Review Article

HIV point-of-care diagnostics: meeting the special needs of sub-Saharan Africa

Godwin A Aleku[1,2], Moses P Adoga[3,4], Simon M Agwale[5,6]

[1] *Manchester Institute of Biotechnology, University of Manchester, Manchester, United Kingdom*
[2] *Directorate of Enforcement, National Agency for Food and Drug Administration and Control (NAFDAC), Lagos, Nigeria*
[3] *Microbiology Unit, Department of Biological Sciences, Nasarawa State University, Keffi, Nigeria*
[4] *Computational and Evolutionary Biology/Bioinformatics, Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom*
[5] *Innovative Biotech, Keffi, Nassarawa State, Nigeria*
[6] *Innovative Biotech USA Inc, Federick, MD, United States*

Abstract

Sub-Saharan Africa, accounting for 70% of the 35 million people living with HIV worldwide, obviously carries the heaviest burden of the HIV epidemic. Moreover, the region's poor health system occasioned by limited resources and inadequate skilled clinical personnel usually makes decentralization of HIV care difficult. Therefore, quality diagnostics that are easy to use, inexpensive, and amenable for use at point of care (POC) are a dire necessity. Clearly, such diagnostics will significantly lessen the pressure on the existing over-stretched centralized HIV laboratory services. Thankfully, some POC diagnostics are already being validated, while others are in the pipeline. As POC test kits emerge, implementation hurdles should be envisaged and planned for. This review examines emerging HIV diagnostic platforms, HIV POC product pipelines, gaps, perceived POC implementation challenges, and general recommendations for quality care.

Key words: point of care; diagnostics; resource-limited settings; sub-Saharan Africa

J Infect Dev Ctries 2014; 8(10):1231-1243. doi:10.3855/jidc.4664

(Received 04 January 2014 – Accepted 26 May 2014)

Introduction

Sub-Saharan Africa carries the heaviest burden of the global epidemic of HIV. The UNAIDS 2013 report revealed that over 35 million people are infected with HIV worldwide, with sub-Saharan Africa accounting for 70% of the epidemic [1]. Sadly, the region also faces other equally serious challenges, such as poverty, famine, infrastructural decay, political instability, and other debilitating diseases [2], which directly or indirectly drive the epidemic.

Paradoxically, the health systems in sub-Saharan Africa are ill-equipped to contend with emerging threats [3,4]. This is further worsened by inadequate healthcare professionals. Only 1.3% of the world's healthcare workforce caters to this region that experiences 25% of the global disease burden [5]. Consequently, delivery of quality healthcare in sub-Saharan Africa is still far from ideal. This is particularly worrisome in chronic disease management such as HIV/AIDS treatment.

However, the global response to the epidemic by all stakeholders has been impressive and

commendable. For example, in 2003, the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) launched the "3 by 5" program to scale up antiretroviral (ARV) coverage in sub-Saharan African countries [6]. The results have been encouraging. Today, over 9.7 million infected persons are estimated to be receiving these life-saving medications in low- and middle-income countries, representing 61% ARV coverage [1], a huge difference from the 2% coverage in 2003 [6].

Despite this historic achievement gained in the scale-up of ART services, infected people requiring ARVs exceed those currently on ART, based on the WHO's 2013 revised guideline [1,7]. Accordingly, emphasis now centers not only on the numbers of patients enrolled in care, but more importantly on quality of care for enrolled patients [8,9]. This brings

to mind the enormous task that lies ahead. On one hand, there is the need to continue the scale-up of ART enrolment to guarantee universal access. On the other hand, there is a critical need to effectively monitor and retain ART and would-be ART patients.

The birth of HIV rapid diagnostic tests (RDTs) and their successful incorporation into HIV testing at the point of care (POC) in resource-limited settings (RLS) led to the massive enrolment into HIV care [1,10]. However, clinical diagnostic monitoring tools, such as those for CD4 cell enumeration, viral load measurement, and early infant diagnosis are largely laboratory based, distant from the point of care, costly, and require skilled personnel [8-10]. This presents a special challenge on how scale-up and decentralization of ART services can be suitably accompanied with laboratory services in RLS to guarantee quality in HIV care.

To resolve this issue and to also provide robust HIV care, quality HIV diagnostics and monitoring tools that are affordable, sensitive, accurate, easy to use, rapid, portable, and deliverable at the point of HIV care are being developed [9,11,12]. These advances in rapid diagnosis and clinical monitoring technologies offer the promise of increasing access to treatment and improving clinical outcomes for people living with HIV/AIDS.

However, it is important to note that translating technological successes to improved clinical care in resource-limited, geographically remote, and harsh climate regions may not always be smooth. A viable implementation model adaptable to this region worst hit by the epidemic must accompany the future deployment of these technologies to ensure that the real values of such innovations are captured.

Here, the leveraging of portable device technologies to bring HIV laboratory services to the patient through innovative point-of-care diagnostic technologies is reviewed. The perceived implementation hurdles are appraised. Also, the need to develop a viable and adaptable model that allows the incorporation of evolving POC devices into existing healthcare structures and systems in sub-Saharan Africa for improved care is discussed.

HIV diagnostics: current landscape and pipeline

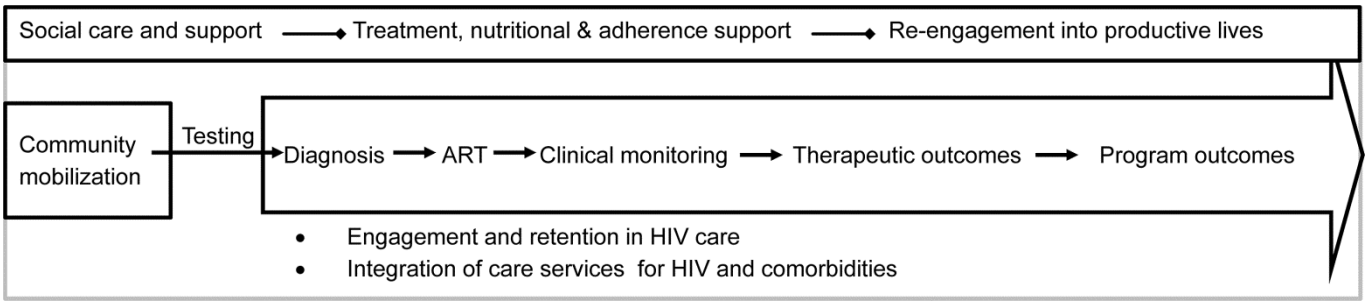
HIV care involves a coordinated and linked spectrum of services encompassing diagnosis, treatment, monitoring, and support in such a structure that guarantees and optimizes access and adherence to the treatment continuum of HIV care (Figure 1). As a gateway and monitoring tool in the HIV continuum of care program [11], the HIV diagnosis spectrum encompasses testing, early infant diagnosis, disease staging, treatment monitoring, and drug resistance assay, all of which contribute towards goals engendered in the holistic care package.

Technologies for HIV testing

HIV testing programs often accompanied by prevention campaigns are largely decentralized, and HIV+ persons are appropriately triaged to facilities with the capacity to provide a comprehensive ART program.

