was reported for CD4 counts at the 350cells/µl level and included sensitivity and specificity (including 95% CI) for completeness. Instrument errors were recorded. Functions were performed using STATA 12. Scatter plots were used to represent outliers in the clinically relevant ranges for each analyte. Normal ranges for each analyte were determined by the NHLS reference technology as follows: Hb 12–18g/dl; ALT 10-40U/l; Cr 64-104umol/l, lactate <2.2mmol/L. Royal College of Pathologists of Australasia (RCPA) allowable differences [45] were also applied to determine outliers: Hb ±0.5 <10g/dl and ±5% >10g/dl, ALT ±5 ≤40 U/l and ±12% >40U/l, Cr±8 <100umol/l and ±8% ≥100umol/l, lactate ±0.5mmol/L ≤4mmol/L and ±12% >4mmol/L.

Qualitative data (measured prior to and during the study) of the multiple POC implementation process addressed workflow and feasibility issues, rather than simply comparative laboratory data. These variables included: training and additional consumable needs, length of time and number of added duties required to perform multiple POCT and number of transcription errors during manual result recording.

Results

Data summary

A total of 324 patients were approached to participate over a 6 month period at CHRU (December 2010 to June 2011) and 469 patients at CCMT over 1 month (January 2012 to February 2012). No patient declined participation. Clinical data was available for 305 CHRU study participants as follows: 65% (n=198) of the patients were female; mean age of females was 39.8 [21-61] years and males was 43.2 [26-61] years; mean number of days on ART at the time of POC testing was 833 days for men and 764 days for women. Patients on first line ART: n=175; the remainder were either not on therapy (being initiated on ART) or had missing demographics at time of blood draw.

The number of diagnostic tests requested by HCWs for patients at a particular visit attending the CCMT site is represented in the pie chart in Figure 1 and shows that 70% (325/464) of patients required 3 or more POCT to be performed per visit.

Method comparison of POCT versus laboratory testing

All POCT platforms placed in both clinics passed verification using 25 laboratory specimens (and clinical specimens for lactate). No QC failures were observed on any of the instruments and 100% compliance was obtained by the POC nurses in performing instrument QC as reflected in Table 1. EQA material tested on the PIMA, HemoCue and Accutrend showed results to be within acceptable limits despite material not being specific for all POC instruments. One sample for each instrument tested flagged outside the reference range according to the Z-score of >2, but the values were not in the clinically relevant range.

Table 2 and Figure 2 detail the method comparison statistics and scatter plots of nurse operated POCT compared to laboratory reference results. Pima CD4 testing performed at CHRU had a bias of 26cells/µl which showed better accuracy than testing performed at CCMT (54cells/µl), in spite of their similar CD4 results (similar CD4 median and range). CD4

testing performed at CCMT also yielded more misclassification at the 350cells/μl threshold than testing performed at CHRU however at both sites, misclassification of CD4 using Pima would have resulted in more patients identified for ART initiation. The Pima CD4 error rates at both sites were similar.

The performance of ALT and lactate POCT was variable and significantly different between the sites (CI did not overlap), but the majority of specimens were within the clinically relevant range, and would not have resulted in a change in clinical management.

The bias for Hb and creatinine testing was accurate and similar at both sites. POC testing of CD4, Hb and ALT using the VacuDrop at CHRU generated acceptable bias values similar to the main study at this site.

Qualitative analysis multi-disciplinary POCT

POCT ease of use: Practical training for the nurses on all the POCT instruments took approximately half a day per instrument and included sample testing, performing QC, instrument maintenance and troubleshooting. Additionally, the POC nurse had to be trained on general laboratory safety, handling of a pipette, waste disposal and laboratory spill clean-up. Apart from hard copy standard operating procedures provided to each POC testing laboratory, it was found that quick reference charts containing visual aids were preferred. Upon interview of the nurses after the study, no difficulties in performing the individual POCT were reported. Both the Reflotron pipettes and Vacudrop were easy to use. However, with the Vacudrop there was no guarantee or quality measure to ensure single use only.

***The need for additional consumables to perform multiple POCT*:** Several POCT consumables were required which were not typical to the clinic environment. These were: pipettes (10-100μl) (requiring calibration) and sterile pipette tips (universal: 10-100μl) for blood sample dispensing, parafilm/plastic for Hb microcuvette filling and specimen racks for transport of blood tubes to the POCT laboratory. To ensure safety, several additional components were required: laboratory coat, non-powdered gloves, suitable disinfectant,

ethanol and bleach for instrument cleaning, measuring cylinder for preparing cleaning reagents, wash bottles for storing reagents and paper towel. Miscellaneous items required were: multi-plugs, network cables, fridge for EQA/QC material storage, paper for result printing.

***The number of added duties required to perform multiple POCT*:** Table 3 lists the general responsibilities (duties) required by nurses in HIV ART clinics (not specific to the two clinic sites in our study) and then the additional list of duties that the nurse would perform if they were responsible for POCT, as seen in our study.

The length of time to perform the multiple POCT: This was measured at the CHRU site (n=160) and showed the earliest time a POCT was performed was 9:30am (median 11:00; which did include patient consent and enrolling). The latest time a POCT was performed was 16:26pm (median 12:25). The median time from starting the first POCT to starting the last POCT varied, depending on the number and type of tests requested; when CD4 was included, 4 tests took 1hr47minutes; when CD4 was not included, 3 tests took 6minutes. The median number of patients that could be consented, enrolled, bled and tested by one study nurse in one day was 7 (minimum 2, maximum 12). It should be noted that this was under circumstances where dedicated nursing staff were placed at sites to only perform this study.

***POCT errors*:** At the CHRU site, the PIMA CD4 instrument reported 9 errors (6%). Channel filling error was reported once and device application errors were reported 8 times. CCMT reported an error rate for the PIMA CD4 up to 4.3% (13/302) of which only one specimen could not be resulted on repeat testing. No errors or invalids were reported for the HemoCue, Reflotron and Accutrend. A total of five (5/469) transcription errors (incorrect value) were discovered during the statistical analysis at the CCMT site and three (3/324) at the CHRU site, totalling 1%.

Discussion

A plethora of POC technologies are in the HIV and TB diagnostic pipeline which will require rigorous evaluation to assess performance in the field. This is the first study in South Africa to investigate nurse operated multi-disciplinary POCT for ART initiation and monitoring in a clinical site. In our setting, nurses were easily trained on multiple POCT platforms placed in a dedicated POC testing room, with minimal disruption to clinic workflow. The combination of training materials developed was effective in ensuring competency.

Although POCT has been shown to reduce errors in only a few steps of the entire testing process compared to laboratory testing, quality and risk management is still required [46]. To this end, our dedicated nurses showed 100% compliance on the test specific QC procedures from each of the POC manufacturers. In addition, NHLS EQA material was also trialled on the Pima, HemoCue and Accutrend and demonstrated suitability on POC instruments tested performed by non-laboratory trained staff. EQA in future will be an important component of ensuring quality management of the entire POCT process but may require development or modification before scale up of current services for POCT sites. In South Africa, the South African National Accreditation System (SANAS) has ISO guidelines for medical testing laboratories (ISO 15189) and more recently, specifically for the implementation of POCT (ISO 22870).

Overall, 70% of our study patients required 3 or more tests per visit in both clinic settings. This raises several issues if multiple POCT is to be implemented. Firstly, if a venepuncture specimen is used, which has been shown in some circumstances to be more accurate [39], this could be used for repeat testing and/or referral of residual blood to the laboratory in the case of test failures. Venepuncture will however, require skill in phlebotomy and qualification as HPCSA/SANAS/National Department of Health/NHLS regulated personnel (at least in South Africa where the study was conducted), as well as extra training on opening blood specimens in a “non-laboratory” environment (or use of the Vacudrop). A further challenge will be to minimise duplication in services. Capillary sampling is easier to perform by “non-

regulated" operators and will allow task shifting and decentralisation [47], but would require multiple finger sticks per patient per clinic visit a process which has been shown to be accurate [39].

The accuracy of nurse operated multiple POCT was demonstrated. Hemocue Hb testing in particular, showed excellent agreement with predicate methodology and the same was true for CD4 and ALT. Cr and lactate at POC showed increased variability. This may be due to variability between operators (two different nurses performed POCT at CCMT site) and some seasonal variability (some testing done in winter at CHRU site, possibly leading to colder hands, poorer blood flow) [30]. In spite of quality systems in place at the POCT sites, error rates (4.3-6%) were evident from the PIMA CD4 instrument only, but were in line with other studies [38, 48]. This together with characteristics of the CD4 technology platform (Pima CD4 over-misclassification) will need to be taken into consideration before implementation.

The length of time taken to perform and result multiple POCT would require workflow considerations within the clinic. In this study, at least 22 extra duties were required by a NIMART (nurse initiated management of antiretroviral treatment) trained nurse [49]. This will further increase with the addition of screening tests such as cryptococcal antigen and hepatitis B antigen [25]. An increase in workload leads to increased transcription errors and even in this study, where dedicated nurses performed POCT without any NIMART duties, transcription errors occurred. The need for centralized data monitoring and the ability to interface with information systems is required [10, 50] to ensure that data can be audited and managed.

For wide-scale implementation of POCT in South Africa, the first step will need to be identification of appropriate clinics where there are gaps in service delivery. This is already being done through Geographical Information System (GIS) mapping tools, not only to

identify gaps but also decide on the most cost-effective implementation strategies. The role of connectivity, cost-effectiveness and overall impact of POCT remain to be addressed through a randomised controlled trial.

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Tables

Table 1: Internal quality control (QC) results for CD4, Hb, ALT, Creatinine and lactate analytes performed by nurses at two POCT sites and external quality assessment (EQA) performed at one POCT site.

Table 2: Method comparison summary statistics of nurse operated POCT for CD4, Hb, ALT, Creatinine and lactate versus laboratory generated reference results. The sections highlight independent studies performed at two different clinic sites (CHRU and CCMT) using venepuncture derived specimens. A section is included for the use of the Vacudrop at one site.

Table 3: List of general clinic duties for nurses in HIV ART clinics and the added duties required for performing multiple POCT.

Figure Captions

Figure 1: A pie chart representing the percentage of tests requested by HCW on their patients (n=469) attending the CCMT clinic. The pie chart reflects the number of POCT tests required per visit from 464 patients (n=5 not recorded).

Figure 2: Scatter plots of method comparison of POC testing arms for the two sites compared to routine laboratory results. The vertical axis in each plot is either percentage similarity (A) or difference (B-E) between POC and routine and the horizontal axis is the absolute value of predicate tests: A) Percentage similarity for CD4. Red dashed line indicates the 350 cells/ul threshold. B-E) Bland Altman difference scatter plots for POC versus predicate methodology for Hb (B), ALT (C) and Cr (D) and lactate (E). Grey areas indicate normal ranges for analytes based on reference method.

Tables

Table 1: Internal quality control (QC) results for CD4, Hb, ALT, Creatinine and lactate analytes performed by nurses at two POCT sites and external quality assessment (EQA) performed at one POCT site.

Analytes	CHRU		CCMT
	QC	EQA	QC
PIMA CD4	Daily low (n=26), high control (n=26), 0 failures, 100% compliancy	Survey 1 Trial 1 sample A: Error (internal cartridge error) Survey 1 Trial 1 sample B: 110c/ul (Z-score -0.68) Survey 2 Trial 2 sample A: 573c/ul (Z-score -2.05)*	Daily low (n=33), high control (n=33), 0 failures, 100% compliance
HemoCue Hb	Weekly High (n=5), normal (n=6), low (n=6)., 0 failures, 100% compliancy	0710W: 9.3g/dl (Z-score 1.94) 0710X: 8.0g/dl (Z-score 2.46) 0810W: 9.5g/dl (Z-score 0.12) 0810X: 11.2g/dl (Z-score 1.65)	Weekly High (n=7), normal (n=7), low (n=6). 0 failures, 100% compliance
Reflotron Cr	One monthly universal control (n=4) 0 failures, 100% compliancy	No POCT EQA material available from NHLS	One monthly universal control (n=7) on each instrument, 0 failures, 100% compliance
Reflotron ALT	One monthly universal control (n=4), 0 failures, 100% compliancy	No POCT EQA material available from NHLS	One monthly universal control (n=7) on each instrument¥, 0 failures, 100% compliance
Accutrend Lactate	Monthly low (n=3), high (n=3) controls, 0 failures, 100% compliancy	Sample 1: 3.8mmol/l (Z-score 1.7) Sample 2: 0.8mmol/l (Z-score 0.64) Sample 3: 4.5mmol/l (Z-score 2.5)	Monthly low (n=32), high (n=32) controls per instrument¥, 0 failures, 100% compliance

Values underlined are>z-score 2, however *not in the clinically relevant range; ¥ two instruments were used during the study time frame

Table 2: Method comparison summary statistics of nurse operated POCT for CD4, Hb, ALT, Creatinine and lactate versus laboratory generated reference results. The sections highlight independent studies performed at two different clinic sites (CHRU and CCMT) using venepuncture derived specimens. A section is included for the use of the Vacudrop at one site.

Venipuncture POCT at CHRU	**CD4 (cells/ul)**	**Hb (g/dl)**	**ALT (U/l)**	**Cr (umol/l)**	**Lactate (mmol/l)**
n	152	157	146	156	93
Median routine lab	361	14	23	68	2.3
Bias* (95% CI)	26 (16; 36)	-0.3 (-0.36; -0.14)	7.4 (5.6; 9.0)	4.5 (2.09; 6.95)	-0.01 (-0.13; 0.1)
Mean % similarity (CV)	97 (8.1%)	101 (3.1%)	90 (11.5%)	97 (10.9%)	104 (14.7%)*
CD4 total miss-classification	9.85% (false positive 9.2%), 6% errors				
n (using a VacuDrop)	82	71	22*	Not done	Not done
Median routine lab	432	13.5	18.5		
Bias* (95% CI)	-7.2 (-15.8; 1.5)	-0.3(-0.43; -0.2)	9.62(1.79; 17.4)		
Mean % similarity (CV)	103 (11%)	101 (2.0%)	86 (13.3%)*		
CD4 miss-classification	7.3% (over = false positive 3.6%)				

Venipuncture POCT at CCMT	**CD4 (cells/ul)**	**Hb (g/dl)**	**ALT (U/l)**	**Cr (umol/l)**	**Lactate (mmol/l)**
n	276	309	310	320	192
Median routine lab	379	12.8	23	65	1.2
Bias* (95% CI)	54 (45; 63)	-0.16 (-0.19; -0.13)	3.1 (2.11; 4.14)	5.5 (4.49; 6.42)	-1.1 (-1.18; -1.04)
Mean % similarity (CV)	94 (10.3%)	101 (1.2%)	95 (9.5%)	96.5 (6.3%)	148 (19.4%)*
CD4 total miss-classification	15.6% (false positive 14.1%), 4.3% errors				

*smaller sample size

Table 3: List of general clinic duties for nurses in HIV ART clinics and the added duties required for performing multiple POCT.

Current clinic duties	**POC DUTIES (pre-analytical, analytical, post-analytical)**
Patient registration	Additional finger stick/venepuncture
History taking	Specimen labeling
Physical examination	Multiple instrument QC testing (~ four instruments)
Counseling	Multiple instrument maintenance (~four instruments)
Rapid HIV testing (HCT)	Perform POCT (ALT, Creat, Hb: <2minutes; PIMA = 20 minutes; Xpert MTB/RIF =2 hours*)
Pregnancy testing	Result recording/printing/reporting
Phlebotomy	External quality assessment (EQA)
Treatment	Infection control
Return visit booking	Spill cleaning
	Waste disposal
	Additional skills required: Phlebotomy and pipetting skills
	Additional duties: Operator certification and on-going monitoring, managing test failures, instrument downtime, stock and waste control, specimen storage.

*GeneXpert test added since this could also be included for POCT in ART clinics.

Figure 1
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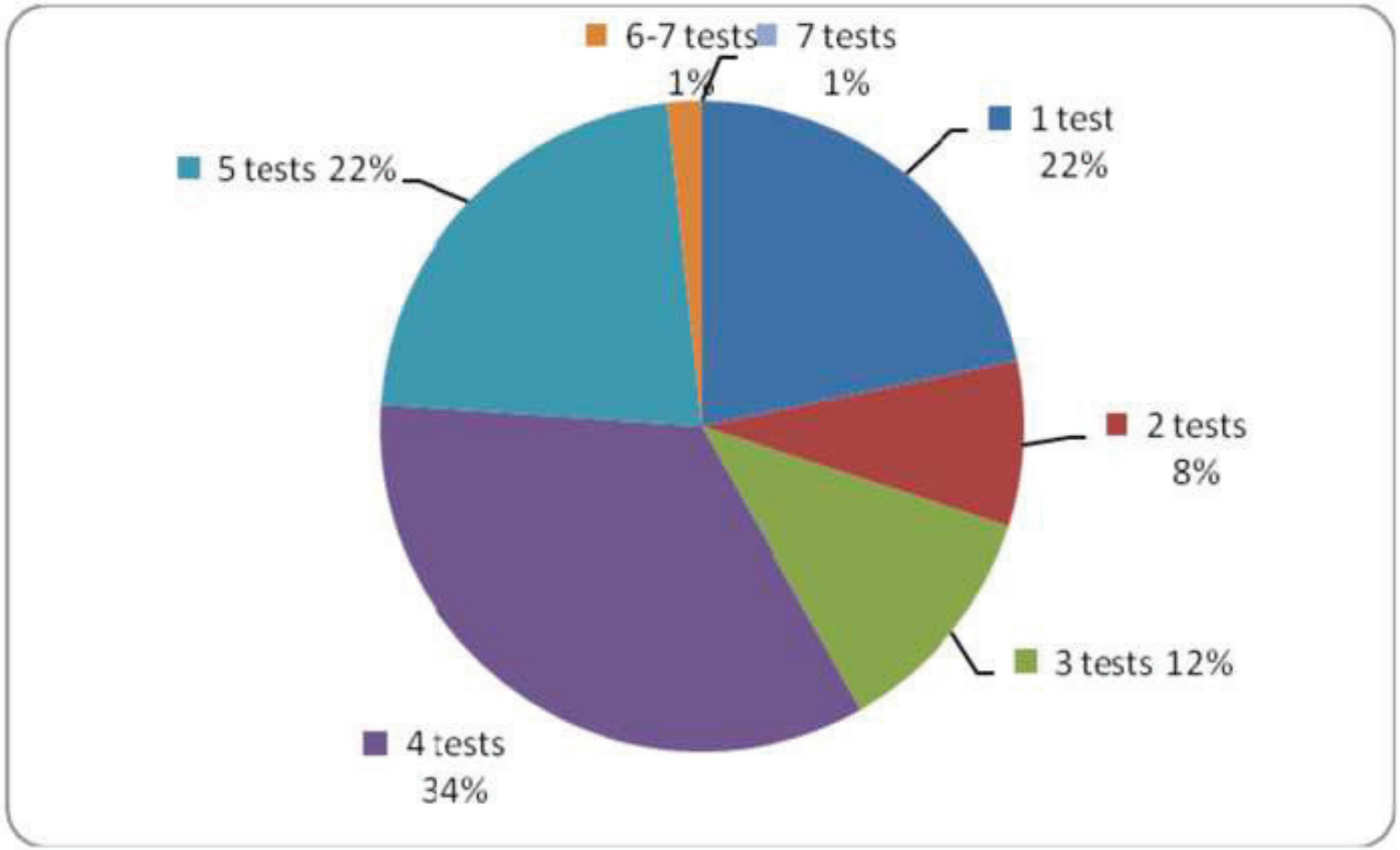

Figure 1: A pie chart representing the percentage of tests requested by HCW on their patients (n=469) attending the CCMT clinic. The pie chart reflects the number of POCT tests required per visit from 464 patients (n=5 not recorded).

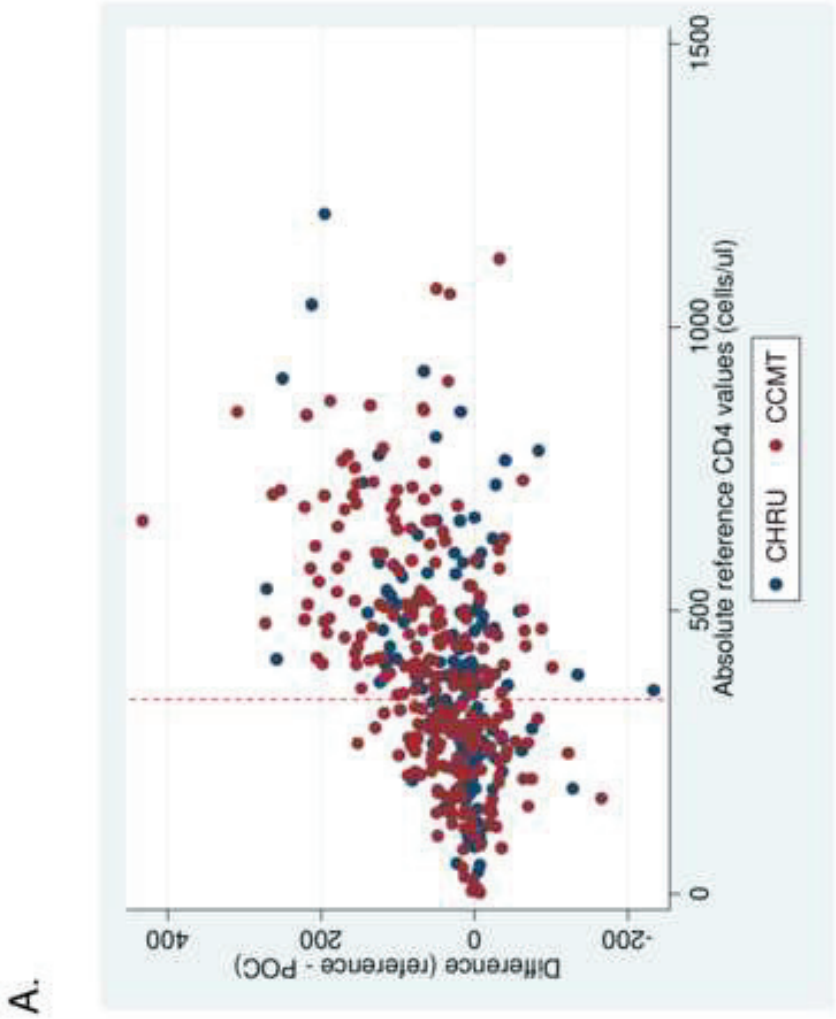

Figure 2: Scatter plots of method comparison of POC testing arms for the two sites compared to routine laboratory results. The vertical axis in each plot is either percentage similarity (A) or difference (B-E) between POC and routine and the horizontal axis is the absolute value of predicate tests: A) Percentage similarity for CD4. Red dashed line indicates the 350 cells/ul threshold. B-E) Bland Altman difference scatter plots for POC versus predicate methodology for Hb (B), ALT (C) and Cr (D) and lactate (E). Grey areas indicate normal ranges for analytes based on reference method.