Technologies for HIV testing in adults and children above 18 months of age include devices that detect a specific viral antigen (*e.g.*, p24 antigen) [13], HIV antibody-based tests kits (*e.g.*, western blots), and nucleic acid amplification tests (*e.g.*, reverse transcription polymerase chain reaction amplification) [14,15]. HIV quantitative antibody-based testing methods (*e.g.*, western blot and enzyme-linked immunosorbent assays [ELISAs]) are now for reserved use, usually to validate the more cost-effective, stable, and portable HIV rapid test kits in RLS. While nucleic

Figure 1. Linked services in HIV continuum of care program. POC devices will reduce turnaround time between initial testing and treatment and ensure effective clinical monitoring of treatment at the community level. Incorporation of POC devices in the cascade will reduce loss to follow-up, facilitate the integration of care services for HIV and other co-infections, and improve engagement and retention in HIV care. ART: antiretroviral therapy.



acid amplification and p24 antigen test methods detect early HIV infection, they are complex, expensive, and require skilled technicians and laboratory settings, and thus are not routinely deployed in HIV testing in RLS.

As progress in HIV testing technologies was made, enzyme immunoassay-based methods were formulated into POC RDT kits. Most HIV RDTs are immunochromatographic-lateral flow devices and offer the advantages of quicker turnaround time, cost effectiveness, user friendliness, and heat stability [16], hence the incentives for their successful scale-up and wide application in HIV testing and surveillance in RLS.

Although there has been consensus on the high accuracy of RDTs [16], flaws in their performance have been highlighted even where the recommended use of two sequential RDT test kits is practiced. Some of these flaws have been attributed to individual or regional variations of circulating HIV subtypes and immune cross-reactivity [17]. For example, a study in the Democratic Republic of Congo reported a 10.5% false-positive rate despite the use of two sequential RDTs in HIV testing [17]. This suggests the need to incorporate a quality assurance system, and where significant performance flaws are observed and verified, alternative testing strategies should be employed in such affected regions.

RDT technologies have evolved to include non-blood fluid samples such as saliva and urine. Oral mucosal fluid-based RDTs show comparable accuracy to blood tests [18], and their use has been demonstrated to have a positive impact in reducing mother-to-child transmission in a rural hospital in India [19]. However, in a recent evaluation of OraQuick RDT, clinical sensitivity of 86% on oral fluid samples was reported [20], and a cohort study in Nigeria with OraSure showed low accuracy, especially in diagnosing HIV acute infection [21].

Notwithstanding the downsides, oral fluid RDTs hold potential for application in home-based HIV self-testing programs, as highlighted in a study in Africa [18]. There is, however, reluctance to adopt HIV self-testing surveillance because the linkage to HIV care for self-testers is poorly defined. The legal, ethical, gender, human rights, and public health implications relating to HIV self-testing have been weighed, and countries are now encouraged to scale up HIV self-testing as a complimentary strategy to increase access to HIV testing [22]. In parallel, ongoing efforts to provide practical logistic systems in RLS and the growing relevance of telemedicine [23] may provide platforms for wide acceptance and scale-up of self-testing programs in the foreseeable future. Ultimately, more evidence on the performance of the rapid oral test and other non-blood based tests will be needed in countries that are considering their implementation, even as more FDA-approved oral fluid RDT kits are launched.

POC fourth-generation multiplex HIV RDTs are being developed and are uniquely capable of simultaneously detecting HIV p24 antigen and antibodies to HIV-1 and HIV-2 (*e.g.*, ARCHITECT HIV Ag/Ab Combo Assay), as well as detecting other viral co-infections such as hepatitis B and C (*e.g.*, Multiplo) [15]. A recent performance evaluation by Pilcher *et al.* demonstrated that a new fourth-generation POC antigen-antibody combo rapid immunoassay (ARCHITECT) was comparably effective to the HIV RNA test method in detecting HIV acute infection [20], and hence may be employed as primary screening assay in high-risk HIV testing. Evidence on the test performance in clinics of these methods is limited, but the advantage of simultaneous detection of two or more acute infections is attractive and offers convenience to patients and care providers.

Technologies for disease staging and treatment monitoring CD4 count technologies

The debate on when to start antiretroviral drugs (ARVs) followed the introduction of the first ARV in 1987, and expert opinions on the subject still vary greatly. In a recent debate, Franco and Saag articulated the urgency to start ART early, regardless of CD4 cell level, citing the following reasons: that a better understanding of HIV biology has been gained, newer and safer drugs options have been launched, evidence from cohort studies is supportive, and that the public health implications of delayed therapy are critical [24]. Conversely, Lundgren *et al.* cautioned against initiating ART in the early phase of HIV infection on the premise that favorable evidence from risk-to-benefit ratio analysis drawn from large randomized trials are lacking; as such, they support the use of ART only in moderate HIV-induced immunodeficiency or severe HIV complications [25].

Amidst vigorous debate and increasing research evidence on when to initiate ART, the WHO recently raised the eligibility requirement from the initial CD4 count cut-off mark of 350 cells/μL to 500 cells/μL [7], thus establishing a new global consensus on when patients should initiate therapy. Judging from the continuous upward revision of the CD4 cell eligibility criterion and the improvements in HIV therapeutics and monitoring, it may be predicted that ART

eligibility status may be granted to all HIV+ patients irrespective of CD4 count in the next few years. Needless to say, for this to be the case, there must be convincing research-based evidence to support it.

Notwithstanding the swing direction of the ART eligibility pendulum, CD4+ T cell enumeration will continue to remain an important biological indicator in the staging of HIV disease progression, in decisions on ART eligibility and selection, in monitoring the effectiveness of treatment, and also as a guide to the diagnosis of opportunistic infections. Prompt availability of CD4 test results has been demonstrated to increase ART initiation and retention of patients in care [26,27].

Traditional, expensive, and cumbersome laboratory-based flow cytometric technologies for CD4 T cell count have been very reliable as clinical monitoring tools, but they are largely centralized, requiring laboratory infrastructure, skilled personnel, and frequent equipment maintenance. As such, their use in resource-constrained and remote regions of sub-Saharan Africa presents challenges [4]. The need to decentralize ART laboratory monitoring for better care, and the success recorded in the scale-up of malaria and HIV RDTs testing kits [28] in RLS together make clearer the inherent benefit of developing cost-effective, user-friendly, accurate, and rapid POC CD4 T cell testing options. While some are already in the market (Partec CyFlow miniPOC, PointCare Now/HumaCount CD4 Now, and Alere Pima Analyser) [12,29], more are in the pipeline (Visitect CD4, Daktari CD4 counter, Mbio Diagnostics's POC CD4 device, Zyomyx CD4 kit, and BD FACSPresto) [15].

POC technologies for CD4 count include miniaturized flow cytometric devices, cartridges with microfluidic adaptations to flow cytometry (*e.g.*, microfluidic image cytometers), or immunochromatographic strips [29-31]. Newer POC options incorporate automatic biochips, eliminating the manual sample preparation step, which was a limitation with earlier versions of CD4 POC devices [32].

Evidence of the impact of POC CD4 testing kits on therapeutic outcomes is limited but positive. A study in Mozambique reported a reduction in total loss-to-follow-up before initiation of antiretroviral treatment from 64% to 33% [27]. In another study in South Africa, the introduction of POC CD4 enumeration devices increased the proportion of ART-eligible patients [33].

However, reports on the sensitivity and performance of the available POC test kits are divergent. A peer-reviewed assessment of the Poincare NOW revealed low sensitivity; 53% and 61% of adult patients at the 350 and 200 cells/μL thresholds, respectively, were misclassified [34,46]. The CD4 POC PIMA analyzer (Alere, Inc.) evaluated in South Africa and in Zimbabwe, however, performed well compared to the standard protocol [33,35].

As more POC technologies for CD4 count emerge from a robust pipeline [15], improvements in first-generation CD4 POC diagnostics are anticipated. Unbiased performance evidence in different countries will help to sieve the good from the not-so-good and to guide scale-up decisions.

Technologies for early infant diagnosis (EID)

Early infant diagnosis (EID) of HIV infection confers substantial benefits to HIV-infected and HIV-exposed infants, to their families, and to programs providing prevention of mother-to-child transmission (PMTCT) services [36].

Marston *et al.* analyzed pooled data from 12 studies in sub-Saharan Africa and estimated that without ARV treatment, the annual net survival would be 52% among infants infected perinatally [37]. While early ART initiation in children dramatically decreases morbidity and mortality, ARV coverage of HIV-infected children remains low [38].

The persistence of maternal antibodies in the child's system makes antibody-based tests unsuitable for exclusion or confirmation of HIV infection in children less than 18 months of age [39]. Therefore, technologies for EID are based on detection of viral components in the infant's blood, including cell-free RNA, viral DNA incorporated into host cells, or the viral capsid p24 antigen [39-41]. Polymerase chain reaction (PCR)-based methods typically demonstrate higher sensitivities across HIV-1 than do p24-based tests, and thus are preferred. Qualitative DNA PCR assays have traditionally been preferred for EID over quantitative RNA PCR assays [41], with the latter often used for viral load monitoring after a diagnosis has been made. Multiplex RNA and DNA PCR assays are now being used for EID in resource-limited settings. Ultrasensitive (immune complex-dissociated) p24 antigen assays may represent an accurate, low-cost method for EID [39], and recombinase polymerase amplification-based POC test devices have shown promising preliminary results [40].

Despite significant improvement in EID diagnostics, many infants enrolled into care are lost

from care at each step in the EID cascade and many more do not have access to care [37,41]. The EID point-of-care testing offers the promise of improving access to treatment and optimizing retention in remote clinics. For example, a Nigerian EID study reported a median EID result of 47 days, with only 25% of infected infants in the study center enrolled into ART care [41]. Quick and cost-effective POC test options (*e.g.* rapid ultrasensitive assay, p24 antigen assay) are being evaluated for EID in RLS [38-40], and some may become available in the next few years [15].

Technologies for HIV viral load (VL) measurement

It is consensually held that viral load measurement remains the most informative and reliable biological indicator for timely detection of treatment failure. Hence, it has been adopted as a gold standard for monitoring clinical prognosis in patients receiving ART in the developed world [42,43]. Also, it is routinely deployed as a tool for monitoring treatment adherence, diagnosing HIV infection during early infancy, and conducting HIV sentinel surveillance [42].

Studies in South Africa highlight the positive impact of viral load monitoring in conjunction with targeted adherence monitoring for conserving first-line drug regimens [44] and in early detection of treatment failure [45]. In addition, the limitation of CD4 cell count in diagnosing treatment failure [46] calls for the adoption of viral load measurement as the standard of care in resource-limited settings. At present, routine HIV viral quantification remains unaffordable, unsustainable, centralized, and is only done periodically in the great majority of RLS [12,47].

Technologies for viral load measurement include nucleic acid-based tests (*e.g.*, Roche Amplicor HIV-1 Monitor test), in-house nucleic acid tests [47], non-nucleic acid-based tests (*e.g.*, Cavidi ExaVir Load: HIV-specific reverse-transcriptase activity assay that shows good correlation with viral load measurement) [48], and ultrasensitive p24 antigen detection assay [47].

Typically, viral load testing protocols require continuous power supply, air conditioning, centrifugation facilities, a cold chain system, and other infrastructures, which are not available in remote regions in sub-Saharan Africa. As such, nucleic acid-based HIV load measurement in this region is performed at reference laboratories, making loss-to-follow-up inevitable due to the long turnaround time [45,49]. Non-nucleic acid-based assays have also been developed, but their clinical validity has been questioned [48]. In-house real-time PCR for HIV quantification is being validated in RLS [47] and, with appropriate logistics, may become an affordable option for viral load measurement and EID. Generally, in-house testing methods will need continuous validation and quality management if they are to be scaled up in clinical laboratory monitoring of infected patients. A robust and efficient system is needed to incorporate in-house testing systems into mainstream diagnostic services.

Computational models capable of predicting virological response to ART are being developed and evaluated [50], and the operationalization may include an accessible online treatment support link [50]. Computer model prediction requires thorough calibration and validation at the community level. This model may prove useful in the future; however, baseline and periodic follow-up viral load investigations will need to be conducted using the traditional laboratory services.

Although no POC devices for viral load assay are commercially available, a number of assays are progressing rapidly towards a platform for evaluation in RLS [15]; if they are successfully validated, viral load measurement may become a gold standard in treatment monitoring in RLS in the near future.

The POC diagnostics product pipeline for viral load includes an amplification-based assay being developed by the University of Cambridge, a nucleic acid-based test kit, the LiatAnalyser, the NAT system and the EOSCAPE-HIV HIV Rapid RNA Assay System, among others [15].

Technologies for HIV resistance testing and HIV companion diagnostics

The high genetic diversity of HIV and its variability in selecting drug-resistant strains when subjected to ARV is well documented [51-54]. Unfortunately, one of the consequences of ART scale-up is an increasing incidence of treatment failure and an increasing spread of drug-resistant viral strains. A review published by Stadeli and Richman revealed that acquired resistance to ARVs was detected in 20.7% of patients on ART for ≥ 36 months in sub-Saharan Africa [51]. A similar review by Gupta *et al.* showed that East Africa and southern Africa had annual increases in drug-resistant HIV prevalence of 29% and 14%, respectively [52].

A predictive genotypic resistance profile, as demonstrated in adult patients cared for in the private sector of Cameroon or in the public sector in Bangui,

Central African Republic [53], can potentially guide regional and individualized treatment selection to second-line ARVs and to conserve effectiveness of first-line ARVs [44]. Pre-treatment resistance testing seems to be the preferred solution to decreasing incidences of treatment failure; however, this solution is not useful in RLS. Drug resistance testing is not yet recommended for individual ART monitoring in resource-limited settings; it is only available in regional or national reference laboratories because it is prohibitively expensive [54]. POC technologies for HIV resistance testing is still a far cry, and investment into POC technology should be intensified.

Similarly, the gradual roll-out of pharmacogenomics technologies (companion diagnostics) enables prior identification of patients at risk of atypical adverse drug reactions [55] and determination of individual responses to drugs before drug exposure [56]. This facilitates tailoring drug administration to suit individuals' genetic dispositions to such drugs (personalized medicine). For example, companion diagnostics for abacavir (abacavir/HLA B*5701) have been demonstrated to eliminate the risk of life-threatening hypersensitivity associated with the use of abacavir, a first-line ARV [57]. HLA B*5701 is already being extensively used in some countries (*e.g.*, Australia, United Kingdom, and Ireland). Also, the cell-based tropism diagnostic test is used to determine the tropism status of patients to be placed on maraviroc (HIV CCR5 co-receptor antagonist) [58]. The promotion of the concept of co-development of companion diagnostics alongside their drug candidates [56] may make inroads to improving access to this technology, as they are unavailable in RLS. As this new class of diagnostic evolves, research into companion diagnostic POC options should be encouraged to meet the special circumstances in RLS.