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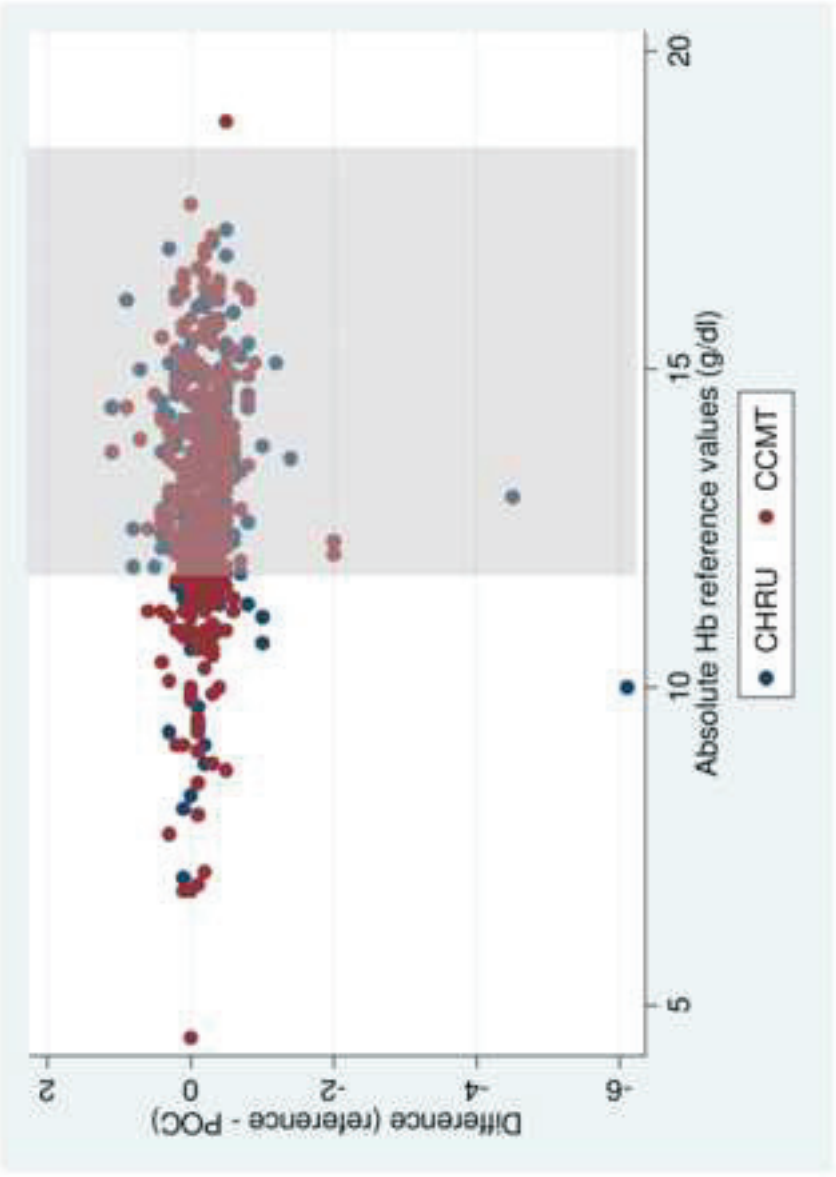

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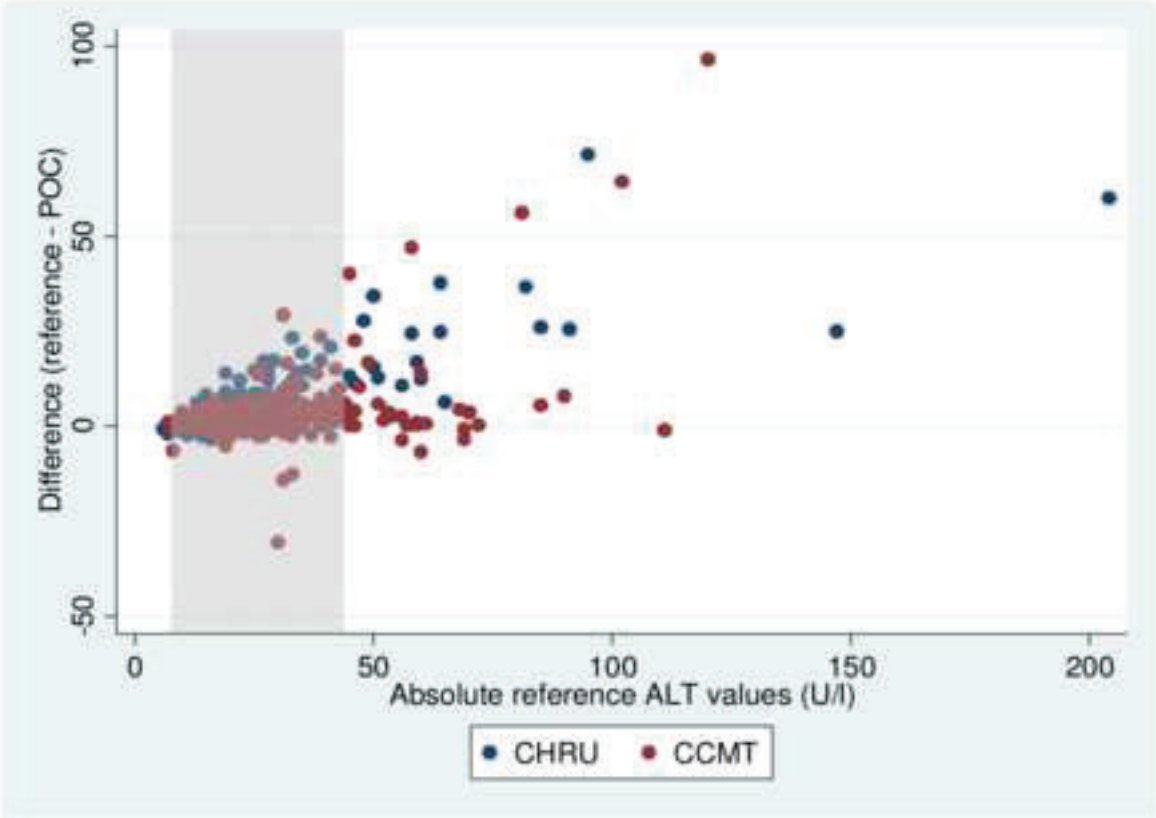

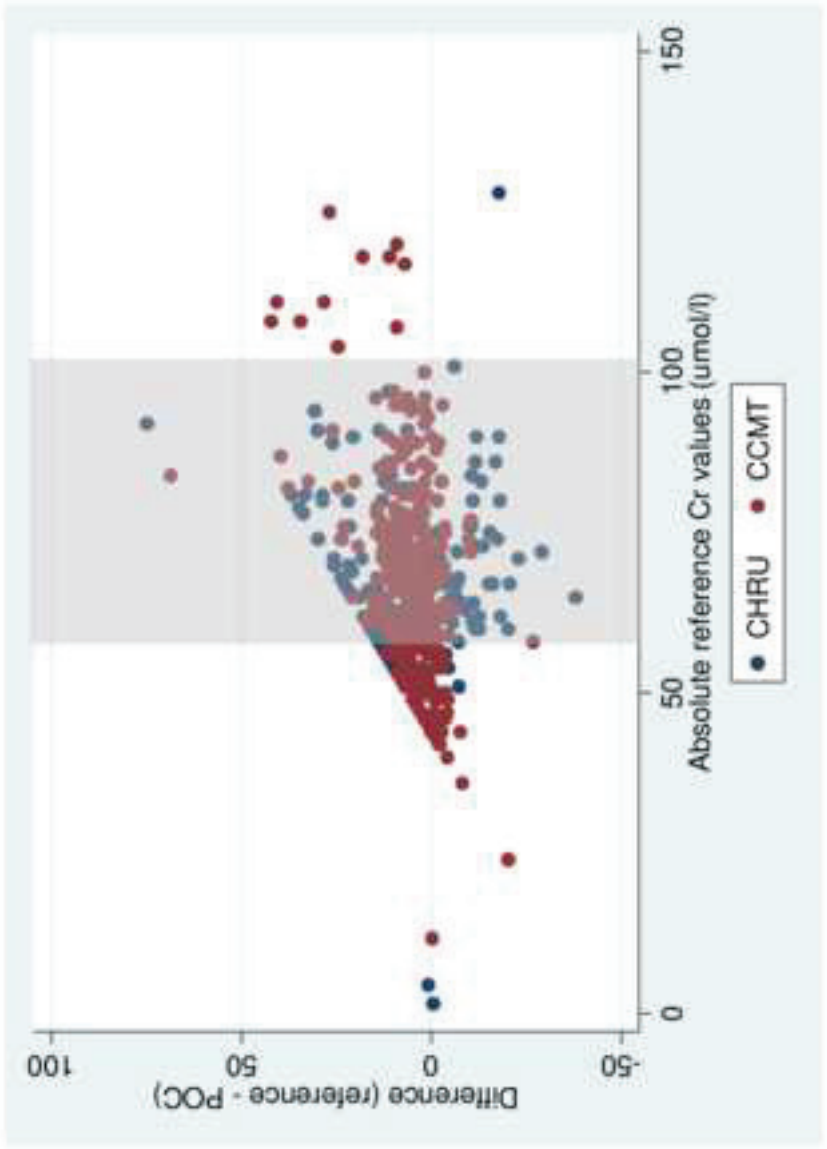

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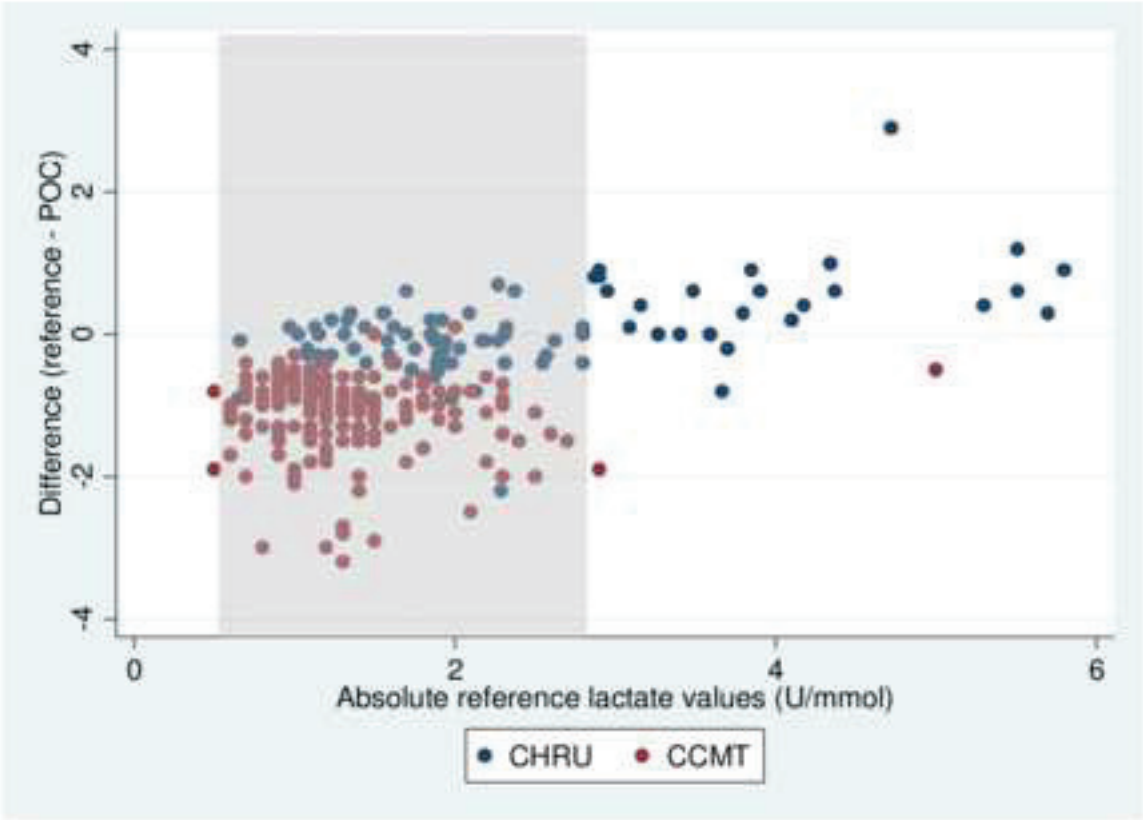

5.2 FEASIBILITY OF PERFORMING MULTIPLE POINT OF CARE TESTING FOR HIV ANTI-RETROVIRAL TREATMENT INITIATION AND MONITORING FROM MULTIPLE OR SINGLE FINGERSTICKS

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PLOS ONE

Feasibility of Performing Multiple Point of Care Testing for HIV Anti-Retroviral Treatment Initiation and Monitoring from Multiple or Single Fingersticks

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Abstract

Background: Point of Care testing (POCT) provides on-site, rapid, accessible results. With current South African anti-retroviral treatment guidelines, up to 4 fingersticks /patient/clinic visit could be required if utilizing POC. We determined the feasibility and accuracy of a nurse performing multiple POCT on multiple fingersticks followed by simplification of the process by performance of multiple POC on a single fingerstick.

Method and Findings: Random HIV positive adult patients presenting at a HIV treatment clinic in South Africa, for ART initiation/ monitoring, were approached to participate in the study between April-June 2012. Phase I: n=150 patients approached for multiple POCT on multiple fingersticks. Phase II: n=150 patients approached for multiple POCT on a single fingerstick. The following POC tests were performed by a dedicated nurse: PIMA (CD4), HemoCue (hemoglobin), Reflotron (alanine aminotransferase, creatinine). A venepuncture specimen was taken for predicate laboratory methodology. Normal laboratory ranges and Royal College of Pathologists Australasia (RCPA) allowable differences were used as guidelines for comparison. In 67% of participants, ≥3 tests were requested per visit. All POCT were accurate but ranged in variability. Phase I: Hemoglobin was accurate (3.2%CV) while CD4, alanine aminotransferase and creatinine showed increased variability (16.3%CV; 9.3%CV; 12.9%CV respectively). PIMA generated a misclassification of 12.4%. Phase II: Hemoglobin, alanine aminotransferase and creatinine showed good accuracy (3.2%CV, 8.7%CV, 6.4%CV respectively) with increased variability on CD4 (12.4%CV) but low clinical misclassification (4.1%). No trends were observed for the sequence in which POC was performed on a single fingerstick. Overall, PIMA CD4 generated the highest error rate (16-19%).

Conclusions: Multiple POCT for ART initiation and/or monitoring can be performed practically by a dedicated nurse on multiple fingersticks. The process is as accurate as predicate methodology and can be simplified using a single fingerstick.

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Introduction

The most important determinants of success in anti-retroviral treatment (ART) programs in South Africa rely on rapid HIV diagnosis, linkage to care, timely treatment initiation, and long-term retention of patients in care [1]. By 2012, South Africa had scaled up its ART services to access approximately 7.1 million people [2] and has 2.5 million people currently receiving treatment [3]. This rapid scale up has led to large investment in developing laboratory capacity in the public sector through the expansion of the National Health Laboratory Services (NHLS) centralised laboratory infrastructure (currently 62 CD4 and 17 HIV viral load centralised laboratories). According to the Department of Health's National Strategic Plan for 2012/2013–

2016/2017, emphasis will be placed on the need for universal annual screening of HIV and TB, thus further increasing testing requirements [4]. Many HIV infected patients who need access to laboratory services for management, live in remote areas with limited access to even basic healthcare services [5]. To meet these demands, decentralisation of laboratory testing through the implementation of Point-of-Care (POC) may provide a solution particularly, for those clinics that are low volume sites and are serviced by laboratories more than a few hours drive from the clinic. The vast numbers of patients in South Africa requiring ART initiation and monitoring however, increases the volumes of tests required, thus challenges of the feasibility of wide-scale implementation of multiple POC assays.

POC or near-patient testing, employs small, simple-to-use, portable technologies for low volume settings [6] and are designed to allow rapid pathological sample analysis at the point of care [7], on easily available specimens such as fingerstick blood (capillary) or sputum. Fingerstick blood collection for POC testing has advantages over venous blood draw in that it is less invasive, faster to perform and provides results immediately [8]. In remote settings, phlebotomy skills are also a limiting factor to improving access to laboratory tests. In this context, the use of fingersticks and heel pricks has gained momentum in two scenarios: 1) HIV rapid diagnostic testing for adults and older children performed by lay counsellors, 2) collection of blood by heel sticks for dried blood spots, to be processed in central laboratories, for HIV exposed infants for HIV PCR assays [9,10].

In South Africa, the National Department of Health (NDoH) is calling for the strengthening of primary healthcare through systems re-engineering [11]. POC testing as an extension of laboratory systems and services may have a place in this process [12]. Advances in the POC testing arena may help to alleviate many of the problems faced by low resource, high HIV and TB burden settings, by providing on-site, rapid accessibility to laboratory tests and timely treatment initiation. The use of POC testing devices has previously proven feasible and accurate on fingerstick blood [13] and CD4 at POC has been shown to reduce pre-treatment patient loss to follow up and improve overall ART initiations [13-16].

According to the South African treatment guidelines at the time of the study [17] the initial laboratory tests needed for initiation of ART included a CD4, followed by creatinine (Cr), alanine aminotransferase (ALT) and hemoglobin (Hb). Indeed, the use of multiple POC testing platforms for this group of patients may prove beneficial, especially in terms of improving turnaround times to clinical decision-making and decreasing loss to follow up. In the scenario of a patient initiating ART as per current in-country treatment guidelines, this could require up to 4 fingersticks per visit if utilising POC testing, over and above the initial two fingersticks needed for HIV counselling and testing. An obvious way of overcoming the multiple fingerstick hurdle, would be to perform POC testing on a venepuncture specimen. This would have cost implications for added materials and require the cadre of POC staff to be trained phlebotomists. One research study has shown acceptable performance of multiple POC (up to three POC tests) for ART initiation [13], but little is known about the feasibility and accuracy of performing multiple POC testing on multiple fingersticks on a single patient and no data is available on whether this process can be simplified by performing multiple POC testing from a single fingerstick specimen. This study therefore investigated the following issues: (i) Can multiple POC testing be performed from multiple fingersticks in terms of operational practicality and still yield accurate results?; (ii) Can multiple POC testing be simplified by performing all POC testing from a single fingerstick specimen?; and (iii) Does the sequence of POC tests performed contribute to result variability?

Methods

Ethics statement

The study was approved by the Faculties of Health Sciences ethics committees at both the University of the Witwatersrand, (protocol number M120143) and the University of Pretoria/ Tshwane (CD4 and Hb POC protocol number 151/2010; chemistry POC protocol number 240/2010).

Patient enrolment

Patients visiting the Comprehensive Care Management and Treatment (CCMT) clinic, Tshwane District Hospital in Pretoria, South Africa, were consented (written consent) and enrolled into the study between the periods of April to June 2012, by two trained study nurses. Criteria for inclusion in the study included: individuals >18 years of age, with known HIV positive status and presenting for either ART initiation or monitoring at designated time points.

Study procedures

The doctor requested the test repertoire as per National ART treatment guidelines at the time of the study [17]. The study staff collected all fingerstick specimens and performed POC testing in a designated POC testing room in the clinic. POC results were not acted on for clinical management. The POC instruments available for the study were the PIMA (Alere Inc., Waltham, MA, USA) for CD4, the HemoCue 201+ (HemoCue AB, Ängelholm, Sweden) for Hb and the Reflotron®Plus (Roche Diagnostics, GmbH, Germany) for ALT and Cr (age and sex can be used to calculate Cr clearance). These were selected based on current testing guidelines for HIV initiation and monitoring as well as instrument availability at the time of the study. An additional EDTA tube (for CD4 and Hb) and/or clotted blood (for ALT and Cr) was collected by venepuncture for predicate laboratory testing as per routine standard-of-care (SOC) and used for clinical decision-making. All blood specimens were transported to the laboratory and tested within 6 hours post-venepuncture. The NHLS laboratories performing the predicate testing all comply to Good Laboratory Practice standards and are SANAS accredited (South African National Accreditation system) [18].

Quality control (QC) on all the POC instruments was performed as per manufacturer's instructions using supplier recommended material and briefly described: PIMA - daily QC

Table 1. Supplier recommended lancets for multiple POC arm based on supplier information/recommendations [19-22].

POC instruments	Supplier recommended lancets	Lancet Specifications
PIMA	Sarstedt safety lancet	1.6mm depth
HemoCue	HemoCue safety lancet	2.25mm depth
Reflotron	Roche AccuChek Softclix Pro lancet	1.7mm depth

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with a low and high control cartridge (Alere Inc.); HemoCue – weekly QC with 3 Hemotrol controls namely, low, normal and high (Eurotrol); Reflotron – weekly QC with a universal control, Precinorm U, for Cr and ALT (Roche Diagnostics). A log sheet was used to record QC values.

Multiple POC testing from multiple fingerstick specimens

According to the study objectives, two phases were carried out.

Phase 1, performance of multiple POC testing on multiple fingersticks: POC nurses recruited 150 patients into the multiple fingerstick phase of the study. This phase followed each POC test manufacturer's standard operating procedures for blood collection by fingerstick. Each POC test was performed on a separate fingerstick using supplier recommended lancets (Table 1) [19-21].

Multiple POC testing from a single fingerstick specimen

Following on the first phase, phase 2 measured the performance of multiple POC tests on a single fingerstick. 150 patients were enrolled into this phase, which followed a simplified version of the manufacturer's standard operating procedures for each POC test; if multiple POC tests were requested, all tests were performed on a single fingerstick (depending on the amount of blood available) using a single lancet. The lancet chosen for this arm was the PIMA Sarstedt safety lancet (Sarstedt Group), as it uses a blade to produce a finger slice as opposed to a traditional fingerstick and thus produces a larger amount of blood [22]. If insufficient blood was available to perform all the tests requested on the single fingerstick, a second fingerstick was performed.

The sequence of POC tests from a single fingerstick

The sequence of blood collection and testing was changed during the second phase of the study to allow for the different instruments to be tested first on the single fingerstick (n = 50 for each sequence). Sequence 1: Reflotron followed by HemoCue and PIMA; Sequence 2: HemoCue, Reflotron, PIMA; Sequence 3: PIMA, Reflotron then HemoCue.

Any POC instrument/cartridge errors/failures in either phase were only repeated with an additional fingerstick if the patient was willing.

Predicate laboratory testing procedures

The predicate methodology used by the Core Laboratory included: PLG CD4 using the FC 500 (Beckman Coulter, Miami, FL); Hb using Advia 120 and 2120 analysers (Siemens Diagnostic Solutions, Tarrytown, NY); ALT and Cr on the Synchron DXC 800 (Beckman Coulter, Miami, FL). The normal ranges for each analyte using predicate methodologies were used as a reference for determining potential clinical changes in decision-making if POC results had been used. These reference ranges were: Hb 12g/dL - 18g/dL; ALT 10-40U/l; Cr 64-104umol/l; a cut off of 350 cells/ul for CD4 was applied for clinical misclassification. The Royal College of Pathologists of Australasia (RCPA) allowable differences [23] were also used as guidelines for assessing performance of each of the phases, these were: Hb ±0.5<10g/dL and ±5%>10g/dL, ALT ±5≤40 U/l and ±12%>40U/l, Cr±8<100umol/l and ±8%≥100umol/l.

Comparison was also made in terms of the sequence in which analytes were tested on POC instruments from a single fingerstick (phase 2).

Statistical analysis

The numbers of tests (fingersticks required) per patient and test (or instrument) errors per phase were quantified. T-tests were used to determine any difference in age and CD4 count and a Chi-squared test was used to determine any difference in gender between the two groups with 95% confidence. Assay performance (precision and accuracy) and method comparison (agreement) between POC and predicate methodology was measured using mean, median, range, percentage similarity (using percentage similarity standard deviation [SD] and coefficient of variation [7] [24]) and Bland-Altman (using bias) [25]. Functions were performed using STATA 12. Scatter plots were used to represent outliers and included the normal reference ranges. Similar scatter plots were used to visualize sequence of testing during the single fingerstick phase. Misclassification for CD4 was determined using the 350 cell/ul threshold and sensitivity and specificity were calculated.

Results

Patient demographics

The mean age of all participants consented and enrolled between the 30th April and 15th June 2012 was 35.5 years (n=299) of which 75.6% were female (n=226). There was no significant difference in the two groups (multiple or single fingerstick) for patient age (p=0.64), gender (p=0.24) or CD4 count (p=0.65). Of the total patients enrolled into the study, 67% required three or more tests per single visit based on standard ART guidelines at time of study (South African 2010 guidelines). A schematic of the study design is shown in Figure 1.

Multiple POC testing from multiple fingerstick specimens

One hundred and fifty patients were approached for this first part of the study. Two patients, both requiring four POC tests withdrew from the study; one after receiving two fingersticks

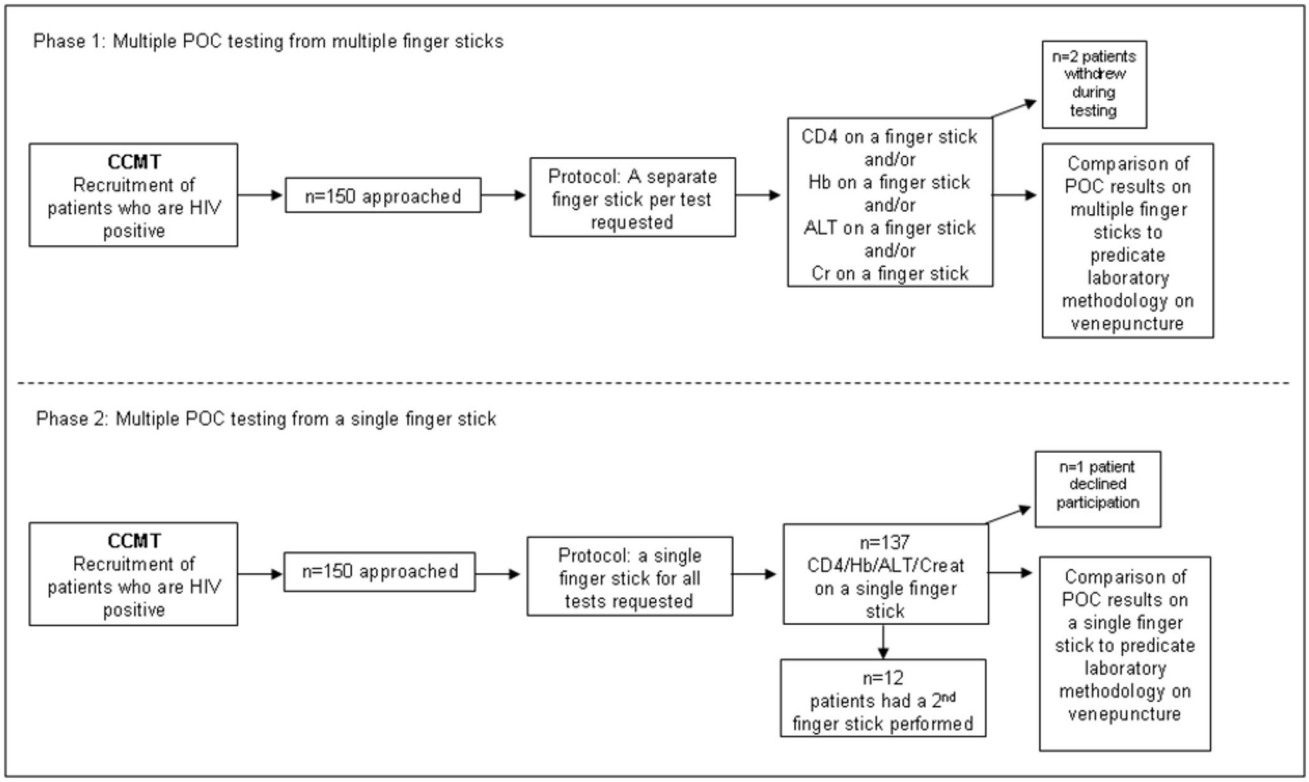

Figure 1. Schematic of study design.

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and the other after receiving three fingersticks (Figure 1). Six percent (9/150) of patients required a 5th or 6th fingerstick to complete the POC test repertoire, mostly for CD4 and Cr due to poor blood flow from the fingerstick. The total number of tests requested during phase 1 was 475 with Cr the most frequently requested analyte. The PIMA instrument reported the highest error rate at 16.33% (n=16) (7 exposure control, 3 cell movement control, 1 reagent control, 4 image control, 1 gating control error), of which 6 were repeated on another fingerstick. The Reflotron had 2 operator errors and 4 POC tests could not be completed due to insufficient blood flow. Three venepuncture samples were rejected from laboratory testing during this phase.

Method comparison of the POC test results on multiple fingerstick specimens versus predicate laboratory methodology is shown in Table 2 and Figure 2A-D. PIMA CD4 demonstrated good accuracy (mean percentage similarity of μ=100.7%) but increased variability (percentage similarity SD 16.5%), compared to the laboratory predicate assay. Bias was acceptable (32 cells/ul) for the data set (~median of 380 cells/ul). The scatter plot in Figure 2A demonstrated a downward trend in PIMA CD4 results indicating that the PIMA reads higher in the low CD4 count (<350 cells/ul) range and lower in the high CD4 count (>500 cells/ul) range, compared to the predicate laboratory method. A few outliers are visible, but all are below the <350 cells/ul category. The overall misclassification of PIMA CD4 at the 350 cells/ul threshold was 12.4% giving a sensitivity of 86.4% (Table 3).

HemoCue Hb showed good accuracy (mean percentage similarity of 100.9%), precision and overall agreement (percentage similarity SD and %CV of 3.2). Figure 2B shows random scatter of outliers (16.5%, 19/115) based strictly on the RCPA guidelines. As the bias is low (-0.19) compared to predicate methodology, it would not alter clinical decision-making.

ALT testing on the Reflotron was 100% accurate for phase I when compared to predicate method and showed good precision (9.3%) and overall agreement (percentage similarity CV 9.3%). Random scatter of values across the data set with very few outliers are visible (1.7%, 2/115) using RCPA allowable differences (Figure 2C). As both these outliers were above the normal predicate reference range of 40U/l, they may have affected clinical decision-making. The mean negative bias of -0.58U/L was low.

The mean percentage similarity for Cr measurements generated lower values than predicate methodology (97.4%) and showed variability (percentage similarity of SD12.6%) attributable to outliers (19.1%, 25/131). The scatter plot (Figure 2D) also shows a trend due to the minimum cut off of 44.2umol/l on the Reflotron instrument, as well as a trend towards reading lower than predicate methodology as Cr levels increase. However, an overall acceptable bias (within RCPA limits) was observed.

Table 2. Method comparison of POC results for all analytes versus predicate laboratory methodology.

Variables	CD4 (cells/ul)	Hb (g/dl)	ALT (U/l)	Cr (umol/l)
Phase 1: Multiple fingersticks vs predicate				
N	98	115	115	131
Mean (range)	384 (36-917)	12.9 (3.8-18.3)	35.3 (5-775)	59.6 (44.2-528)
Median	380	13.1	22	52.1
Bias* (95% CI)	32 (12; 53)	-0.19 (-0.34 ;-0.05)	-0.58 (-2.11;0.95)	5.32 (3.14;7.5)
Bias SD	101.33	0.8	8.3	12.6
Mean % similarity	100.7	100.9	100	97.4
% Similarity SD	16.5	3.2	9.3	12.6
% Similarity CV	16.3	3.2	9.3	12.9
Phase 2: Single fingerstick vs predicate				
N	73	94	97	129
Mean (range)	402 (42-824)	12.5 (6.1-17.1)	27.8 (5-165)	53.8 (44.2-97)
Median	398	12.5	21	49.4
Bias* (95% CI)	30 (-3; 63)	-0.3 (-0.49 ;-0.19)	0.64 (-0.69;1.97)	4.95 (3.68;6.22)
Bias SD	143.7	0.7	6.6	7.3
Mean % similarity	99.4	101.5	99.1	96.4
% Similarity SD	12.4	3.2	8.6	6.2
% Similarity CV	12.4	3.2	8.7	6.4

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Multiple POC testing from a single fingerstick specimen

One hundred and forty nine patients where consented for this part of the study, with one individual declining to participate. The total number of tests requested was 407 with Cr again the most frequently tested analyte. All the POC tests requested could be completed on 91.9% (137/149) of patients from a single fingerstick specimen and 8.1% (12/149) required a second fingerstick (Figure 1). PIMA generated the highest error rate of 19.18% (n=14) (9 cell movement control, 1 insufficient volume, 3 exposure control, 1 gating control error), of which only one could be repeated on a second fingerstick specimen. One CD4 POC test could not be completed due to insufficient blood flow. No errors were observed from any other POC instruments.

Method comparison of analyte results from phase II are reported in Table 2 and Figure 3A-D. From a single fingerstick, PIMA CD4 demonstrated good accuracy (mean percentage similarity μ=99.4%) and a bias of 30 cells/ul (median of 398 cells/ul) but increased variability of 12.4% percentage similarity SD. One outlier is visible above 350 cells/ul, but would not have changed clinical patient management as both PIMA and predicate technologies identified this patient as not suitable for ART initiation. The overall misclassification of PIMA CD4 at the 350 cells/ul threshold was 4.1%, giving a sensitivity of 97% (Table 3).

Hb results also showed good accuracy (mean percentage similarity of 101.5%), precision and overall agreement (percentage similarity SD and %CV of 3.2) and low bias compared to predicate, however applying strict RCPA limits, 20.2% (19/94) of results would be considered outliers (Figure 3B).

ALT measurements from a single fingerstick showed good accuracy and precision (99.1% and 8.6% respectively) and similar overall agreement (percentage similarity CV 8.7%). A few random outliers (using RCPA limits) are highlighted in Figure 3C (5.2%, 5/97), all above 40U/l, which potentially could have affected clinical decision-making. Single fingerstick testing showed a positive but low bias that was different to the negative but low bias in multiple fingerstick testing.

Cr testing generated lower values than predicate methodology (μ=96.4%) with low variability (percentage similarity SD6.2%) and bias of 4.95umol/l (within RCPA limits). A trend however, is present in Figure 3D with Reflotron generating lower values as Cr levels increase, resulting in potentially 19 outliers (14.7%).

Sequence analysis of multiple POC testing on a single fingerstick

Evaluation of the POC test results across the sequence of testing from a single fingerstick specimen showed random distribution of outliers and no trends were evident across assay performance (Figure 4A-D).

Quality control

The quality control material on all the POC instruments performed within acceptable limits, with only one outlier occurring on the PIMA low control. After repeat testing, this control performed within acceptable manufacturer's standards.

Discussion

Many potential benefits are reported after introduction of POC testing into non-laboratory environments in low resource countries, such as reduced skill requirements, faster turnaround times, better patient management and resource utilization [6] and improved staff satisfaction [26]. In South

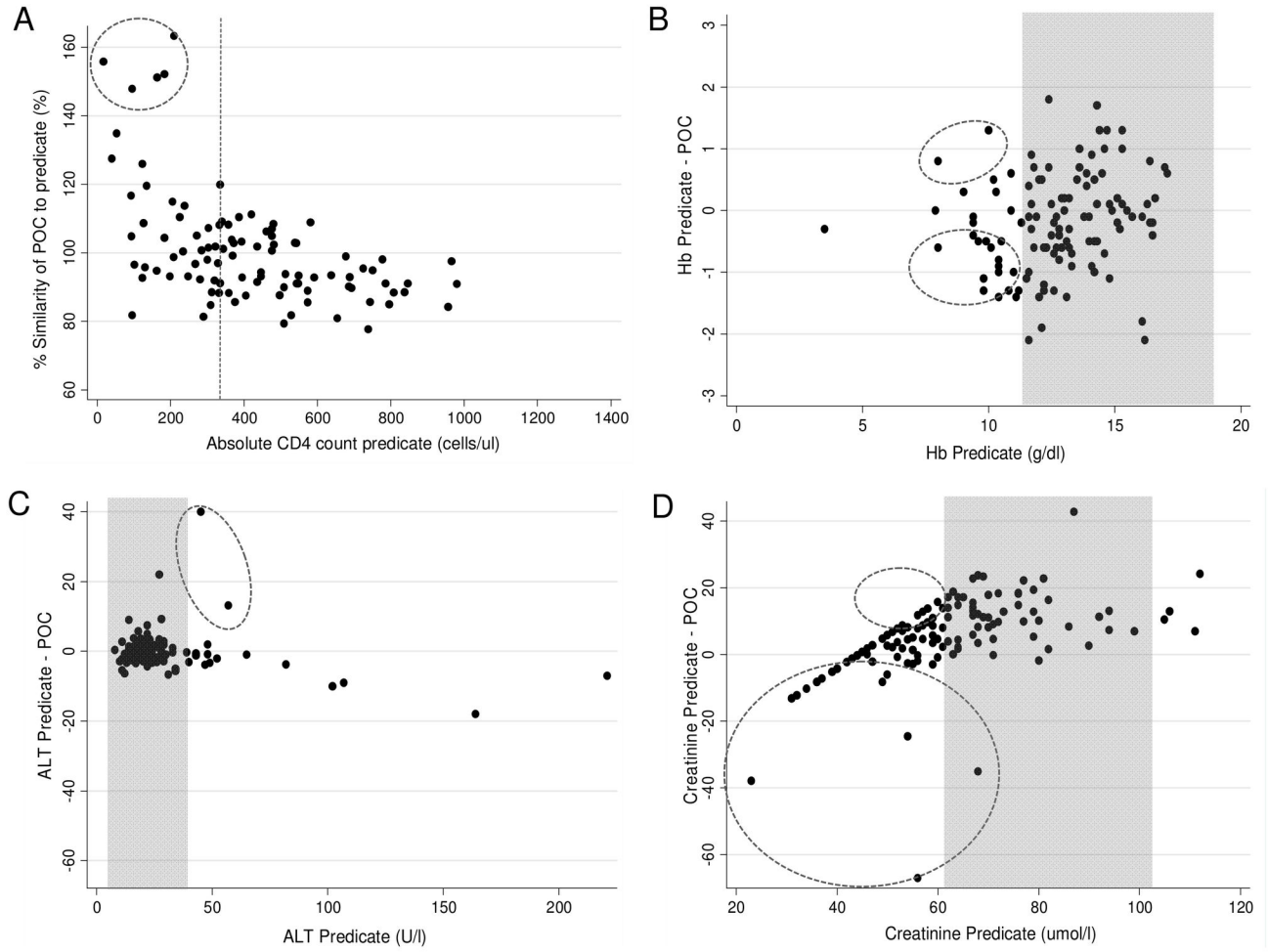

Figure 2. Scatter plots of method comparison for POC testing from multiple fingersticks compared to routine laboratory methodology (Phase I). The vertical axis in each plot is either percentage similarity (A) or difference (B-D) between POC and predicate and the horizontal axis is the absolute value of predicate tests: A) Percentage similarity for CD4. Red dashed line indicates misclassification point of 350 cells/ul and circle highlights outliers. B-D) Bland Altman difference scatter plots for POC versus predicate methodology for Hb (B), ALT (C) and Cr (D). Grey areas indicate normal ranges for analytes based on predicate method and circles highlight outliers mostly based on RCPA guidelines.

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Table 3. ART eligibility misclassification, sensitivity and specificity of PIMA based on a CD4 threshold of 350 cells/ul for phase I (multiple fingerstickS) and II (single fingerstick).

	True Positive	False Positive	True Negative	False Negative	Total misclassification	*Sensitivity at 350 cells/ul (95% CI)*	*Specificity at 350 cells/ul (95% CI)*
Multiple (n=96)	38 (39.6%)	6 (6.2%)	46 (48%)	6 (6.2%)	12.4%	86.4% (72; 94)	88.5% (76; 95)
Single (n=73)	30 (41.1%)	2 (2.7%)	40 (54.8%)	1 (1.4%)	4.1%	97.5% (81.4; 99.8)	95% (82.5; 99.1)

doi: 10.1371/journal.pone.0085265.t003

Africa, which has the largest ART roll out program in the world, the implementation of multiple POC testing for monitoring could be highly beneficial for detecting acute and chronic adverse events associated with ART [27]. It has been proposed that POC should be an extension of the laboratory network using the tiered approach, as proposed by the Maputo declaration

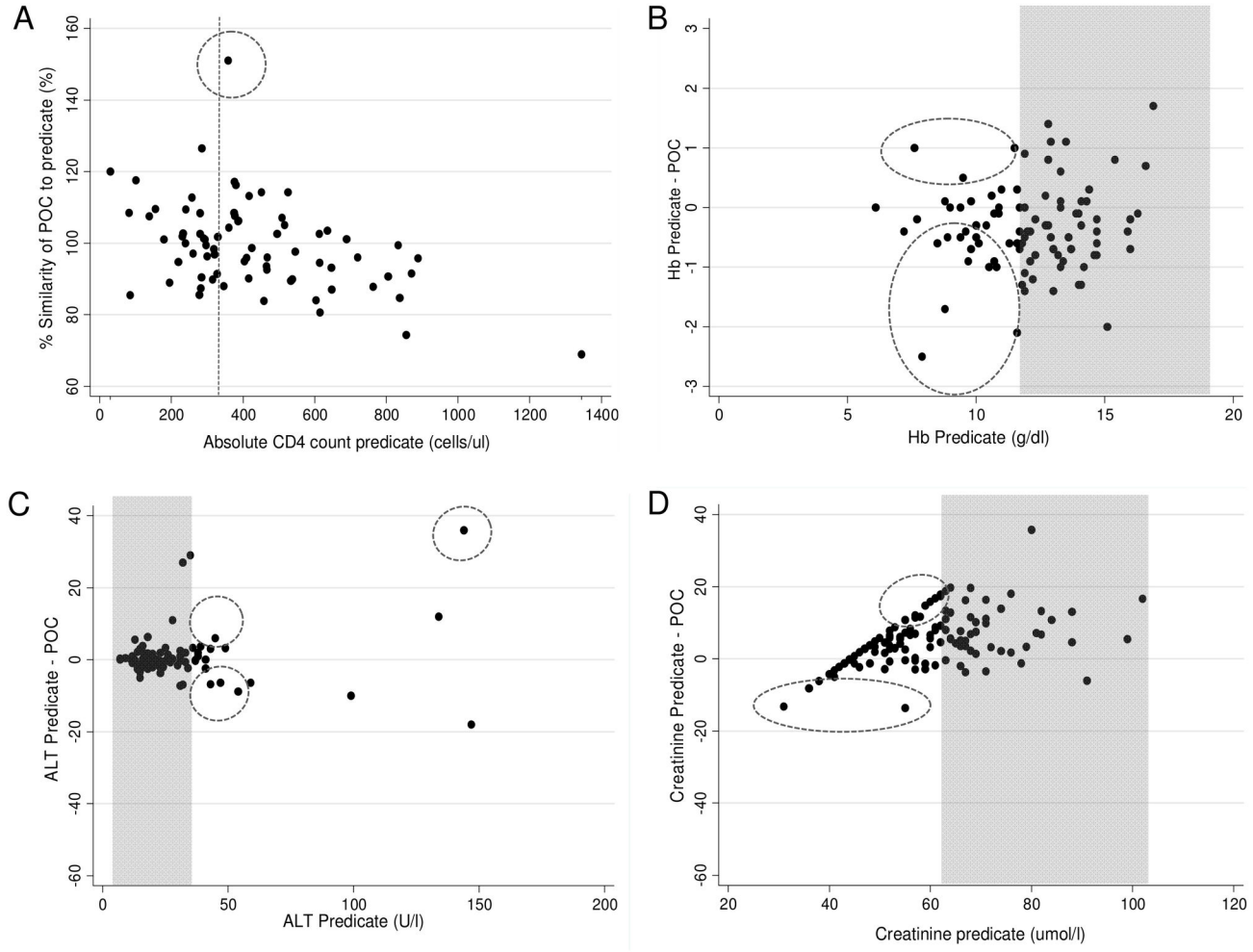

Figure 3. Scatter plots of method comparison of POC testing from a single fingerstick compared to routine laboratory methodology (phase II). A) Percentage similarity for CD4. Red dashed line indicates misclassification point of 350 cells/ul and circle highlights outliers. B-D) Bland Altman difference scatter plots for POC versus predicate methodology for Hb (B), ALT (C) and Cr (D). Grey areas indicate normal ranges for analytes based on predicate method and circles highlight outliers mostly based on RCPA guidelines.

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[28]. However, this small study highlights that more than half of patients attending the clinic for routine ART monitoring/ initiation, required 3 to 4 tests per visit. This equates to multiple fingersticks per patient, per visit if POC is introduced. The operational capacity and feasibility of this practice on a national scale needs further analysis. Study findings demonstrated that multiple POC tests performed as per manufacturer's instructions (i.e. single fingerstick using supplier recommended lancet) can practically be performed by a dedicated nurse. Only 6% of subjects needed a 5th or 6th fingerstick to complete the POC test repertoire in this cohort. However in more remote settings where individuals undertake manual labour such as in farming or mining communities, obtaining a fingerstick specimen from calloused fingers may be more difficult. This is being investigated in a further study. Overall, the performance of the POC analytes, compared to predicate laboratory methodology, was accurate for Hb and ALT. Increased variability was more evident with PIMA CD4 and Reflotron Cr.