Diagnostics for HIV/AIDS-related opportunistic infections

Immunological suppression with progressing HIV infections increases the vulnerability of HIV+ persons to certain infections when compared to the background population. These infections can cause varying degrees of morbidity that may eventually lead to death if undiagnosed and untreated. Life-threatening opportunistic infections are common in patients with significantly compromised immunity (CD4 T cells below 200 cells/uL); these infections threaten the success of ART programs [59,60]. Among the several opportunistic infections that invade these immuno-compromised persons, cryptococcosis and tuberculosis (TB) are leading causes of death in HIV co-infections [59]. This is particularly severe in sub-Saharan Africa, where these diseases are endemic [61,62].

The TB diagnostic platform in RLS is weak. In addition to the four-point clinical symptom score (presence of cough, fever, night sweats, and/or weight loss), the readily available method for diagnosing TB in RLS is smear microscopy. Unfortunately, both clinical symptom score and smear microscopy may give misleading results in severely immuno-compromised individuals. Low sensitivity of 20% for smear microscopy and 50% specificity for the four-point symptom score have been reported [60]. Also, smear microscopy protocol for TB diagnosis requires the collection of three samples, presenting a logistic challenge to both patients and service providers [12].

The evolving TB diagnostic platform includes nucleic acid amplification-based test devices. An example is Xpert MTB/RIF, which possesses attractive features of accuracy, user friendliness, and dual functionality of detecting *Mycobacterium tuberculosis* and rifampicin resistance; however, it is unaffordable in many settings and requires sophisticated equipment [12,63,64]. Other technologies include antibody-based serological testing devices, which are also relatively expensive [65] and have been demonstrated to perform poorly in immuno-compromised HIV+ individuals, and are thus not recommended for routine TB testing [12]. Antigen-based test devices (*e.g.*, Determine TB-LAM) demonstrated low sensitivity but proved valuable when used in combination with sputum smear microscopy in screening for TB among severely immuno-compromised HIV-infected patients [64], but this evidence is limited and requires further investigation.

While an ideal conceived TB POC has not been determined, the progress in TB molecular testing technologies promises the development of such desired POC for TB in the foreseeable future.

Similar to TB, POC diagnostic option in RLS for diagnosing cryptococcal infection is not available. The most commonly used serum-based screening tests for *cryptococcosis,* the latex agglutination test and the enzyme immunoassay, require complex laboratory infrastructure including a spectrophotometer and skilled personnel; as such, they are not implementable in RLS [66]. A recently developed monoclonal antibodies-based device had > 95% sensitivity when evaluated against cryptococcal latex agglutination and culture and has been licensed for use using serum and cerebrospinal fluid samples [67]. The lateral flow

immunoassay device is an attractive POC kit in RLS as it can also be used with urine or whole blood, eliminating the need for centrifugation, and it is relatively cost effective [66]. However, evidence of performance in urine and serum specimens in asymptomatic HIV and cryptococcosis co-infected individuals is limited.

The positive impact of service integration of HIV care and other health services in reducing total cost of care and improving overall health outcomes was advanced by Sweeney *et al.* in a systematic review [68]. The potential gains from integrating ART services with those of TB and cryptococcal infections have further been stressed [69].

The availability of POC devices for HIV, TB, and cryptococcosis may facilitate the integration of these services and maximize the potential values from such integration. Conceivably, a POC diagnostic toolbox containing POC devices for the spectrum of HIV testing (including CD4 cell count and viral load measurement) and for the diagnosis of TB and cryptococcosis should be envisioned, as it may dramatically improve care retention and overall clinical outcomes for co-infected persons. A mutiplex device capable of testing HIV, TB/cryptococcosis simultaneously is a big ask but an attractive incentive for scale-up and integration of HIV, TB, and cryptococcosis care services.

Sample collection and transportation

In RLS, laboratory services for viral load testing, p24 antigen quantitation, and HIV drug resistance studies (when necessary) are delivered at sub-regional, regional, and national reference laboratories [70]. Hence, collection, storage, and transportation of samples from point of care to these reference service centers in an uncompromised manner constitute a huge challenge due to infrastructural, logistical, economic, and personnel limitations. The use of dried fluid spot cards has been suggested to bridge this gap. Already, the dried blood sampling method has been successfully implemented and widely used in many RLS providing EID services. In addition, these cards have been used in the spectrum of diagnosis in HIV care including serologic testing, p24 antigen quantitation, and more recently, viral-load determination and resistance genotyping [71]. Snijdewind *et al.* reviewed the application of dried blood spot (DBS) testing in other viral diseases [72].

The limitations of the DBS sampling technique have been appraised by Hamers *et al.* [73] and Snijdewind *et al.* [72]. These limitations include low sample volume (50 microliters), likelihood of contamination and possible degradation of HIV RNA in events of prolonged storage at room temperature under high humidity, and the inefficiency of extraction procedures to retrieve samples from the dried spot [71,73]. However, the authors of both studies argued that the caveats of DBS notwithstanding, its role in improving clinical outcomes of HIV care programs in RLS should necessitate its wide adoption. Moreover, some of the limitations can easily be overcome: automated RNA extraction in a reference laboratory may eliminate human error and inconsistencies [73], prompt dispatch of collected DBS can eliminate the need for long storage, and high sensitivity of newer diagnostics should address the concern of small sample size in DBS [71]. DBS sampling techniques and implementation framework may be optimized to address the aforementioned limitations. This may widen its application in the scale-up of laboratory services in resource-limited settings.

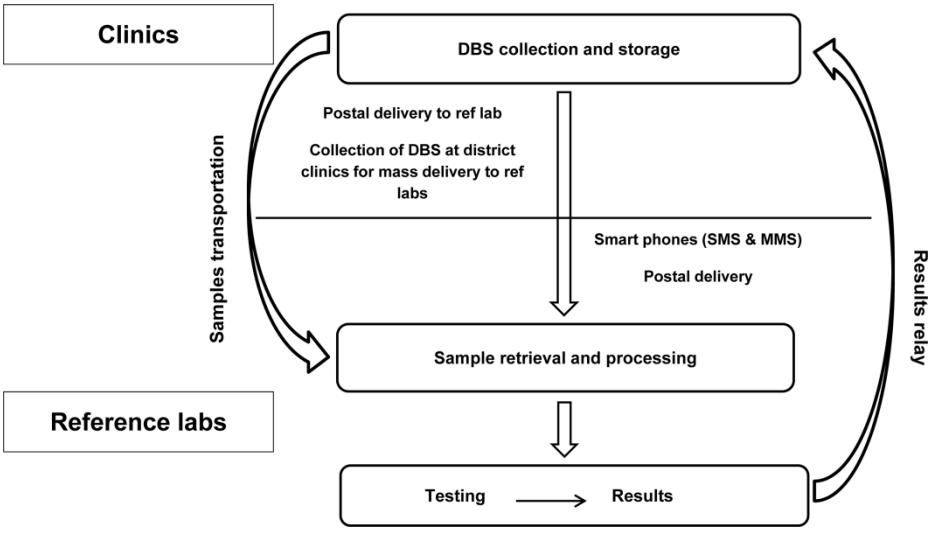


Figure 2. Implementation of dried blood sample (DBS) in HIV care in resource-limited settings. DBS reduces the difficulty with sample preparation and storage. DBS is stable at room temperature, can be sent through the post, and results may be relayed to clinics via SMS or scanned copy with smartphones to reduce turnaround time. While POC devices are rolled out, DBS can augment laboratory services that are centralized and are likely to remain centralized over the coming years.