In an attempt to simplify the POC testing process from necessitating multiple fingersticks and reduce discomfort to patients, we performed multiple POC tests on a single fingerstick specimen. This process was found to be practical and simple and only a small percentage of patients (8%, 12/149) required a second fingerstick to complete the requested POC testing repertoire. Advantages of this simplified testing would be reduction in the number of fingersticks per patient and thus a significant reduction in nurse exposure to blood and other contaminants, reduced discomfort to the patient, as well as a reduction in consumables used. The overall performance of the various POC tests from a single fingerstick was acceptable for all analytes tested compared to predicate methodology. Based on the RCPA guidelines, some

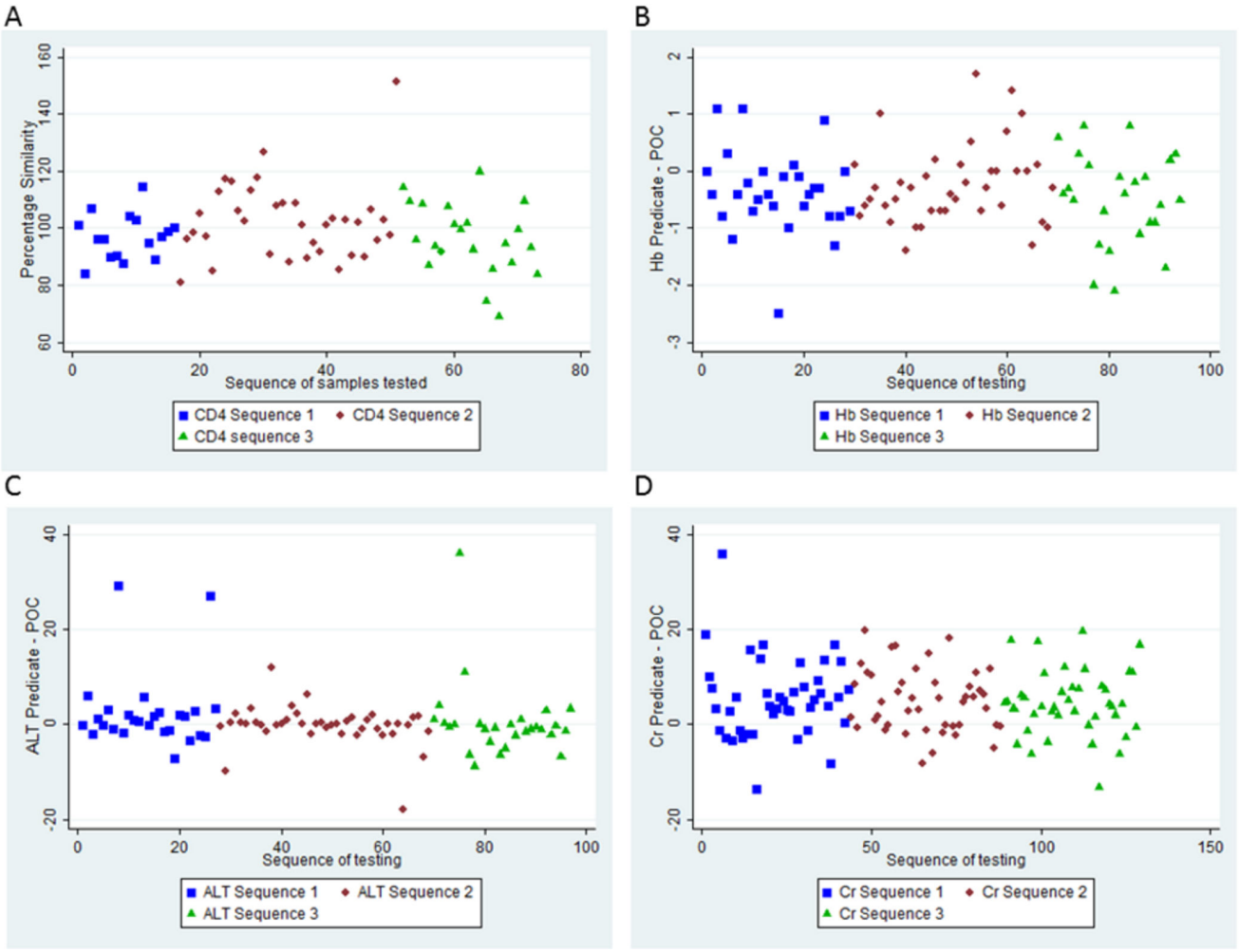

Figure 4. Scatter plots showing sequence of POC testing from a single fingerstick (phase II) compared to routine laboratory methodology. A) Percentage similarity for CD4. B-D) Bland Altman difference scatter plots for sequence of POC testing versus predicate methodology for Hb (B), ALT (C) and Cr (D). Blue indicates sequence 1: Reflotron followed by HemoCue and PIMA; Red indicates Sequence 2: HemoCue, Reflotron, PIMA; and green indicates Sequence 3: PIMA, Reflotron then HemoCue.

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values may be considered outliers on the plots for Hb, ALT and Cr, however no trends or differences were visible between the sequence in testing protocol from a single fingerstick.

In scrutinizing the performance of each platform in more detail, starting with the CD4 test, examples of within technology variability such as FACSCount and PLG studies report acceptable variability (%CV) on repeat venous sample testing of 5%-9.3% [29] [30]. PIMA within variability (and across 5 different instruments) on venous samples (range in CD4 of 44 -504 cells/ul) shows higher but acceptable variability ranging from 4.2% to 15.5% respectively [31].

The PIMA CD4 evaluation in our study (across technology) on fingerstick specimens generated values at this upper venous limit (16.3%CV for phase 1 and 12.4%CV for phase 2), reflecting the PIMA acceptability for ART initiation, but less assurance from this study data if this technology were to be used for monitoring (unless venepuncture specimens are tested). This latter application however, is of less concern with the new South African and WHO ART 2013 guidelines using CD4 for initiation and a reduction in CD4 monitoring after the first year, with greater emphasis on VL monitoring. The alternative would be to use venepuncture for PIMA CD4 monitoring. Increases in variability of capillary PIMA CD4 testing have previously been documented, where % similarity CV's ranged from 11% in a Johannesburg Clinic, to as high as 28.8% in an antenatal hospital clinic [32]. Irrespective of this variability, the overall bias in PIMA CD4 versus predicate testing in both the phases is acceptable (~30 cells/ul) for the range in CD4 count (median >350 cells/ul), however the variability (SD) of this bias was broad (~100 cells) probably

indicative of a small sample size. The total misclassification of 12.4% for multiple fingersticks and 4.1% for testing on a single fingerstick has similarly been documented in other studies and shows more patients would be initiated on ART than missed if testing were done on a single fingerstick specimen [33-35]. Concern is noted over the high PIMA CD4 error rate (16% and 19%), which was higher than any other POC instrument used in our study, and was slightly higher during the single fingerstick phase. Similar error rates for PIMA CD4 have been observed in other reports [32,34].

The HemoCue instrument's good performance for Hb measurements on fingerstick, whether multiple or single (bias of -0.2±0.8g/dL multiple and -0.4±0.9g/dL for single) is in contrast to literature which demonstrates significant variability in capillary blood measurements [36] and a tendency for POC to increase at higher Hb values [37,38]. Bland Altman difference plots showed random distribution of outliers but more outliers (16% versus 20%) are visible when POC was performed from a single fingerstick specimen.

Reflotron performed well for ALT measurements, regardless of whether single or multiple fingersticks were performed. These findings are in contrast to Grounden et al [27] who found significant biases for Reflotron ALT. The better performance in our study for both phases could be attributed to the experience and thorough training of the nurse operators that may have resulted in better sample collection. With creatinine measurements however, which are known to be challenging due to haematocrit variations and interference by bilirubin [39], the Reflotron was found to read lower than predicate method as creatinine levels increased, regardless of whether POC was performed from multiple or a single fingerstick. Similar findings have been observed in other studies where Reflotron was found to underestimate creatinine measurements at concentrations between 90 and 150umol/L [39]. One factor that may result in overestimation of creatinine in the routine laboratory specimens is sample haemolysis [40] which is mainly due to poor sample collection. However, we do not believe this to have influenced our results, as these samples are generally checked and rejected by the routine laboratory.

In summary, this study demonstrates the feasibility of performing multiple POC testing on multiple fingersticks to accurately monitor ARV treatment. We also demonstrated that a single fingerstick produces sufficient blood to accurately perform up to four POC tests (approximately 95ul of blood), to simplify the testing process. This may be the preferred method to ensure quality testing if multiple POC tests are to be introduced for ART initiation in South Africa.

POC testing can reliably and accurately be performed on fingerstick blood thereby minimizing potential bio-hazardous risk introduced by uncapping EDTA tubes and pipetting of venepuncture blood, does not require a trained phlebotomist, generates minimal biological waste, is minimally invasive and relatively easy to perform [41] but multiple POC will need dedicated staff. Patient acceptance of multiple fingersticks for POC testing is also a consideration for uptake of POC and is being evaluated in a further study. We envisage that a new cadre of staff would need to be trained for POC operation, one that has both technical skills and clinical knowledge. Monitoring of quality POC testing will have to be a component of implementation, as previously outlined for these issues around HIV rapid testing [42].

Limited guidelines on multiple POC testing for ART initiation and monitoring are available, so field testing studies such as this are important to understanding how POC performs in the field. Many other obstacles to implementation will need clarification before POC can be implemented. Depending on where POC is placed, different facilities will likely have differing needs and resources; throughput of POC instruments will have to be taken into consideration; management of stock control and quality control; the cadre of POC staff needed; management of testing volumes and results; impact on patient care.

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Author Contributions

Conceived and designed the experiments: NG LS JP LN WS. Performed the experiments: NG SE RN. Analyzed the data: NG LS. Contributed reagents/materials/analysis tools: JP WS. Wrote the manuscript: NG LS JP LN WS. Revision of manuscript: LS JP LN SE RN WS. Final approval of manuscript: LS JP LN SE RN WS.

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5.3 AN INVESTIGATION OF FINGERSTICK BLOOD COLLECTION FOR POINTOF-CARE HIV-1 VIRAL LOAD MONITORING IN SOUTH AFRICA

An investigation of fingerstick blood collection for point-of-care HIV-1 viral load monitoring in South Africa

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Background. Viral load (VL) quantification is an important tool in determining newly developed drug resistance or problems with adherence to antiretroviral therapy (ART) in HIV-positive patients. VL monitoring is becoming the standard of care in many resource-limited settings. Testing in resource-limited settings may require sampling by fingerstick because of general shortages of skilled phlebotomists and the expense of venepuncture supplies and problems with their distribution.

Objective. To assess the feasibility and ease of collecting 150 µL capillary blood needed for the use of a novel collection device following a classic fingerstick puncture.

Methods. Patients were recruited by the study nurse upon arrival for routine ART monitoring at the Themba Lethu Clinic in Johannesburg, South Africa. Each step of the fingerstick and blood collection protocol was observed, and their completion or omission was recorded.

Results. One hundred and three patients consented to the study, of whom three were excluded owing to the presence of callouses. From a total of 100 patients who consented and were enrolled, 98% of collection attempts were successful and 86% of participants required only one fingerstick to successfully collect 150 µL capillary blood. Study nurse adherence to the fingerstick protocol revealed omissions in several steps that may lower the success rate of capillary blood collection and reduce the performance of a subsequent VL assay.

Conclusion. The findings of this study support the feasibility of collecting 150 µL of capillary blood via fingerstick for point-of-care HIV-1 VL testing in a resource-limited setting.

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The World Health Organization (WHO) has estimated that about 34 million individuals are infected by the current HIV/AIDS pandemic.[1] Although much progress has been made in controlling this disease, in sub-Saharan Africa approximately 23 million people remain infected,[1] with South Africa (SA) alone contributing about 11 087 cases/100 000 population.[1]

The monitoring of the HIV viral load (VL) in patients receiving antiretroviral therapy (ART) is critical to ensure treatment success, identify problems with treatment adherence, and identify HIV drug resistance to inform the decision to switch to second-line or future third-line therapies.[2] Currently there is much discussion regarding the role of VL monitoring in the care of HIV/AIDS patients. The WHO recommends VL monitoring as the preferred approach because of its ability to identify treatment failure earlier than immunological and clinical modalities.[2] Treatment failure is defined by the WHO as a plasma VL >1 000 copies/mL after two consecutive measurements during a 3-month interval accompanied by adherence support.[2] However, despite the updated WHO recommendations, poor access to VL testing often persists in resource-limited settings owing to simple logistical issues such as the collection and transportation of specimens.

Recently, alternatives such as dried blood spots (DBSs) and point-of-care (POC) devices are being investigated as potential ways to increase access to VL testing in low- and middle-income countries (LMICs). While standard laboratory platforms typically retain high accuracy when utilising the recommended threshold of 1 000 copies/mL, both DBS and POC devices may need to utilise a higher limit (3 000 - 5 000 copies/mL has been suggested) until better sensitivity is established at the lower limit of detection.[2] A study by Viljoen *et al.*[3] in Durban using DBS HIV-1 RNA testing appeared accurate and feasible down to approximately 3 000 copies/mL. In a study conducted in southern India by Neogi *et al.*,[4] DBS HIV-1 RNA testing revealed 100% sensitivity and specificity at 5 000 copies/mL, but only 50% sensitivity with 100% specificity at 1 000 copies/mL. Most recently, when Kleshik *et al.*[5] quantified HIV-1 RNA in single 50 µL DBSs and limited incubation time prior to sample preparation to 30 minutes, a limit of detection of 866 copies/mL was reported.

An important concern regarding the use of DBSs for HIV-1 RNA quantification is the amplification of cell-associated HIV nucleic acid in whole blood, leading to a falsely high VL measurement when compared with the amplification of viral nucleic acid in plasma specimens. Several recent studies using DBSs have shown reasonable correlation for VLs >3 000 copies/mL, but significant over-quantification has been observed in specimens with <3 000 copies/mL.[6-9] Unless this over-quantification is addressed, the usefulness of DBSs for VL monitoring may be limited in samples containing <3 000 copies/mL.

DBSs may serve to improve access to VL monitoring by linking any existing central laboratory infrastructure to regions with poor access to VL testing, where transport delays and centrifugation to plasma are not feasible. However, blood collection and the shipment of DBSs, with subsequent VL quantification and reporting of results, is not easily achieved during the same day in order to impact on patient care on the same visit. Rapid POC testing may address this logistical shortcoming by quantifying VL on site during the same visit. Subsequent ART intervention may then take place during the same day, as patients with poor adherence to treatment or those with newly developed drug resistance are screened earlier in the process.

Shortages of skilled phlebotomists and the expense of venepuncture supplies have contributed to the development of a POC VL quantification device for use in resource-limited settings that may utilise sampling by fingerstick instead of venepuncture. Fingerstick specimens are currently used for a wide range of tests for haematology, chemistry and serology.[10-13] Recently the University of the Witwatersrand, Johannesburg, SA, investigated the feasibility and accuracy of performing multiple point-of-care tests (POCTs) on fingersticks. This study found that capillary blood for up to four POCTs (95 µL) could be obtained from a single fingerstick in 92% of the subjects.[14] A collaboration between the Quidel Corporation and the Northwestern Global Health Foundation (NWGHF), USA, is developing a POC RT-PCR testing platform and VL assay that will require a volume of 150 µL capillary blood to reach a sensitivity with a lower limit of detection of 1 000 copies/mL.[12] The 150 µL whole blood will be converted to plasma using sample preparation materials provided by the NWGHF.[12] A significant barrier to implementing this platform in the future will be overcome if 150 µL capillary blood can be reliably collected following a fingerstick. In order to facilitate the collection of capillary blood for this study, a novel EDTA-treated capillary blood collection device with a capacity of 150 µL was developed.

The aim of this study was to assess: (*i*) the proportion of collection attempts that obtain 150 µL capillary blood using a newly developed fingerstick-based collection device; (*ii*) the number of puncture sites required to obtain 150 µL blood; and (*iii*) study nurse adherence to the fingerstick and blood collection protocol.

Methods

Setting and participants

The study was conducted at the Themba Lethu Clinic at Helen Joseph Hospital, Johannesburg, where a medical student from the Feinberg School of Medicine in Chicago, USA, observed a study nurse perform fingerstick punctures and collect capillary blood specimens from 100 patients having routine blood tests for ART monitoring. Each patient routinely received one venepuncture for blood collection during their visit. Fingerstick punctures were not performed at this clinic for the routine blood tests involved in ART monitoring. For the purposes of this study, a phlebotomist first performed a venepuncture on each patient for their routine blood tests and then one or more fingersticks were performed by the study nurse. The study nurse was highly experienced, with over 1 000 venepunctures and 1 000 fingersticks performed during her career.

Eligible patients were HIV-positive individuals currently receiving ART who had previously been tested for CD4 and/or HIV VL. Primary exclusion criteria included the presence of heavy callouses, severe dehydration, clinically identifiable illness and/or opportunistic infection, and persistently cold fingers after a warming attempt. Suitable participants were recruited from the blood collection room after a phlebotomist had administered venepuncture and collected the requested routine standard-of-care blood specimens. Each patient was asked to sign an informed consent waiver before enrolling in the study and receiving a fingerstick. Ethics approval for this study was granted by the Institutional Review Board at Northwestern University (ID: STU00076689) and the Human Research Ethics Committee at the University of the Witwatersrand (Protocol M120143).

Data collection and measurements

Fingerstick punctures were delivered using a device with a blade depth and width of 2.0 mm and 1.5 mm, respectively. The BD Microtainer Contact-Activated Lancet (BD Diagnostics, USA) was initially used to deliver fingerstick punctures until a stock shortage necessitated the use of the BD Genie Lancet (BD Diagnostics), with identical blade depth and width specifications. The study nurse was not given explicit instructions on how to perform the fingersticks; instead, she was simply asked to perform them according to her usual methods until blood collection was complete. A fingerstick and blood collection protocol checklist was created for the purpose of this study to assess baseline study nurse adherence to the protocol without training or specific instructions provided. The study nurse was blinded with respect to the fingerstick and blood collection protocol checklist used to assess adherence (Appendix 1) for the fingerstick and blood collection protocol in its entirety.

A novel blood collection device capable of holding 150 µL was used to discriminate between successful and unsuccessful collection attempts. The device contained several layers of EDTA-treated membrane strips designed to wick exactly 150 µL capillary blood. A complete collection was described to the nurse as the moment when both the front and rear of the membrane strips in the collection device appeared solid red in colour. Each step of the protocol checklist was observed, and completion or omission of any step was recorded on a template for every patient. A stopwatch was started immediately after the fingerstick to time the duration of the fingerstick procedure followed by blood collection. When more than one fingerstick was necessary, the study nurse obtained verbal consent before proceeding with each additional fingerstick. The result of each collection attempt was recorded. The study nurse performed translations as needed. Any unique insights offered by her were documented.

Results

A total of 132 patients were approached for participation in this study. Twenty-nine patients refused to give consent: 18 offered no reason for their refusal to do so, 7 stated that they were in a hurry, 2 did not want additional tests performed, and 2 stated that they were scared of receiving a fingerstick. Of the remaining 103 patients, 3 were excluded from the study because of the presence of callouses and/or extremely thick skin, self-described as relating to their respective occupations. One hundred remaining patients participated in the study.

Ninety-eight out of 100 collection attempts were successful, and 86% required only one fingerstick to successfully collect 150 µL of capillary blood (Table 1). The two failed collection attempts were in adult men without callouses, exceptionally thick skin

Table 1. Number of fingersticks required to obtain 150 µL blood

Fingersticks received, *n*	Patients (*N*=100), *n*
1	87*
2	10
3	2
4	1†

*One collection attempt failed to obtain 150 µl blood.
†The combined collection from four fingersticks failed to produce 150 µL blood.

Table 2. Nurse adherence to protocol

Protocol steps	Adherence, % (*N*)
Pair of gloves worn by nurse	0 (0)
Patient sitting	100 (100)
Patient's fingers warmed in advance	7 (7)
Puncture site disinfected with alcohol pad	86 (101)
First drop of blood wiped away	5 (109)
Hand positioned palm down	100 (100)
Hand positioned below elbow	56 (65)
Collection device held above skin; scraping avoided	96 (104)
Gentle pressure applied; strong milking avoided	95 (103)
Pressure applied after collection	100 (100)

or persistently cold fingers after a warming attempt. After four consecutive fingersticks were conducted on the first patient, he refused additional attempts. In the second patient, slow blood flow was observed after the first fingerstick. He declined to give consent to perform any additional fingersticks. Neither patient exhibited a negative response to receiving their fingerstick(s), as neither was observed to wince in pain, pull away or cry out. Neither patient exhibited physical signs of dehydration, but further questioning revealed a history of possible low fluid intake.

The mean time to perform one fingerstick followed by a successful collection was 76 seconds (range 27 - 225). Study nurse adherence to the fingerstick and blood collection protocol is summarised in Table 2.

Discussion

The successful monitoring of VL in patients receiving ART is critical in identifying treatment failure resulting from adherence issues or the development of HIV drug resistance. The world's largest population of HIV-positive individuals resides in sub-Saharan Africa, yet this region has variable and often limited access to VL testing. Development of a simple, cost-effective and readily accessible VL assay with high sensitivity is therefore needed. Currently, DBSs and novel POC platforms are being investigated as opportunities to expand access to VL monitoring in LMICs. Failure to access VL testing is frequently due to simple issues such as sample collection and transport. This study aimed to assess the feasibility of collecting 150 µL of capillary blood following a fingerstick puncture for use in a POC rapid RT-PCR testing platform and VL assay.

Although 86% of collection attempts successfully achieved a complete collection from a single fingerstick, the study nurse adherence to the protocol (Table 2) revealed omissions in several key steps that may adversely affect the success of capillary blood collection and/or the sensitivity of a subsequent VL assay. However, it should be noted that fingerstick device training may occur in an informal manner that fails to emphasise strict adherence to every step of the detailed manufacturer's protocol for fingerstick blood collection. Notably, with 0% adherence, the study nurse was never observed wearing a pair of gloves during this study. While wearing gloves ultimately has no effect on the success of blood collection, repeated omission of this step may inform the subsequent design of a blood collection device that minimises the risk of healthcare worker contact with the collected blood specimen.

The two most commonly omitted steps that may adversely affect the success of blood collection were: (*i*) patient's fingers warmed in advance by any method; and (*ii*) positioning of the patient's hand below elbow level, with 7% and 56% adherence, respectively (Table 2). Occasionally a patient with cold fingers was asked to rub their hands together quickly to generate heat. A warm cloth, which would have been ideal, was not readily available for the purpose of warming fingers. Placement of the patient's hand below the level of their elbow also presented a significant challenge in many cases. Patients were seated in a chair rather than on an elevated examination table because they were subjected to phlebotomy immediately before fingerstick testing, and the routine practice in this setting was for phlebotomy to be done with the patient in a chair. Placing the patient's hand below the level of their elbow while seated in a chair meant that the study nurse would have to bend over and painstakingly reach down in order to perform the fingerstick and observe progress in filling the collection device. Lastly, the first drop of blood was wiped away from the puncture site in only 5% of all fingersticks performed in this study (Table 2). It is hypothesised that the first drop of blood may contain interstitial fluid that could adversely affect the results of a subsequent VL assay, but this has yet to be confirmed.

Omissions in potentially important steps of the protocol suggest that when a POC VL platform and novel VL assay are first introduced to clinics, supplemental quick reference materials and/or brief maintenance training may improve the quantitative performance of a POC VL assay. When training healthcare personnel or preparing a protocol checklist for them in the future, special attention should be given to those frequently omitted steps that may adversely affect the outcome of a subsequent assay. The need for ongoing quality monitoring and training has been reported for performing rapid HIV testing and is a critical component of successful diagnostics.[15] Additionally, all necessary fingerstick materials should be conveniently located to facilitate optimal adherence to the manufacturer's fingerstick protocol.

The unique perspective of the study nurse highlighted several important benefits of performing a fingerstick over venepuncture. First, nurses or community healthcare workers with minimal training can perform fingersticks, potentially resulting in increased access to VL monitoring for patients. Nursing assistants in SA, for instance, receive 1 year of formal training and are not qualified to perform venepuncture on patients. A POC VL quantification assay relying on fingersticks rather than venepuncture could be widely utilised by this workforce. Second, fingersticks result in fewer blood spills and so decrease biohazard risk to healthcare workers, and require far less blood than venepuncture. In some cases a dehydrated and/or sick patient will provide an insufficient quantity of blood by venepuncture, requiring the test(s) to be completed again at a later time. Fingerstick blood collection may be more successful than venepuncture in certain patients. Finally, fingersticks require less counter space, fewer waste bins and less disposal of packaging materials.

The transition to fingerstick blood collection for VL testing may initially complicate the workflow in clinics that require other routine tests for ART monitoring. Venepuncture blood collection is often used for a variety of laboratory tests, including but not limited to CD4, a full blood count, liver function tests, and haemoglobin, creatinine, cholesterol and triglyceride measurements. A transition to fingerstick blood collection for VL testing would initially require phlebotomists to perform one or more fingersticks in addition to venepuncture for most patients. The overall utility of fingerstick blood collection would therefore increase if multiple POC tests could be performed simultaneously for ART monitoring.

Conclusions

Capillary blood collection was highly successful in this study, with the vast majority of patient encounters yielding 150 µL blood after only one or two fingersticks. The widespread implementation of a POC VL assay in a resource-limited setting would not be hindered by the ability to collect the targeted volume of 150 µL capillary blood when using the appropriate lancet, but would require training and ongoing quality monitoring.