Overall, improvements in sampling techniques have aided in addressing logistic challenges in RLS. DBS is an immediate example; DBS specimens are heat stable, non-infectious, and can be shipped via mail or courier, thus reducing the cost of specimen transport to reference laboratories from remote clinics [71,72] (Figure 2). The reference laboratory relays test results to point-of-care delivery almost immediately after tests are completed, using smart phones (Figure 2). In this way, the turnaround time is minimized.

As ART and PMTCT services are being scaled up and decentralized to include primary healthcare facilities in sub-Saharan Africa, accompanying laboratory services have largely remained centralized. Transportation of samples and relays of test results to and from remote treatment centers and reference laboratories is challenging and costly. Recently, Kiyaga *et al.* reported how Uganda's new coordinated sample transport model increased access to EID services from 36% to 51% [74]. The system significantly reduced transportation costs by 62%, reduced turnaround time by 46.9%, and by a further 46.2% by introducing SMS printers [74]. Improvising near-efficient transport systems and the scale-up of DBS may significantly improve access to laboratory services and HIV care in sub-Saharan countries.

HIV diagnostics platforms: gaps and needs

Unmet needs in HIV diagnosis could be seen firstly in the lack of simple, affordable, quick, accurate, equipment-free, and reliable POC options for early infant diagnosis and for viral load measurement [4,12,43]; equally important, however, is the gap created by the poorly defined implementation logistic system in RLS [75].

POC options for resistance assays have the potential to bring personalized medicine to RLS and to guide decisions on HIV programmatic projects, but resistance testing remains extremely expensive and centralized, and it runs only as part of clinical studies [12]. In a similar vein, advances in pharmacogenomic technologies should be leveraged in developing companion diagnostic POC options as newer ARVs are being developed. These are capable of predicting patients' responses to drugs prior to administration, thereby reducing incidences of life-threatening toxicities and therapeutic failure, and bringing personalized medicine to HIV+ people in RLS.

In addition, POC technologies for population incidence studies and HIV surveillance have the potential to dramatically improve the effectiveness and ease of HIV epidemiological studies. Such assays are being developed, though challenges are currently being faced in their development [76].

Increasing TB drug resistance and the high number of patients co-infected with TB and HIV buttress the urgent need for POC diagnostic devices for TB and other life-threatening opportunistic infections (*e.g.*, cryptococcosis). Portable and sturdy devices for hematology and physio-chemistry testing (*e.g.*, full blood count, serum creatinine and others) will be immensely beneficial to ART programs.

Furthermore, parallel advances and growth in mHealth and mobile telemedicine can be leveraged into HIV care programs to improve communication and linkages [23].

Arguably, the diagnostic portfolio for HIV testing is impressive; however, uptake of tests and scale-up remain unsatisfactory in RLS. Forty-nine percent of infected persons are still unaware of their status in Sub-Saharan Africa [1]. Current logistic structures for the implementation of innovative technologies are complex and ineffective [8] and will urgently need to be addressed to allow the successful incorporation of the POC testing portfolio into existing diagnostic systems in sub-Saharan Africa.

As attention gradually shifts towards implementation and subsequent scale-up of POC devices in RLS, understanding and preparing for the implementation challenges is imperative in increasing access to the right populations in the right way.

Incorporating POC into HIV care in sub-Saharan Africa

Advances in rapid diagnosis and portable device technologies are being leveraged into POC diagnostics to bring laboratory services to patients' bedsides, thus bypassing the requirement for sophisticated laboratory systems. Existing HIV laboratory monitoring services in RLS are largely centralized, often distant from ART care delivery points, or inaccessible to remote community dwellers. Geographical, socio-economic, and skilled personnel limitations constitute barriers to accessing quality HIV care. An estimated 40% of persons diagnosed with HIV infection in sub-Saharan Africa either do not provide a blood sample for CD4 cell evaluation or do not return to obtain their CD4 count results because access routes are complex [8,77]. With the existing diagnostic platform in sub-Saharan Africa, it becomes difficult to recruit more patients into care while effectively monitoring patients already in care. Obviously, access to POC technologies in RLS is pivotal to improving ART

program outcomes in geographically remote and resource-constrained regions.

Importantly, the clinical impact of POC CD4 technologies is being evaluated, and their potential to reduce loss-to-follow-up and improve retention in care are being carefully examined. For example, the Pima Analyzer (Alere's rapid POC CD4 device) has been validated in various settings [33]. However, the mere availability of high-performing POC devices does not automatically translate into successful implementation in the field; the experience from the roll-out of HIV RDT testing devices is an example [17]. Despite the decentralization of HIV testing protocols and the availability of HIV RDT test kits in RLS, 49% of persons living with HIV in sub-Saharan Africa do not know their status [1], and many present to care late. The implication is an increased cost to health systems, higher risk of transmissions of the infection, and a possible slack in reducing the mortality rate with ART [80].

A range of perceived barriers to successful implementation of evolving POC technologies may include economic, regulatory, and policy-related factors [75,78,79]; implementation plans should therefore devise strategies to overcome these perceived hurdles. Regulatory affairs agencies in sub-Saharan African countries need to ensure that performance evaluation of emerging POC kits are carried out locally prior to the registration of such products. A quality assurance system should be efficient to ensure continuous quality control of registered products. One key area which perhaps is as important as the technology itself is documentation. Systems that ensure immediate reporting of test results to care providers and a mechanism to link test results to appropriate counseling and treatment must accompany the implementation of the of technology.

Also, POC implementation programs require viable business models to ensure sustainability and affordability in low-income countries [75]. These strategies may include designing economic structures to strengthen private-public sector partnership. National governments in sub-Saharan Africa, donor agencies, and partners may consider subsidizing costs for POC devices and should invent harmonized pricing strategies to address the huge disparities in the cost of receiving HIV care between public and private health facilities. The biopharmaceutical and diagnostic industries may adopt a differential pricing method for HIV POC diagnostics to encourage high patronage in RLS. This may be in a form of corporate social responsibility gestures to guarantee affordability of newly launched HIV POC kits.

In addition, the leveraging of mobile smart phones and the development of telemedicine can help address challenges of POC implementation in regions where access to computing systems is limited [23,77]. For example, telephonic counseling has been shown to improve outcomes of HIV in-home testing [23].

If the real value behind the innovation of POC technologies is to be captured, it is necessary to understand current diagnostic practices and the peculiar health system structures of regions in which POC technology implementation is planned [78]. Logistic management systems will need to be robust and efficient. The supply chain of diagnostic commodities should be strengthened, and inventory control systems should be well managed to avoid stock-out of such commodities. Referral and transfer systems should be as simple as possible and well defined, and personnel should be well acquainted with such procedures. Implementation strategies may include: building health facilities with internal and external linkages to ensure effective service referral and transfer systems; strengthening capacity for forecasting; effective inventory management, reporting, and documentation; and enabling reliable quality assurance and management systems. Training and retraining of implementers should be regular.