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Appendix 1

Fingerstick and blood collection protocol

1. Assemble materials
- Disposable gloves
- 70% isopropyl alcohol pads
- Lancets
- Blood collection device
- Sterile gauze pad
- Warming device (moist towel or sodium acetate hand warmer)

2. Wash hands and put on pair of disposable gloves
3. Position patient and select the fingerstick puncture site
- Patient should be sitting or lying down
- Patient should have their hand in a downward position, allowing gravity to increase blood supply to the hand
- Middle or ring finger is preferable; fifth finger should not be punctured, because tissue depth is insufficient to prevent bone injury

4. Warm the site
- Use a warm, moist towel or other appropriate warming device (not exceeding 40°C/105°F) for 3 minutes; alternatively, have the patient vigorously rub their hands together to generate heat

5. Disinfect the site
- Cleanse the site using a 70% isopropyl alcohol pad
- Allow the site to air dry in order to provide effective disinfection and to prevent possible haemolysis or erroneous results from residual alcohol

6. Perform the puncture
- Have the patient hold their hand below elbow level
- Turn the patient's hand palm down
- Hold the lancet with two fingers
- Position the lancet firmly against the puncture site
- Press lancet against puncture site until release mechanism is activated

7. Discard used lancet into a sharps container
8. Collect the blood specimen
- Wipe away the first drop of blood, as this drop may contain an excess of tissue fluids that may cause erroneous results
- Position the collection device directly beneath the puncture site and avoid scraping across skin
- Gently apply intermittent pressure along finger capillaries and open the puncture slightly to maximise blood flow
- Avoid strong repetitive pressure or 'milking', as this may cause haemolysis or tissue fluid contamination of the specimen
- Blood collection is complete when both sides of the collection device appear solid red in colour

9. Cover the puncture site and dispose of all materials
- Wipe the site dry and apply direct pressure with a sterile gauze pad until bleeding has stopped
- Place all used materials in appropriate biohazard containers

5.4 EXTENDING LABORATORY-BASED PLASMA HIV VIRAL LOAD (VL) TESTING TO CLINIC-BASED WHOLE BLOOD TESTING AT POINT OF CARE: AN EVALUATION OF THE PROTOTYPE LIAT™ HIV QUANT BLOOD ASSAY (IQUUM) (SUBMITTED TO J CLIN MICRO)

Title page

Extending laboratory-based plasma HIV viral load (VL) testing to clinic-based whole blood testing at point of care: An evaluation of the prototype Liat™ HIV Quant blood assay (IQuum).

Short title: Liat POC Viral load monitoring on whole blood

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Abstract

Background: Plasma viral load (VL) testing is recommended at 6months and yearly intervals thereafter for HIV antiretroviral treatment (ART) monitoring. VL testing on plasma is currently laboratory-based using high throughput platforms, which limits access and relies heavily on specimen integrity. A recent laboratory evaluation of the Liat™ platform (IQuum, Inc) for HIV VL testing showed the Liat HIV Quant plasma assay can be used interchangeably with existing VL technologies in South Africa. We evaluated the clinic performance (nurse operated at point of care (POC)) of this platform for VL testing from whole blood using the Liat HIV Quant blood (IQuum, Inc) assay.

Method: HIV-positive patients attending the Themba Lethu clinic in Johannesburg, South Africa for routine ART monitoring were recruited. Finger stick blood was collected in an EDTA microtainer tube and tested on the Liat HIV Quant blood assay within 5minutes. POC VL results were compared to the Roche COBAS CAP/CTM v2.0 (Roche) on plasma EDTA specimens, collected at the same visit.

Results: Of 101 patients (mean age 41.4yrs and 62.4% females), 82% (81/99) generated quantifiable plasma VL values (median 251c/ml [2.4log copies/ml]) and 24% of these patients (19/81) had a VL>1000 cp/ml. The Liat HIV Quant blood assay was easy to perform by a nurse at POC, but generated 97% (96/99) quantifiable results (median VL 5370c/ml [3.73log cp/ml]) of which 92% patients (88/96) VL were >1000cp/ml. The Liat HIV Quant blood assay misclassified 70% patients (69/99) at 1000cp/ml (100% sensitivity [confidence interval (CI):82.4%, 100%]); 13.8% specificity [CI:54%, 94%)]). If the VL ART monitoring threshold were raised to 5000copies/ml, the specificity improved to 53.8% (CI: 42.2%, 65%), and 53% patients (52/99) generated VL >5000cp/ml, resulting in 41% patients (41/99) misclassified.

Conclusion: The total nucleic acid extraction methodology of the Liat HIV Quant blood assay, increases the number of patients identified as ART failure at both 1000c/ml and 5000c/ml thresholds compared to plasma testing. Whole blood VL testing using the Liat HIV platform in niched clinic settings (such as maternity wards) would, however, be advantageous in the diagnosis of HIV.

Introduction

By the end of 2013, 12.9 million HIV infected people had accessed life-saving antiretroviral therapy (ARVs) globally (1). The Joint United Nations Programme on HIV/AIDS (UNAIDS), recently released the ambitious new treatment targets for 2020, called '90-90-90', which aim to place 90% of all HIV-positive people on ARV (1). For limited resource, high HIV burden settings, meeting these goals will require substantial speed in resource scale up and programmatic adaptation (2).

With the recent changes to the ARV treatment guidelines, a viral load (VL) is now the preferred method for treatment monitoring and is recommended at month 6 following treatment initiation and then at 12 monthly intervals thereafter for identifying treatment failure and non-adherence (3). The current threshold for identifying virological treatment failure is a plasma VL result of more than 1000 copies/milliliter (cp/ml) or 3 log cp/ml. Anything above this threshold will result in the patient receiving adherence intervention (check for adherence compliance, tolerability and drug- drug interaction or psychological issues) (4) and a follow up plasma VL test 2 months later, which if confirmed at >1000 cp/ml, results in treatment switch to second line therapy (3).

At present, VL testing on plasma is laboratory-based and relies on high throughput instrumentation, infrastructure and trained technologists/technicians (5-7). Besides these constraints, logistical challenges are also present, such as the need for phlebotomists to draw blood and efficient specimen transport with cold chain storage (8), thus limiting access in many low- and middle-income countries (LMICs). A recent survey by the World Health Organisation (WHO) found that on average, one VL instrument was servicing 8,706 persons on ART in LMICs, demonstrating the need to not only improve access to VL testing but also scale up and/or extend existing capacity (9).

The need to diagnose high burden diseases in low resource settings where laboratory facilities may not be accessible is driving the need for alternative solutions to plasma VL testing. Dried Blood Spots (DBS), already used for early infant diagnosis (EID) (10-13), are being investigated to improve access to VL testing for treatment monitoring (14-18) as they reduce the complexities associated with plasma transport (19). DBS make use finger or heel stick blood specimens, thereby foregoing the need for trained phlebotomists. DBS are easy to transport at ambient temperature (20), but have reduced sensitivity due to small specimen input testing volumes (50-100µl for DBS versus 200-600µl for plasma) (15, 16) or may over-quantify below a plasma VL of 1000cp/ml due to assay extraction and amplification of integrated VL DNA and cell associated viral RNA (3). If a 5000 cp/ml threshold is applied, however, DBS have been shown to be reliable indicators of true virological treatment failure (20).

VL point-of-care testing (POCT) offers a further alternative to extend access to testing services (21, 22). Until recently, commercially available POC VL tests did not exist for HIV but major technological developments in the field have now seen many new assays and platforms in the pipeline (22, 23). One such POC VL technology under development is the Liat™ HIV Quant

100 Assay (developed by IQuum, Inc, Marlborough, MA, USA) which was acquired by Roche
101 Molecular Systems, Inc. (Branchburg, NJ, USA) in 2014, to ensure scale up and market, and is
102 being renamed the COBAS Liat analyser (24).
103 Based on automated sample preparation, total nucleic acid extraction, amplification and real-time
104 detection, the assay provides a quantitative, interpreted VL result in 35 minutes on whole blood
105 (25). An initial laboratory evaluation in South Africa on the HIV Quant plasma and whole blood
106 assays gave 100% sensitivity versus plasma VL with decreased specificity on whole blood
107 (41.2%) (26).
108
109 To date, the Liat™ analyser has not been evaluated within a clinical setting by the intended
110 operator. The objective of this study was to investigate the option of extending laboratory plasma
111 based VL testing with finger stick whole blood POCT testing, through: 1) evaluating the
112 feasibility of nurse operated POC VL testing, within a busy ARV treatment clinic; 2) assessing
113 the performance of the Liat™ Quant blood assay on whole blood specimens derived from finger
114 stick, in comparison to plasma VL at the 1000 cp/ml and 5000 cp/ml thresholds.
115
116 **Methods**
117 **Ethics statement**
118 The study was approved by the Human Research Ethics Committee of the University of the
119 Witwatersrand, Johannesburg, South Africa (protocol number M120143).
120 **Study site and patient enrolment**
121 HIV-positive patients attending the Themba Lethu ARV treatment clinic (Right to Care, Helen
122 Joseph Hospital), Johannesburg, South Africa, for routine ART monitoring, were recruited by a
123 study nurse when they presented at the phlebotomy room requiring a VL test.
124 **Study procedures**

After patients received their venepuncture for standard-of-care (SOC), an additional finger stick specimen was obtained by the study nurse using the BD Microtainer® Contact-Activated lancet (21 G x 1.8 mm) (Becton Dickinson Diagnostics, Franklin Lakes, NJ, USA). The manufacturer recommended collecting finger stick blood directly into the supplied pasteur pipette and transferring into the Liat™ cartridge immediately, but due to the clinic workflow and need for patient enrolment and consent, this was not possible. Finger stick blood was therefore collected in an $EDTAK_3$ microtainer tube (Becton Dickinson) and taken directly to a designated POC laboratory (adjacent to the phlebotomy room) within the clinic for POC VL testing on the Liat™ HIV Quant blood assay within 5 minutes of blood collection. The POC laboratory was established in order to avoid disruption of clinic workflow and because of space constraints within the phlebotomy room. Two Liat™ analysers were available for the study.

Briefly, testing on the HIV Blood Quant assay involved transferring 75µl of whole blood from the $EDTAK_3$ microtainer, using the supplied pasteur pipette (IQuum, Inc.), directly into the Liat™ cartridge opening, closing the cartridge cap, scanning the assay cartridge barcode and loading it into the Liat™ instrument. Quantitative whole blood results were available in 35 minutes. No POC VL results were acted on, and patient management was per SOC.

Predicate laboratory testing

All routine SOC VL testing was performed on plasma specimens at a SANAS (South African National Accreditation Service) accredited laboratory, the National Health Laboratory Service (NHLS) PCR laboratory, Johannesburg, on the Roche COBAS AmpliPrep/COBAS Taqman (CAP/CTM v2) (Roche Molecular Systems, Inc., Branchburg, NJ, USA). These results were used as the reference method for comparison of the Liat™ HIV Quant whole blood results.

Statistical Analysis

Bland Altman (27) and percentage similarity (28) using MS Excel, STATA 12 and Medical Calculator (29) were used to determine assay accuracy (agreement) between the Liat™ HIV Quant blood assay and the CAP/CTM v2.0 on plasma. The sensitivity, specificity and misclassification of blood specimens on the Liat™ was also calculated compared to the CAP/CTM v2 using the 1000cp/ml and 5000cp/ml WHO cut-off values for virological failure (3). Qualitative characteristics of the assay were reported as: ease of use, error rates, space requirements and waste disposal.

Results

Patient demographics

A total of 101 patients were approached for inclusion into the study between the 2nd August and 28th October 2013. The mean age of all participants consented and enrolled was 41.4 years (n=101) and 63 participants were female (62.4%). The median time participants had received ART at the time of enrolment was 3 years (mean 3.36 years). One patient was excluded from analysis due to insufficient blood flow from the finger stick and the Liat HIV Quant blood reported one invalid result (not repeated on a second finger stick as patient had left the clinic).

Description of study data

Overall the Liat HIV Quant blood assay generated higher VL values compared to plasma based testing, with 65.7% (65/99) generating an absolute difference of >1.0 log cp/ml.

Plasma specimens tested on the Roche CAP/CTMv2 plasma-based assay yielded 13% (13/99) as lower than detectable limit (LDL), 5% (5/99) as target detected but not quantified (<20 cp/ml) and 81.8% (81/99) generated a quantifiable result. Of those specimens generating a quantifiable result, 19 had VL>1000 cp/ml (3 log cp/ml) and would therefore have required adherence

175 counselling and a follow up VL in 8 weeks. The median plasma VL on the Roche CAP/CTMv2
176 was 2.40 log cp/ml (or 249 cp/ml).
177 Whole blood specimens tested on the Liat HIV Quant blood assay yielded 3% (3/99) specimens
178 (as target not detected (TND) and 97% (96/99) had a quantifiable result, of which 91.6% (88/96)
179 had VL>1000cp/ml. The median VL on the Liat HIV Quant blood assay was 3.73 log cp/ml (or
180 5592 cp/ml).
181 The distinction between the plasma and whole blood assays, visualised in figure 1, shows that
182 13% of specimens reported by Roche CAP/CTMv2 as LDL were detectable by the Liat HIV
183 Quant blood assay and all were reported as VL>1000 cp/ml. The Liat HIV Quant blood assay
184 reported 3% of specimens as TND but these were all detected by plasma based testing on Roche
185 as VL<1000 cp/ml (38, 102 and 320 cp/ml respectively; median 3.6log cp/ml). This confirms the
186 Liat HIV Quant blood assay did not miss any patients with plasma VL>1000c/ml.
187
188 As described in Figure 2, at the current 1000 cp/ml treatment failure threshold, plasma VL
189 results categorised 19 patients as treatment failures, whilst blood-based (total nucleic acid
190 extraction methodology) testing categorised 88 patients as treatment failures. This generated a
191 Liat HIV Quant blood assay sensitivity of 100% but a poor specificity of 13%, resulting in
192 69.7% (69/99) total misclassification, all of which were upward misclassified compared to
193 plasma VL. If the treatment failure threshold was adjusted to 5000 cp/ml [as for DBS (20)],
194 based on Liat Quant blood testing, 52 patients (52.5%) would have required a follow up plasma
195 VL. At this threshold, the Liat HIV Quant blood assay sensitivity reduced to 79% but specificity
196 improved (53.8%). The total misclassification at the 5000 cp/ml threshold was 41%; 37 patients
197 were upward misclassified and 4 patients were downward misclassified (plasma VL values were:
198 1008c/ml, 1882c/ml, 2139c/ml and 2628c/ml).
199

Analytical Performance of the Liat™ HIV Quant blood assay

Overall, the Liat HIV Quant blood testing showed a high (21.6%CV) percentage similarity coefficient of variation and a mean log bias of -1.0 log cp/ml (SD 0.91c/ml). Figure 2 further illustrates the analytical performance of the Liat HIV Quant blood compared to the Roche CAP/CTMv2 on plasma, categorized according to the 1000 cp/ml and 5000 cp/ml treatment failure thresholds. The overall mean bias for the Liat™ HIV Quant blood assay was acceptable at values >1000 cp/ml, however the absolute numbers of specimens in this data set is low for typical evaluation studies.

Qualitative results

Installation of two Liat analysers was completed by the study nurse and training using instructions provided by the manufacturer was completed within an hour by a scientist. The analysers have a small footprint of approximately 11.4 cm (wide) by 19 cm (high) by 24.1 cm (deep) and weighed approximately 3.8kg, requiring little bench space. All test cartridges were stored in a 4°C fridge available within the POC laboratory for general use. The study nurse was able to test 4-6 specimens per analyser per day, but this also included patient consenting and enrolment into the study. Two indeterminate VL results were reported on the Liat HIV Quant blood assay, but only one could be repeated on a second finger stick specimen as the patient was still available. No errors were reported, and all Liat HIV Quant blood assay cartridges were easily disposed in the standard clinic biohazard waste removal bins.

Discussion

A laboratory evaluation of the Liat HIV Quant plasma assay demonstrated that the technology is interchangeable with existing high throughput VL platforms in terms of performance but due to the total nucleic acid extraction protocol which detects cell associated DNA and RNA, the whole blood assay over quantified VL in <4 log cp/ml range (26). This current study aimed to quantify the performance of the Liat™ HIV Quant blood assay in a clinic setting, receiving majority ART experienced patients, and determine the feasibility of a nurse performing POC VL testing. Similar to laboratory findings, in this urban clinical study setting, the Liat HIV Quant blood assay operated by a nurse on finger stick derived whole blood specimens, showed an overall increased detection of VL below the 5000cp/ml threshold compared to plasma VL on the Roche CAP/CTMv2. This was exacerbated by the fact that 19% patients visiting this clinic would be required to have follow up VL testing using existing plasma based technologies, but 88% on whole blood VL testing. Overall agreement of the Liat HIV Quant blood assay compared to plasma testing on this patient group (ART experienced) was above the acceptable level of 2.9% similarity CV (30)(21.56% CV) with increased variability (SD of the bias of 0.91log c/ml). When categorised according to the two treatment failure thresholds, Liat HIV Quant blood showed acceptable bias with plasma >1000 cp/ml and >5000 cp/ml.

Three patient specimens were undetectable by the Liat HIV Quant blood assay but all were below the 3 log c/ml threshold on plasma based testing and would therefore not have had any effect on the clinical management of these patients. The Liat HIV Quant blood assay did, however, detect 13 patients as containing quantifiable HIV which were all lower than detectable limit by plasma-based testing.

The specimens collected in this study typically represent those from ART monitoring patients visiting an urban clinic in South African. Within this setting, the Liat HIV Quant blood assay clinically misclassified 69.7% of VL results as treatment failures (non-adherence) at the 1000 cp/ml threshold (88 patients identified as treatment failures by Liat HIV Quant blood assay versus 19 patients truly identified by plasma VL) but had 100% sensitivity compared with plasma VL. Thus, use of the Liat HIV Quant blood assay using the current guidelines (4) would translate into a substantial increase in the number of patients requiring adherence counselling and return clinic visits for follow up plasma VL testing, which would increase costs to patients, impact patient clinic flow, and increase laboratory plasma testing costs and testing volumes. The Liat HIV Quant blood assay, however, did not miss any patients that based on plasma testing would truly require treatment switching. If the virological treatment failure threshold were to be elevated to 5000 cp/ml for whole blood monitoring, 52.5% of the Liat HIV Quant blood VL results would have been misclassified, 4% of these patients who were true virological failures would have been missed, although their plasma VL values did not exceed 3000c/ml.

In our setting, the study nurse performing the POC VL testing had no prior laboratory experience and was easily trained on the operation of the Liat platform (including finger stick collection, waste disposal, spill clean-up and safety) within an hour. Besides the instruction provided by the supplier, an easy to understand standard operating procedure was also developed and found to be valuable. The Liat analyser can be accommodated into the clinic workflow by either being placed directly into the phlebotomy room or placement elsewhere and finger stick blood collected into a microtainer EDTA tube, as was the case with our study. The study nurse was able to test 4-6 patients per instrument per day (including patient enrolment and consent) but the Liat has the potential to perform 15 samples per 8 hour day (22), and includes battery functionality and full connectivity. As most patients attend the clinic in the morning the majority

of POCT would need to be performed by midday (manuscript under review), which would require reengineering the clinic workflow to accommodate VL testing and ensure optimal patient throughput. The only current aspect of Liat HIV POC testing (either plasma or blood) which could potentially be seen as a drawback is the need for cold-chain storage of the test cartridges, requiring access to a fridge, which will require strict control in a clinic environment.

In South Africa, the National Health Laboratory Service (NHLS) provides testing services to more than 80% of the population and currently has 17 high throughput, centralised VL laboratories that manage ~3million VL tests per year. POC testing would never replace this current workflow, but certainly can extend this service and thereby help to meet the '90-90-90' targets. Provision of a VL result in 35 minutes (as for Liat HIV Quant blood protocol) directly in the clinic will allow clinicians to decide on the need for appropriate adherence counselling and follow-up visit for VL testing or immediate discharge of the patients from the clinic until their annual routine next visit. However, this study has alerted the fact that VL tests for monitoring that are based on total nucleic acid extraction, performed on whole blood specimens, will increase the misclassification rate of patients identified as ART failures. Alternatively, introducing plasma-based Liat HIV Quant plasma POC VL testing which is interchangeable with existing VL platforms in terms of performance (26) may be more effective, even though it will require the need for specimen centrifugation. Extending current plasma based VL services, would therefore need to take these pros and cons into consideration.

The ability of the Liat HIV Quant blood assay to measure proviral DNA as well as cell associated RNA would, however, make it advantageous in diagnosing HIV in key populations such as babies at birth from HIV-positive mothers (26) and may warrant a place as a niched POC VL test. Currently, VL testing for early HIV infant diagnosis is fraught with challenges (20); laboratory testing for infants is not always available and even though qualitative testing is

299 becoming more accessible through the use of DBS, turnaround times may take weeks (31). This
300 together with full costing remains to be addressed.
301
302 **Acknowledgments**
303 We wish to thank the patients of Thembu Letu (Right to Care) clinic for participating in this
304 study.
305
306

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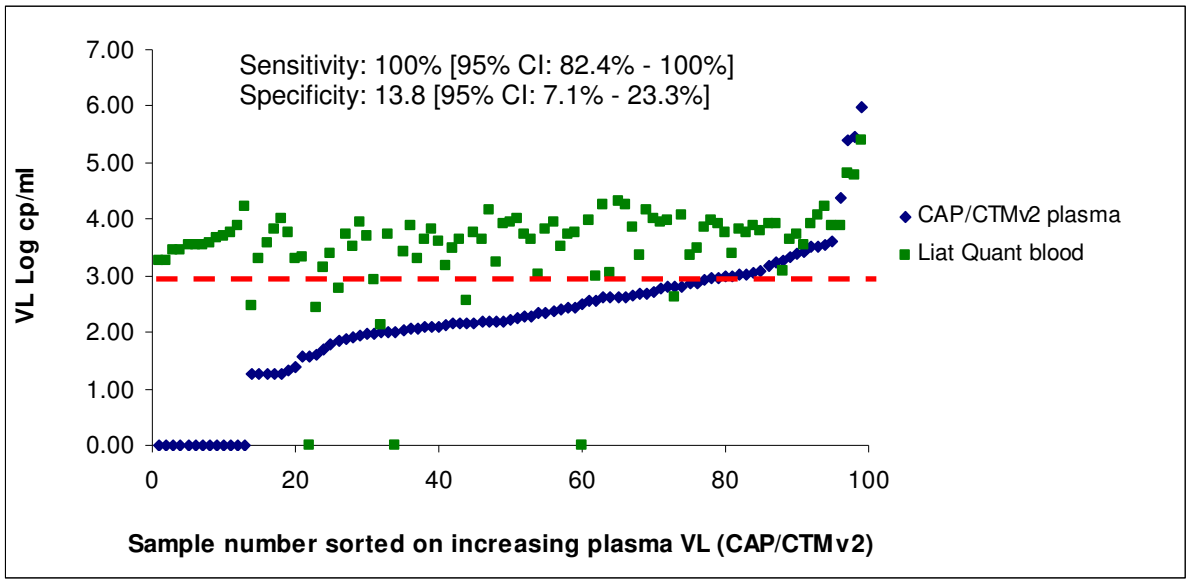
List of figures

A.)