Ultimately, the goal of HIV diagnosis is to provide accurate, efficient, cost-effective, and accessible diagnostic services throughout the spectrum of testing required to diagnose, stage, and monitor ART effectively. As POC testing options for different diagnostic stages emerge in batches, the dried fluid sampling method can fill the gap and augment diagnostic services that are likely to remain centralized in RLS (*e.g.*, resistance assays).

Eventually, a framework design that allows the use of both centralized and POC testing systems to run complementarily or supplementally would be necessary. HIV national coordinating centers will need to devise policies and implementation strategies that allow the incorporation of POC devices into the existing laboratory systems in sub-Saharan countries. Such strategies will need to address community-level implementation hurdles on a case-by-case basis, ensuring that the peculiarities of these communities (*e.g.*, perceptions and beliefs, conflict status) are addressed.

Conclusions

The goal of "test and treat" and the campaign for universal access to HIV diagnosis and treatment are realizable with the advent of POC technologies and the decentralization of laboratory services in sub-Saharan Africa. However, the success or failure will depend on whether the implementation strategies are adaptable and implementable in RLS.

Key gaps remain in the performance validation of emerging CD4 POC technologies, and in the lack of POC devices for viral load measurement and EID. Other gaps include the lack of availability of POC testing options for HIV drug resistance assays, multiplex HIV/TB/cryptococcosis diagnostics, and population incidence surveillance tools. Also, POC devices for other opportunistic infections that can potentially improve the quality of HIV care and the management of co-infections are lacking. It is also not too early to ask for POC options for pharmacogenomic testing (companion diagnostics), as HIV+ patients in sub-Saharan Africa will benefit greatly from personalized medicine delivered at the community level.

Finally, collaborative efforts built around combining technical expertise, regulatory capacity, and implementation strategies might speed up the development of the product pipeline, performance evaluation, and implementation at the country and community level. In the end, it is about creating an environment where HIV-infected persons can live near-normal lives and the society can be freed of the risk of contracting the disease.

References

1. UNAIDS (2013) UNAIDS Report on Global AIDS Epidemics 2013. Available: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf. Accessed 15 December 2013.
2. United Nations (2009) The Millenium Development Goals Report 2009. Available: http://www.un.org/millenniumgoals/pdf/MDG_Report_2009_ENG.pdf. Accessed 20 November 2013.
3. Harries AD, Jensen PM, Zachariah R, Rusen ID, Enarson DA (2009) How Health systems in Sub-Saharan Africa can benefit from Tuberculosis and other infectious disease programmes (Unresolved issues). Int J Tuberc Lung Dis 13: 1194-1199.
4. Bélec L, Bonn J (2011) Challenges in Implementing HIV Laboratory Monitoring in Resource-constrained Settings: How to do more with less Future. Microbiol 6: 1251-1260.
5. Naicker S, Plange-Rhule J, Tutt RC, Eastwood JB (2009) Shortage of Healthcare Workers in developing countries--Africa. Ethn Dis 19: 60-64.
6. World Health Organization (2003) Treating 3 million by 2005: making it happen: the WHO strategy. Available: http://libdoc.who.int/publications/2003/9241591129.pdf. Accessed 15 December 2013.
7. World Health Organization (2013) Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Available: http://www.who.int/hiv/pub/guidelines/arv2013/download/en/index.html. Accessed 18 December 2013.
8. Rosen S, Fox MP (2011) Retention in HIV Care between Testing and Treatment in Sub-Saharan Africa: a Systematic Review. PLoS Med: e1001056.
9. Zachariah R, Reid SD, Chaillet P, Massaquoi M, Schouten EJ, Harries AD (2011) Viewpoint: Why do we need a point-of-care CD4 test for low-income countries? Trop Med Int Health 16: 37-41.
10. Libamba E, Makombe SD, Harries AD, Schouten EJ, Yu JK, Pasulani O, Mhango E, Aberle-Grasse J, Hochgesang M, Limbambala E, Lungu D (2007) Malawi's contribution to "3 by 5": achievements and challenges. Bull World Health Organ 85: 156-160.
11. Wu G, Zaman MH (2012) Low-cost tools for diagnosing and monitoring HIV infection in low-resource settings. Bull World Health Organ 90: 914-920.
12. Pai PN, Pai M (2012) Point-of-Care Diagnostics for HIV and Tuberculosis: Landscape, Pipeline, and Unmet Needs. *Discov Med* 13: 35-45.
13. Spacek LA, Lutwama F, Shihab HM, Summerton J, Kamya MR, Ronald A, Laeyendecker O, Quinn TC, Mayanja-Kizza H (2011) Diagnostic accuracy of ultrasensitive heat-denatured HIV-1 p24 antigen in non-B subtypes in Kampala, Uganda. Int J STD AIDS 22: 310-314.
14. Crump JA, Scott LE, Msuya E, Morrissey AB, Venter WF, Stevens WS (2009) Evaluation of the Abbott m2000rt RealTime HIV-1 assay with manual sample preparation compared with the ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR v1.5 using specimens from East Africa. J Virol Methods 162: 218-222.
15. Murtagh MM (2012) HIV/AIDS diagnostics Technology Landscape. UNITAID. Available: http://www.unitaid.eu/images/marketdynamics/publications/UNITAID_2012_Semi-