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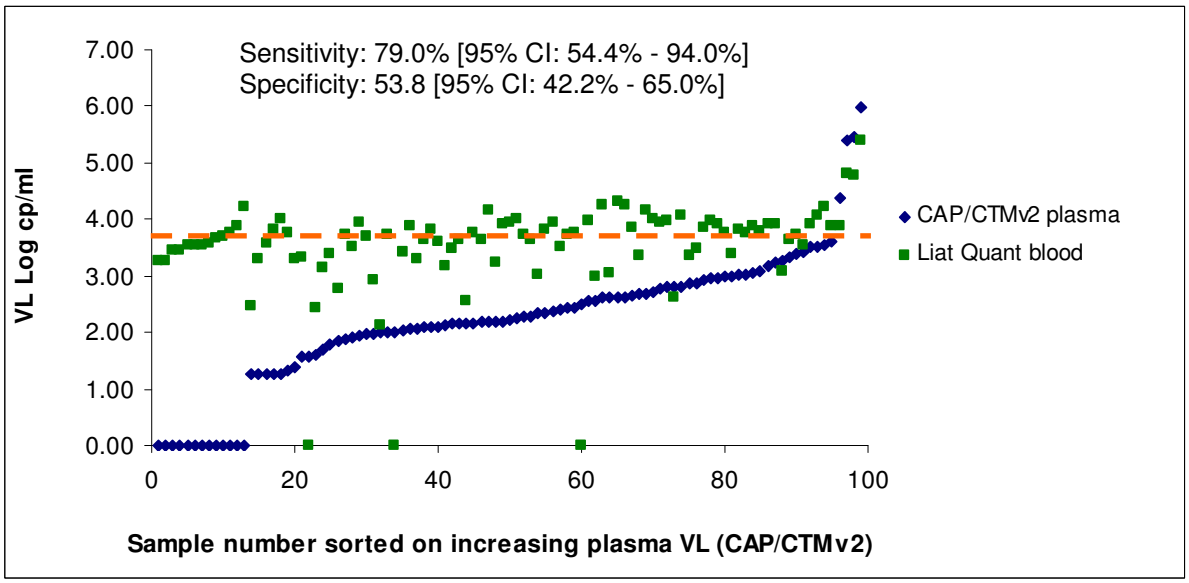

Figure 1: Scatter plot of log transformed VL values (y axis) for the Liat™ HIV Quant whole blood and the Roche CAP/CTMv2 assays, sorted according to increasing VL on plasma VL (x axis). a) Red dotted line indicates the current 1000 cp/ml (3 log cp/ml) treatment failure threshold used for plasma VL and b) the orange dotted line indicates the 5000cp/ml (3.7log cp/ml) threshold recommended for dried blood spot testing (20).

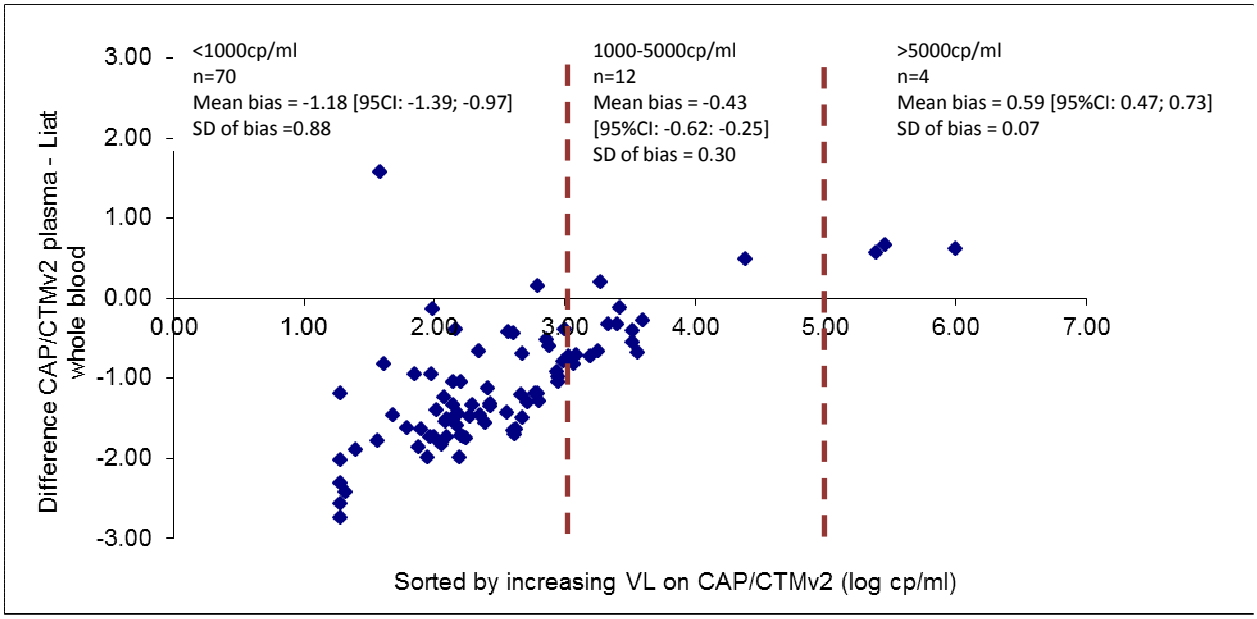

Figure 2: Bland-Altman difference plot of Liat™ HIV Quant blood assay versus Roche CAP/CTMv2 showing the mean difference and SD in the <1000c/ml, 1000-5000c/ml and >5000cp/ml ranges.

CHAPTER 6: POLICY DEVELOPMENT

All the components described above have contributed to the development of the current status of POCT in South Africa (2013), entitled ‘Point of Care Testing: Position paper Forum Report to support National strategic Plan for POCT for the management of HIV and TB in South Africa’. This paper initiated the national policy discussion with a scientific basis. (Complete document available on request from the candidate).

POINT OF CARE TESTING:

Position paper Forum Report to support National strategic Plan for POCT for the management of HIV and TB in South Africa

An overview of the Point-Of Care Forum hosted by the National Department of Health

24TH AND 25TH JUNE 2013

The National Department of Health thanks the following speakers for their valuable contributions to the Point of Care Testing Forum:

Dr Yogan Pillay (National Department of Health); Sagie Pillay (National Health Laboratory Service); Professor Wendy Stevens (National Health Laboratory Service); Dr Maurine Murtagh (Murtagh Group); Dr Regina Osih (Consultant Clinician); Professor Sydney Rosen (Health Economics & Epidemiology Research Office; Universities of Boston and Witwatersrand); Dr Amir Shroufi (Médecins Sans Frontières); Dr Andrew Black (Wits Reproductive Health & HIV Institute); Henry Julius (National Health Laboratory Service); Y Tsibolane (Free State Department of Health); Dr Ilesh Jani (National Institute of Health, Mozambique); Professor Lesley Scott (National Health Laboratory Services), Natasha Gous (University of Witwatersrand); Brad Cunningham (University of Witwatersrand); Dr Thato Chidarikire (National Department of Health); Dr Tim Tucker (Strategic Evaluation, Advisory and Development Consulting); Dr Adrian Puren (National Institute for Communicable Diseases); Dr Jaya George (National Health Laboratory Service); Tope Adepoyibi (Program for Appropriate Technology in Health (Seattle, USA); Dr Dusty Gardiner (Council for Scientific and Industrial Research); Dr Sergio Carmona (National Health Laboratory Service); Professor Debbie Glencross (National Health Laboratory Service); Lousanne Oosthuizen (University of Stellenbosch); Kate Schnippel (Health Economics & Epidemiology Research Office); Jonathan Lehe (Clinton Health Access Initiative); Emily Hyle (Campaign to End Paediatric HIV/AIDS); Dr Rosanna Peeling (LSHTM: London School of Hygiene and Tropical Medicine); Dr Trevor Peter (African Society for Laboratory Medicine and Clinton Health Access Initiative); Dr Varough Deyde (Centre for Disease Control).

POINT OF CARE TESTING:

Position paper Forum Report to support National strategic Plan for POCT for the management of HIV and TB in South Africa

An overview of the Point-Of Care Forum hosted by the National Department of Health

24TH AND 25TH JUNE 2013

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Session 4:

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ABBREVIATIONS

ABBREVIATIONS

ART:	Anti-Retroviral Treatment
ASLM:	African Society for Laboratory Medicine
CCPLP:	Combined Clinical POC Laboratory Platform
CEPA:	Campaign to End Paediatric HIV/AIDS
CHC:	Community Health Centre
CHRU:	Clinical HIV Research Unit
CLIA:	Clinical Laboratory Improvement Amendments of 1988
CrAg:	Cryptococcal Antigen
DBS:	Dried Blood Spot
EF7:	European Union's Seventh Framework Programme
EID:	Early Infant Diagnosis
FBC:	Full Blood Count
FDA:	Food and Drug Administration, USA
FIND:	Foundation for Innovative Diagnostics (Geneva, Switzerland)
GCC:	Grand Challenges Canada
HCT:	HIV Counselling and Testing
HCW:	Health Care Worker
Hb:	Haemoglobin
LTFU:	Loss to Follow Up
MSF:	Médecins Sans Frontières
NHLS:	National Health Laboratory Service
NDOH:	National Department of Health (South Africa)
NIMART:	Nurse Initiated Management of ART
PATH:	Program for Appropriate Technology in Health (Seattle, USA)
PHC:	Primary Health Care
POCT:	Point-Of-Care Testing
QA:	Quality Assurance
QC:	Quality Control
RNA:	Ribonucleic Acid
VL:	Viral Load

EXECUTIVE SUMMARY

Point-of Care testing (POCT) refers to testing that is performed near or at the site of the patient with the result leading to a possible or immediate change in patient management or outcome [19].

There is an ever-expanding pipeline and strong advocacy for POCT emerging from various groups on a global basis who maintain that universal access for HIV and TB care requires the use of POCT for earlier testing and improved retention in care. Cited advantages of POCT include improved turnaround time, greater accessibility, potentially improved patient retention and possible reduction in overall health care costs. However, despite the rapid growth and interest in POCT, many aspects remain controversial, in part because this process challenges the conventional approach to laboratory testing, and specifically for South Africa, the prevailing paradigm. Significant laboratory infrastructure currently exists in South Africa in both the public and private sectors and sheer volumes of testing may make total de-centralization prohibitive in terms of instrumentation and human resources required.

Major Issues surrounding the implementation of POCT exist such as poor regulatory control, difficulties in ongoing monitoring of quality and that limited guideline documents are currently available for the safe implementation of POC devices. In addition, there are few studies that report data on full economic costing for POC [13], which is likely to vary depending on tests used and diseases investigated.

In order to address these controversies, a Point-of-Care Forum hosted by the National Department of Health, was held on the 24th and 25th June 2013 to determine the process by which South Africa could adopt Point-of-Care testing (POCT) in clinics and how it could be institutionalised. The forum was to address issues not only related to HIV and TB, but also for various non-communicable diseases such as diabetes and cancer.

The desired outcomes of the forum were to establish current, urgent and feasible clinical testing needs for POCT in South Africa as well as to establish feasible models for implementation, focusing on quality concerns, regulatory hurdles and research gaps.

There is a dearth of well -designed randomized, controlled clinical trials to evaluate the outcomes and impact of the implementation of POCT. Various clinical experiences were presented at the forum from sub-Saharan Africa. A number of these studies, such as the Home-based Care Plus trial in KZN, Rapid Initiation of Anti-retrovirals in Pregnancy (RAP) study in Cape Town, RCT – GCC and RapIT (Midrand PHC), are still a while away from informing policy and have shown that POCT is just one step in a multi-step process along the continuum of care. Other experiences showed that POCT had great potential for certain populations such as migrants where loss to follow up is high and where immediate results would add value.

A pilot study involving implementation of PIMA CD4 POC testing in the Free State showed that time to initiation was reduced, however, challenges were identified in that some nurses viewed POC implementation as additional workload, and that some patients migrated from facilities before the facilities were able to track, record and file the results in patient's folders. Experiences from Mozambique showed that after the introduction of POC CD4 the loss to follow up before CD4 staging dropped, ART initiation rate increased and time to ART initiation was reduced from 48 days to 20 days. Retention rates in care however, remained the same. It was recommended by this group that deploying POC should be done in co-existence with conventional testing as part of a total laboratory network and there was acknowledgement that POC testing is far from error -proof. There was a warning that simple implementation is not always efficient: access does not necessarily mean that the patient gets care (approximately 25% of patients did not get CD4 testing even with POCT on site). It was highlighted that significant health systems strengthening is needed.

Breakout discussions included the clinical perspectives on POCT in South Africa to address which tests were needed and where and when they needed implementation. Recommendations included POCT for the following tests: CD4 (same day result not essential, but important in some populations such as hard to reach areas, areas where turn-around times (TATs) are outside of the norm, or a high pre-ART loss to follow up, creatinine, tuberculosis GeneXpert (only if TAT >2days or areas where multi-drug resistant high), reflex CrAg testing if CD4 count <100, paediatric patients (5-

15yrs): Rapid HIV test and CD4 as per adults, early infant diagnosis (EID) for younger children. Viral load testing at POC was not recommended by the group as it was believed that there is no evidence to support the benefit. HbA1c for diabetes management was also recommended for POCT.

For POC implementation: discussions were held as to whether total coverage should be initiated through an expansion of the existing tiered laboratory framework or whether total decentralization should be chosen as the implementation model. This would include two types of facilities that could be accredited for POCT, either those that are able to manage only rapid strip-based testing; or those that are able to manage more sophisticated tests. Should large scale implementation of POCT be considered, the following factors need to be taken into account:

1. Feasibility in approximately 4,000 clinics;
2. Costs of the impact of introduction against the loss to follow up (LTFU), whether "Test and treat" may be a future approach to increasing ARV initiation;
3. The ability to have connectivity going forward with POCT;
4. Additional human resources; and
5. Regulations

In the South African primary healthcare environment, POC assays should be rapid and easy to perform, require minimal training and no specialized laboratory setup, and reagents should be stable and temperature independent, if possible. Few tests actually meet all these criteria or the FDA specifications for a simple test. Simplicity does not, however, always lead to improved quality of care and thus implementation needs to be facilitated in a staged fashion with careful monitoring of performance and ongoing quality at each implementation step. It should also be noted that the transfer of assays from a centralized testing facility to POC does not ensure improved clinical outcome and the value needs to be proven in properly controlled clinical studies [54].

The upcoming diagnostic pipeline for TB POCT includes Cepheid, Molbio, Ustar as well as analysers from Epistem (Genedrive) and Eiken. Interestingly, the Xpert assay has received FDA approval as a moderate complexity assay, excluding it from CLIA-waived POC assays. For the HIV diagnostic pipeline certain CD4 platforms are already in the market and include PointCare Now, the Partec mini-cyflow and the Alere PIMA CD4 test (Alere since 2009). A plethora of fast followers are available for validation such as MBio, Daktari, FACSCpresto, Visitect, Zyomyx, Omega Diagnostics/Burnet, among others. For viral load testing, three assays are close to market, including LIAT (IQUUM), Alere Q, (Alere) and SAMBA (Real World Diagnostics). Few have adequate validation data and uncertainty exists around scaling up production.

Various operational experiences were presented throughout the forum and highlighted important areas for discussion including blood specimen collection methodologies (fingerstick versus venipuncture), who would be responsible to perform POC testing in clinics, the need for implementation checklists and the importance of connectivity to ensure data retention. A South African experience showed that clinic infrastructure for POC testing is varied and would require certain re-organisation. Quality systems would need to be adapted from laboratory systems to POC testing to ensure good clinical POC practice, and training for computer literacy was emphasized .

SANAS requirements are now detailed in ISO guidelines 22780 where POC exists as an extension of the laboratory. Nurses had limited time to perform POC testing which would add additional tasks to their already busy schedules.

Several connectivity options were presented highlighting software solutions which are currently in use in the laboratories for high-throughput fully automated analysers as well as SMS printers for improved TATs in patient result delivery, to future solutions that would be needed for POC connectivity. It was clear that any implementation for POC would require a solution to manage the result reporting and quality in order to collect the data for central review, analysis and reporting.

Two systems were evaluated to meet the centralized management of POC testing, Conworx and AegisPOC. Both cover the required mechanisms to allow central management of decentralized testing with existing infrastructure available to interface to the current NHLS Laboratory Information System (LIS) system. The link between instrument and data management platforms and clinical and patient management solutions however, still needs to be addressed in order to complete the process from sample resulting to patient treatment.

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Cost and cost models for Point-of-Care Technology were presented during the forum. Importantly, it was noted that without a consistent and transparent methodology, estimates of the costs of CD4 tests using POC technology are likely to be difficult to compare and may lead to erroneous results about costs and cost-effectiveness. It was emphasized that context matters, particularly with respect to labour (i.e. level of staff) and test volumes. Key cost drivers were found to be labour, consumables and volume of tests, and it was noted that systems and roll-out costs also need to be included in cost models. Overall, POCT has been found to be more costly than laboratory testing, however, answers as to whether the incremental costs of POCT implementation will have added value requires measurement and comparison of patient outcomes and impact.

Breakout discussions from an implementation perspective on POCT in South Africa highlighted the need for a technical working group to be formed that would be responsible for expert advice surrounding implementing POC policies as well as for training, quality assessment, mentorship, SOP's and on-going monitoring of testing services.

From a POCT quality perspective, results from implementation of HIV rapid tests in South Africa were presented from the SEAD study and showed that enhanced national quality assurance programmes were critical, training and mentoring needed to be expanded and ongoing, and that procurement and distribution needed serious attention. South African experiences also emphasized the importance of post-marketing surveillance and that lot-to-lot variation needs to be monitored; appropriate training material needs to be implemented; and that internal quality control measures need to be put into place for successful POC implementation. These findings were mirrored in a presentation highlighting the challenges experienced with POC diabetes testing in Ethiopia. Challenges relating to the development of appropriate algorithms and protocols; the maintenance and calibration of instruments; the quality of testing; inadequate staff training and supply and stock issues were discussed, emphasizing the critical need for quality control at every step of the process.

Discussions from the breakout session for quality perspectives on POCT in South Africa suggested that the current standards may be too strict for POC and need to be modified to fit within a South African clinical context. The consensus was that quality systems are essential to implement, but that this should be conducted in a phased manner taking into account LIS management and gate keeping, cadre of staff, training, supply chain management and safety. It was recommended that an advisory committee be established for overall quality management.

Regulatory control surrounding POCT was also discussed at the forum showing differences in legislation for different countries. It was noted that new concepts in quality control technology need to be reviewed in light of the new generation of POC assays and instrumentation. In South Africa specifically, no regulations are currently in place for the control of medical devices. There is no Medical Devices Act governing the implementation and use of laboratory assays or for POCT. The accrediting body in South Africa, SANAS, has recently issued an ISO guideline governing the use of POC assays: ISO/FDIS 22879 entitled: Point of care testing (POCT)- Requirements for quality and competence [50]. This standard can be applied when POCT is conducted in hospitals, clinics or organizations providing ambulatory care and specifically excludes patient self-testing in home or community.

There are a number of regulatory hurdles in getting a product from "bench to bedside" with processes that are very fragmented and lacking in co-ordination. Ultimately, the primary goal is the protection of public health and safety. It was acknowledged that regulatory oversight of In Vitro Diagnostics (IVDs) is highly variable in the developing world, and that approval processes are often lengthy, costly and non-transparent. The Global Harmonization Task Force (GHTF) was established in 1992, driven by the growing need for international harmonization in the regulatory process for medical devices. In 2013, GHTF will transition to a purely regulatory body called the International Medical Devices Regulatory Federation (IMDRF), which will continue to promote the principles of harmonization.

Feedback from the regulatory breakout session indicated that it is clear that regulatory control is a barrier to POC implementation in South Africa and that there needs to be a balance between control and implementation. Recommendations included that a legal framework be established to allow for the approval of good quality tests, that processes be transparent and that regulations should be enabling and allow for innovation, rather than be a barrier. For

EXECUTIVE SUMMARY

POC validations, it was proposed that they should be conducted in the population for which they are intended and that foreign data should be accepted for approval. Policies and procedures should be standardized through the national guidelines and regulatory bodies and these can be enforced through the South African Health Products Regulatory Agency (SAHPRA).

From the data presented at this forum, the main starting point for POCT implementation in South Africa is believed to be CD4. A meta-analysis was presented for data collected from various publications on evaluations of the PIMA (Alere) analyser, since this was most likely the only test currently available for POC CD4 implementation at the time of this publication. Performance of the PIMA analyser was shown to have improved over time (2009–2012) which may indicate improved instrument and assay versions as well as improved training modules and better lancets. PIMA showed good agreement with other technologies at CD4 counts less than 100cells/ µl, but increased variability at 350cells/ µl. This increased variability leads to an overall 12% misclassification, but in favour of patient treatment initiation. Overall variability was found to increase with the use of capillary specimens, but again in favour of patient treatment initiation.
In order to proceed with POCT, the first steps of this process will be to identify appropriate clinics some of which has already been done through GIS mapping to identify areas where there are gaps in service delivery, but also to determine the most cost-effective way to provide analytical capacity for each of these areas and suitable instruments for POCT. Thereafter, it will be critical to ensure quality at all sites, to assign dedicated technical staff of a correct cadre to be placed within existing clinic NDoH structures and with correct reporting management lines. Additionally, data capture which requires LIS connectivity and a license fee will be required. Once these POC sites are in place, the service may then be extended to offer other POC testing such as ALT, Creatinine, Hb and TB.

Final remarks from the forum had strong emphasis on the establishment of a technical working group with various different stakeholders from NDoH, NHLS, NGO's and partner organisations as well as from clinical advisory groups in order to formulate policies and implementation plans for processes moving forward. This document serves as assistance for the technical working group, among others, for making informed decisions and being instrumental in choosing the appropriate way forward for POCT in South Africa.

CHAPTER 7: CONCLUDING CHAPTER

7.1 DISCUSSION

Great strides have been made in the fight against the HIV/AIDS epidemic. As a consequence of the success of the ART program, there was a resultant 44% decline in HIV infection rates between 2001 and 2012 and two of the hardest hit areas, Southern Africa and Central Africa, have seen declines of 48% and 54% respectively (1). Despite these achievements, there were still 2.1 million new HIV infections reported globally in 2013; 1.5 million of these were reported in sub-Saharan Africa (1), where it is estimated that three quarters of HIV infected adults have not achieved viral suppression due to gaps and shortfalls in service delivery (2). Expansion of ART programs will be imperative to achieving the '90-90-90' treatment goals, which in turn will require expanded laboratory capacity.

South Africa is unique in terms of both the high prevalence of HIV and TB co-infection rates and the availability of centralized laboratory infrastructure and capabilities to address high testing demands. Although capacity in these centralized facilities can handle high throughput volumes through the use of sophisticated laboratory equipment, expertise and resources, there is still an uneven distribution of these tertiary reference laboratories throughout the country, with most servicing industrialized urban areas (3). Approximately 19 million South Africans live in rural areas according to the World Bank database (4), where barriers to healthcare access include high travel costs and travel distances to clinics (5), long queues in clinics (6) and disproportionate healthcare costs (5). Many of these rural areas are serviced by PHC clinics that lack infrastructure, skilled workers and resources and are only capable of performing technically non-demanding tests (such as rapid HIV tests and pregnancy tests). In theory these tests, while easy to perform, are notorious for their poor quality (7) and the improvement of testing quality is now a national focus.

To address these challenges, new testing strategies such as POCT, which look beyond expansion of centralized testing capacity to improve access to healthcare services and achieve the global '90-90-90' goals, are being investigated (8). In South Africa, these challenges may need to be addressed through a hybrid model, much like for CD4 testing (9). In preparation for this, the current work, as part of a GCC funded project, evaluated new diagnostic technologies for HIV and TB, developed essential quality components for POCT and determined the feasibility of multiple POCT in the field through the presentation of 11 conference abstracts, 9 publications published in peer reviewed journals, two submitted and under review and 1 contribution to a policy document. This work culminated in support of a randomized clinical study assessing the feasibility of using multi-disciplinary POCT versus standard of care to support ART treatment for HIV infected individuals in PHC clinics (to be reported on in 2015).

7.1.1 ENGAGING GOVERNMENT AND ASSESSING CLINICAL NEED

Before embarking on widespread POC implementation in South Africa, the NDoH required a concrete recommendation for the country on what POC tests would best address the clinical needs of the country and how best to implement them. A POCT forum was organized by the NHLS and hosted by the NDoH and involved key stakeholders (NDoH, NHLS, non-governmental organizations (NGO's), clinical advisory groups and partner organizations) in an effort to establish the context for POC implementation in South Africa.

The key challenges likely to emerge during large-scale POCT implementation efforts were presented by the literature review in Chapter 2. The review addressed the clinical needs based on in-country treatment guidelines at the time and placed a strong emphasis on CD4 and VL testing. The evolving role of VL testing for treatment monitoring and the potential of moving to 'test and treat' strategies however, has led to scale up of VL testing services

becoming even more important in achieving the '90-90-90' treatment goals (10). The necessity of other core tests (haematology, biochemistry and opportunistic infections, especially TB) was also acknowledged for the management of HIV-positive individuals, and hence formulated the need to develop a 'multiple POCT' policy for HIV and TB integration of services.

7.1.2 THE EVALUATION OF NEW TECHNOLOGIES FOR THE DIAGNOSIS AND/OR MONITORING OF HIV AND TB

Challenges that may hinder national POC implementation efforts will be the selection and evaluation of suitable POC tests from the plethora of upcoming HIV and TB technologies in the development pipeline. To this end, two plasma-based VL POC technologies were evaluated. The Liat™ HIV-1 Plasma Quant VL assay (IQuum Inc, now Roche Molecular) (presented in Chapter 3.1) was evaluated against two in-country VL predicate platforms. The Liat™ plasma assay showed good performance with 100% sensitivity at the 1000 copies/ml treatment failure threshold and no virological failures were missed. The second plasma based POC VL assay evaluated was the Xpert® HIV-1 VL on the GeneXpert® platform (Cepheid, Sunnyvale, CA). Preliminary evaluation data on the Xpert® HIV VL has been presented at various forums (11, 12) and shows acceptable performance in comparison to the Roche COBAS® CAP/CTM version 2 and does not miss any true virological failures. Both the Liat™ and Xpert® assays thus appear promising candidates and could be interchangeable with existing in-country predicate technology in terms of performance, but have limitations. As both are plasma-based, the requirement for a centrifuge make neither suitable for a 'true' POC environment and thus may be better placed within a district or community level facility.

Two further POC VL assays, both blood-based, were therefore also investigated; the Liat™ HIV-1 Blood Quant VL assay (presented in Chapter 3.1) and the Alere™ q HIV-1/2 assay (Alere™) (13). Although these assays were easy to perform, both overestimated VL (much like DBS,) due to their total nucleic acid (TNA) extraction protocols. One can therefore expect a 10.6% and 45% upward misclassification with the Liat™ and Alere™ q assays, respectively (13). The implications of such overestimation would lead to more patients identified as treatment failures, thus increasing the need for follow-up plasma VL testing and increased programmatic costs. This suggests that a change in the clinical on-site treatment algorithm would probably be required if used for ART monitoring but would likely be difficult to implement. The application of these POC VL assays could therefore be in a niched environment; providing a blood-based POC VL test within a maternity ward to diagnose HIV in new-borns at risk of further transmission from HIV-positive mothers, and plasma based POCT for mothers to reduce risk of HIV transmission.

When investigating options for increasing access to VL testing, it should be acknowledged that POCT may not be the only option. Alternative strategies to improve the logistical challenges around transportation of blood specimens from clinics to laboratories are also warranted as centralized testing is always more controlled and affordable in the laboratory environment. The use of DBS for ART treatment monitoring has been investigated on a longitudinal cohort of patients and proven as valuable as plasma VL for detecting patients failing treatment at the 1000 copies/ml threshold (14). Blood collection, transport and storage technologies, such as Primestore media (Longhorn Vaccines and Diagnostics, San Antonio, TX, USA) may also present a viable option (15).

In terms of TB diagnosis, limitations to conventional testing methods have made it notoriously difficult to diagnose, especially among HIV-positive individuals (16). In 2010, the world geared up for a game-changer in TB diagnosis, the molecular-based GeneXpert® MTB/RIF assay. The first publications on GeneXpert® described its analytical performance

(17, 18) and the demonstration study by Boehme (19) illustrated the performance of the GeneXpert® in 10 countries. There was however, little guidance on the implementation of the Xpert® MTB/RIF assay into national TB programs, particularly in high HIV and TB endemic regions such as South Africa. To address this, the Xpert® MTB/RIF assay was evaluated in Johannesburg in order to inform policy development for TB diagnosis in South Africa (Chapter 3.2). The laboratory performance of the Xpert® MTB/RIF against several current TB diagnostic assays on a single sputum specimen, demonstrated the superiority of the Xpert® MTB/RIF. More importantly, the Xpert® MTB/RIF did not show reduced sensitivity in HIV-positive specimens. These findings gave confidence to the South African NDoH to proceed as the first country with a single, national, phased implementation plan to replace smear microscopy with the Xpert® MTB/RIF as the first line TB diagnostic (20, 21). Even though the GeneXpert® was placed into smear microscopy centres due to the prohibitive cost of placement at POC (22), many groups have provided compelling data on use of the Xpert® MTB/RIF in clinical settings, showing same day treatment initiation and shorter time to treatment (23-25).

Use of the Xpert® MTB/RIF has also expanded to paediatrics/childhood TB, a process which has historically been hampered by the type of specimen which can be collected, multiple samplings over several days (26), poor quality and low volume of specimens (27), and facilities which are ill-equipped to perform induced sputum collection. Initial evaluation studies using the Xpert® MTB/RIF in paediatrics, performed testing on laboratory decontaminated and concentrated specimens (27-31). Through a study performed by the candidate, the technicalities of using the Xpert® MTB/RIF for pediatric TB diagnosis within a clinical setting, at the POC, on raw sputum specimens was investigated (32) and showed that the majority (67%) of “routinely obtained” sputum specimens from children (≤14; n=484) were below the required volume for Xpert® testing and required ‘topping up’ with saline. Even though the Xpert® MTB/RIF outperformed smear microscopy, it would be difficult to

implement as a replacement for smear and culture in paediatrics due to the high rejection rate of low volume specimens and lower sensitivity compared to culture (32). Numerous studies have since been presented on implementation of GeneXpert® for pulmonary (33-37) and extra-pulmonary (38-40) TB diagnosis in children.

In an attempt to address the lack of GeneXpert® testing at POC in the South African model, the candidate investigated an “equipment-free” TB diagnostic assay that appeared close to market, the EasyNat® MTB detection Kit (Ustar Biotechnologies, Hangzhou, Ltd). An initial laboratory evaluation of the technology yielded excellent sensitivity and specificity but the testing process was found to be very laborious and in the current format and not suitable for POCT (41). The difficulty too, within a program that is GeneXpert® dominated, is the inability of some POC tests to simultaneously investigate RIF (and INH) drug susceptibility, which the Xpert® MTB/RIF does for RIF.

7.1.3 DEVELOPING THE PRINCIPLE COMPONENTS TO ENSURE BEST PRACTISE FOR MULTI-DISCIPLINARY POCT: QUALITY, SITE READINESS, TRAINING

There is a general lack of guidelines detailing the quality, infrastructure and training requirements for POC implementation (42), specifically for South Africa. To address this need, the candidate investigated these requirements in order to inform best practise for quality POCT to complement exisiting laboratory testing.

Ensuring the quality of POCT results in the field is a major challenge, especially when current HIV rapid testing in clinics is fraught with problems (43). As a starting point, the candidate presents the development of two distinct but compatible quality monitoring programs in Chapter 4, one for molecular HIV VL platforms (Chapter 4.1) and the other for molecular TB platforms (Chapter 4.2). The SAVQA (South African Viral Quality Assurance)

panel was originally developed in response to the need for ensuring newly placed high throughput VL testing platforms in centralized laboratory facilities were ‘fit for purpose’. The usefulness of this standardized HIV-1 panel was quickly realized as a tool for assessing the performance of newly developed VL POC technologies (44-47) against existing in-country technologies. To date, the panel has been successfully used to verify the Liat™ HIV-1 Quant (IQuum, Inc), Alere™ q HIV-1/2 (Alere) and the Xpert® HIV-1 VL (Cepheid) assays prior to laboratory evaluation (48, 49). Through collaboration with numerous VL technology development groups, this panel will continue to be manufactured and supplied to aid developers in assessing their product for the South African market, and will also be further developed for use by healthcare workers at POC.

The rapid national implementation of the GeneXpert® technology into smear microscopy sites informed the need for a quality monitoring system (21), particularly since its use by numerous NGO’s (Wits Reproductive Health and Research Institute, Right to Care, Aurum Institute) at the POC and expansion of the program by the NHLS into correctional services and mobile vans (50). The Dried Culture Spot (DCS) verification program was developed in 2011 to ensure that newly placed GeneXpert® instruments were ‘fit-for-purpose’ and has successfully been used to verify >4,600 GeneXpert® modules at laboratory and clinical sites. Through a public/private partnership between the University of the Witwatersrand and Cepheid, verification panels are now shipped together with new GeneXpert® instruments to test every module installed in the field, both nationally and internationally. The DCS program used for verification of the GeneXpert® was endorsed by the WHO and the Global Laboratory Initiative in 2014. Further to this, the DCSs were launched as an EQA program in 2013, to ensure ongoing quality and accuracy of Xpert® MTB/RIF patient results (51). The EQA program is offered as three panels per year and currently supports 207 NHLS, 2 private and 2 AIDS Clinical Trial Group (ACTG) laboratories in South Africa. Additionally, the program is also supporting 289 sites in 20 different countries worldwide.

The success and versatility of the DCS program in the field is apparent by its use in remote settings and mobile laboratories by non-laboratory trained staff (Chapter 4.3), and in its application to other TB diagnostic technologies, most notably the Genotype MTBDR*plus* LPA (Chapter 4.4). In light of this work, a collaboration has been established with the National Institute of Health funded ACTG in the United States, in order to set up a pilot trial of the DCS material for ACTG sites performing line probe testing. Interest in the product has also been expressed by other TB platform/assay developers, such as Abbott Molecular (Des Plaines, IL, USA) for their upcoming high throughput MTB assay. This work has proven the versatility and ease of use of the matrix from centralised laboratories to decentralised POC sites.

The Research and Development team involved in the development of the DCS program, has been acknowledged for their work through three awards: the NHLS Top Award for Innovation in 2013 (at the National Innovation Annual Awards Ceremony), the Gauteng Accelerator Program (GAP) Biosciences Award in 2014 (52) and a special Social Impact award for Africa held in Morocco in 2015 (53).

To ensure the appropriate infrastructure and training needs for POCT are met, the candidate developed a POC implementation checklist specifically designed to assess the South African PHC clinic readiness prior to multidisciplinary POC placement for HIV and TB service integration. This checklist assesses variables such as clinic space and storage availability, infrastructure, security, ventilation, power supply and connectivity (54, 55) (Appendix C) (not ISO requirements) and has been adopted and modified for use by the NHLS NPP for assessment of POC laboratory sites prior to GeneXpert® MTB/RIF installation. In terms of training of POC operators, the golden rule is to always assume no prior experience (56). The candidate developed a simple training package with the non-laboratory user in mind, which included easy-to-follow standard operating procedures, quick reference and workflow charts

(Appendix D1 and D2), maintenance and stock templates, a practical training module and a 'clinic starter' kit (including basic consumbles required to perform POCT) (57). The standard operating procedures and reference charts developed are currently being used by the NPP trainers for GeneXpert® training.

Although not part of the candidates work, central to ensuring the quality of POCT results will be the need for connectivity (58). POCT will need to fit within the current national LIS which connects diagnostic instruments in the public sector directly and then stores all results generated in a central data warehouse to allow central management and monitoring. Connectivity will also allow for a centralised system to coordinate and ensure stock supply.

7.1.4 DETERMINING THE FEASIBILITY OF MULTIPLE POCT FOR HIV AND TB SERVICE INTEGRATION IN THE FIELD

In Chapter 5.1, the feasibility and accuracy of nurse performed multi-disciplinary POCT within a clinical setting is demonstrated. A key component to ensuring the success of this study was the selection of appropriate clinics using the site assessment checklist and thorough training of non-laboratory staff using the developed POC training material. This was the first study in South Africa to demonstrate how a dedicated nurse was able to perform multidisciplinary POCT as accurately as laboratory testing on multiple analytes. This study highlighted the fact that introduction of POCT into a clinic led to a notable increase in daily duties for the POC staff. With clinic nurses already experiencing work-related stress and burnout (59, 60), a new cadre of technical staff specifically for POCT will be needed and this level of staff will depend on the site and complexity of the POC tests required. In this study, all POCT was performed on a single venous specimen. POC tests are however, designed for use on minimally invasive specimen types such as finger sticks (61) in order to reduce complexity of the testing process. In South Africa, which has multiple testing requirements for ART initiation and monitoring and more than 50% of patients attending an

ARV treatment clinic for routine monitoring require three or more tests per clinic visit, this creates unique challenges. Subsequently, the feasibility and acceptability of performing multiple finger sticks for multidisciplinary POCT was addressed in two further studies (Chapter 5.2 and 5.3). The first, investigated whether performing multiple POC tests (CD4, Hb, Cr and ALT), each on a separate finger stick, would be feasible for the nurse and acceptable to the patient. Not only was a dedicated nurse able to perform multiple finger sticks and the relevant POCT required on a patient easily and accurately, but the process could be simplified by performing all POC tests on a single finger slice (lancet designed for CD4 testing to prevent lymphocyte degradation). Interestingly, patients also preferred having even multiple finger sticks to having a venepuncture.

This study provided important insight for POC platform developers in designing their assays and determining the maximum blood volume that could be collected from a single finger stick. As a direct consequence of this work, a collaboration was established with Northwestern University and NWGHF to initiate a second study to investigate maximum blood volumes from a single finger stick (Chapter 5.3). Findings have influenced the design of a blood collection device for a new POC VL platform under development by Northwestern, namely the Savanna POC VL (45).

The CD4 venous and capillary performance evaluation (nurse versus laboratory) results from Chapter 5.2 have been included in a meta-analysis of the PIMA CD4 platform (62) and the PhD candidate is subsequently a member of the PIMA CD4 consortium.

The feasibility of a nurse performing POC VL testing on a finger stick specimen within a busy ARV treatment clinic was later demonstrated to determine whether the technology has the potential to extend existing laboratory VL testing (presented in Chapter 5.4). In the field, the assay was easy to perform but showed greatly increased detection of VL specifically at the 1000 copies/ml treatment failure threshold (70% misclassification); this effect was minimised

but still apparent when the threshold was brought up to 5000 copies/ml (41% misclassification). Again, this highlights the potential value of the assay for diagnosis of HIV in key populations, rather than use for treatment monitoring.

7.1.5 POLICY DEVELOPMENT

Much of the work presented by the candidate contributed towards the development of a draft National policy document (Chapter 6) in support of the national strategic plan for POCT for the management of HIV and TB in South Africa (63).

7.2 CONCLUSION

Multidisciplinary POCT for HIV ART (initiation and monitoring) and TB (diagnosis) service integration is feasible and accurate, if performed in a well-managed clinic by dedicated, well-trained staff. To achieve quality POCT results, appropriate training, quality and data management systems need to be in place. Although the current work details the laboratory and clinical evaluation of a few POC technologies, ultimately the choice of platform to implement will depend on the needs of the population in which they are intended as well as the ability of the technology supplier to meet testing demands. Ideally, the chosen POC technologies should be rapid and easy to perform, require minimal training and no specialized laboratory set up, and reagents should be stable and temperature independent, if possible. Few technologies currently meet these criteria and based on South Africa's testing volume needs, a hybrid model that includes POCT to extend the existing laboratory service footprint, could help achieve the '90-90-90' goals. Performance evaluations of new POCT technologies will need to be ongoing based on availability. Alternative models which will improve logistics around specimen collection and transport and mobile health (mHealth) solutions which utilise mobile phones to increase access to patients, are also providing promise as a strategy for health systems strengthening (64).

Although the focus of the current work was on HIV and TB, in reality the life expectancy of HIV-positive individuals is increasing, but so too are the frequency of co-morbidities such as diabetes, non-AIDS defining cancers, cardiovascular and metabolic diseases (65, 66). These will also need to be taken into account when expanding and integrating the repertoire of POC tests to be implemented.

Beyond the scope of the current work, the need for cost effectiveness and clinical effectiveness studies cannot be overlooked. Overall, POCT has been found to be more costly than laboratory testing (67), but answers as to whether the incremental costs of POCT

implementation will have added value requires measurement and comparison of patient outcomes and impact through clinical trials.

In a country such as South Africa, which has unprecedented numbers of HIV and TB-positive persons, the ‘90-90-90’ goals will require multiple, integrated interventions and significant investment and innovations to reach the objectives by 2020. POCT will form ‘part of’ the plan, but will also require innovative strategies around linkage to care.

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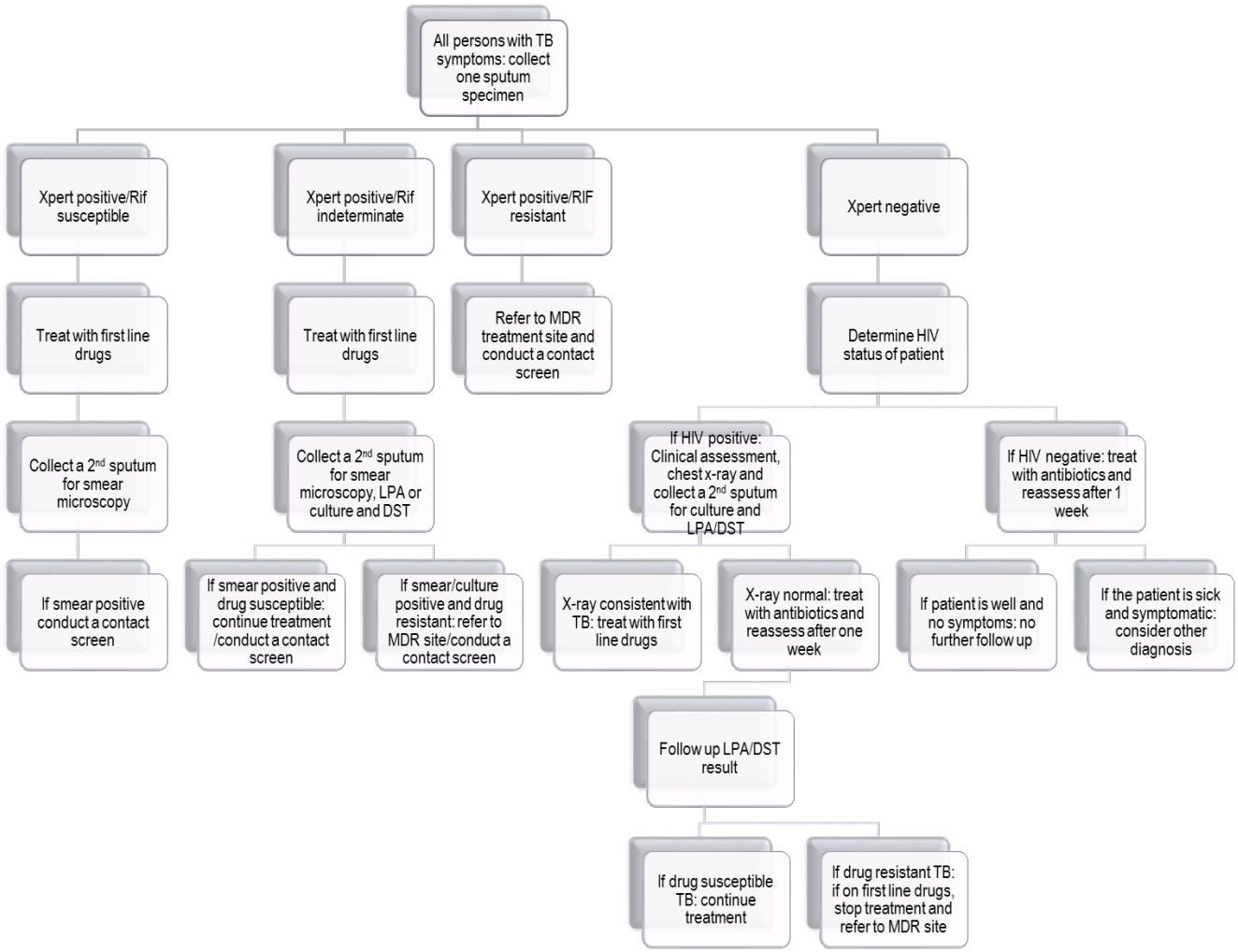
7.3 APPENDICES

7.3.1 APPENDIX A

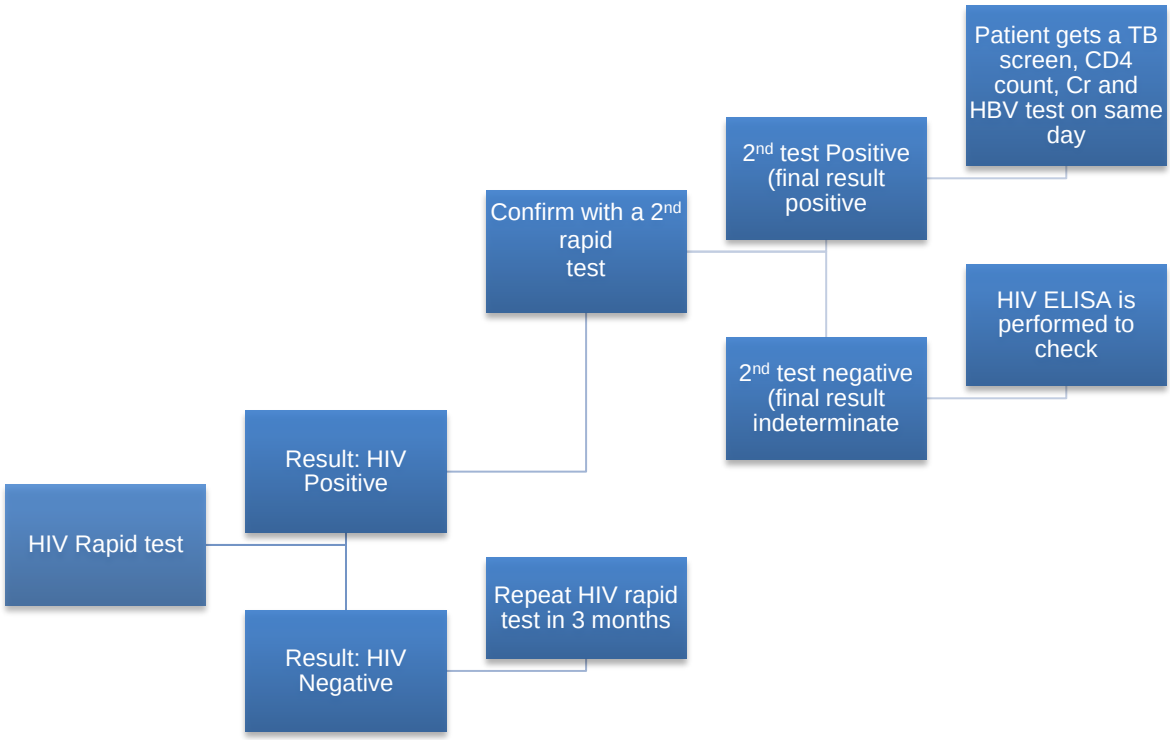
The South African national adult pulmonary TB diagnostic algorithm and interpretation.