annual_Update_HIV_Diagnostics_Technology_Landscape.pdf. Accessed 22 December 2013.
16. Delaney KP, Branson BM, Uniyal A, Phillips S, Candal D, Owen SM, Kerndt PR (2011) Evaluation of the performance characteristics of 6 rapid HIV antibody tests. Clin Infect Dis 52: 257-263.
17. Klarkowski DB, Wazome JM, Lokuge KM, Shanks L, Mills CF, O'Brien DP (2009) The evaluation of a rapid in situ HIV confirmation test in a programme with a high failure rate of the WHO HIV two-test diagnostic algorithm. PLoS ONE 4: e4351
18. Choko AT, Desmond N, Webb EL, Chavula K, Napierala-Mavedzenge S, Gaydos CA, Makombe SD, Chunda T, Squire SB, French N, Mwapasa V, Corbett EL (2011) The Uptake and Accuracy of Oral Kits for HIV Self-Testing in High HIV Prevalence Setting: A Cross-Sectional Feasibility Study in Blantyre, Malawi. *PLoS Med* 8: e1001102.
19. Pai NP, Barick R, Tulsky JP, Shivkumar PV, Cohan D, Kalantri S, Pai M, Klein MB, Chhabra S (2008) Impact of round-the-clock, rapid oral fluid HIV testing of women in labor in rural India. *PLoS Med* 5: e92.
20. Pilcher CD, Louie B, Facente S, Keating S, Hackett J Jr, Vallari A, Hall C, Dowling T, Busch MP, Klausner JD, Hecht FM, Liska S, Pandori MW (2013) Performance of Rapid Point-of-Care and Laboratory Tests for Acute and Established HIV Infection in San Francisco. PLoS One 8: e80629.
21. Luo W, Masciotra S, Delaney KP, Charurat M, Croxton T, Constantine N, Blattner W, Wesolowski L, Owen SM (2013) Comparison of HIV oral fluid and plasma antibody results during early infection in a longitudinal Nigerian cohort. J Clin Virol 58 Suppl 1: e113-118.
22. World Health Organization (2013) Report on the First International Symposium on Self-Testing for HIV. Available: http://apps.who.int/iris/bitstream/10665/85267/1/9789241505628_eng.pdf. Accessed 21 December 2013.
23. Estrin D, Sim I (2010) Health care delivery. Open mHealth architecture: an engine for health care innovation. *Science* 330: 759-760.
24. Franco RA, Saag MS (2013) When to start antiretroviral therapy: as soon as possible. BMC Med 11: 147.
25. Lundgren JD, Babiker AG, Gordin FM, Borges AH, James D, Neaton JD (2013) When to start antiretroviral therapy: the need for an evidence base during early HIV infection. BMC Med 11: 1741-7015.
26. Faal M, Naidoo N, Glencross DK, Venter WDF, Osih R (2011) Providing Immediate CD4 Count Results at HIV Testing Improves ART Initiation. J Acquir Immune Defic Syndr 58: e54-59.
27. Jani IV, Sitoe NE, Alfai ER, Chongo PL, Quevedo JI, Rocha MB, Lehe JD, Pete TF (2011) Effect of point-of-care CD4 cell count tests on retention of patients and rates of antiretroviral therapy initiation in primary health clinics: an observational cohort study. Lancet 378: 1572-1579.
28. Thiam S, Thior M, Faye B, Ndiop M, Lamine-Diouf M, Diouf MB, Diallo I, Fall FB, Ndiaye JL, Albertini A, Lee E, Jorgensen P, Gaye O, Bell D (2011) Major reduction in antimalarial drug consumption in Senegal after nationwide introduction of malaria rapid diagnostic tests. PLoS One 6: e18419.
29. Boyle DS, Hawkins KR, Steele MS, Singhal M, Cheng X (2011) Emerging technologies for point-of-care CD4 T-lymphocyte counting. Trends Biotechnol 30: 45-54.
30. Moon SJ, Keles HO, Ozcan A, Khademhosseini A, Hæggstrom E, Kuritzkes D, Demirci U (2009) Integrating microfluidics and lensless imaging for point-of-care testing. Biosens Bioelectron 24: 3208-3214.
31. Manabe YC, Wang Y, Elbireer A, Auerbach B, Castelnuovo B (2012) Evaluation of portable point-of-care CD4 counter with high sensitivity for detecting patients eligible for antiretroviral therapy. PLOS One 7: e34319.
32. Watkins NN, Hassan U, Damhorst G, Ni H, Vaid A, Rodriguez W, Bashir R (2013) Microfluidic CD4+ and CD8+ T Lymphocyte Counters for Point-of-Care HIV Diagnostics Using Whole Blood. Sci Transl Med 5: 214ra170.
33. Glencross D, Coetzee L, Faal M, Masango M, Stevens W (2012) Performance evaluation of the point-of-care CD4 analyser Pima using capillary blood sampling in field tests in South Africa. J Int AIDS Soc 15: 3.
34. Bergeron M, Daneau G, Ding T, Sitoe NE, Westerman LE, Stokx J, Jani IV, Coetzee LM, Scott L, Weggheleire AD, Boel L, Stevens WS, Glencross DK, Peter TF (2012) Performance of the PointCare NOW System for CD4 Counting in HIV Patients Based on Five Independent Evaluations. PLoS ONE 7: e41166.
35. Sukapirom K, Onlamoon N, Thepthai C, Polsrila K, Tassaneetrithep B, Pattanapanyasat K (2011) Performance Evaluation of the Alere PIMA(TM) CD4 Test for Monitoring HIV-Infected Individuals in Resource-Constrained Setting. J Acquir Immune Defic Syndr 58: 141-147.
36. Guay L (2010) Scaling up early infant diagnosis as the bridge between prevention, care, and treatment. International AIDS Society, Vienna 2010. Available: http://pag.aids2010.org/session.aspx?s=150. Accessed 22 February 2013.
37. Marston M, Becquet R, Zaba B, Moulton LH, Gray G, Coovadia H, Essex M, Ekouevi D, Jackson D, Coutsoudis A, Kilewo C, Leroy V, Wiktor S, Nduati R, Msellati P, Dabis F, Newell M, Ghys PD (2011) Net survival of perinatally and postnatally infected children: a pooled analysis of individual data from sub-Saharan Africa. Int J Epidemiol 40: 385-396.
38. Interagency Task Team (2012) Early Infant Diagnosis. Available: http://www.zero-hiv.org/wp-content/uploads/2013/03/EID-TaskTeam-background-paper.pdf. Accessed 23 December 2013.
39. Wittawatmongkol O, Vanprapar N, Chearskul P, Phongsamart W, Prasitsuebsai W, Sutthent R, Chokephaibulkit K (2010) Boosted p24 antigen assay for early diagnosis of perinatal HIV infection. J Med Assoc Thai 93: 187-190.
40. Boyle DS, Lehman DA, Lillis L, Peterson D, Singhal M, Armes N, Parker M, Piepenburg O, Overbaugh J (2013) Rapid detection of HIV-1 proviral DNA for early infant diagnosis using recombinase polymerase amplification. MBio 4 pii: e00135-13.
41. Anoje C, Aiyenigba B, Suzuki C, Badru T, Akpoigbe K, Odo M, Odafe S, Adedokun O, Torpey K, Chabikuli ON (2012) Reducing mother-to-child transmission of HIV: findings from an early infant diagnosis program in south-south region of Nigeria. BMC Public Health 12: 184.
42. Aldous AL, Haubrich RH (2009) Defining treatment failure in resource-rich settings. Curr Opin HIV AIDS 4: 459-466.
43. Usdin M, Guillerm M, Calmy A (2010) Patient needs and point-of-care requirements for HIV load testing in resource-limited settings. J Infect Dis 201 Suppl 1: S73-S77.
44. Orrell C, Harling G, Lawn SD, Kaplan R, McNally M, Bekker L, Wood R *(2007)* Conservation of first-line

antiretroviral treatment regimen where therapeutic options are limited. *Antivir Ther* 12: 83-88.
45. Keiser O, Chi B H, Gsponer T, Boulle A, Orrell C, Phiri S, Maxwell N, Maskew M, Prozesky H, Fox MP, Westfall A, Egger M (2011) Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in Southern Africa. *AIDS* 25: 1761-1769.
46. Kankor R, Diero L, DeLong A, Kamle L, Muyonga S, Mambo F, Walumbe E, Emonyi W, Chan P, Carter EJ, Hogan J, Buziba N (2009) Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings. Clin Infect Dis 49: 454-462.
47. Stevens WS, Scott LE, Crowe SM (2010) Quantifying HIV for monitoring antiretroviral therapy in resource-poor settings. J Infect Dis 201: 16-26.
48. Wang S, Xu F, Demirci U (2010) Advances in developing HIV-1 viral load assays for resource-limited settings. Biotechnol Adv 28: 770-781.
49. Puren A, Gerlach JL, Weigl BH, Kelso DM, Domingo GJ (2010) Laboratory operations, specimen processing, and handling for viral load Testing and surveillance. J Infect Dis 201: 27-36.
50. Revell AD, Wang D, Wood R, Morrow C, Tempelman H, Hamers R, Alvarez-Uria G, Streinu-Cercel A, Ene L, Wensing A, Reiss P, van Sighem AI, Nelson M, Emery S, Montaner JS, Lane HC, Larder BA (2014) An update to the HIV-TRePS system: the development of new computational models that do not require a genotype to predict HIV treatment outcomes. J Antimicrob Chemother 69: 1104-1110.
51. Stadeli KM, Richman DD (2013) Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review. Antivir Ther 18: 115-123.
52. Gupta RK, Jordan MR, Sultan BJ, Hill A, Davis DH, Gregson J, Sawyer AW, Hamers RL, Ndembi N, Pillay D, Bertagnolio S (2012) Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. Lancet 380: 1250-1258.
53. Charpentier C, Talla F, Nguepi E, Si-Mohamed A, Bélec L (2010) Virological failure and HIV-1 drug resistance profiles among patients followed-up in private sector, Douala, Cameroon. AIDS Res Hum Retroviruses 27: 221-230.
54. Kozal MJ (2013) Overview of HIV drug resistance testing assays. In Hirsch MS and Mitty J, editors. UpToDate. Available: http://www.uptodate.com/contents/overview-of-hiv-drug-resistance-testing-assays. Accessed 22 December 2013.
55. Phillips KA, Veenstra DL, Oren E, Lee JK, Sadee W (2001) Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. JAMA 286: 2270-2279.
56. Valenti WM (2007) Companion Diagnostic Tests in HIV Medicine: The Road to Personalized Medicine. AIDS Read 17: 546-549.
57. Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, Sayer D, Castley A, Mamotte C, Maxwell D, James I, Christiansen FT (2002) Association between presence of HLA-B* 5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-I reverse transcriptase inhibitor abacavir. *Lancet* 359: 727-773.
58. Poveda E, Briz V, Quinones-Mateu M, Soriano V (2006) HIV tropism: diagnostic tools and implications for disease progression and treatment with entry inhibitors. *AIDS* 20: 1359-1367.
59. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R *(2008)* Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* 22*:* 1897-1908.
60. Lawn SD, Wood R (2011) Tuberculosis in antiretroviral treatment services in resource-limited settings: addressing the challenges of screening and diagnosis. J Infect Dis 204 Suppl 4: S1159-S1167.
61. Jarvis JN., Boulle A, Loyse A, Bicanic T, Rebe K, Williams A, Harrison TS, Meintjes G (2009) High ongoing burden of cryptococcal disease in Africa despite antiretroviral roll out. AIDS 23: 1182-1183.
62. World Health Organization *(2013)* WHO Report: Global Tuberculosis Control 2013. Available*:* http://www.who.int/tb/publications/global_report/en/. Accessed 18 December 2013.
63. Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, Cuevas LE, McHugh TD, Zijenah L, Kapata N, Abubakar I, McNerney R, Hoelscher M, Memish ZA, Migliori GB, Kim P, Maeurer M, Schito M, Zumla A (2013) Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis 13: 349-361.
64. Lawn SD, Wood R (2012) Point-of-care urine antigen screening tests for tuberculosis and cryptococcosis: potential for mortality reduction in antiretroviral treatment programs in Africa. Clin Infect Dis 54: 739-740.
65. Dowdy DW, Steingart KR, Pai M (2011) Serological testing versus other strategies for diagnosis of active tuberculosis in India: a cost-effectiveness analysis. PLoS Med 8: e1001074.
66. Roy M, Chiller T (2011) Preventing deaths from cryptococcal meningitis: from bench to bedside. Expert Rev Anti Infect Ther 9*:* 715-717.
67. Lindsley MD, Mekha N, Baggett HC, Surinthong Y, Autthateinchai R, Sawatwong P, Harris JR, Park BJ, Chiller T, Balajee SA, Poonwan N (2011) Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. Clin Infect Dis 53: 321-325.
68. Sweeney S, Obure CD, Maier CB, Greener R, Dehne K, Vassall A (2012) Costs and efficiency of integrating HIV/AIDS services with other health services: a systematic review of evidence and experience. Sex Transm Infect 88: 85-99.
69. Vijayan T, Klausner JD (2013) Integrating clinical services for HIV, tuberculosis, and cryptococcal disease in the developing world: a step forward with 2 novel diagnostic tests. J Int Assoc Provid AIDS Care 12: 301-305.
70. Bélec L, Bonn J (2011) Challenges in Implementing HIV Laboratory Monitoring in Resource-constrained Settings: How to Do More With Less Future. Microbiol 6: 1251-1260.
71. Zazzi M (2012) Dried blood spot testing: filling the gap between antiretroviral treatment & monitoring in India. Indian J Med Res 136: 903-905.
72. Snijdewind IJ, van Kampen JJ, Fraaij PL, van der Ende ME, Osterhaus AD, Gruters RA (2012) Current and future applications of dried blood spots in viral disease management. Antiviral Res 93: 309-321.
73. Hamers RL, Smit PW, Stevens W, Schuurman R, Rinke de Wit TF (2009) Dried fluid spots for HIV type-1 viral load and resistance genotyping: a systematic review. Antivir Ther 14: 619-629.

74. Kiyaga C, Sendagire H, Joseph E, McConnell I, Grosz J, Narayan V, Esiru G, Elyanu P, Akol Z, Kirungi W, Musinguzi J, Opio A (2013) Uganda's New National Laboratory Sample Transport System: A Successful Model for Improving Access to Diagnostic Services for Early Infant HIV Diagnosis and Other Programs. PLoS One 8: e78609.
75. Palamountain KM, Baker J, Cowan EP, Essajee S, Mazzola LT, Metzler M, Schito M, Stevens WY, Young GJ, Domingo GJ (2012) Perspectives on introduction and implementation of new point-of-care diagnostic tests. J Infect Dis 205 Suppl 2: S181-S190.
76. Incidence Assay Critical Path Working Group (2011) More and better information to tackle HIV epidemics: towards improved HIV incidence assays. PLoS Med 8: e1001045.
77. Govindasamy D, Van Schaik N, Kranzer K, Wood R, Mathews C (2011) Linkage to HIV Care from a Mobile Testing Unit in South Africa by Different CD4 Count Strata. J Acquir Immune Defic Syndr 58: 344-352.
78. Engel N, Kenneth J, Pai M (2012) TB diagnostics in India: creating an ecosystem for innovation. Expert Rev Mol Diagn 12: 21-24.
79. Pai NP, Vadnais C, Denkinger C, Engel N, Pai M (2012) Point-of-Care Testing for Infectious Diseases: Diversity, Complexity, and Barriers in Low- And Middle-Income Countries. PLoS Med 9: e1001306.
80. Fisher M (2008) Late diagnosis of HIV infection: major consequences and missed opportunities. Curr Opin Infect Dis 21: 1-3.

Corresponding author
Godwin A Aleku
Manchester Institute of Biotechnology, University of Manchester
131 Princess Street, Manchester
M1 7DN, United Kingdom
Phone:+44 (0) 7423168532
Email: godwin.aleku@postgrad.manchester.ac.uk

Conflict of interests: No conflict of interests is declared.