Adapted from the National Tuberculosis Management guidelines 2014 (68).

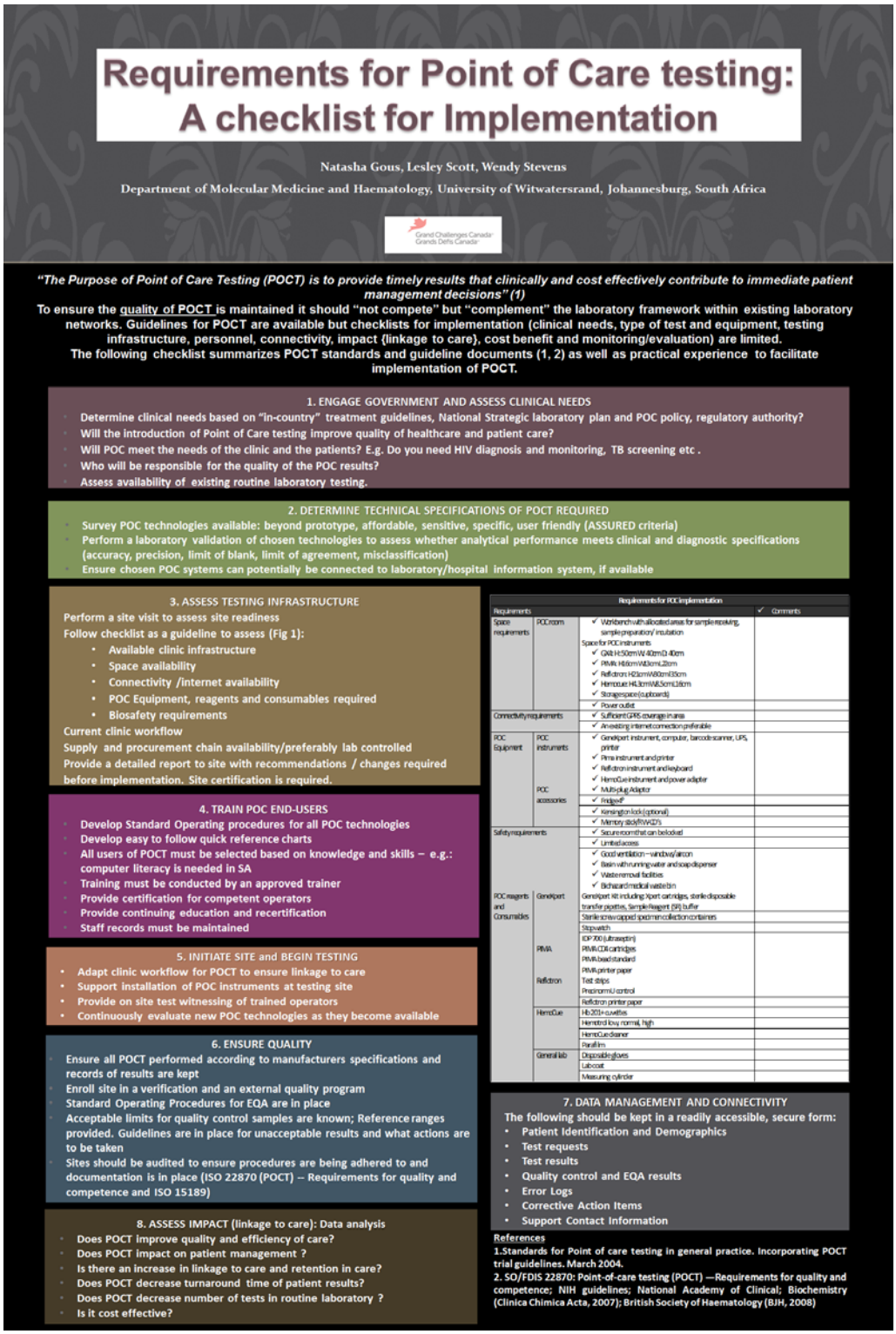


7.3.2 APPENDIX B

The national HCT algorithm according to the South African NDoH (adapted from (69)).



7.3.3 APPENDIX C



Requirements for Point of Care testing: A checklist for Implementation

Natasha Gous, Lesley Scott, Wendy Stevens

Department of Molecular Medicine and Haematology, University of Witwatersrand, Johannesburg, South Africa

Grand Challenges Canada · Grands Défis Canada

"The Purpose of Point of Care Testing (POCT) is to provide timely results that clinically and cost effectively contribute to immediate patient management decisions" (1)

To ensure the quality of POCT is maintained it should "not compete" but "complement" the laboratory framework within existing laboratory networks. Guidelines for POCT are available but checklists for implementation (clinical needs, type of test and equipment, testing infrastructure, personnel, connectivity, impact {linkage to care}, cost benefit and monitoring/evaluation) are limited.
The following checklist summarizes POCT standards and guideline documents (1, 2) as well as practical experience to facilitate implementation of POCT.

1. ENGAGE GOVERNMENT AND ASSESS CLINICAL NEEDS

- Determine clinical needs based on "in-country" treatment guidelines, National Strategic laboratory plan and POC policy, regulatory authority?
- Will the introduction of Point of Care testing improve quality of healthcare and patient care?
- Will POC meet the needs of the clinic and the patients? E.g. Do you need HIV diagnosis and monitoring, TB screening etc .
- Who will be responsible for the quality of the POC results?
- Assess availability of existing routine laboratory testing.

2. DETERMINE TECHNICAL SPECIFICATIONS OF POCT REQUIRED

- Survey POC technologies available: beyond prototype, affordable, sensitive, specific, user friendly (ASSURED criteria)
- Perform a laboratory validation of chosen technologies to assess whether analytical performance meets clinical and diagnostic specifications (accuracy, precision, limit of blank, limit of agreement, misclassification)
- Ensure chosen POC systems can potentially be connected to laboratory/hospital information system, if available

3. ASSESS TESTING INFRASTRUCTURE

Perform a site visit to assess site readiness
Follow checklist as a guideline to assess (Fig 1):

- Available clinic infrastructure
- Space availability
- Connectivity /internet availability
- POC Equipment, reagents and consumables required
- Biosafety requirements

Current clinic workflow
Supply and procurement chain availability/preferably lab controlled
Provide a detailed report to site with recommendations / changes required before implementation. Site certification is required.

4. TRAIN POC END-USERS

- Develop Standard Operating procedures for all POC technologies
- Develop easy to follow quick reference charts
- All users of POCT must be selected based on knowledge and skills – e.g.: computer literacy is needed in SA
- Training must be conducted by an approved trainer
- Provide certification for competent operators
- Provide continuing education and recertification
- Staff records must be maintained

5. INITIATE SITE and BEGIN TESTING

- Adapt clinic workflow for POCT to ensure linkage to care
- Support installation of POC instruments at testing site
- Provide on site test witnessing of trained operators
- Continuously evaluate new POC technologies as they become available

6. ENSURE QUALITY

- Ensure all POCT performed according to manufacturers specifications and records of results are kept
- Enroll site in a verification and an external quality program
- Standard Operating Procedures for EQA are in place
- Acceptable limits for quality control samples are known; Reference ranges provided. Guidelines are in place for unacceptable results and what actions are to be taken
- Sites should be audited to ensure procedures are being adhered to and documentation is in place (ISO 22870 (POCT) -- Requirements for quality and competence and ISO 15189)

8. ASSESS IMPACT (linkage to care): Data analysis

- Does POCT improve quality and efficiency of care?
- Does POCT impact on patient management ?
- Is there an increase in linkage to care and retention in care?
- Does POCT decrease turnaround time of patient results?
- Does POCT decrease number of tests in routine laboratory ?
- Is it cost effective?

Requirements for POC implementation

Requirements			✓ Comments
Space requirements	POC room	✓ Workbench with allocated areas for sample receiving, sample preparation/ incubation	
		Space for POC instruments:	
		✓ GX4: H:50cm W:40cm D:40cm	
		✓ PIMA: H:60cm W:30cm L:20cm	
		✓ Reflotron: H:20cm W:80cm H:35cm	
		✓ Hemocue: H:13cm W:35cm L:16cm	
		✓ Storage space (cupboards)	
		✓ Power outlet	
Connectivity requirements		✓ Sufficient GPRS coverage in area	
		✓ An existing internet connection preferable	
POC Equipment	POC instruments	✓ GeneXpert instrument, computer, barcode scanner, UPS, printer	
		✓ Pima instrument and printer	
		✓ Reflotron instrument and keyboard	
		✓ HemoCue instrument and power adapter	
		✓ Multiplug Adaptor	
	POC accessories	✓ Fridges[2]	
		✓ Kensington lock (optional)	
		✓ Memory stick/RW CD's	
Safety requirements		✓ Secure room that can be locked	
		✓ Limited access	
		✓ Good ventilation – window/aircon	
		✓ Basin with running water and soap dispenser	
		✓ Waste removal facilities	
		✓ Biohazard medical waste bin	
POC reagents and Consumables	GeneXpert	GeneXpert Kit including Xpert cartridges, sterile disposable transfer pipettes, Sample Reagent (SR) buffer	
		Sterile screw capped specimen collection containers	
		Stopwatch	
	PIMA	IDP 700 (Ultrasept in)	
		PIMA CD4 cartridges	
		PIMA bead standard	
		PIMA printer paper	
	Reflotron	Test strips	
		Precinorm U control	
		Reflotron printer paper	
	HemoCue	Hb 201+ cuvettes	
		Hemotrol low, normal, high	
		HemoCue cleaner	
		Parafilm	
	General lab	Disposable gloves	
		Labcoat	
		Measuring cylinder	

7. DATA MANAGEMENT AND CONNECTIVITY

The following should be kept in a readily accessible, secure form:

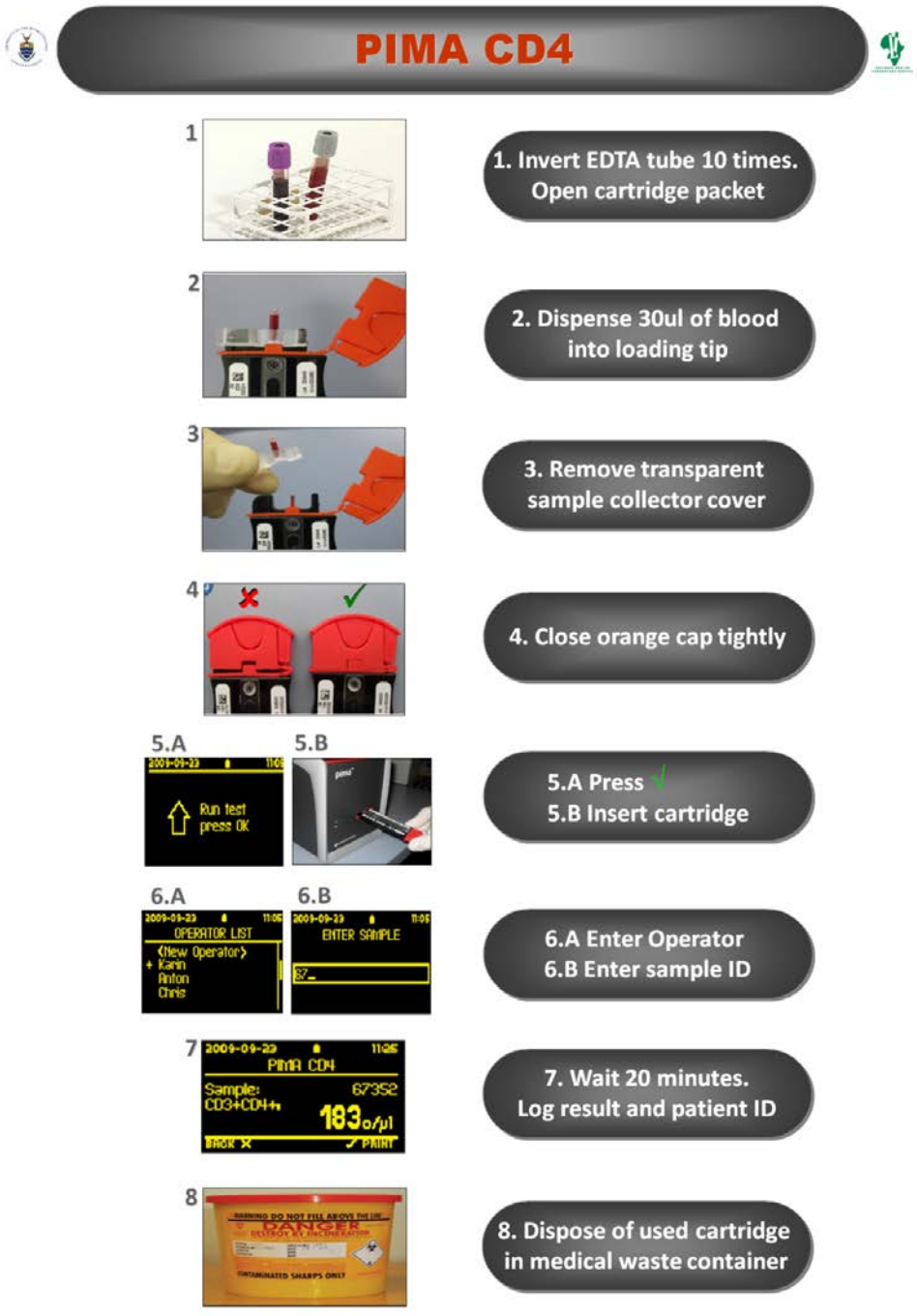
- Patient Identification and Demographics
- Test requests
- Test results
- Quality control and EQA results
- Error Logs
- Corrective Action Items
- Support Contact Information

References

1.Standards for Point of care testing in general practice. Incorporating POCT trial guidelines. March 2004.
2. SO/FDIS 22870: Point-of-care testing (POCT) —Requirements for quality and competence; NIH guidelines; National Academy of Clinical; Biochemistry (Clinica Chimica Acta, 2007); British Society of Haematology (BJH, 2008)

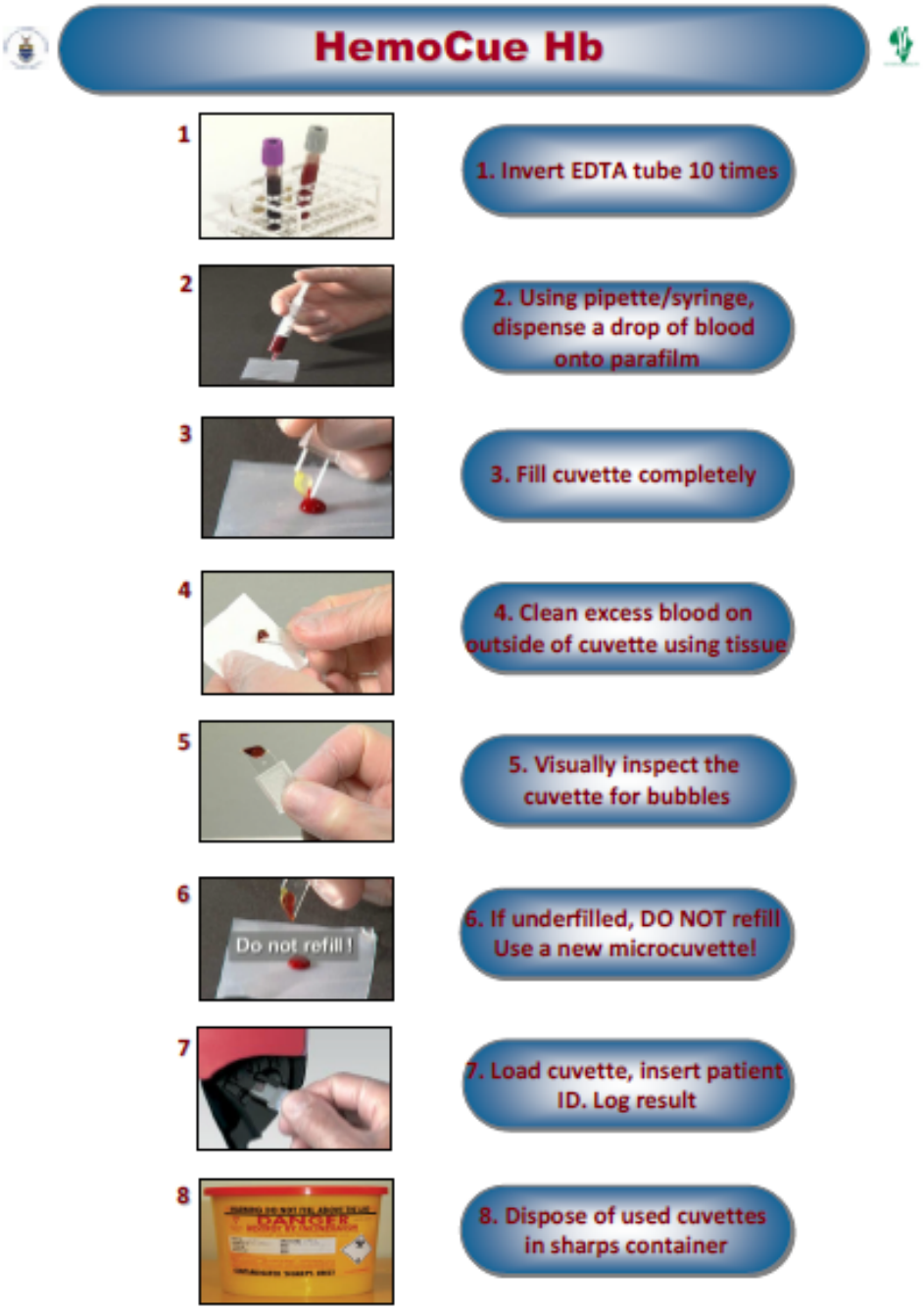
7.3.4 APPENDIX D1

An example of a quick reference chart developed for use of the PIMA CD4 at POC.



7.3.5 APPENDIX D2

An example of a quick referecne chart deveopled for use of the HemoCue Hb at POC.



7.3.6 APPENDIX E1

Ethical clearance certificates.

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)
Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-0709
Private Bag 3, Wits 2050, South Africa

23 January 2012

Professor Lesley Scott
Senior Medical Scientist
Department of Molecular
Medicine & Haematology
Faculty of Health Sciences
Medical School
University

Sent by e-mail Lesley.Scott@mhls.ac.za

RE: **Protocol M110139: 'Evaluation of Sputum PCR and the Development of Novel Screening Strategies by Flow Cytometry for the Diagnosis of Tuberculosis**
Protocol amendment

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has reviewed and approved your request " perform the same study in the Joubert Park Clinic" on the abovementioned protocol as detailed in your letter dated 20th December 2011.

Thank you for keeping us informed and updated.

Yours sincerely,

Anisa Keshav
Secretary
Human Research Ethics Committee (Medical)

7.3.7 APPENDIX E2

Study — Essell

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Noble

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M070826**
PROJECT	Evaluation of Sputum PVR and the Development of Novel Screening Strategies by Flow Cytometry for the Diagnosis of .....
INVESTIGATORS	L Noble
DEPARTMENT	Molecular Medicine
DATE CONSIDERED	07.08.31
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY

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

DATE 07.11.01 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof W Stevens

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

7.3.8 APPENDIX E3

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Prof Wendy Stevens

CLEARANCE CERTIFICATE	M10333
PROJECT	Investigation of the Feasibility of Implementation of Multi-Disciplinary Point of Care (POC) Testing in an HIV Treatment Clinic
INVESTIGATORS	Prof Wendy Stevens.
DEPARTMENT	Molecular Medicine & Haematology
DATE CONSIDERED	26/03/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 29/03/2010 CHAIRPERSON .....
(Professor PE Cleaton-Jones)

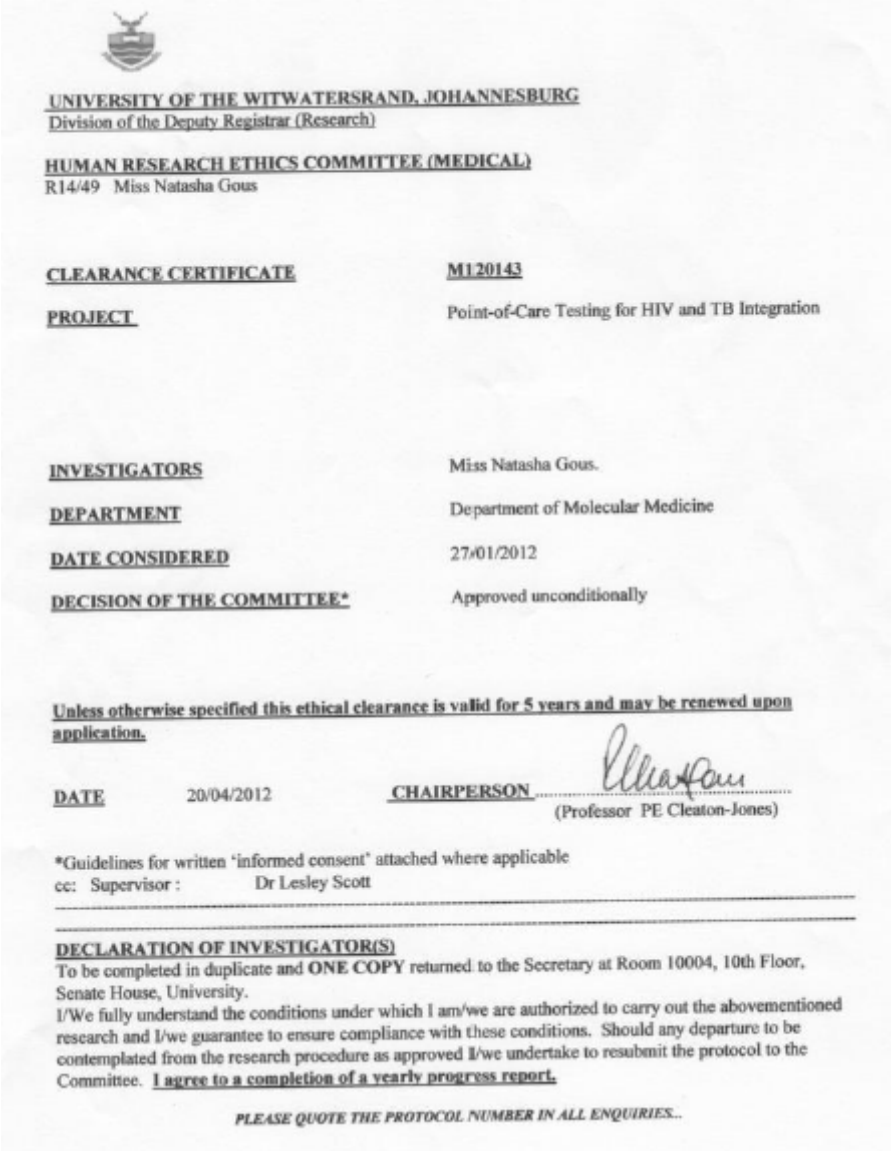
*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

7.3.9 APPENDIX E4



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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Miss Natasha Gous

CLEARANCE CERTIFICATE	M120143
PROJECT	Point-of-Care Testing for HIV and TB Integration
INVESTIGATORS	Miss Natasha Gous.
DEPARTMENT	Department of Molecular Medicine
DATE CONSIDERED	27/01/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 20/04/2012 CHAIRPERSON .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Lesley Scott

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES..