UNITAID
TECHNICAL
REPORT

HIV/AIDS
Diagnostic Landscape

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please note correction in price for the CyFlow ® CD4 miniPOC (pages 27&67)

Executive Summary

Knowledge of one's HIV status is the gateway to care and treatment for HIV/AIDS, and it is also potentially a critical prevention measure. Recognizing the importance of HIV testing in achieving the goal of universal access to antiretroviral therapy (ART), voluntary counseling and testing (VCT) has been scaled-up in resource-limited settings. In another effort to improve uptake of HIV testing, the World Health Organization (WHO) has advocated provider-initiated testing in generalized epidemics and in certain concentrated and low-level epidemics.* Despite these efforts, there is still poor HIV testing coverage in resource-limited settings. For example, the results of recent population surveys in 10 countries in sub-Saharan Africa indicate that more than 60% of individuals did not know their HIV status.**

It is now generally recognized in the international community that there is a need to significantly increase the level of access to robust, high-quality diagnostics in resource-limited settings in order to facilitate early detection and treatment of HIV/AIDS. At the same time, there is growing demand to find ways over the next few years to simplify and improve the efficiency of diagnostics for HIV/AIDS without diminishing the quality of patient care. In order to appreciate what it will take to accomplish this, it is first necessary to understand the testing continuum required for the HIV patient, the current diagnostic market landscape and the existing barriers to diagnostic access.

Testing in connection with HIV infection begins with the initial diagnosis of the disease. Following a positive diagnosis, recommended testing includes CD4 testing for staging and monitoring the progress of the disease before initiation onto ART, and viral load testing for monitoring patients after initiation onto treatment.

For initial diagnosis of HIV, there are a large number of tests available from a variety of manufacturers, of which rapid HIV antibody tests are most commonly used for routine diagnosis of patients older than 18 months of age because they are inexpensive, accurate and can be used in decentralized settings without laboratory infrastructure. For CD4 testing and virological testing, which in addition to monitoring the patient on treatment (viral load) is used for detection of HIV infection in infants under 18 months of age (EID), the market is more circumscribed, with a handful of manufacturers dominating the market in each category. Further, the great majority of testing options available today for CD4 and virological testing are laboratory-based platforms with assays performed on sophisticated instrumentation requiring dedicated laboratory space and trained laboratory technicians. In many cases laboratory-based testing is expensive, and in almost all cases, requires sample transport networks to enable access to such testing for patients in peri-urban and rural settings.

Given this diagnostic landscape for HIV, it is generally accepted that the market for diagnostics for initial diagnosis of the disease is robust and efficient, but in order to improve access to CD4, viral load and EID testing in resource-limited settings and to bring down the cost of these tests, such testing needs to be simplified and brought closer to the point of patient care. This report therefore examines the new diagnostic technologies in the pipeline, most of which are tests designed to be delivered at the point of care (POC) and considers to what degree they have the desired characteristics for such diagnostics, including that they are sensitive, specific, affordable and user-friendly.

With respect to CD4 testing, the general conclusion is that currently there are a number of good laboratory-based platforms using proven flow cytometry technology many of which can be efficient and cost-effective when performed by well-trained laboratory technicians and when combined with good sample transport systems (which, in turn, can be improved with the use of mobile technologies for swift return of laboratory test results). However, in order to improve access, especially for rural

* World Health Organization and UNAIDS. Guidance on provider-initiated HIV testing and counseling in health facilities. Geneva: World Health Organization, 2007. http://whqlibdoc.who.int/publications/2007/9789241595568_eng.pdf. Accessed: 18 May 2011.
** World Health Organization. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector: progress report. Geneva: World Health Organization, 2010. http://www.who.int/hiv/pub/2010progressreport/summary_en.pdf. Accessed 18 May 2011.

patients, and to reduce loss to follow-up of patients, there is still a need for good and cost-effective POC CD4 testing options. Several such options are already on the market, and others are under development with anticipated release in late 2011 and beyond, at least one of which will be a disposable POC CD4 test. Assuming that performance of these POC tests stands up to robust evaluation, there is real promise here.

With respect to viral load testing, there are also a good number of sophisticated laboratory-based platforms for measuring viral load. However, despite the clinical consensus on the importance of viral load testing particularly for detecting treatment failure, with few exceptions, notably South Africa and Brazil, there is limited access to such testing in resource-limited settings. Factors restricting access include the need for sophisticated laboratory capacity and instrumentation, along with training for laboratory technicians and well-functioning sample transport networks. In addition, the cost of the testing is considerably higher than that for CD4. Viral load testing that could be conducted at the point of patient care would reduce the need for infrastructure and training as well as lowering the cost of testing. Although there are currently no POC viral load assays in the market, there are a number of platforms/assays in development, at least two of which may come to market in late 2011 or early 2012.

Finally, with respect to testing for infants under 18 months of age, the most widely-used test for EID is a DNA PCR molecular test, which is also performed on sophisticated laboratory-based instruments; alternatively, EID can be done on viral load platforms as well. In either case, the DNA PCR test is subject to some of the same drawbacks and limitations as viral load testing with respect to implementation in resource-limited settings. However, facilitated by funding from UNITAID and with the help of its implementing partner, the cost of the testing has come down, sample transport networks have been developed, and training has been implemented. As a result and due to the urgent need for infant testing, there has been considerable uptake of EID in-country. Despite this, the availability of EID at the point of care, could drive access to more hard-to-reach areas in-country, improve loss to follow-up of patients, and could bring down the cost of testing. Because viral load platforms can be used for EID, the new technologies in that testing area are viable options as well. But, in addition, there are at least two POC assays being developed specifically for EID. At least one of these may be launched in late 2011 or early 2012.

Advances in access to tests for infant diagnosis, as well as for ART staging and monitoring are needed in resource-constrained settings, and new technologies are in the pipeline that are likely to bring about significant changes to how these tests are delivered. At the same time, new platforms for high volume testing are also becoming available, allowing cost-effective consolidation of testing in high volume centers (super-labs). The level of CD4, viral load and EID testing required in resource-limited settings over the coming years will likely necessitate scale-up in centralized testing facilities, including in some cases super-labs, and at the same time, a drive towards POC testing to improve access, especially for hard-to-reach populations. The appropriate mix of high volume laboratories and POC testing will be country-specific, and will depend on such factors as the urban/rural split of the country, the expected volume of each category of testing and the ability to effectively transport samples between collection sites and laboratories as well as the efficiency of return of laboratory results to collection sites. Realistically, it will also depend on the comparative all-in cost of centralized versus decentralized testing. Ultimately, the landscape for HIV/AIDS diagnostics in resource-limited settings is unlikely to be either all laboratory-based or all POC.

The pace at which countries implement an optimized mix of high volume, centralized and low volume, POC diagnostic services tailored to their individual diagnostic needs will be key to the impact on access, efficiency and quality of diagnostics in resource-constrained settings over the next decade. Strategic funding on the part of UNITAID and other funders could make a difference in a number of areas, including in the acceleration of new diagnostic technology introduction, especially for POC technologies.

Introduction

In the interest of improved efficiency and better value for money with respect to antiretroviral therapy (ART) over the next 5 to 10 years, there is growing demand to find ways to streamline diagnostics for HIV/AIDS. The question is being asked whether there are ways to simplify diagnostics that will help to make ART more scalable and that will allow ART service delivery to be significantly decentralized at the community level, while at the same time reducing the cost of diagnostics for HIV/AIDS without diminishing the quality of patient care.

In order to answer those questions, it is necessary to understand the current landscape for HIV diagnostics, including the algorithms and tests required in the care and treatment of the HIV/AIDS patient, both before and after treatment initiation; the platforms used and price points of that testing; and the ways in which testing is delivered. With an eye to maintaining acceptable standards of patient care, it is then important to consider the future landscape of HIV diagnostics and what efficiencies might be achieved in terms of test algorithms, the cost of testing and decentralized service delivery, especially with respect to the introduction of diagnostics to be delivered at the point of patient care.

The initial hypothesis is that there is a need to significantly increase the level of access to robust, high-quality diagnostics in resource-limited settings because they will play a crucial role both in facilitating early detection and treatment of HIV/AIDS. This, in turn maximizes the preventive impact of treatment, and helps to ensure an appropriate and rapid response to drug resistance, a problem likely to grow substantially over the coming years.

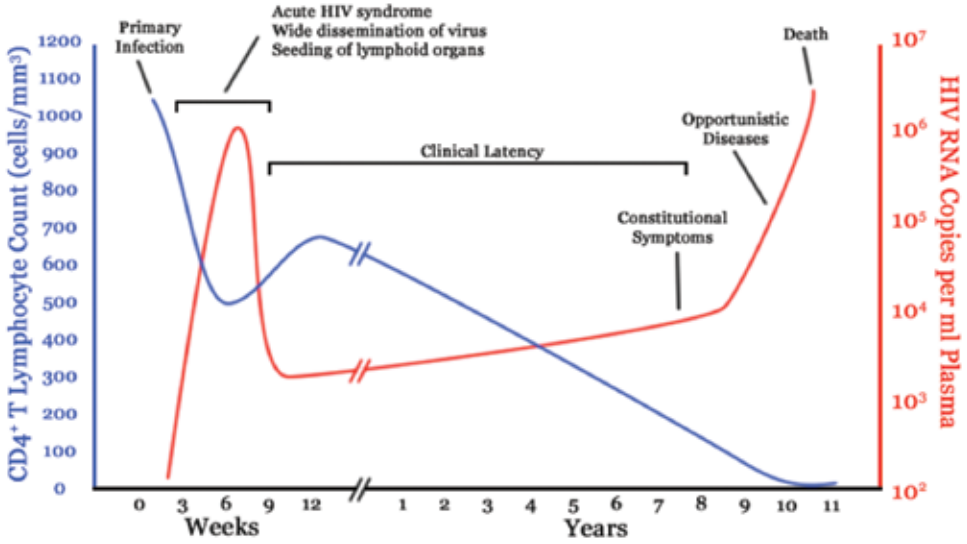
The challenge is how to most effectively ensure this access to high-quality diagnostics for HIV/AIDS. What is likely to be the most efficient diagnostic landscape over the next 5 to 10 years? For example, will improved access to diagnostics be achieved through a mix of diagnostic services in most countries that combines sophisticated, high-volume, low unit-cost laboratories in high-density areas, and lower-volume, simpler, POC or near-POC platforms in less densely populated regions? Or is some other model likely to prevail? This background will be essential in order to help UNITAID and other potential funders determine what investments could best move the diagnostics market towards an efficient and effective landscape and which of these investments could be transformational in effecting improved and lower cost access to diagnostics for HIV/AIDS in resource-limited settings over the long run.

This report reviews the current landscape for HIV diagnostics, including the algorithms and tests required in the care and treatment of the HIV/AIDS patient, both before and after treatment initiation; the price points of that testing; and the ways in which testing is delivered, including the technology platforms in use today. With this background, the report then reviews the current technologies and diagnostic platforms in three key testing areas for the HIV/AIDS patient, CD4 and viral load testing for adults and children, as well as EID, including EID run on viral load platforms, all of which are today typically accessed in resource-limited settings through sophisticated laboratory-based testing platforms. The report then goes on to consider POC CD4 or near POC, viral load and EID platforms either on the market or in the development pipeline and considers the implications of the landscape, what efficiencies might be achieved with respect to test algorithms, the cost of testing and decentralized service delivery.

Current Diagnostic Landscape[1]

Diagnostics for HIV/AIDS can generally be divided into three test categories: (i) tests to facilitate initial diagnosis, (ii) tests to stage the patient and (iii) tests to monitor the patient, both before and after initiation of ART. There are generally accepted algorithms and tests used at each stage as discussed below [1].

HIV disease involves a continuum of progressive damage to the immune system from the time of infection to the manifestation of significant immunologic damage by various opportunistic infections, wasting or CD4 lymphocyte count that marks the development of full-blown AIDS [2]. The period of time from infection to the development of AIDS is known as the incubation period, and it can vary significantly from person to person, but is generally quite long (years) rather than the short period (days or weeks) common to many other viral infections (e.g., the common cold or influenza) [3]. See below a typical, but approximate, clinical disease progression showing the relationship between the levels of HIV (viral load) and CD4+ T cell counts over the usual course of untreated HIV infection [4].



HIV infection is generally characterized by a spike in HIV antigens during the first few weeks after infection. Subsequent to that early period of acute infection, antibodies produced as a result of HIV infection appear and are then present throughout the course of the disease. The detection of these antibodies to HIV is the most common means to identify the infection, and HIV rapid tests for initial diagnosis of infection target this antibody response.

The extended incubation period of AIDS means that laboratory tests are required to identify persons at high risk of disease progression in order to guide clinical decision-making in asymptomatic seropositive patients, such as when to begin ART. Because depletion of CD4+ T lymphocytes is the hallmark and the apparent source of the central immune defect of HIV disease, determination of the CD4 lymphocyte count (or percentage) has been the most important laboratory marker of disease progression [1].

Tracking the course of the HIV virus itself by accurate measurement of the quantity of viral RNA in the patient's plasma has become as important a laboratory marker as CD4 lymphocyte count and is considered the best marker to use for ART decision-making post initiation onto therapy [1]. The measurement of the number of viral copies per milliliter of plasma (commonly known as "viral load") provides a clinically useful range of values that can indicate the effectiveness of ART in HIV disease.

[1] This section owes much of its content to data gathered by the author and the laboratory services team of the Clinton Health Access Initiative (CHAI) in 2010. Portions of this section are drawn from an unpublished report, entitled "ART 2.0 – Implications for Diagnostics in Resource-Limited Settings," co-authored with Dr. Trevor F. Peter of CHAI. Additional sources for this section are cited below.

Initial Diagnosis

There are a number of tests available to determine whether a person is infected with HIV, the virus that causes AIDS. These include HIV antibody tests (measured in blood, saliva or urine), p24 antigen tests and PCR tests. Of these, HIV antibody tests are most commonly used for routine diagnosis of patients older than 18 months of age because they are inexpensive and accurate. For the patient older than 18 months of age, HIV rapid disposable tests, using blood or saliva, are most commonly used for screening in decentralized settings without laboratory infrastructure, and if the patient is positive for HIV/AIDS on the initial test, a second test will be used to confirm the diagnosis. Generally speaking, in almost all resource-limited settings, the confirmatory test is also done using a rapid disposable test.[2] However, in some settings, the confirmatory test is an Enzyme-Linked Immunosorbent Assay (ELISA) and/or Western blot conducted in a central laboratory. If the two screening tests are discordant, a tie-breaker test is used, which is also generally an HIV rapid disposable.[3]

HIV rapid tests come generally in the form of a lateral flow strip or cassette, which, when performed correctly, are convenient, self-contained tools for HIV serologic testing. They are generally easy-to-use, can usually be performed on fingerstick blood, contain built-in quality controls, and can be administered by technicians and non-technicians alike, including community health workers. Further, as a rule, tests can be completed in less than 10 minutes. The cost of these HIV rapid tests in resource-limited settings, excluding any distributor mark-ups, generally ranges from about US$0.50[4] per test to about $1.60 per test.[5] ELISA testing is lab-based and generally costs $1.50 to $2.00 per test, including consumables, but is no longer widely used for HIV screening.

Because of the persistence of maternal antibodies in infants under the age of 18 months, the use of antibody tests, like commercially-available HIV rapid disposable tests, cannot be used to accurately screen infants for HIV/AIDS. Instead, DNA PCR or RNA PCR testing (i.e., virological testing), which detects the genetic material of HIV, should be used to determine the HIV status of infants in that age group.[6]

The most widely-used test for EID is the DNA PCR molecular test. It is also possible to use RNA detection methods (e.g., viral load) or p24 testing[7] for this purpose, but these methods are used in very few settings. In either case, the test itself is laboratory-based and requires relatively sophisticated instrumentation and a trained laboratory technician. In order to reach the broader in-country population efficiently, blood collection for the DNA PCR test has been decentralized to clinics, PMTCT centers and the like. The infant's blood is collected on filter paper (known as dried blood spots or DBS), which is transferred via couriers to the laboratory for testing, and test results are then returned to the clinic or other collection site for dissemination to care givers. Because this process can sometimes be slow, especially the return of results from laboratories, some countries have introduced SMS printers (or other mobile technologies) in order to achieve markedly improved return of results from laboratory to collection sites.

DNA PCR testing can be run on either low-throughput or high-throughput instruments depending on the needs in any individual country. A single instrument platform and related equipment (e.g., centrifuge, bio-safety cabinet, freezer, etc.) can cost from about $100,000 to more than $200,000, depending on the throughput of the platform. The cost of the test itself ranges from about $10.00 per test on low-throughput platforms to about $12.00 to $20.00 per test on high-throughput platforms. This cost covers the test reagents and associated supplier-provided, non-commodity consumables only and does not include DBS collection supplies, which cost from about $1.40 per test to about $2.75 per test, depending on bundle configuration. It also does not include more general laboratory consumables (e.g., gloves, pipettes, etc.), which cost from about $0.35 per test to $4.00 per test depending on the instrument platform chosen.

[2] Some countries run the screening tests in parallel and all patients will therefore get two tests; most countries run the screening tests serially as described. In addition, some countries only use ELISA tests for initiation screening (e.g., Vietnam) and, as indicated, some still use an ELISA test for confirmatory testing.
[3] Some countries use an ELISA test in the case of discordant results.
[4] All figures in the document are in US$.
[5] In this report, the fully-loaded cost of testing, including the cost of human resources and overhead associated with testing, is not considered. These costs can vary considerably from country-to-country. Also, none of the cost data discussed includes distributor mark-ups, which can range from a low of 5% of the cost of the test to as much as 30% of the cost of the test or more, nor does the data include freight, insurance, taxes or other such ancillary charges, again which vary country- to-country. Costs for instruments and reagents in this document are ex works pricing, unless otherwise noted. With respect to distributor costs, it is important to keep in mind that for platforms based on laboratory instruments, distributors play an important role in service and maintenance of the instruments, and in managing the supply chain. The distributor margin covers most of this cost. However, for disposable tests (e.g., HIV rapid tests and some POC CD4 tests being developed), there is no instrument and the margin is used to cover the costs of importation, storage and handling.
[6] Per the World Health Organization (WHO) 2010 Guidelines on ART initiation for infants and children (the "WHO 2010 Guidelines"): "It is strongly recommended that HIV virological testing be used to diagnose HIV infection in infants and children less than 18 months of age."
[7] Viral load testing is discussed in more detail below in connection with monitoring the HIV+ patient who is on treatment.

Patient Staging

Once an adult is diagnosed as HIV positive, CD4 testing is used together with clinical staging to determine whether the patient is eligible for treatment [6]. This is because after a primary HIV infection, the virus directly attacks CD4 T lymphocyte cells (which effectively coordinate the body's immune response) and begins to destroy them while at the same time using them as host cells for replication. Billions of CD4 T lymphocytes may be destroyed each day, eventually overwhelming the immune system's ability to regenerate them. In HIV-infected adults, the measure of an individual's CD4 T lymphocytes, or absolute CD4 count, is the most robust surrogate marker for immune competence [7]; for children under 5 years of age, the %CD4 measure is considered more reliable.[8] Clinicians therefore seek to routinely test CD4 count in order to monitor disease progression and to determine when an individual should be initiated on ART. Per the WHO 2010 Guidelines on ART Initiation, if the absolute CD4 count of an adult or a child over 5 years of age is below a defined threshold (currently less than or equal to 350 cells/mm^3), ART should be initiated.[9] For children between the ages of 24 and 59 months, the guideline is to initiate ART at an absolute CD4 count of ≤750 cells/mm^3 or at a %CD4+ T-cell ≤25, whichever is lower, irrespective of WHO clinical stage of HIV infection.

Whether in low- or high-throughput settings, CD4 testing is primarily conducted on lab-based instruments, although there are three POC CD4 test platforms currently available in the market. In rural settings, peri-urban settings and even in some urban settings, blood collection is done at clinics and blood samples are transported (via courier, post or other services, including motorcycle services) to labs for testing; results are then returned, generally via the same mechanism, although recently mobile technologies (e.g., SMS) have been introduced at some sites for this purpose. For CD4 testing, it is not currently recommended to use DBS for sample collection.[10]

The cost of lab-based CD4 testing varies based on testing volumes, reagents used and whether testing is conducted on high- or low-throughput instruments. The cost of CD4 reagents usually varies from a low of about $2.00 to approximately $14.00 per test, excluding collection and laboratory consumables. The cost of consumables will add between $1.00 and $2.00 per test to the cost. Instruments range in price from about $25,000 for low-throughput devices to $90,000 for high-throughput instruments.

The cost of currently-available POC CD4 testing ranges from just under $6.00 per test to about $10.00 per test for the test reagents alone, with associated sample collection consumables, adding approximately $1.00 per test. The instruments cost from $5,000 to $8,000 per device. As additional POC CD4 products enter the market, including at least one disposable test, prices will likely fall. It is possible that a disposable CD4 test could ultimately cost between $2.00 and $3.00 per test, but early pricing will be higher.

It is important to note that for infants, no staging is required following an initial HIV positive diagnosis. Per the WHO, infants are to be initiated on ART immediately [5]. If a follow-up diagnosis proves negative, ART would be ceased. Countries are currently at various stages of adopting this recommendation.

Monitoring the Patient

Prior to initiation on ART, the current WHO recommendations are to repeat CD4 testing approximately every 6 months (and more frequently as patients approach the threshold to initiate ART), or as needed based on clinical symptoms [6]. WHO guidance indicates that CD4 testing is required to identify whether patients with HIV and WHO clinical stage 1 or 2 disease need to start ART. Similarly, following initiation on ART, the WHO recommends CD4 testing every six months if the patient is stable, but more frequently if needed for deciding when to initiate or switch ART [6]. It is worth noting that CD4 testing, along with clinical symptoms, is also being used to diagnose treatment failure in most resource-limited settings.

[8] The absolute CD4 cell count of healthy infants who are not infected with HIV is considerably higher than in adults who are HIV negative. These cells slowly decline to adult levels by the age of about 6 years. Percentage CD4+ T-cell values vary less with age. Per the WHO 2010 Guidelines, relative to the measurement of absolute CD4 count, "the measurement of %CD4+ T-cell is thought to be more valuable in children under 5 years of age."

[9] In the absence of CD4 testing, the WHO recommends ART initiation for all patients with WHO clinical stage 3 or 4 disease.

[10] Recently, the use of DBS as a possible alternative for CD4 testing in resource-limited settings has been investigated (Redd et al), but the variability in the results and the failure to detect immature lymphocytes suggest the need for more research before the use of DBS in connection with CD4 testing should be considered a viable alternative to extant methods. [7, 8]

Clinical chemistry and hematology tests are routinely used to monitor toxicities associated with ART. From the wide range of tests available, only a limited number are considered essential, according to recent WHO Guidelines, which generally base chemistry and hematology test recommendations on ART regimens. For example, for AZT-containing regimens, hemoglobin measurement is recommended before initiation (and at weeks 4, 8 and 12 after initiation), while for TDF-containing regimens, creatinine clearance calculation is recommended both before initiation and every 6 months thereafter. Additional tests and test panels are recommended as required, depending on patient symptoms. These comprise full chemistry panels, including, but not limited to, ALT, other liver enzymes, renal function, glucose, lipids, amylase, lipase, lactate and serum electrolytes [6].

The technology options available for multi-parameter chemistry and hematology testing range from manual, to semi-automated to fully-automated low- and high-throughput lab-based instruments, and the cost of these platforms has a broad price range, from about $9,000 to $32,000 for hematology instruments and from about $3,000 to almost $60,000 for chemistry instruments. A number of low-volume, low-cost, robust, automated hematology analyzers designed for low-end laboratories are widely available and are becoming a standard option. Similarly, semi-automated spectrophotometers for chemistry analysis have been traditionally placed in low-end laboratories and remain in widespread use today.

In addition, for high volume settings, high-throughput chemistry and hematology instruments (large bench-top or floor-standing models) are available. Significant dedicated laboratory space is required, typically with features such as large reagent storage capacity and air-conditioning, dedicated UPS, and well trained, computer-literate technicians.

The technology options available for POC chemistry or hematology are not widely available in resource-limited settings. Nevertheless, simple hand-held instruments exist for tests such as blood glucose, and hemoglobin, as well as for fixed ranges of 3-6 chemistry parameters. These are mobile units, which cost approximately $1,000 - $5,000, and were designed for doctors' offices, home-use or bedside testing in patient wards. There are also a limited number of POC chemistry and hematology platforms that are less mobile, larger in size and capable of running a wider range of tests. With price ranges of approximately $3,000 to $10,000, depending on the features available, these are designed to be placed in a clinical care setting, such as a patient ward, outpatient clinic, or doctor's office, and can be operated by non-laboratory health care workers after minimal training.

The average cost of the basic full blood count is approximately $1.15 per test, while consumables average approximately $2.00 per test. For chemistry testing, the costs vary per test run and on average range from $0.10 per test to $0.45 per test. Consumables average approximately $1.50 per test.

Finally, post-initiation onto ART, viral load testing should ideally be used to monitor patients, especially to detect early signs of treatment failure. Left untreated, HIV virus replication can produce billions of new HIV copies daily. Plasma HIV RNA (viral load) testing quantifies the HIV viral burden in plasma. Where it is available, viral load testing is a standard tool for monitoring the patient's response to ART and, in conjunction with CD4 testing, to assess HIV progression.[11] However, due to the cost and complexity of the test, the implementation of viral load testing in resource-poor settings has been relatively limited. This situation persists despite current WHO guidelines on viral load testing that recommend its use every 6 months to detect viral replication and confirm treatment failure. These recommendations, however, stop short of urging all countries to implement viral load testing and instead encourage its use only where routinely available. Per the WHO, treatment failure is deemed to occur at persistent viral load readings above 5,000 copies per milliliter [6].[12]

[11] The analogy of a train on a track (attributed to John Coffin of Tufts University, circa 1996) has been helpful in illustrating the independent contributions of CD4 count and HIV viral load in an individual person. If the infected individual is imagined as being on that train traveling toward a clinical event--such as dying from AIDS--the CD4 count provides information on the distance of the train from that destination, whereas the viral load provides information on the speed at which the train is reaching the destination.

[12] It should be noted that in the absence of viral load testing, the WHO suggests the use of immunological criteria to confirm clinically-diagnosed treatment failure. It is also well established that viral load detects treatment failure well before CD4 count or clinical signs [9]. Recent research in South Africa, Kenya, Uganda and Botswana has also demonstrated that WHO CD4 count and clinical criteria have low sensitivity and specificity for diagnosing virological failure, both prematurely declaring patients as failing and missing patients who are truly failing [10 - 15].

At the present time, viral load testing is exclusively laboratory-based. Most testing is done on sophisticated, high-throughput instruments. No viable POC testing options are currently available, although several are under development. Viral load samples have to be collected and transported to central laboratories for testing, and although DBS has recently been introduced for several of the viral load platforms, it has yet to be taken up in-country to any degree.

One of the most important barriers to implementing viral load testing in resource-limited settings is the current high cost with prices for reagents and non-commodity test consumables averaging about $28 to $29 per test.[13] To put this in perspective, these costs are about 4 to 5 times more than CD4 testing and do not include the large upfront investment required to establish viral load-ready laboratories and purchase instruments for testing, which costs generally ranges from about $100,000 to $225,000, including installation and training. In addition, at the present time, collection consumables and laboratory consumables for viral load testing are not bundled and must be purchased separately. These items add approximately $2.75 per test and $1.50 per test, respectively, to the cost.

[13] The $28-$29 figure is a weighted average test price, including non-commodity consumables, offered by major suppliers across sub-Saharan Africa, excluding South Africa, for testing in the public health system. Reagent pricing is higher in Asia-Pacific and Latin America where tests often exceed $40 per test.

The Diagnostic Platforms in Depth

Of the various tests required/recommended for initial diagnosis, staging and ongoing monitoring of HIV, the tests that present the most persistent challenges to improved access and efficiency are CD4, viral load and EID. As discussed above, rapid assays for detecting the specific HIV antibody are accurate, when used correctly, are low cost and are already readily available at the point of patient care. Because chemistry and hematology testing is generally symptom- or regimen-based for the HIV patient and because there are already a number of technologies available at the point of care, these tests do not represent a significant barrier to access. The primary barriers to diagnostic access are tests for staging and monitoring of HIV patients (CD4 and viral load) and for the diagnosis of infants under 18 months of age (EID) in resource-limited settings. These are the test areas where POC technologies could potentially make the biggest difference in improving access and, as such, are the primary subjects of this report.

Factors to Consider in Diagnostic Platform Selection

This report considers each one of the CD4, viral load and EID test categories, discussing the underlying technologies used for each test, the current laboratory-based and/or POC platforms currently available for each test as well as the pipeline of POC technologies for each test category. Before discussing the diagnostic platforms in depth, it is important to review the characteristics of diagnostic platforms/devices that should be considered when decisions are being made as to the choice of diagnostic platform. The following operational characteristics are important to consider [1,16]:

- Type of technology (including whether for laboratory or POC) and output (test parameters measured);
- Throughput and turnaround time;
- Sample needed and sample stability (e.g., venous blood, plasma, capillary blood);
- Protocol complexity;
- Reagent stability;
- Cost of instrumentation and cost per test for reagents;
- Environmental requirements of the instrumentation, including power supply, ability to withstand heat and humidity and tolerance of altitude;
- If instrument-based, size and weight of instrument and associated devices (e.g., data station, printer);
- Supplies (and cost thereof) required from parties other than the manufacturer of the instrument/test (e.g., vortex, pipettes, etc.)
- Recommended or required instrumentation beyond the analyzer itself – e.g., data station, printer bar-code scanner;
- Training required;
- Availability of QC reagents and compatibility with EQA programs; and
- Where the technology is recommended for use (e.g., hospitals, clinics, etc.).

For each of the platforms currently available for CD4, viral load and EID testing and for each such technology in the diagnostic pipeline discussed in this report where sufficient information is available from the developer, these operational characteristics are set out in Appendix 1.

In addition to the operational characteristics of the various platforms/devices, it is also important to consider the performance of the platform. In general, performance refers to the ability of the technology to give accurate and reproducible results. Both the accuracy and precision of a quantitative test need to be evaluated.[14]

The accuracy of a technology is a measure of the degree of closeness of the reported value to the true value, and is evaluated by comparing results obtained by the test under evaluation with those

[14] Note, however, that for a qualitative test – e.g., HIV rapid tests and DNA PCR – accuracy and performance are not the relevant measures. Rather, sensitivity and specificity, as well as negative/positive predictive values are needed.

obtained for the same samples using a reference technology. Although correlation of those results is one measure of accuracy, it is generally not a sufficient measure. It is important to measure bias and misclassification of the test results as well. Bias, which may be reported using Bland-Altman analysis, reflects the average/mean difference between the results of the technology under evaluation and the comparator or reference technology [17]. Misclassification probabilities, which may be upward misclassification probability or downward misclassification probability, describe the likelihood that a test will incorrectly categorize a result as higher or lower than a given cut-off value, respectively.

The precision of a test answers the question how close the results are when testing is repeated using a single technology. It is a particularly important measure when used in the context of following a patient's serial measurements using the same technology – e.g., the level of a patient's absolute CD4 count or viral load from test to test. Data on precision are often reported as the coefficient of variation (CV), which is a measure of dispersion. A lower CV indicates less variation and greater assay reproducibility.

CD4 Performance

While it is important to consider the performance (accuracy and precision) of diagnostic systems when making decisions about which diagnostic platforms to implement in-country, this is particularly challenging with respect to CD4 testing platforms as "no gold standard technology or internationally recognized reference preparation exists for CD4" [7,16].

With respect to CD4 assay accuracy, neither correlation nor Bland-Altman plots alone are sufficient. Misclassification probabilities provide more clinically relevant information about the CD4 test being evaluated, with the upward misclassification around a treatment threshold perhaps being of most clinical importance (as it may lead to a delay in the initiation of ART or prophylactic treatment in some patients) [7]. On the other hand, downward misclassification may result in the decision to treat large numbers of patients who have CD4 counts above the ART initiation threshold when using the reference test.[15] As to the precision of CD4 tests (i.e., the reproducibility of results), the %CV may be badly underestimated if it is based on too few replicates; a minimum of 8 replicates should be used [18].

The WHO has recently conducted a systematic review of the available literature on CD4 performance. To date, it has been concluded that it is difficult to answer clinically relevant questions from such a review [7]. For example, studies may conclude that a method is an acceptable alternative to a reference technology based on correlation alone, or based on "mean difference" between the two, which gives no indication of the maximum differences observed, which could be large, despite a small mean difference. Further, the maximum differences could vary at different levels of absolute CD4 count, even within the clinically relevant range [7,16]. Misclassification, especially misclassification down, is likely to be an underestimate since none of the studies in the literature is restricted to the most clinically relevant range.

The overall conclusions are that [7,16]:

- There is both physiological and technology-related variability associated with CD4 measurement no matter which technology is used;
- Different technologies are associated with different performance characteristics in terms of both misclassification and precision and these characteristics have important implications for patient management and HIV care and treatment programs;
- Although test performance (accuracy and precision), especially misclassification, should be considered when choosing to introduce and implement a CD4 technology, the data are not always available, and when available, are not robust enough to give a clear idea of the comparative merit of different technologies; and
- Given the potential for error described above, access to quality control (QC) reagents and participation in external quality assurance (EQA) programs are very important.

Diagnostic manufacturers routinely publish information on their technology's accuracy and precision. However, this is often self-reported data. Independent, peer-reviewed evaluations are a more reliable source of performance information for diagnostics. For each platform/device considered in this report, an indication of performance and/or performance data availability is provided.

[15] Glover [16] notes that a more important measure might be the probability that a patient with an absolute CD4 count well below the ART initiation threshold might be incorrectly classified as above the threshold, but that such data are rarely available in the published literature.

CD4+ T-Cell Counting Technologies

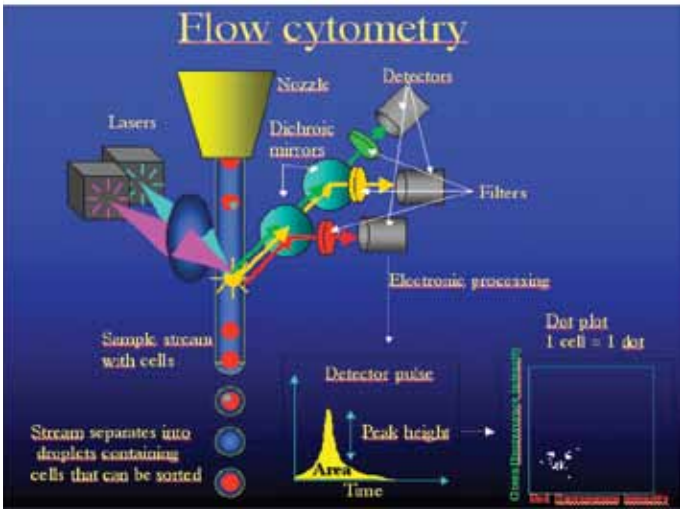
Introduction to Flow Cytometry

Flow cytometry is a method to differentiate and count cells and microparticles. It is considered the gold standard technique for CD4+ T-cell enumeration [19,20], and is the underlying technology for most of the current CD4 diagnostic platforms in use today in resource-limited settings, including the instruments manufactured by such suppliers as Becton Dickinson, Beckman Coulter, Partec and Guava.

Flow cytometry is a member of a family of technologies known as automated, analytical or quantitative cytology. As the term implies, flow cytometry refers to measuring ("metry") the properties of cells ("cyto") while in a fluid stream ("flow"). The most important feature of flow cytometry is that it allows for the analysis of a large number of particles (100,000 or more) within a short period of time, generally within minutes. It is the only technique capable of quick quantitative measurements of multiple features of individual cells, including a cell's (or a particle's) relative size, granularity or internal complexity.

An important requirement of flow cytometry is the need to specifically label cell constituents with fluorescent molecules, which are then used to identify cells carrying this "label". Cell constituents can be made up of a number of cellular components, including DNA, which can be labeled by different dyes/stains. Unique markers or proteins on the cell surface can be labeled with monoclonal antibodies conjugated with one of many fluorescent dyes (fluorochromes). But, perhaps the most important property of flow cytometry is the ability of certain flow cytometers to separate individual cells as a function of the different physical and biological characteristics of the cells being analyzed. This is referred to as flow cytometric cell sorting.

Flow cytometers can be considered to be specialized fluorescence microscopes. At the most fundamental level in a flow cytometer, cells in suspension flow single file (fluidics) past a focused laser where they scatter light and emit fluorescence (optics) that is filtered and collected (interrogation). The cells are then converted to digitized values that are stored in a file (electronics) that can be read by specialized software (interpretation) [21,22]. The fluidics, optics and electronics systems work together to determine how cells or particles scatter incident laser light and emit fluorescence as they pass through the interrogation point [23]. See below a schematic representation of a classical laser-based flow cytometer depicting the major components for cell flow, laser excitation and measurement of fluorescence and light scattering.

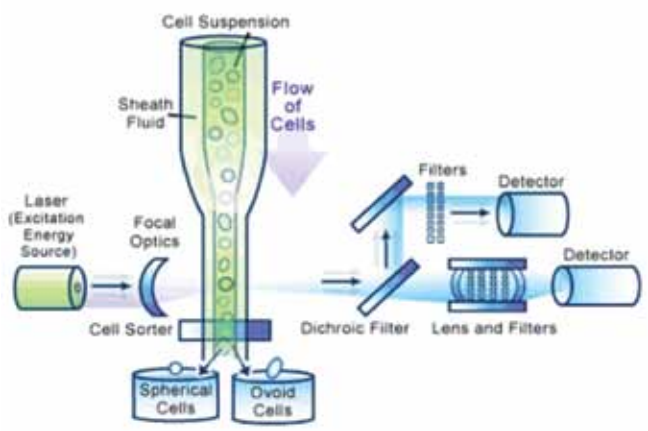


Schematic courtesy of Jan Grawé, BioVis, Uppsala Univeristy; http://www.scilifelab.uu.se/Technology_Platforms/BioVis.

Each of the three main component systems of a flow cytometer, fluidics, optics and electronics is discussed in more detail below.

Fluidics System

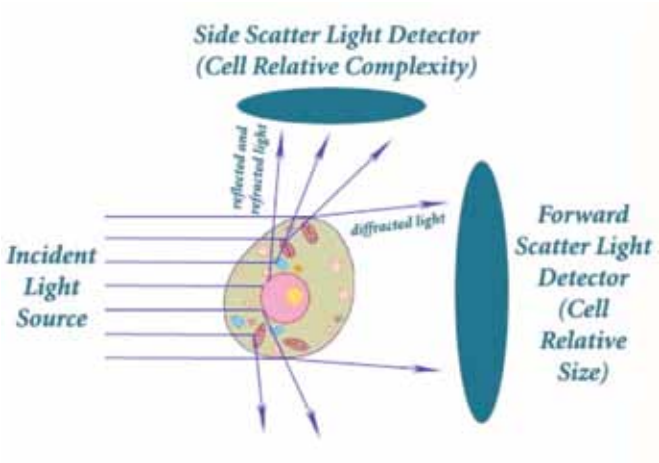
The fluidics system (an example of which is pictured below [24]) transports particles/cells in a fluid stream to a laser beam for interrogation. The fluid, called sheath fluid, is usually a saline solution. The portion of the fluid stream where particles are located is called the sample core. The flow of sheath fluid accelerates the cells and constrains them to the center of the sample core where the laser beam will then interact with the cells. Typically, cells are ejected through the flow chamber at a rate of about 1,000 cells per second [25].



Optics System

Flow cytometry optics systems consist of a complex system of lenses made up of excitation/ illumination options and collection components. The excitation components include lasers, lenses and filters to route the laser beams to the flow cell, while the collection components consist of a collection lens to amass light signals emitted from the cells.

When particles pass through the laser intercepts (or interrogation points), they scatter light (both in a forward direction and in a side direction). Light that is scattered in the forward direction (along the same axis the laser is traveling) is detected in the Forward Scatter Channel (FSC). Light scattered at 90 degrees to the axis of the laser path is detected in the Side Scatter Channel (SSC). See the diagram below. The intensity of the FSC depends on the size of the cell and not its refractive index. The intensity of the SSC is proportional to cell granularity or complexity. Because FSC is related to cell size and SSC is related to its internal structure, a correlated measure between the two can allow for differentiation of cell types in a heterogeneous cell population. For example, larger and more granular granulocyte cells produce a large population with high SSC and FSC. Monocytes, on the other hand, are large cells, but with less granularity, and they produce a separate population with high FSC and lower SSC. Therefore, these cells can be separated into different populations based on their FSC and SSC alone.



Schematic courtesy of Dorothy Kratochwil-Otto, Flow Cytometry Lab, University of Alberta, Canada (http://www.flowcytometry.ualberta.ca).

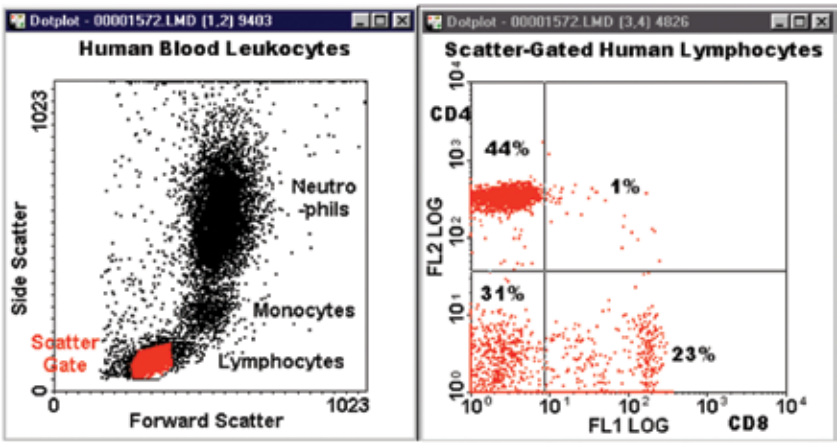
Finally, as the laser interrogates the cell, fluorochromes on or in the cell (either intrinsic or extrinsic) may absorb some of the light and become excited. As those fluorochromes leave their excited state, they release energy in the form of a photon with a specific wavelength, longer than the excitation wavelength. These fluorescent stained particles or cells can be detected individually.

Forward and side-scattered light and fluorescence from stained cells are split into defined wavelengths and channeled by a set of filters (e.g., dichroic) and mirrors within the flow cytometer. The fluorescent light is filtered so each sensor will detect fluorescence only at a specified wavelength. These sensors are called photomultiplying tubes (PMT's).

Electronics System

In a flow cytometer, as the fluorescing cells pass through the laser beam, they create a peak or pulse over time in the number of photons. The PMTs detect and collect these photons of light and convert them to current (voltage). The electronics system must then process that light signal and convert the current to a digitized value or number that a computer can graph. This is done by using a series of linear and log amplifiers. Linear amplification is frequently used to amplify FSC and SSC light signals of cells; logarithmic amplification is most often used to measure fluorescence in cells.

Electronic signals are then further processed (by an analog to digital converter) and sent to a computer so that the results can be interpreted. These profiles of cells may be displayed in a number of formats, including dot plots, contour plots and density plots. Below is an example of a dot-plot quadrant analysis for human blood lymphocytes [26].



Diagrams courtesy of Professor Eric Martz, University of Massachusetts, Amherst, MA.

The Primary CD4 Platforms Currently Used Today in Resource-Limited Settings

There are currently a handful of platforms that account for virtually the entire market share for CD4 testing in resource-limited settings. These are lab-based platforms from BD Biosciences, a division of Becton Dickinson (BD), Beckman Coulter (Coulter), Millipore (formerly Guava and now a division of Merck), Partec and Apogee. In the developing world, BD and Coulter have the largest CD4 testing market share.[16]

However, before considering these platforms in depth, it is important to note that there are other methods of CD4 enumeration available in the market. First among these is what is known as the dual platform approach. In this approach, three measurements are obtained from two different instruments, a flow cytometer and a hematology analyzer. With dual platform methodologies, either the total lymphocyte count (using the traditional method) or total white cell count (using the PanLeucogating method) is obtained from the hematology analyzer. The CD4 T lymphocyte percentage is obtained (in the traditional method) or the white cell lymphocyte percentage is obtained (in the PanLeucogating method) using the flow cytometer. In both cases, the absolute CD4 count is then derived using a mathematical formula. The dual platform approach introduces variability into CD4 enumeration because it combines results from two platforms into a single calculation [18]. However, the PanLeucogating method is producing improved performance over the traditional approach [27]. In general, the dual platform method for CD4 enumeration is not particularly well-suited to resource-limited settings because it is complex and requires significant training.

In addition to dual platform approaches to CD4 cell enumeration, there are also manual methods available. These methods involve the use of both a light or fluorescence microscope and a hemocytometer. The Manual CD4 Count Kit from Coulter (using CD4 Cyto-Spheres Reagents) and the Dynal T4 Quant Kit (Dynabeads) are assays that can be used in manual methods. The methodology requires the user to count cells labeled with beads in a defined area on slides. While such manual bead-based assays have low upfront capital costs, they are quite labor intensive, can be slow and require experienced and capable microscopists to obtain accurate results [28,29,30]. These characteristics make manual methods of CD4 cell enumeration less than ideal for resource-limited settings.

Finally, it is also possible to enumerate CD4 cells with reagents designed to be used on hematology analyzers (without the need for a microscope). For example, Dynabeads can be used in conjunction with the POCHi-100 hematology analyzer from Sysmex, and a team from Chiang Mai University has developed reagents, called CD4 Select, that can be used to enumerate CD4 cells on a hematology analyzer alone. Moderate training is required for this method of analysis, and there are currently no peer-reviewed, independent evaluations of these technologies available.

In resource-limited settings, single platform methods for CD4 cell enumeration have become the methodology of choice. Single platform methods provide absolute CD4 (and in most cases, %CD4) measurements using a single instrument. In these assays, CD4 T lymphoctyes can be counted in a precisely-determined volume of blood or by using known numbers of fluorescent microbeads "admixed" to a known volume of CD4-stained blood [25]. There are several single-platform technologies, including the platforms from BD and Coulter, each of which is a bead-based technology, and those from Millipore/Guava and Partec, each of which uses volumetric methods.

[16] Unless otherwise noted, information on each of the CD4 technologies described below has been taken from company materials generally available on the respective company websites and/or from direct discussions with each of the manufacturers/developers of such technologies. Images used herein have been reproduced with the permission of each of the respective companies/developers.

Some of these single platform systems, including the BD FACSCalibur and the Coulter Epics, are open platforms. This means that the platforms will accept a variety of reagents. For example, TruCount reagents from BD can be used on the Epics platform. Cytognos beads (from Cytognos SL) can be used on Coulter Epics or BD FACSCalibur. However, each time different reagents are used on any of these platforms, the instrument must be re-calibrated. The remaining single platform systems commonly used in resource-limited settings, including the Guava Auto CD4/CD4% and PointCare NOW platforms, are closed systems. This means that they can only use reagents manufactured by the platform manufacturer; reagents from other manufacturers are not inter-changeable.

Each of these lab-based, single platform CD4 testing systems is discussed in some detail below. They are presented in order of their throughput capabilities, which also influences the level of the healthcare system in which the instruments can and should be used.

High Throughput Systems

Both BD and Coulter manufacture open platform, high-throughput flow cytometry systems, the BD FACSCalibur™ Flow Cytometer and the Epics XL™ or XL MCL™, respectively. These systems can be, and are, used for CD4 testing, but are not dedicated CD4 testing platforms. Each of these systems is most appropriate for national and central laboratories. Partec also manufactures a high-throughput CD4 platform, and due to its relative simplicity, it can be used in small hospitals at the provincial and district level.

BD FACSCalibur™ System

BD (http://www.bdbiosciences.com) manufactures the BD FACSCalibur™ system (pictured below), which is a large, bench-top, automated, multicolor flow cytometry system that can perform both cell analysis and cell sorting (for research use) in one system. The technology is bead-based which means that the cytometer employs scatter and fluorescence detection and known concentrations of reference beads in each sample to obtain absolute T cell concentrations [31]. In order to maximize the information obtainable from limited samples, the FACSCalibur uses multiple fluorochromes to identify and isolate subset cell populations in a single sample. The system can quickly do a number of routine tasks, including both absolute CD4 counts in cells/μL, which is the international standard for such measurement, and percentage CD4 counts (using BD TruCount reagents); it can also do immunotyping, residual white blood cell enumeration, stem cell analysis and DNA analysis. The FACSCalibur is a flexible and upgradeable modular system, with software that can be customized per the needs of the user.

While the FACSCalibur system is relatively easy to use, with walk away automation via a loader option or a high-throughput sampler that can handle assays in 96 or even 384 microtiter plates, it is a sophisticated, high performance system engineered for use both for *in vitro* diagnostics and for research laboratories. It is especially useful in settings that can take advantage of its capabilities for assay development, verification, and identification of cellular populations of interest.

Although most experts agree that there is no true reference standard for CD4 testing, many consider the FACSCalibur system to be the "gold standard" for CD4 counting. It is the platform against which the performance of other CD4 systems is most frequently compared and there is at least one published, peer-reviewed evaluation of the platform using TruCount reagents [32]. It is in use in resource-limited settings, but is generally only appropriate for central/national reference laboratories where its high throughput (approximately 200 samples per day or 40 samples per hour) and sophisticated capabilities can be used appropriately.

The cost of the FACSCalibur instrument is about $75,000, but can be higher depending on the country/region, options chosen and whether there are any special negotiated prices available. For the basic three-color reagent test (TruCount) used by most laboratories in resource-limited settings, the cost of reagents is volume-dependent and ranges from about $3.00 per test at volumes of more than 75,000 tests per instrument per annum to as much as $7.00 per test at significantly lower annual volumes.

Coulter EPICS XL™ or EPICS XL-MCL™ System

Like the BD FACSCalibur, the Epics XL™ or Epics XL-MCL™[17] (pictured below), manufactured by Coulter (http://www.beckmancoulter.com/products/market-segmentation/clinical-diagnostics.asp), is a large, bench-top flow cytometer. The system is automated and can analyze up to 4 colors of immunofluorescence from a single laser. Epics is a bead-based system that can perform absolute and percentage CD4 counts (using FlowCARE™ PLG or TLG reagents), but can also perform multi-parametric DNA analysis, platelet studies, reticulocyte enumeration, cell biology/functional studies as well as a broad range of research applications. The instrument is self-contained and biohazard safe. It is sufficiently flexible that the operator can change filter elements for versatility in research settings.

The Epics XL is the only flow cytometer to offer a state-of-the-art Digital Signal Processing (DSP) for reliable linearity and drift-free amplification and compensation. The single laser design eliminates concerns regarding multi-beam stability, signal delay and alignment.

Like the FACSCalibur system, Epics is relatively easy to use, and with the addition of the MCL, provides walk away automation, but it is also a high volume (approximately 150 - 200 samples per day without the MCL and, on average, 47 samples per hour, or about 375 samples per day, with the MCL), high performance system that is geared for use in busy reference laboratories where in addition to CD4 counting, it can be employed for additional analyses, including diagnosis of acute and chronic leukemias, lymphomas and platelet disorders, among others.

Assuming certain test volume commitments, the cost of the Epics XL-MCL instrument is about $90,000. For the basic FlowCare PLG or TLG reagents used by most laboratories in resource-limited settings, the cost of reagents is volume-dependent and ranges from about $2.50 to $4.50 per test at volumes of more than 75,000 tests per instrument per annum to about $5.00 to $8.00 per test at volumes under 11,000 tests per instrument per annum.

[17] The Epics XL-MCL includes a multi carousel loader ("MCL") and an integrated vortex, which makes the system fully-automated, allowing the operator to load the carousel and walk away. Because of its automation capabilities, it is the only system sold by Coulter in sub-Saharan Africa.

The Epics system is a well-established platform, which is used in resource-limited settings, including South Africa. Published, peer-reviewed evaluations of the Epics, using FlowCount beads, are available [33].

Partec CyFlow® Counter

The CyFlow® Counter[18] from Partec (http://www.partec.com/cms/upload/CyFlowCounter.pdf) is a portable, compact desk-top flow cytomer designed for routine CD4 and %CD4 counting (as well as total lymphocyte and WBC counting) in a single, dedicated platform. It is pictured below on the left. The Partec CyFlow is a volumetric system, measuring cell counts by mechanical means, rather than by calibration and beads. The CyFlow Counter also has what the company calls "alignFree™" technology, meaning that the system does not require optical alignment and laser adjustment, which are required on the larger lab-based systems like FACSCalibur and Epics.

The CyFlow Counter can be combined with a CyFlow sample preparation and autoloading system (pictured above on the right). This station is intended for use with Partec dry CD4/CD4% reagents (Partec also offers liquid CD4/CD4% reagents for use without the loading system). The system allows 10, 20, 30 or 40 samples at a time to be loaded on a tray; alternatively, 96 well plates can be used. Whereas typical CyFlow Counter thoughput is about 250 samples per day, the company indicates that this added capability allows for acquisition of up to 400 samples per day, making the system a compact, but high-throughput option. Further, because the reagents are available in a dry/lyophilized form in ready-to-use test tubes, there is no need for cold chain and refrigeration of reagents.

Since the CyFlow Counter is relatively compact but has high throughput, it can be used not only at national and reference laboratories, but also in hospitals at the provincial and district level. The device is also small enough to be used in mobile laboratories. Further, the instrument can be run off of a car battery or solar panels, if needed.

The cost of the CyFlow Counter instrument alone is about €17,000 (~$27,000), but the total cost will be higher with the addition of the sample preparation and auto-loading system. Reagents are available both in dry and liquid form, the pricing for which is not dependent on volumes. Absolute CD4 reagents cost approximately €1.75 ($2.40) per test, while %CD4 reagents for pediatric use cost approximately €2.50 (~$3.40 per test).

With respect to the performance of CyFlow, there is published, peer-reviewed literature available [28, 34], although the WHO indicates that there is limited independent data on misclassification rates around the current WHO treatment threshold of 350 cells/μL [7, 35].

[18] Note that Partec also manufactures another device, the CyFlow® SL_3, which performs volumetric absolute counting of CD4 and CD4% for pediatric patients, total lymphocyte count and WBC. The instrument costs about €22,000 (~ $30,000) and uses the same reagents as the CyFlow Counter. The SL_3 operates on the same principles as the CyFlow counter, which is a newer generation device from Partec.

Medium to Low Throughput Systems

BD FACSCount™ System

The BD FACSCount™ system (pictured below) is a complete, dedicated system for measuring both absolute and percentage CD4 counts or CD4, CD8 and CD3 T-cell counts (http://www.bdbiosciences.com). It is the platform that is most widely used in resource-limited settings. The system is made up of a relatively compact bench-top instrument, reagents and controls.

The FACSCount system uses a whole blood sample, eliminating lyse and wash steps, which, in turn, simplifies sample preparation for the operator. Fluorescence reference beads, included in a reagent tube, ensure accurate enumeration of the lymphocyte populations of interest; no operator intervention is required. The software in the instrument can calculate automatically both absolute CD4 counts and CD4 percentages (important for use on children under 5 years of age, as discussed earlier in this report) using a single-tube (pictured below) assay.

The FACSCount system is generally considered to be robust, and due to relatively simplified sample preparation and the degree of automation of the instrument, requires minimal operator training. The system has been used in CD4 monitoring for HIV/AIDS care and treatment programs in resource-limited settings for more than a decade; its performance is considered to be reliable, and independent performance data is available [36, 37]. The FACSCount is used in a wide range of laboratory settings, including central laboratories as well as district hospitals/laboratories. As a medium- to low-throughput system, it is generally appropriate for use where sample load is less than fifty samples per day, which is likely to include district hospitals, for example. BD has established a comprehensive network of support resources, including service and maintenance resources, for resource-limited settings.

The FACSCount platform is a closed system, although BD recently announced a strategic collaboration with ReaMetrix, a privately-owned biotechnology company based in Bangalore, India, to develop dried reagents for the FACSCount system. The cost of the FACSCount instrument is about $30,000. Pricing for reagents depends on test reagents chosen (single tube absolute CD4 only, single tube absolute CD4 and percentage% CD4, or double tube) as well as volume per annum per instrument. The general range of pricing for the reagents alone ranges from approximately $3.50 per test for test volumes of more than 10,000 tests per instrument per annum, and up to $10.00 per test for test volumes up to 4,500 tests per instrument per annum.

Millipore-Guava® Auto CD4/CD4% System

The Guava® Auto CD4/CD4% system (pictured below), manufactured by Millipore (a division of Merck, http://www.millipore.com/flowcytometry), is a small, bench-top instrument that provides the ability to measure both absolute and percentage CD4 counts as well as total lymphocyte count. The Guava system uses volumetric sampling through a syringe-pump, which eliminates the need for reference beads during cell counting. In addition, the sample is aspirated through a microcapillary flow cell instead of using sheath fluid to focus the sample, which eliminates the need for complex fluidics and large volume storage. The elimination of beads and the use of a flow cell also reduce the costs per assay run and reduces the size of the system, which is about a quarter of the size of typical flow cytometers.

The Guava software module provides automated data acquisition, gating and analysis, which leads to ease of use and simplicity. The company estimates that the system can be learned in about a day's training. In addition, the Guava system is generally rugged because of its simplified fluidics, self-aligning lasers and user-changeable microcapillaries. In turn, this means that the Guava system is relatively easy to maintain.

The Guava system, which is a closed system, is a medium- to low-throughput platform, allowing for approximately 50 samples per day to be processed. Like the FACSCount, the Guava instrument can be used in a wide range of settings. In recent years, the company has expanded its ability to provide service and maintenance on the instruments through a network of local distributors. The cost of the Guava instrument is about $33,000. The pricing for the reagents (combined CD4 cell count, CD4% and total lymphocyte count) is $2.00 per test, regardless of volume.

With respect to the performance of the Guava system, WHO concludes that it is difficult to place it in the platform hierarchy. Although there were studies on the earlier version of Guava reagents (Easy CD4) [38,39], there is a dearth of evidence on the performance of the Guava Auto CD4/CD4% reagents, with no peer-reviewed studies having been published to date [7].

Apogee Auto40 Flow Cytomer

The Apogee Auto40 Flow Cytometer, manufactured by Apogee Flow Systems (http://www.apogeeflow.com/auto40_flow_cytometer.htm), pictured below, is a bench top, volumetric flow cytomer capable of performing both absolute and percentage CD4 counts as well as total and percentage total lymphocytes, CD8 count and CD4:CD8 ratio. The system is not bead-based, but rather uses a precision syringe sampling system that delivers sample to the flow cell at a precisely controlled rate.

The Apogee system was designed for both military environments and resource-limited settings. Accordingly, the instrument is rugged. Sample preparation is similar to that for FACSCalibur and requires vortexing as well as 25-minute incubation in a dark room. Sample run time is approximately 120 seconds, but can be longer for samples with low CD4+ cells. Data is stored in the Apogee's internal hard drive for immediate or later analysis by the operator.

The Apogee Auto40 is a medium-throughput system that can run a maximum of 20 samples per hour. Although it is an automatic instrument, it also offers an option to manually analyze difficult or damaged samples. The cost of the Apogee Auto40 is about $30,000. The pricing for reagents is approximately $2.50 per test for absolute CD4 counts and $3.50 per test for % CD4.

Although the Apogee Auto40 has been evaluated against competing systems (e.g., study done at The Infectious Diseases Clinic in Milan, Italy and a study done at Le Dantec Hospital in Dakar, Senegal), there are no published, peer-reviewed studies currently available on the platform. To date, there has been little uptake of this system in resource-limited settings.

CD4 Testing at the Point of Care

Each of the high, medium and low throughput platforms discussed above are systems primarily designed for use in laboratory settings. A number of them, including the FACSCalibur, Epics and FACSCount are used in developed as well as developing world settings. However, as discussed earlier in this report, it is generally accepted that in order to improve access to CD4 testing in resource-limited settings and in order to bring down the cost, CD4 testing needs to be brought closer to the point of patient care.

Although flow cytometry has been the standard for CD4 counting for almost 30 years now, it is not inherently well suited for use in decentralized testing. Point-of-care CD4 testing is likely to require new technologies. To date, CD4 assay development approaches include selective cell staining, followed by capture or count by digital photography, measuring CD4 molecules instead of cells, or measuring proxy molecules of CD4. Both instrument-based and disposable tests are in the CD4 development pipeline. Such POC CD4 tests would preferably meet the ASSURED criteria for the ideal rapid test, which was developed by WHO [40]. The ASSURED criteria are as follows:

A = Affordable
S = Sensitive
S = Specific
U = User-friendly (simple to perform in a few steps with minimal training)
R = Robust and rapid (results available in less than 30 minutes)
E = Equipment-free
D = Deliverable to those who need the test

Below, POC diagnostics for CD4 testing that are either on the market or in development are discussed in some detail, including technical specifications as well as advantages and disadvantages of each. Three of these technologies are already on the market: PointCare NOW™, the Pima™ CD4 Analyzer and the CyFlow™ CD4 miniPOC. The remaining technologies discussed, Daktari, Burnet, Zyomyx, MBio and others are not yet available on the market. See Appendix 2 for the current CD4 POC pipeline.

PointCare NOW™

The PointCare NOW™ system (pictured below) was developed by PointCare Technologies, Inc. (http://www.pointcare.net/products) specifically for decentralized and low-resource settings. It is a compact, tabletop system that measures CD4 absolute count and %CD4, WBC count and hemoglobin, as well as total count and percentage lymphocytes, monocytes, neutrophils and eosinophil. The system uses forward light scattering (rather than the fluorescent dyes used in some systems) to distinguish lymphocytes from white blood cells, and then uses a colloidal gold label[19] to change the natural light scatter characteristics of the CD4 subclass of lymphocytes in order to perform the CD4 enumeration.

The PointCare NOW instrument is considered to be robust due to its modular, injection-molded housings with few moving parts. The system also has solid-state electronics, and comes pre-calibrated from the factory, which eliminates the need for calibration by the instrument operator. In addition, the system has the advantage of being fully automated. There are no manual sample preparation steps for pipetting, incubation, vortexing and the like. The operator is able to take a capped phlebotomy blood-sample tube and, with the cap still in place, insert it into a receiving slot in the PointCare NOW instrument for analysis, thus eliminating operator contact with blood. The operator can, in fact, walk away from the instrument at this point in the process. Results are available in 8 minutes.

[19] The label consists of anti-CD4 antibodies coupled with nano-sized gold particles.

The PointCare NOW system is a medium- to low-throughput platform that can handle about 50 samples per day and is appropriate in settings with that level of volume. The system is closed and requires the use of PointCare reagents. The cost of the PointCare NOW instrument is about $25,000. The pricing for reagents is approximately $10 per test.

To date, no peer-reviewed, independent performance evaluations of PointCare NOW have been published. Such reviews are expected to be published soon. There is, however, summary performance data from a CDC evaluation of the instrument available on the company's website at: http://www.pointcare.net. In addition, a recent unpublished evaluation conducted at military clinics in Uganda indicated good clinical alignment between each of two PointCare systems when compared separately to a BD FACSCalibur.

The Pima™ Analyzer

The Pima™ Analyzer is a small bench-top, fixed volume cytometer manufactured by Alere™ Inc. (http://www.alere.com/en/global-products-services.html). The Pima employs the same static image analysis and counting principles as existing CD4 enumeration technology, in a compact, portable (can be carried in a backpack), and robust housing. A separate printer is also available. The Pima Analyzer and printer are pictured below.

The Pima system is made up of the Analyzer and a disposable Pima CD4 test cartridge containing dried reagents, pictured below. As such, it is a closed system. It is capable of measuring absolute CD4 counts in whole blood, but it cannot currently determine percentage CD4 counts for pediatric use. This capability could be added to the system, along with other cell type counts. The cartridge is able to take up approximately 25 µL of blood, which is then combined in the cartridge itself with the dried reagents necessary to run the test. The Pima CD4 test is actually performed within the cartridge and no part of the Pima Analyzer comes into contact with the blood sample during processing, which minimizes the risk of Analyzer contamination.

The Pima Analyzer is equipped with miniaturized multi-color fluorescence imaging optics. Fluorescence signals are detected by an on-board camera and analyzed using proprietary software algorithms on board an embedded computer. T-helper cells carry both CD3 and CD4 surface antigens and therefore emit light at wavelengths specific for both antibody-dye conjugates. This allows the specific differentiation of T-helper cells from other blood cell types carrying only one of the two surface antigens. Results are also stored in an on-board archive and are assigned to a sample ID that has been entered into the Pima Analyzer by the operator along with the date/time the test was carried out. Data can be retrieved and down-loaded by the operator at any time after the test has been completed. An external Pima Printer can be attached via USB to the Pima Analyzer to print test results.

To date, since its introduction into the market in late 2009, the Pima system has proven to be robust and requires minimal operator training. It can perform a maximum of 20 tests per day. As a simplified, low-throughput POC system, it can be used appropriately at all levels of the healthcare system where high-volume throughput is either not required or for use in situations where same day results are particularly important, even in high-volume settings.

The cost of the Pima Analyzer is about $5,500, and the cost per test is about $6.00. Maintenance of the instrument is simplified because failed instruments can be swapped out for new systems. The company will maintain stock of new instruments in-country for this purpose.

Two peer-reviewed, independent evaluations of the Pima system have been published. One study, which was conducted in Zimbabwe and was based on a study of 165 patients, found that the Pima performed well against the reference technology, FACSCalibur, with no significant difference in mean absolute CD4 counts between the two platforms [41,42]. Additionally, the study found no significant difference in CD4 counts between the platforms whether the test was run by a nurse or a laboratory technician. The study concluded that: “POC CD4 testing can be conducted in a voluntary testing and counseling setting for staging HIV-positive clients” [41]. The second study, from Mozambique, found that primary health nurses operating the Pima produced results with low levels of bias for CD4 counts, that CD4 cell counts in paired specimens of finger prick and venous blood tested on Pima were close in agreement and that the repeatability of CD4 cell counting was similar to that observed with laboratory instruments [42].

CyFlow® CD4 miniPOC

Partec has introduced a very compact, portable CD4 counter (pictured below) that uses flow cytometry, including laser modules, optics, fluidics and electronics, to provide CD4+ T-cell and %CD4 enumeration. The company emphasizes that the device can measure the total range of CD4 absolute counts starting as low as 10 CD4 cells/µL and ranging up to 3,500 CD4 cells/µL and CD4 percentages from 0 to 70%. The device is used with Partec dry CD4 reagents (making it a closed system), which eliminates the need for cold chain or cold storage. Like its larger sibling, the Partec CyFlow Counter, the device can run up to 250 CD4 tests per day, but can also be used in small health centers and other sites with a lower daily volume of testing. (http://www.partec.com/cms/upload/CyFlowCD4miniPOC.pdf)

The CD4 miniPOC requires only 20µL of blood, which is added to a Partec reagent-filled tube and incubated for 15 minutes. Buffer is added, and ultimately the sample blood is drawn up into a syringe to a precise fill line. The operator then places that syringe onto the POC device and the instrument slowly injects the processed sample into the instrument, where CD4 detection takes place. Sample processing, which is automated in some systems, is stripped from the Partec device. Sample processing takes place outside of the device.

The cost of the CD4 mini POC instrument, which is a closed system, is approximately €8,890 (~$12,790), but may be less than $8,000 per device in bulk procurement. The system uses the same dried reagents as its larger sibling, the CyFlow Counter, the cost of which is €1.75 (~$2.40) per test for absolute CD4 count and €2.50 (~$3.40 per test) for %CD4.

To date, no peer-reviewed, independent performance evaluations of the Partec CD4 miniPOC device were found in a literature review.

CD4 Technologies in the Diagnostic Pipeline

The following CD4 diagnostics are still under development and have not yet been introduced into the market, but are expected to be evaluated and/or launched in 2011, 2012 or 2013.

Daktari™ CD4 Counter

Daktari Diagnostics, Inc. (http://daktaridx.com) is developing a portable and robust CD4 device, pictured below with its associated cartridge. Currently, the product can only do absolute CD4 counting, but the system could be adapted to do other cell counts, including malaria or parasitic infections. The product has not yet been launched, but is likely to begin clinical trials in the second quarter of 2011, with commercialization expected in the fourth quarter of 2011.

Intended for use at the point of patient care, the Daktari eliminates complex sample preparation through technology known as “microfluidic cell chromatography,” which isolates cells and other particles in a miniature sensing chamber. No pipetting, labels or reagents are required. Similarly, the Daktari device does not require fragile and expensive optical sensors, but rather uses a second innovation, “lysate impedance spectroscopy,” which employs a simple sensor to count captured CD4 cells by measuring their internal contents electrically. The Daktari instrument then interprets the electrical signal and reports the CD4 count in less than 10 minutes.

The Daktari CD4 device base model, currently under development, will include a data management system that will have a keypad user interface and a back-end data package that will come built-in to the device. However, the company will also maintain a version of the device without keypad entry. Both systems will also enable data to be downloaded to a PC and uploaded wirelessly for central aggregation (but without patient identification).

The anticipated cost of the Daktari CD4 counter is $800 for the device. Per test cost is expected to be approximately $8.00. If the device is damaged, the low cost and portability of the instrument would allow it to be swapped out with a replacement device rather than being repaired on-site.

There is currently no performance data available for the Daktari CD4 system; clinical trials are expected to commence in 2011.

MBio

MBio Diagnostics, Inc. (http://www.mbiodx.com/) is developing a POC CD4+ T-cell counting system that utilizes single-use disposable cartridges and a simple reader instrument (pictured below). The instrument functions as a two color fluorescence imaging cytometer and delivers absolute CD4 counts based on immunostaining and direct cell counting. Although initially configured for absolute CD4 count, as a fluorescence imaging system, it can be readily configured for other marker combinations (CD3, CD8, etc.). The disposable cartridge is an all-plastic design with no pumps, valves, or complex fluidic features. Finger-stick or venipuncture whole blood samples are processed on the cartridge and then inserted in the instrument for reading, providing a throughput of over 10 samples per hour. The high sample throughput will allow healthcare settings with large POC CD4 testing requirements to meet demand with fewer instruments. Blood and assay fluids stay on the sealed device, minimizing biohazard handling.

The MBio CD4 system is based on the company's proprietary LightDeckTM technology, which takes advantage of the enormous advances driven by the consumer electronics industry in recent years. LightDeckTM is a patent-protected fluorescence assay illumination method that is a variation on planar waveguide technology. The method allows low cost lasers such as those found in DVD players to replace the expensive, fragile lamp sources used in conventional fluorescence microscopes. According to the company, LightDeckTM solves the light coupling reproducibility problem that has limited the use of waveguide technology in low cost disposable devices. The imaging optics and sensor are custom-designed for cell counting, but utilize components from the cell phone industry, where volume manufacturing and quality demands have resulted in extraordinary performance.

MBio Diagnostics is currently developing the system using HIV-positive donor samples from the Antiviral Research Center at the University of California, San Diego (UCSD) Medical Center. The system shows excellent correlation with flow cytometry in the critical range of 50 to 500 cells/μL; no peer reviewed published data is yet available. Pre-regulatory clinical field testing is scheduled for spring 2011, followed by field evaluations in southern Africa later in the year.

Zyomyx

Zyomyx, Inc. (http://www.zyomyx.com) has developed a fully quantitative CD4 readout in a device-free POC format. The system consists of a cartridge, pictured below, along with a small, mechanical mixer/spinner used in the test procedure. Inside the cartridge, the CD4 cells of a given blood sample specifically bind to heavy, anti-CD4 antibody coated particles. The cartridge is subsequently spun slowly in the mixer/spinner whereby only the conjugated cells penetrate into a high density medium, forming a cell stackwidth in a small micro-capillary. The CD4+ T-cell count is proportional to the stacking height of the cells in that capillary, which can be visually read without the need for an electronic reader. The Zyomyx system has not yet been launched, but market introduction could take place as early as 2012.

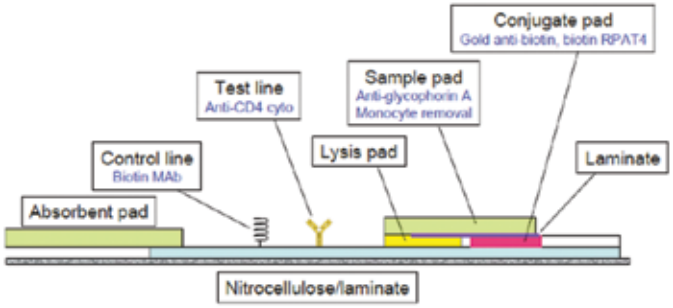
The anticipated cost of the Zyomyx assay is between $6 and $7 per test, and there will likely not be a separate cost for the mechanical mixer/spinner. The mixer/spinner is expected to be capable of performing 1 - 4 test preparations every 10 minutes and will support at least 10,000 tests.

There is currently no performance data available for the Zyomyx system; clinical trials are expected to commence in 2011

Burnet Institute

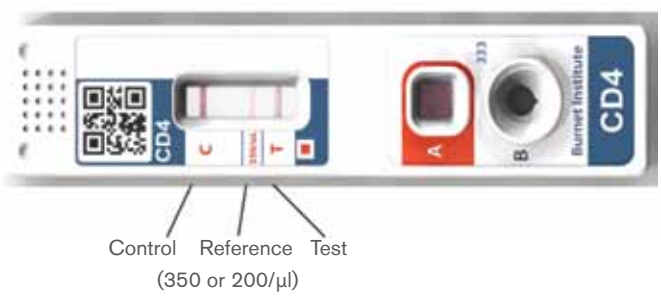
A CD4 Initiative was established in 2005 with a grant from the Bill & Melinda Gates Foundation. The objective of the CD4 Initiative, which was managed by the Imperial College of Medicine in London, was to develop a low-cost, rapid POC test for measuring absolute CD4 counts. Burnet Institute, in collaboration with the Rush University Medical Center and Duke University, participated in that Initiative, and although the Initiative ultimately chose to support the Zyomyx product, discussed above, Burnet and its partners have continued with the development of a rapid CD4 test (http://www.burnet.edu.au/home/cvirology/clinicalresearchlab/projectsix). The approach of the test is to measure CD4 protein on T-cells, rather than to directly measure CD4 cells. Since the amount of CD4 per CD4+ T cell is constant throughout HIV, the total cell-associated CD4 should correlate with CD4+ T-cell count. Burnet used a laboratory-based test (ELISA) as proof of concept, which supported this hypothesis.

While currently being tested in ELISA format, the Burnet CD4 test will ultimately be incorporated into a lateral flow strip (similar to an HIV rapid diagnostic test) with traditional rapid test format, including monocyte removal pad and immunogold conjugate – as illustrated below.



The test, which is expected to cost about $2.00, is semi-quantitative. It will be able to determine, by the presence of a line on the test, whether a patient's CD4 count is above or below a set threshold – e.g., 200 CD4 cells/µL or 350 CD4 cells/µL (the WHO recommendation for treatment) – but it will not give a fully quantitative result. Preliminary independent performance data on the lab-based ELISA format test from Australia and the United States have been promising, with between 96% and 100% discrimination of patient samples falling above or below 200 CD4 cells/µL – i.e., the "treat" decision point in many resource-limited settings – when compared with flow cytometry.

Because of some concerns about the ability of users to read the results of the test, which requires operators to identify the result line and compare it with the reference and controls lines on the strip (see below, top), Burnet has developed a reader for the device (see below, bottom). The reader, which is being developed in collaboration with Axxin Ltd. (Australia), is in the final stages of development and is expected initially to cost about $1,200, but may decline to about $400 over time.



Burnet is currently seeking commercial, manufacturing and philanthropic not-for-profit partners to further the development of its CD4 test. Burnet is re-optimizing the device to change the reference cut-off from 200 CD4 T-cells/µL (the original design level) to 350 CD4 T-cells/µL. Clinical trials of the actual POC test (as opposed to the ELISA test) are planned at 2 sites in the U.S. and in Malawi in May/June 2011. The product launch date is dependent on funding and on the results of the clinical trials.

Other Possible CD4 POC Tests

In addition to the POC CD4 tests/devices discussed above, there are a few other research and development groups working on platforms/devices that could potentially be used for CD4 counting. One of these is discussed below.

Palo Alto Research Center

A group (Peter Kiesel, Joerg Martini, Markus Beck, Malte Huck, Marshall Bern and Noble Johnson) at The Palo Alto Research Center (PARC) has redesigned the optical detection system for flow cytometry. Using "spatially modulated emission," the technique achieves high discrimination of particle signals from background noise, without the use of precision optics, thereby improving robustness, compactness and ease of use, as well as lowering cost. To date, using off-the-shelf components, PARC has assembled and tested a handheld flow cytomer based on the spatial modulation technique. The components cost a few hundred dollars.

To test the technology, the PARC group performed absolute CD4+ T-cell counts in human blood, benchmarking against the FACSCount. The group reports that the measured concentration of CD4 per µL of whole blood as measured by the PARC device was in "excellent agreement" with the CD4 per µL determined by the FACSCount [43,44].

The development of this technology is in the research stage and funding is needed to take it further. Additional information is available at: http://www.parc.com.

Conclusions – CD4 Technologies and Future Directions for CD4 Testing

The Technologies

Currently, there are a good number of technology choices for CD4 testing in resource-limited settings. Most of these are laboratory-based platforms using proven flow cytometry methodologies. In reference laboratory settings with well-trained technicians, these technologies function well and can be cost-effective. Many, but not all, of these CD4 testing platforms, including BD FACSCalibur and FACSCount, as well as the Coulter Epics, have been the subject of independent evaluations and have performed well, within the recognized limitations, both physiological and technical, of CD4 performance.

However, in order to reach patients in peri-urban and rural settings with these laboratory-based CD4 tools, it is necessary to set up sample transport networks to transfer patient blood samples to the reference laboratory for testing and to set up a results return system, involving the same transport used for inbound samples (generally courier services of some sort) or mobile technologies, including SMS. This is made more difficult by the fact that the transport of samples for CD4 testing generally requires the transport of whole blood, which has limited stability, as opposed to DBS, which extends the life of samples. Moreover, sample transport is an additional cost to the provision of CD4 testing and prevents the availability of same day results to patients, which can result in loss to follow-up.

Therefore, in order to improve access to CD4 testing in resource-limited settings, there is a need for good and cost-effective POC CD4 testing options. Several such options are already on the market, and others are under development and should become available as early as late 2011 or early 2012. The current options available for POC CD4 testing are device-based, but disposable CD4 testing is on the near-term horizon. To date, the performance data for POC CD4 platforms are limited, although the results of independent evaluations on Pima have been promising. It is anticipated that as more POC CD4 testing devices are introduced, the results of independent clinical evaluations by the United States Centers for Disease Control and Prevention (CDC), the National Health Laboratory Service (South Africa) and others, as well as evaluations performed in-country, both in laboratory settings and in the field, will become available. Indeed, it is important that this data become accessible.

Future Directions for CD4 Testing and Implications for CD4 Technologies

It is expected that staging and monitoring of patients not yet on ART will continue to rely on CD4 testing. This testing is necessary in order to determine when the patient should be initiated onto treatment. However, after ART initiation, if viral load testing becomes more widely used for patient monitoring, there may be a movement away from six-monthly CD4 count testing. This transition would require broader international consensus. Once patients have stabilized, generally after a year on ART, CD4 testing does not demonstrate important, decision-driving changes, except in a small percentage of failing patients [45]. In this context, since viral load testing is a better indicator of treatment failure, the value of routine CD4 testing drops.

On the other hand, it is possible that access to CD4 testing for ART-eligible patients will need to become a higher priority than it is now. To date, countries have been focused on treating the sickest within their populations, and the need to enroll treatment-eligible patients who are healthier has not been as high a priority. However, some countries are already reaching high levels of ART coverage and their enrollment rates may drop over time – not just because there are fewer patients per unit ART

services provided, but because it is harder to find people who are not clinically ill and seeking care from health facilities.

Despite a possible move towards more routine viral load testing for patients on ART, scale-up of CD4 testing is needed. POC technologies will make it possible not only to expand access to CD4 testing for patients in remote/rural areas, but also to return results to patients on the day of testing, which in turn allows patients to be initiated on ART more quickly. The cost of conventional laboratory-based CD4 is unlikely to fall significantly from current levels, except in settings where testing can be made more efficient. Therefore, the opportunities to further lower unit prices rest in new technologies, such as disposable CD4 tests, which may ultimately be priced at less than $3.00 test without the need for investment in instruments/devices

However, the level of CD4 testing access required in resource-limited settings will likely necessitate both a scale-up in centralized testing facilities, including “super labs,” that carry out very high volume testing (similar to what is available in the United States and Europe for routine non-HIV diagnostics, i.e., increased automation and very high volume platforms), and at the same time, a drive towards POC testing. The latter may ultimately include even personal or home-based testing platforms (similar to other dynamic, chronic diseases, e.g., diabetes glucose monitoring) and may become important in test and treat initiatives, helping to identify and focus efforts on the most infected persons. The appropriate strategic mix of high volume labs and POC testing will be country-specific, and will depend on such factors as the urban/rural split of the country, the volume of CD4 testing overall and the ability to effectively transport samples between collection sites and laboratories. Ultimately, the market for CD4 testing and viral load testing (discussed below) between the two extremes of super labs and POC may be relatively small, but in any event, the landscape will neither be all laboratory-based nor all POC-based.

Viral Load Testing

As discussed earlier in this report, viral load testing is the method favored for monitoring HIV patients once they have started ART. High levels of HIV circulating in the bloodstream indicate that the virus is actively replicating, and these levels can be used, with the aid of molecular methods, to provide important information regarding the risk of disease progression and to predict the outcome of infection [46].

Upon entering the body, HIV infects a large number of CD4 T-lymphocytes and rapidly replicates within these cells, which in turn causes a spike in the quantity of viral RNA in the individual's bloodstream (i.e., the individual's viral load rises). However, for a short time after infection, viral proliferation is controlled, probably by a cellular immune response of the CD8 cells and the body's immune system recovers somewhat. During this period of clinical latency, although the person may be relatively disease- and symptom-free, there is still low level, active viral replication. Over a period of time, however, HIV's unrelenting assault on the immune system, through the elimination of CD4 cells and continuous viral replication, destroys the individual's immune system.

Initiation onto ART interrupts viral replication, leading to a decreased level of virions (virus particles) in the host's bloodstream. This slows the progression of the disease and improves the patient's prognosis. Once initiated onto ART, reduction in an individual's viral load levels can be used as an indicator of the efficiency of therapy, along with clinical symptoms and CD4 counts. Viral load testing is used to determine whether the virus is "undetectable" in the patient's blood (below the limit of detection of currently available technologies as measured in copies of the virus per millimeter) and is considered to be the most effective means of identifying treatment failure. Although still being used, especially in resource-constrained settings, clinical signs and immunological (CD4) monitoring are generally lagging indicators of treatment failure, with misclassification of ART failure by these methods as high as 45% [47,48,49].

Identifying treatment failure early enables patient adherence counseling and may enable patients to stay on 1st line ART longer than otherwise, thereby avoiding unnecessary switches to more expensive 2nd line regimens. Viral load testing also enables clinicians to switch failing patients early to new drug regimens before the accumulation of drug resistance mutations, thereby reducing the spread of highly resistant virus. In other words, viral load testing provides benefits that run both ways: it helps to prevent unnecessary switching to 2nd line therapies, but it also supports migration to 2nd line treatment in a timely manner, thus saving patients' lives. It should also be noted that unlike antibody detection of HIV, which is limited by the transfer of maternal antibodies across the placenta to the fetus, viral load testing can also be useful in diagnosing babies born to HIV-positive mothers (which is discussed later in this report).

Despite clinical consensus on the importance of viral load testing, several factors are limiting access to such testing in low resource settings. As indicated earlier in this report, one key barrier is the current high cost of viral load diagnostics. Another barrier to implementation is the complexity of viral load testing assays that demand sophisticated laboratory capacity: instrumentation, supply chains capable of handling labile reagents, effective sample transport systems, and a high degree of training. Finally, another deterrent to viral load testing in resource-constrained settings is WHO guidance, which has counseled caution in the deployment of viral load testing in resource-limited settings, at least partially on the basis of cost.

Viral Load Testing Complexities

The first molecular assay for quantifying HIV viral RNA was approved by the FDA in 1999. Since then, a number of assays have been developed and will be considered here in some detail. Before doing that, however, it is worth considering a number of complicating factors with respect to viral load assays and platforms, complications that should inform the choice of viral load testing platforms for a given setting. These include HIV diversity and certain practical challenges, including laboratory infrastructure and transport of samples.

HIV Diversity

In 1985, several years after HIV was recognized as an infectious agent, a genetically similar virus causing AIDS was discovered in West Africa. As a result, two types of HIV have been classified and characterized: HIV-1, the original virus, and HIV-2, the strain of virus discovered in West Africa. Of the two types of HIV, HIV-1 is predominant and has been most responsible for the HIV pandemic that exists today [46]. Further complicating matters, HIV-1 is divided into four groups, designated M, N, O and P, the main group of which is group M. And, there are also multiple clades, and within each clade, there are sub-clusters of individual strains of the virus that have been isolated around the world. Finally, mutation of the virus and different evolutionary rates have led to extensive genetic diversity, which, in turn has contributed to the divergence of the distinct clades. When viruses from two or more strains exchange their genetic material and become established, they are called recombinant viruses. In all, there are at least 43 circulating recombinant forms (CRF) or inter-subtype recombinant HIV-1.

The high level of genetic heterogeneity of HIV-1 and the emergence of recombinant strains of the virus complicate viral load assay development [50,51]. In an ideal world, viral load assays would detect and quantify all known HIV-1 subtypes, inter-subtype recombinants and emerging variations thereon. Currently, that is not the case, although the assays are able to recognize most HIV-1 subtypes. Therefore, it is important to consider the prevalence of HIV-1 and HIV-2 groups and subtypes in a particular geographical region when choosing a viral load assay.

Laboratory Infrastructure

Currently available viral load platforms are laboratory-based and require significant infrastructure – including continuous power, clean running water and air conditioning. For example, the typical, non-POC viral load platform based on nucleic acid technology (discussed below) will require two to three dedicated rooms in a laboratory.[20] Each room should have minimal dust and preferably will be temperature controlled (air conditioned). The rooms are needed to accommodate the different stages of the testing process: Room 1 would be dedicated to receipt of the patient sample and sample extraction (most of which is done in a bio-safety cabinet). Room 2 (which could be reduced to a Clean-Air Box in Room 1 if space is limited) would be used to prepare the reagents, which are prone to contamination. Finally, Room 3 (which will become highly contaminated through the test process) would be dedicated to amplification and detection of the virus and results processing. In order to avoid contamination, work flow must proceed from Room 1 to Room 2 to Room 3. Each room needs to have 3 – 4 meters (approximately 10 to 13 feet) of bench space. Further, test reagents generally will have to be stored at between 4° and 8° C. And, as mentioned above, steady current is required so that the electrical test equipment is not damaged.

Sample Transport

Most methods of viral load determination require venous blood collection, processing (centrifuging) of that blood to obtain plasma within a certain timeframe, cold chain and storage of specimens by trained personnel. In resource-limited settings where viral load testing will generally take place only in a national reference, or comparable, laboratory, this means that patient samples will have to be transported from urban, peri-urban and rural settings to the laboratory for processing. This is done using sample transport networks in-country, taking advantage of courier or similar services to take samples to the laboratory and to return results at a later date. But, frequently, these services are not well developed, leading to long delays in returning sample results to patients and loss to follow-up.

Therefore, the ability to use DBS samples for viral load is an important consideration in the implementation of the testing because it greatly simplifies the transport of samples, providing enhanced stability and ease of use for health care workers. The use of DBS is also cost effective. There has been some concern about the correlation of viral load measures using DBS as opposed to plasma. But several recent studies have demonstrated good correlation between the two using different viral-load methodologies, with sensitivity ranges close to 3 log HIV-RNA copies/mL [52,53].[21]

[20] Two exceptions to this are the Siemens kPCR Molecular System and the Siemens VERSANT 440 Molecular System, each of which requires only a single room.
[21] Note that although the correlation between plasma and DBS viral load is generally good, for some platforms the correlation falls away at low cp/mL because of interference from non-plasma-associated virus. However, this occurs below 5,000 cp/mL, which is the level which the WHO considers to be the measure of treatment failure. Therefore, for diagnosis failure, the poor correlation may not be a problem. It might mean, though, that DBS viral load should not be used as an adherence monitoring tool where being able to detect 1,000 cp/mL is important [54].

The Viral Load Technologies

HIV viral load technologies can be categorized broadly as nucleic acid based (NAT) and non-NAT based technologies. The technologies differ in the methods used to quantify HIV virions circulating in the body. NAT technologies detect and quantify viral RNA; whereas non-NAT technologies detect and quantify HIV viral enzymes and proteins that can be correlated to the amount of viral RNA.

The currently available NAT-based and non-NAT based viral load technologies are detailed below:

Nucleic-Acid Based Technologies		Non-Nucleic Acid Based Technologies	
TYPE	**ASSAY NAME**	**TYPE**	**ASSAY NAME**
RT-PCR	AMPLICOR HIV-1 MONITOR™ v1.5 (Roche Diagnostics) COBAS® Taqman v 2.0 (Roche Diagnostics) Abbott RealTime HIV-1 VERSANT® HIV-1 RNA 1.0 (kPCR) (Siemens)	Reverse Transcriptase	ExaVir Load version 3.0 (Cavidi)
NASBA	NucliSens EasyQ® HIV-1 v2.0 (bioMérieux)	p24 Antigen	HIV-1 p24 Ultra ELISA (Perkin Elmer)
bDNA	VERSANT® HIV-1 v3.0 (Siemens)		

NAT-Based Technologies

NAT assays have become the core viral load monitoring technology used in both developed and developing countries, and the NAT-based systems manufactured by Abbott, bioMérieux, Roche and Siemens currently dominate the market.

All such technologies incorporate amplification techniques because levels of nucleic acids are otherwise too low to be detected directly. Amplification methods are either aimed at increasing the number of target molecules (viral nucleic acids) to a level that permits detection (target amplification methods) or are aimed at increasing the signal generated by the method (signal amplification methods) [46]. Currently, the bulk of commercially available viral load assays are based on target amplification.

Whether an assay is based on target amplification or signal amplification, the assay will consist of the following common steps: (i) sample preparation and/or viral nucleic acid extraction; (ii) the actual amplification step that is either target amplification- or signal amplification-based; and (iii) detection and/or quantification of the amplified viral nucleic acids.

Pre-amplification methods (sample preparation and/or viral nucleic acid extraction) are critical to the viral load testing process. For each sample to be analyzed correctly and to achieve an accurate result, the nucleic acid must be both available for the reaction and purified. Protocols for the pre-amplification steps include the use of purification methods for cells and virion centrifugation or a capture step for RNA in plasma, followed by an extraction step to free the target viral nucleic acid [46]. Although HIV nucleic acids are relatively stable, molecular detection methods require prompt processing of samples (generally within 6 hours of collection), a rapid extraction method and appropriate storage of plasma or cells prior to assessing.

There are several amplification methods used to detect viral RNA or DNA after preparation of samples. In target amplification, many copies of a portion of the viral nucleic acid are synthesized via an amplification reaction; in effect, this method enhances the ability to detect very low levels of nucleic acids that occur naturally in the blood. These techniques include the reverse transcriptase polymerase chain reaction (RT-PCR) used in the Roche and Abbott assays and nucleic acid-based sequence amplification (NASBA) used in the bioMérieux assay. In signal and probe amplification methods, a probe or a reporter molecule attached to a probe is detected and the signal generated by this reaction

is amplified/increased; in effect, these methods increase the "marker" that shows that the target is present. Signal amplification techniques include branched chain DNA (bDNA), which is used in the VERSANT™ HIV-1 3.0 assay by Siemens.

Finally, post-amplification methods require the detection and/or quantification of either the amplification products (in target amplification methods) or the increased detection of signals that have been amplified (in signal amplification methods) [46]. Detection can be achieved using any one of a number of reagents – e.g., colorimetric, radioactive, fluorescence. Detection can either be done at the endpoint of the process (completion of the run) or in "real-time" (during the production of results as they occur). Real-time techniques, in which amplification and detection occur simultaneously, are now commonly used. For example, the Roche Taqman platform uses real-time detection, which is achieved via specific, fluorescently-labeled probes that bind to the DNA that is generated via the amplification process (called amplicons).

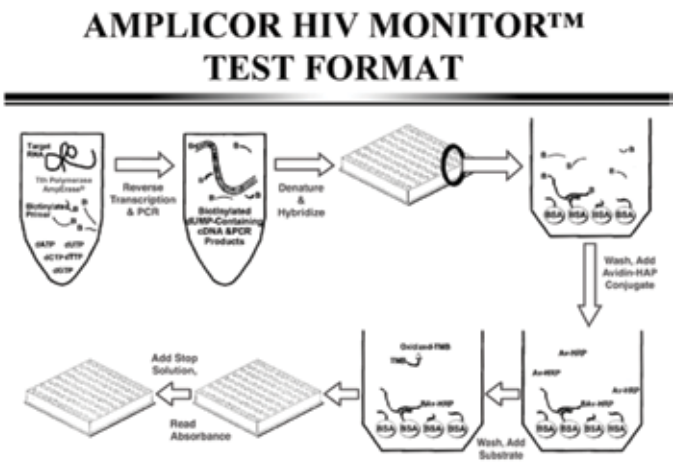
In general, the advantages of NAT-based approaches include that many of the assays have been evaluated and are well-validated; the assays are available in quality-assured kits, and clinicians are comfortable interpreting the results. The assays vary in terms of sample preparation and amplification/detection methodologies, among other things. The major NAT assays and platforms are discussed below.[22]

Platforms Based on RT-PCR

Currently, there are four commercially available RT-PCR based viral load assays: (i) AMPLICOR HIV-1 MONITOR™ v1.5 (Roche Molecular Systems), (ii) COBAS AmpliPrep/ COBAS TaqMan v2.0 (Roche Molecular Systems), (iii) RealTime HIV-1 (Abbott), and (iv) VERSANT HIV-1 RNA 1.0 assay (kPCR) (Siemens). Of these, only the AMPLICOR HIV-1 MONITOR v1.5 is not a real-time assay. There are also a number of in-house procedures and test systems that have good sensitivity and reproducibility and are used in various countries[23], but which will not be touched on in detail in this report.

Roche AMPLICOR System

Roche Molecular Diagnostics produces tests designed, in conjunction with clinical symptoms and other laboratory markers, to monitor HIV patients (http://molecular.roche.com/diseases/Pages/HIV.aspx). Within the AMPLICOR family of tests, there are currently two AMPLICOR HIV-1 MONITOR™ v1.5 assays. The assays are target amplification assays, which target what is known as the gag p24 region of the genome, and differ with respect to the type of equipment used, the degree of automation and the method of sample preparation. Each of the assays can be used with either of two different specimen procedures, UltraSensitive or Standard,[24] and is based on five major processes: specimen preparation to isolate HIV-1 RNA; reverse transcription of the target RNA to generate complementary DNA (cDNA); hybridization of the amplified products to oligonucleotide[25] detection probes specific to the target(s); and detection of the probe-bound amplified products by colorimetric determination. (See the test format below.)



[22] Unless otherwise noted, technical information on the various platforms has been obtained from the online resources provided by manufacturers and/or directly from company representatives. The images used below to illustrate the platforms are being used with the permission of the respective companies/developers.
[23] One example is the Generic HIV Viral Load assay from Bio-Centric (France), which is RUO. This assay can be run on a real-time thermocycler and requires other basic consumables that would cost about $40,000. Time to result is about 4 hours, including RNA isolation. The cost per test is approximately $14.00.
[24] Using the Standard specimen procedure, HIV-1 RNA is isolated directly from plasma by lysis of virions with a chaotropic agent followed by precipitation of the RNA with alcohol. Using the UltraSensitive procedure, the viral particles are concentrated by high speed centrifugation, followed by lysis of the particle with a chaotropic agent and preparation in alcohol. The former has a quantitative range of 400 – 750,000 copies/mL; the latter has a quantitative range of 50 – 100,000 copies/mL.
[25] An oligonucleotide is a short nucleic acid polymer. Oligonucleotides are characterized by the sequence of nucleotide residues that comprise the entire molecule. Oligonucleotides readily bind, in a sequence-specific manner, to their respective complementary oligonucleotides, DNA or RNA to form duplexes or, less often, hybrids of a higher order. This basic property serves as a foundation for the use of oligonucleotides as probes for detecting DNA or RNA.

The AMPLICOR HIV-1 MONITOR Test is the least automated of the assays. This test consists of independent steps for RNA isolation, reverse transcription, and RT-PCR amplification, and detection via a colorimetric readout. Amplification can be done on a variety of PCR analyzers – e.g., GeneAmp PCR System 9700 – which are not supplied by Roche. The instruments required to run this assay include a thermal cycler, an ELISA reader/washer and a microcentrifuge, the total cost of which is approximately $25,000. A full equipment package required for a start-up operation, including the thermal cycler, reader/washer, centrifuge as well as fridge, freezer and other required equipment could cost as much as $100,000.

The COBAS® (comprehensive bioanalytical system) AMPLICOR HIV-1 MONITOR Test requires manual RNA extraction, but uses the COBAS AMPLICOR analyzer for automated RT-PCR amplification, dilution and detection via magnetic particles coated with oligonucleotides specific for the target amplicon. As with the manual system, detection is via a colorimetric readout. This version of the assay offers the advantage of decreased sample manipulation and possibly higher throughput as well as increased consistency and reproducibility because of the automation of dilutions and detection [55]. Since the system is closed, it also allows for greater flexibility with respect to equipment placement in the laboratory. The assay is designed to be run on the Roche COBAS AMPLICOR analyzer, discussed below.

Each of the AMPLICOR assays is an endpoint PCR-based assay. Each is FDA- approved, quantifies HIV-1 Group M (subtypes A-G), and has a limit of detection of 50 RNA copies/mL. The cost per test for the least developed countries is about $17 to $25 per test, and $35 to $90 per test elsewhere. Prices vary considerably depending on volumes, infrastructure and support required, plus any special negotiations with the company.

It is anticipated that the Roche MONITOR assays will ultimately be phased out in favor of real-time assays.

The COBAS® AMPLICOR Analyzer

The COBAS AMPLICOR Analyzer, pictured below, is a bench-top system that fully automates amplification and detection for PCR testing. It combines 5 instruments in one: thermal cycler, automatic pipettor, incubator, washer and reader.

The AMPLICOR Analyzer can analyze 48 samples per run and has a time to result of approximately 8 hours. It is a closed system requiring the use of the COBAS AMPLICOR HIV-1 MONITOR reagents. The cost of the instrument is about $15,000 to $20,000.

Roche COBAS® AmpliPrep/COBAS® TaqMan® System

Real-time PCR technology options are increasingly being used in resource-limited settings because they are faster, have higher throughput, larger dynamic ranges and automate all extraction steps. Roche currently manufactures two real-time PCR assays: the COBAS® AmpliPrep/COBAS® TaqMan® version 1 and version 2. However, because of some reports of under-quantification of viral load using version 1 of the test[26] and because it is not available in all parts of the world, this report will focus on version 2 of the test. The assays use the AmpliPrep instrument for automated viral nucleic acid extraction and the COBAS TaqMan analyzers (TaqMan 48 or TaqMan 96), both of which are discussed below, for automated amplification and detection of the viral nucleic acid target.

[26] Note, however, that the FDA did a comparative study of the COBAS AmpliPrep/COBAS TaqMan HIV-1 test with the COBAS AMPLICOR HIV-1 MONITOR Test, v1.5 and found that the clinical specificity of the former was 99.4% and clinical sensitivity was 98.3%, indicating similar performance on both tests. (http://www.fda.gov/downloads/BiologicsBloodVaccines).

The COBAS AmpliPrep/COBAS TaqMan version 2 test was designed specifically to address HIV-1 mutations, using a dual-target approach. The assay is able to co-amplify two target regions of HIV-1 (known as the gag and Long Terminal Repeat (LTR) regions). The AMPLICOR Monitor assays only target the gag region of the genome. By targeting both regions of the genome simultaneously, the test increases the probability of detection of virus particles.

Like the MONITOR assays, the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2 is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV patients. In addition to plasma specimens, the assay can also be run using DBS, which is an advantage for resource-limited settings. It is able to quantify HIV-1 group M (subtypes A through H) and HIV-1 group O and has a limit of detection as low as 20 copies per mL. At the other end of the spectrum, it can also quantify the amount of HIV-1 in a patient sample up to 10 million copies/mL. The test is FDA-approved for plasma, but is RUO only for use with DBS. Performance of the test has proven to have good correlation with the Roche MONITOR assay version 1.5, which is generally considered to be the gold standard [56].

The cost is approximately $20 to $30 per test in least developed countries; the cost in other parts of the world is about $35 to $90 per test.

The COBAS® AmpliPrep System

The COBAS AmpliPrep instrument is an automated sample preparation technology (pictured below) for use in conjunction with the Roche COBAS TaqMan analyzers discussed below. The company considers the AmpliPrep to provide “walk-away” sample preparation/extraction capability, which can significantly reduce hands-on time of laboratory technicians.

The instrument is large, weighing over 680 pounds. It can process 72 samples per run; each run takes 4 hours. The system is closed and requires the use of test-specific, bar coded, ready-to-use COBAS AmpliPrep kits. The cost of the instrument is approximately $80,000 to $150,000 (with the lowest pricing reserved for lower income countries).

Roche TaqMan Analyzers

Roche manufactures two versions of its TaqMan Analyzer, the COBAS® TaqMan 48 Analyzer and the COBAS® TaqMan 96 Analyzer. Each of the analyzers is a fully automated, closed tube system. The TaqMan 48 (pictured below) is relatively compact and can run from 6 to 48 samples at a time. The instrument is equipped with two thermal cyclers that operate independently and provide run times of 90 to 120 minutes.

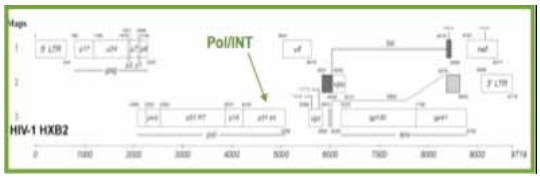
The cost of the COBAS TaqMan 48 Analyzer is approximately $45,000 to $100,000.

In contrast to its smaller sibling the TaqMan 48, the COBAS TaqMan 96, pictured below, is a large instrument, weighing about 485 pounds. It also has higher capacity and can run up to 96 samples at a time, with a run time of approximately 180 minutes.

The cost of the COBAS TaqMan 96 Analyzer is approximately $80,000 to $150,000.

Abbott *m*2000 System

Abbott Molecular (http://www.abbottmolecular.com/index.html) manufactures the Abbott Real Time assay, which is an RT-PCR assay for the quantification of HIV-1 on its automated *m*2000 system. The primers and probes of the assay are targeted to the integrase region of the polymerase (or pol) gene (see below), as opposed to the gag region targeted by the Roche assays, with the aim of minimizing inefficient binding due to sequence mismatch at the probe binding site.



The Abbott Real Time assay can be automated using the Abbott *m*2000sp (or *m*24sp) for sample preparation and the m2000rt for amplification and detection. The assay introduces an RNA sequence that is unrelated to the HIV-1 target into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR, and serves as an internal control to demonstrate that the sample has proceeded correctly through the process. The amount of HIV-1 target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide probes on the *m*2000rt instrument. The probes do not generate a signal unless they are specifically bound to the amplified product. The amplification cycle at which the fluorescent signal is detected by the *m*2000rt is proportional to the log of the HIV-1 RNA concentration present in the original sample.

The Real Time assay has a linear range of 40 copies/mL to 10 million copies/mL and can detect HIV-1 group M (subtypes A – H), group O and group N. The sensitivity of the assay is dependent on specimen volume. The limit of detection is 40 copies/mL for 1.0 mL input, but only 150 copies/mL for 0.2mL input. Performance has been assessed with good results [57]. Like the other assays discussed in this report, it is intended for use in conjunction with clinical presentation and other laboratory markers for HIV disease prognosis and for use as an aid in assessing viral response to ART as measured by changes in plasma HIV-1 RNA levels.

The price per test of the assay ranges from $25 - $40 and is dependent on volumes as well as any negotiations with Abbott.

Sample Preparation with the *m*2000 System

The Abbott Real Time assay is designed to be used with the *m*2000rt amplification and detection instrument as well as with one of three methods of sample preparation: (i) manual (for laboratories with low throughput requirements); (ii) the *m*24sp instrument, which automates sample purification steps; or (iii) the *m*2000sp instrument, which fully automates sample preparation.

The *m*24sp (pictured below) is a bench-top sample preparation and extraction device with a small footprint and is generally appropriate for facilities with medium throughput requirements. It provides a variable extraction system (extraction output can be stored either in deepwell trays or 1.5ml tubes) with ready-to-use and re-usable reagents as well as flexible batch size capabilities.

The cost of the m24sp is approximately $90,000.

The *m*2000sp

The *m*2000sp by Abbott (in the center of the image below), is a larger and more automated sample preparation device than its sibling, the m24sp. With complete automation, comes increased walk-away time for the operator. It is a high- throughput system with a maximum batch size of 96 samples per run. When combined with Abbott *m*2000rt, amplification and detection instrument, the system can provide automation from bar-coded laboratory tube through patient result.

The cost of the *m*2000sp is approximately $120,000.

The *m*2000rt

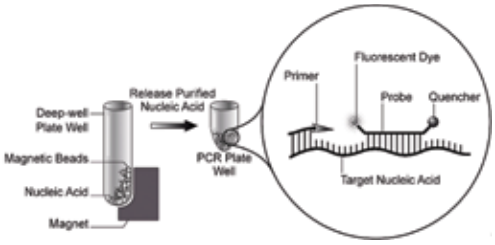
The Abbott *m*2000rt is the amplification and detection platform for use with the *m*24sp and the *m*2000sp, as described above. It is a high-performance system, but is relatively compact, weighing in at just over 75 pounds. The *m*2000rt (pictured below) can run 96 samples at one time in about 3 hours of cycling time (not including time for sample preparation). The system will run both quantitative and qualitative analyses and contains internal controls. Like other laboratory-based viral load systems, the operator must have a thorough knowledge of the applications run on the instrument (and on the sample preparation instrument) and must follow good laboratory practices when operating them. The cost of the *m*2000rt is approximately $38,000 when purchased with the *m*24sp or *m*2000sp, but about $44,000 if manual extraction is used.

VERSANT® HIV-1 RNA 1.0 kPCR Molecular System

The VERSANT® HIV-1 RNA 1.0 kinetic PCR molecular system (kPCR) is manufactured by Siemens (http://www.medical.siemens.com), and because it is CE-IVD-marked but not FDA approved, it is only available outside of the United States. It is the latest real-time PCR assay available on the market for HIV monitoring and is an automated amplification method based on reverse transcription and kPCR technology. The system, pictured below, consists of two modules: the Sample Preparation Module used to extract nucleic acids from plasma samples, and the Amplification Detection Module, along with VERSANT kPCR software. The system is "one-room" technology with no need for clean room operations because of closed-tube processing.

Photo courtesy of Siemens Healthcare Diagnostics.

The Sample Preparation module employed to extract RNA from plasma in the VERSANT system uses magnetic silica beads. Extraction consists of a lysis step that utilizes proteinase K and a chaotropic buffer, several washes to remove non-nucleic acid components of the sample, and elution. In the Amplification Detection Module, the purified RNA is eluted and added to a PCR plate containing an HIV-1 primer/probe mix and the HIV-1 enzyme mix. The wells are then sealed. At that point, HIV and internal control RNA molecules are reverse transcribed to make cDNA and then simultaneously amplified and detected using the kPCR technique. The RT-PCR step uses primers and probes that target a higher conserved region of the pol integrase gene. Below is a schematic representation of the assay principles.



Schematic courtesy of Siemens Healthcare Diagnostics.

Samples on the VERSANT kPCR system are processed in batch mode in a 96-well format, reporting up to 89 sample results per run. Total time to result is less than 6 hours. The linear range of the assay is between 37 HIV-RNA copies/mL and 11,000,000 copies/mL. The assay can detect HIV-1 Group M (subtypes A - G) and Group O variants [58]. Performance of the assay is comparable to its competitors [59].

The price of the kPCR Molecular System ranges from approximately $166,000 to $221,600, and the price per test ranges from $43.25 to $57.70 for reagents only; sample preparation materials will add from $10.80 to $14.40 per test. Actual pricing will depend on volume of testing and negotiated pricing, which can be considerably lower.

NASBA Platform

NucliSens EasyQ® System

The NucliSense EasyQ® system is manufactured by bioMérieux. The EasyQ® HIV-assay v 2.0 is currently available only outside of the United States. The assay targets a well-conserved region of the gag gene and is based on nucleic acid sequence-based amplification (NASBA) that uses real-time technology, allowing for quantification and reporting of as many as 48 specimens in 90 minutes. By analyzing the kinetics of the assay, real-time measurements can be taken during the early phase of the analytical reaction. Fluorescence is produced in association with the amount of RNA generated during amplification and is configured in an automated system, which permits faster time to results. The amplicons produced through this process are detected by molecular beacons (hairpin-shaped molecules with an internally quenched fluorophore whose fluorescence is restored upon binding to a target nucleic acid) [60]. During the amplification process there is a constant growth in the concentration of amplicons to which beacons can bind so that the rate of fluorescence increase is related to the concentration of the amplicons. The kinetics of this curve can then be used to reflect the concentration of HIV RNA in the sample; assay quantification is based on the relative amounts of HIV RNA and a calibrator RNA. The linear range of the assay is from 10 copies/mL to 10,000,000 copies/mL. The assay can detect HIV-1 Group M (subtypes A through J) as well as CRF01_AE and CRF02_AG. Performance of the assay correlates well with assays from Roche, Abbott and Siemens [61,62].

The price of the EasyQ HIV assay v 2.0 varies by volume (and negotiations with the company) and ranges from a low of about €10.5 (~$14.60) to a high of about €19.5 (~$27.20) per test. (http://www.biomerieux-diagnostics.com/servlet/srt/bio/clinical-diagnostics/dynPage?node=infectious_diseases_hiv_aids_2).

NucliSENS® miniMAG® and NucliSENS® easyMAG® Extraction Systems

The NucliSENS® miniMAG® and NucliSENS® easyMAG® extraction instruments make up part of the NucliSENS EasyQ system. The miniMAG is a small semi-automatic generic extraction device for both DNA and RNA in various specimens. It uses proprietary Boom technology with magnetic silica for washing and separation.

Despite its relatively small size, the miniMAG has reasonably high throughput – with 12 extractions in 45 minutes (using 1 miniMAG system) and 24 extractions in 60 minutes (using 2 miniMAG systems). The instrument has one standardized extraction protocol for multiple downstream applications and is considered to have an easy workflow for operators.

The price of the miniMAG extraction device, when purchased with the EasyQ analyzer, is about $12,900.

For higher throughput needs, the easyMAG is a fully automated benchtop extraction device that is able to perform 24 extractions in as little as 40 minutes (and has the possibility of several applications in the same run). The instrument has one generic extraction protocol (DNA/RNA) and one set of reagents for all applications, which together with touch screen technology, makes the process relatively simple. The extraction process is magnetic and is based on Boom technology. The price of the easyMAG instrument, when purchased with the EasyQ analyzer, is approximately $114,000.

NucliSENS EasyQ Amplification and Detection

The NucliSENS EasyQ is a closed system made up of a real-time NASBA amplification step with automated data analysis. No post-amplification steps are required. The risk of contamination is decreased in the system as the tubes containing the amplification product remain sealed throughout the analysis. The viral load of each sample is calculated automatically and displayed on a computer.

The EasyQ analyzer is compact, weighing only about 45 pounds, and can fit easily onto the average laboratory workbench. Further, amplification and real-time detection of 48 samples requires only 90 minutes.

The price of the analyzer is approximately $57,400.

bDNA Technology

Siemens manufactures the VERSANT™ HIV-1 RNA 3.0 Assay, which is a bDNA sandwich nucleic acid hybridization method that targets a well-conserved region of the gag gene and quantifies plasma HIV-1 by amplifying the signal rather than the target RNA. A phosphorescent chemical that binds to the HIV particles is added to the sample. The amount of light is measured and converted into a viral count. This assay does not require viral RNA purification/extraction or PCR amplification steps. The bDNA assay is performed on the VERSANT 440 bDNA analyzer and has a linear range of 50 to 500,000 copies/mL and can detect HIV-1 Group M (subtypes A through G). The performance of the assay correlates well with that of the COBAS AMPLICOR HIV-1 MONITOR assay [63, 64].

The cost of the VERSANT HIV-1 RNA 3.0 assay ranges from about $36 to $72 per test, but through negotiations with the company, and depending on volume, can be considerably lower.

The VERSANT™ 440 Molecular System

As indicated above, the Siemens VERSANT™ 440 Molecular system, pictured below, uses bDNA technology, which eliminates the need for nucleic acid extraction steps. Compared to PCR methods, this lowers the risk of contamination. Further, the technology can be set up in a single room; no separate clean room is required. The technology is also a walk-away system with samples being run in a 96 well format, with automated reagent preparation and delivery that allows processing of up to 168 samples per run. However, the time to result is about 24 hours, including 2.5 hours of hands-on time by the test operator.

The VERSANT 440 analyzer has a relatively compact footprint and costs approximately $55,400.

Photo courtesy of Siemens Healthcare Diagnostics. © 2011 Siemens Healthcare Diagnostics Inc.

Non-NAT Based Technologies

Rather than quantifying HIV RNA, non-NAT technologies quantify proteins and enzymes specific to HIV. These include assays that measure the level of reverse transcriptase activity and assays that measure the concentration of circulating p24 protein.

Reverse Transcriptase Technologies

In the progression of the HIV virus, an enzyme (protein) that is part of that virus reads the sequence of viral RNA nucleic acids that have entered the host cell and transcribes the sequence into a complementary DNA sequence. That enzyme is called “reverse transcriptase.” Without reverse transcriptase, the viral genome could not become incorporated into the host cell and could not reproduce. Reverse transcriptase (RT) assays detect that viral enzyme; the RT activity can be quantified and levels can be correlated to the amount of HIV. Therefore, an assay for RT can reflect the HIV viral load in the patient’s blood.

RT assays originally required radioisotopes, a scintillation counter and an ultracentrifuge for performance, but they have been simplified and made less hazardous. Currently, there is one RT platform available for *in vitro* use – the ExaVir™ Load, manufactured by Cavidi AB (http://www.cavidi.se/ExaVirLoad.aspx).

ExaVir™ Load

The ExaVir™ is a quantitative HIV-RT test that is designed to measure the HIV RT activity in plasma in order to estimate the HIV viral load. The principle is based on the synthesis of a product that can be detected by an alkaline phosphatase conjugated antibody. In the first phase of the assay, virus particles are separated from the plasma in order to remove any disturbing factors present in the plasma, such as antibodies or anti-retroviral drugs. Following this, an ELISA is used to detect and quantify the RT activity by comparison with a recombinant RT enzyme standard of known concentration. The assay is highly manual. See the ExaVir set-up below.

Although the procedures needed for using the ExaVir Load assay are somewhat cumbersome (requiring about 20 steps and 5 hours of hands-on time by the operator), it is generally less expensive than current molecular detection methods. An advantage of the assay is that because the ExaVir Load determines viral load based on quantification of RT activity and does not target a specific nucleic acid sequence, it can measure any HIV type or subtype with high accuracy, including O and N groups. The measuring range of the assay is the equivalent of about 200 to 600,000 copies/mL (or 1 – 3,000 femtograms (fg)/mL). There is performance data available on the ExaVir Load showing good correlation with the COBAS AMPLICOR Monitor assay [65,66].

The ExaVir Load assay requires a vacuum pump (supplied with the first order), a standard ELISA plate reader, a vortex, a 33°C incubator and a freezer, in addition to other basic lab commodities. Further, in order to analyze results, the ExaVir Load Analyzer software is required as well as a computer with Microsoft Excel® version 97 or later and Adobe® Reader®. The assay also necessitates overnight incubation in addition to several hours of incubation during the separation and RT steps; additional incubation time before calculating results means that the total time to result is some 48 hours (including 5 hours of hands-on time for the operator) for 30 tests (or a maximum of 180 tests per week).

The cost of the ExaVir equipment supplied by Cavidi is about $9,000 to $10,000, and the cost per test, which varies according to volume, ranges from about $13 to $15. Despite its reasonable cost and the ability to use the assay in district hospitals and other second tier settings, the ExaVir Load has failed to get significant traction, likely because of its manual nature and relatively long time to result.

p24 Antigen Technologies

HIV-1 infection is generally characterized by an early spike in HIV-1 antigens in the blood. During this period of acute infection or antigenemia, the antigens in the blood are detectable, but in most individuals the antigen levels become undetectable for a period of time after that. It is only later in HIV disease progression, with increasing failure of the patient's immune system and an increasing level of the virus, that the antigen may again become detectable in the blood. One of the viral components in blood during the period of antigenemia is the core protein, p24, the major internal structural protein of HIV-1. The p24 appears within 2 weeks after infection as a result of the initial increase in viral replication and is associated with the period of antigenemia during which the individual is highly infectious.

Testing for p24 antigen can be of value in several circumstances: (i) detecting early HIV infection; (ii) diagnosing infection in infants (which is discussed later in this report); and (iii) monitoring ART. In the past, before the availability of NAT-based technologies, the p24 antigen assay was used for monitoring the development of AIDS and charting disease progression [46]. In particular, the NEN HIV-1 p24 ELISA assay from Perkin Elmer (an ultrasensitive, heat denatured p24 antigen quantification assay) has been used for this purpose. However, the p24 antigen test is not very sensitive and there are concerns about the correlation of p24 with HIV RNA [67]. Moreover, with a linear range of between 10,000 and 30,000 RNA copy equivalents/mL, the assay is of limited utility in detecting early treatment failure and it is not useful in patients with low viral replication [67]. Therefore, the use of p24 antigen testing will not be discussed further in this report in the context of monitoring patients on ART, but will be revisited in the discussion of EID.

New Technologies for Viral Load Testing

Each of the NAT-based viral load systems described above requires testing to be done in a laboratory setting, generally speaking at a central or national reference laboratory, by well-trained technicians. Each requires dedicated space, clean rooms and other specialized and sophisticated infrastructure to diminish contamination and assure accurate testing. Although the Cavidi ExaVir Load assay can be used in less sophisticated settings, it is highly manual and requires 2 days to obtain a result; p24 antigen testing is of limited value in patient monitoring. Viral load testing that could be conducted at the point of patient care with assays meeting the ASSURED criteria would reduce the need for such infrastructure and would reduce the level of training required. In addition, the availability of quality POC viral load testing would ensure that patients on treatment in remote areas would have access to the monitoring tools they deserve with same-day test results, which can minimize loss to follow-up.

Although there are currently no POC viral load assays in the market, there are a number of platforms/assays in development, at least two of which are likely to be launched in late 2011 or early 2012. Described below are new viral load assays in the pipeline. See Appendix 2 for the pipeline.

Alere NAT System

The Alere NAT system is a generic platform for the implementation of different nucleic acid tests. The first test to be commercialized will be an integrated test for quantitative measurement of HIV viral load from approximately 25µL of whole blood. The assay measures total HIV-1 and HIV-2 RNA in whole blood – i.e., combined viral load from both cell-based (proviral DNA) and plasma-based (free viral RNA). The device on which the assay is run, pictured below, is small, portable, affordable and battery-powered and allows for “walk away” testing.

More specifically, the Alere HIV viral load test comprises a disposable cartridge that provides for sample collection, cell lysis, specific target capture, reverse transcription, polymerase chain reaction amplification and real time fluorescence detection based on reporter probe hybridization on an integrated micro probe array. The company expects that the assay will have at least comparable clinical sensitivity and specificity to current virological testing reference technologies (COBAS AmpliPrep/COBAS TaqMan). The assay is expected to detect HIV-1 Groups M, N and O and HIV-2.

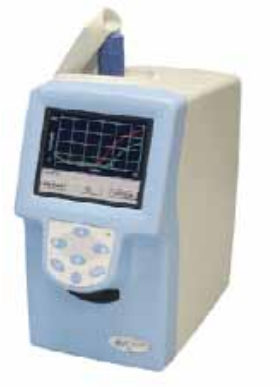
The Alere NAT platform is designed to require no manual sample preparation or pre-treatment, with set-up for each test estimated at 30 seconds or less. Further, dry reagents and buffer are contained in the disposable assay cartridge (pictured below), which is a closed system. Nucleic acid purification, amplification and detection all take place within the instrument, after cartridge insertion. This reduces the opportunity for contamination.

The test workflow for the operator is straightforward and consists of: (i) lancing the patient's finger and wicking whole blood directly into the Alere cartridge, (ii) capping the cartridge and inserting it into the analyzer and (iii) entering the operator and sample ID on the analyzer and pressing "run." At this point, the instrument processes the sample after which the operator removes the cartridge from the instrument and disposes of it. The viral load measurement is shown on the analyzer instrument display and is stored in an on-board archive. The process takes between 30 and 60 minutes.

Product launch could take place as early as 2012. No per-test cost or pricing for the instrument has yet been determined.

Liat™ Analyzer

The Liat™ Analyzer (pictured below), manufactured by IQuum (http://www.iquum.com/products/diagnostic.shtml), is a NAT-based lab-in-a-tube platform using reverse transcription, PCR amplification, and real-time detection to quantify viral load[27]. It uses disposable, flexible Liat Tubes pre-packaged with liquid reagents that automate all NAT processes, including reagent preparation. The analyzer is small and portable and it executes all required assay steps and reports a quantitative test result within 30 minutes to just under 1 hour, depending on the limit of detection specified by the user. For example, if the user wants to measure viral load down to 500 to 1,000 copies per mL, the device takes about 30 minutes to produce a result; if the user wants to measure viral load at 50 copies per mL, the device will take about 55 minutes to arrive at the result.



The testing process on the Liat Analyzer is straightforward. The plasma (or whole blood) is collected into the Liat Tube (pictured below). In the case of whole blood, the blood may be wicked directly from the patient's finger into the tube. The tube is then capped by the operator, who scans the tube's barcode and inserts it into the analyzer. At that point, the analyzer completes the testing procedure with no further operator intervention or interpretation required; it is a closed system, thus minimizing cross-contamination and biohazard risks. Use of the system requires minimal training.

[27] Note that the Liat platform can also be used for the detection of the H1N1 virus as well as influenza A and B strains. Viral load testing is just one application that can be used on the platform.

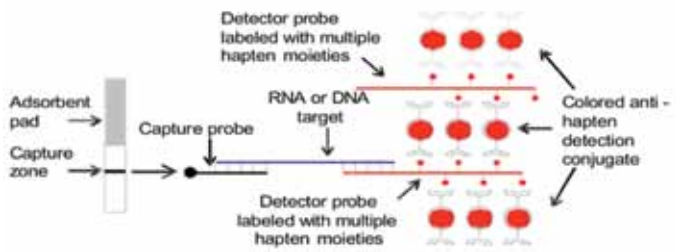
The Liat Analyzer has an internal optical system that provides 4 independent optical detection channels for real-time monitoring and quantification, allowing for internal controls and multiplex detection in each test. It can be powered by AC mains or by battery.

The current list price for the Liat Analyzer is $25,000, but this may go down over time. No price per test been established for resource-limited settings. An evaluation of the analyzer's viral load detection capabilities against the Roche COBAS and the Abbott m2000 system has been done by Robert Coombs at the University of Washington, and the device compared favorably. Additional clinical trials will be done in 2011, with a potential market launch date in 2012.

SAMBA

The Simple AMplification Based Assay (SAMBA) is being developed by a team led by Dr. Helen Lee, Director of the Diagnostics Development Unit (DDU) at University of Cambridge. Two NAT-based HIV assays are being developed: (i) a semi-quantitative test for monitoring of ART and (ii) a qualitative test for use in EID. The first SAMBA HIV assay to be launched will be the semi-quantitative viral load assay. The SAMBA machine will integrate extraction, amplification and detection into a bench-top analyzer with amplification and detection taking place in a closed cartridge.

The SAMBA HIV test uses 200 µL of plasma or 100µL of whole blood. The sample preparation process is an aqueous-based method involving cell lysis and nucleic acid extraction using a solid phase. The amplification and detection process is integrated into a closed cartridge to prevent amplicon contamination and targets the LTR region of the genome. Amplification is based on both target and signal amplification. (See below).



A capture probe is used to capture the target sequence, and a detection probe with multiple hapten labels is subsequently attached to the target sequence, enabling amplification of the signal to improve sensitivity and allow visual reading. The lattice structures, shown above, ensure visual detection of the RNA or DNA target, which can be read off of a test strip visually within 25 minutes. The test strip is based on a nitrocellulose membrane in a dipstick format.

Based on an assessment with the WHO International standard HIV RNA genotype panel, the SAMBA assay was able to detect all HIV-1 subtypes at 400 copies/mL. The SAMBA qualitative assay for EID has been evaluated in-house using 416 clinical samples from the Royal London Hospital and compared to the Roche PCR assay in a blinded fashion. Sample with discrepant results were tested by the Abbott Real Time PCR assay. Using both the Roche and Abbott viral load assays as combined gold standard, the SAMBA qualitative HIV assay was able to detect all samples with viral loads greater than 100 RNA copies/mL and showed a sensitivity of 98.5% and a specificity of 100%.

Currently, the total amplification time of the SAMBA is one hour with throughput suitable for use at a small laboratory – e.g., at district hospital level in sub-Saharan Africa. Diagnostics for the Real World, Ltd, the spinout company of DDU located in California, will be the manufacturer of the SAMBA system.

Additional Technologies in the Pipeline

In addition to the POC diagnostics discussed above for which a specific viral load assay or assays have already been developed, other diagnostics, in the pipeline are not quite as far along in the development of viral load assays. Some of these are discussed briefly below.[28]

Cepheid GeneXpert® System

The Cepheid GeneXpert® System is a fully-automated and integrated system for PCR-based DNA testing. The system currently has applications for anthrax testing, MRSA, MTB/RIF and enteroviral meningitis, among others. Although the GeneXpert platform does not yet have an HIV viral load application, Cepheid (http://www.cepheid.com) recently announced a collaboration with FIND to develop such an assay over the next few years.

The GeneXpert System integrates and automates sample preparation, amplification and detection in a single-use, self-contained microfluidic cartridge, illustrated below. GeneXpert cartridges can handle a variety of sample volumes, which enables a higher concentration of starting target materials. Concentration and purification of the target in the cartridge, in turn, increases the sensitivity of the resulting test.

Once nucleic acid in a sample is extracted, it is moved from the sample processing chamber in the cartridge into the cartridge reaction tube where amplification and detection are effected. The GeneXpert System consists of, a series of from 1 to 16 modules that perform rapid heating and cooling cycles required for real-time PCR in the reaction tube of the cartridge (pictured below is a system with 4 modules). Each module includes a six-channel optics system capable of exciting and detecting multiple fluorescent dyes in the same reaction tube. The modules optically monitor the chemical reactions in each cartridge in order to amplify the sample nucleic acid for reliable and quick measurement; continuous monitoring also allows the software to automatically stop the reaction as soon as the target is detected, which can shorten the time to results. Each of the modules works independently and can be subjected to a different protocol if desired.

[28] This is a non-exclusive list of potential viral load assays.

The company indicates that the GeneXpert System requires little operator handling or specialized knowledge. The operator can simply insert the biological sample for testing into the self-contained cartridge and insert the cartridge into the analyzer. The device has a small footprint and low power requirements, making it portable. Further, the GeneXpert software comes pre-installed on a desktop or laptop computer and can display results for each module in real time.

The current list price of the GeneXpert System (with four modules, as illustrated above) is between $25,000 and $30,000, and the cost per cartridge for MTB/RIF is about $20. It is not known at this time what the cost per cartridge will be for the proposed viral load assay or when the product will become available, although product introduction in 2013/2014 is possible.

Northwestern Global Health Foundation

The Northwestern Global Health Foundation (NWGHF) (http://www.nwghf.org) in collaboration with Quidel Corporation is developing a POC rapid RT-PCR testing platform that will be both easy to use and low cost. The product design calls for a small device (pictured below) that can hold up to 8 samples at a time, but that could process fewer samples than that, with test results in 60 minutes. The system will incorporate internal controls. The proposed viral load assay could be run off of either about 4 drops (about 100 µL) of fingerstick blood or 1m/L of blood from a venipuncture. As with other assays for viral load testing, the difference is that a small amount of blood will likely have a limit of detection of about 500 copies/mL versus 50 copies/mL for the larger sample size. In order to detect down to 50 copies/mL, the test procedure would require the use of an external centrifuge and pipetting of 500 µL of plasma into a card; for a limit of detection at 500 copies/mL, blood can be transferred directly into a card, which is inserted into the device.

The device will run on AC power, but will come equipped with a back-up battery in the event of power loss. Test results will be displayed on a touch screen. No specific cost data is currently available, but NWCHF indicates that pricing is anticipated to be competitive with CD4 testing.

NWCHF/Quidel hope to launch this product in 2013.

WAVE 80 EO-NAT HIV Rapid RNA Assay System

WAVE 80 Biosciences (http://www.wave80.com) is developing a rapid HIV NAT-based POC viral load assay designed for use in resource-limited settings. The company describes the assay technology as similar to branched DNA, employing a combination of direct capture (non-PCR) nucleic acid detection, inorganic (non-protein) signal amplification and whole-blood finger-stick processing within a single-use, enclosed cartridge format. The cartridge will contain all reagents necessary to run the test on-board and the reagents will not require cold chain.

The system (pictured below) currently consists of three parts: (i) the cartridge, which is expected to be integrated with a snap-on sample preparation module; (ii) a small, low cost “run” module that is battery-powered and contains a bar code scanner; and (iii) a small, portable reader, which can run on mains power or a rechargeable 8 hour battery. The system is expected to be very easy to use and to require minimal training of no more than a day for the least trained individual.

The EOSCAPE HIV-1 RNA VL Test prototype. Top right: Sample-ready disposable cartridge; Bottom right: EOSCAPE data scan with touchscreen

The testing process is straightforward. The operator will use a lancet to collect approximately 100µL - 200µL of whole blood from the patient and will use the sample preparation module to wick the blood sample into the cartridge. Then, the operator will simply insert the cartridge into the run module, and after about 30 minutes of run time, the operator will then insert the module into the reader where a result can be read in about a minute. Multiple run modules can be used for parallel processing, for a throughput of ~50 samples per day per technician. The WAVE 80 system will be capable of providing either a qualitative or a quantitative result, with a lower limit of detection threshold of 1,000 copies/mL.

Full scale validation and clinical testing of the WAVE, 80 EO-NAT Assay System (EOSCAPE HIV-1 RNA viral load) is expected to begin in 2012, with in-country testing for market launch in 2014.

Advanced Liquid Logic

Advanced Liquid Logic, Inc. (ALL) (http://www.liquid-logic.com) provides digital microfluidics technology solutions for liquid handling applications. Its approach for liquid handling is used to electrically manipulate discrete droplets with electrodes to independently control each droplet. The company's technology enables lab-on-a-chip devices, which can be configured in software to execute various assay protocols, such as immunoassays, PCR, clinical chemistry, and sample preparation.

The company has developed a compact benchtop immunoassay analyzer, shown below, that is currently being evaluated. The company indicates that it is evaluating various assay formats as well as the portable analyzer.

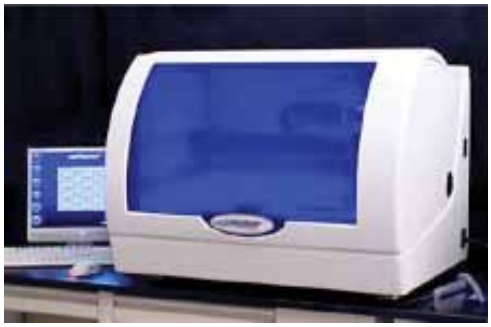
ALL does not currently have a viral load assay, but in 2009 was awarded a four-year, $5.2 million contract from the National Institute of Allergy and Infectious Diseases (NIAID) for the development of a rapid, POC diagnostic device for the detection of HIV in low-resource settings. The development of a viral load assay may flow from this work.

Other Possible Platforms for Viral Load

In addition to the platforms discussed above, there are a few platforms, either already used for other indications or of technological interest, which could also be used for viral load testing at some point in the future. However, the companies that manufacture the systems enumerated below do not currently have specific plans to develop a viral load assay on these platforms. In addition to the companies/systems detailed here, there may well be additional companies/developers with platforms that could be applied to viral load testing, including, but not limited to, systems from Shanghai Semi-Bio, Eiken Chemical and Carpegen. But, because these technologies seem less likely to be applied to viral load testing in the near term, they are not discussed further in this report.

BD MAX™ System

The BD MAX™ System (previously known as the HandyLab Jaguar System) (http://www.bd.com/geneohm/english/products/max/instrument/) is a fully-automated platform for molecular diagnostics. The system, pictured below, automates cell lysis, nucleic acid extraction, PCR set-up, amplification and detection. It is designed to reduce the time, complexity and cost of molecular testing.



All reagents and consumables required for lysis, extraction and PCR, which is optional, are loaded into a Unitized Reagent Strip URS, which simplifies instrument set-up.

The BD MAX uses microfluidic-based real-time PCR, with PCR reactions being performed individually in disposable microfluidic cartridges. The company indicates that small reaction volumes together with microthermal circuits allow for short thermocycling times. Detection is based on multi-wavelength fluorescence detection.

Currently, the BD MAX System is cleared for use with the BD MAX GBS (Group B Streptococcus) Assay. Other assays are being developed, but there is no current indication from the company that a viral load assay is planned.

Enigma Diagnostics Limited

Enigma Diagnostics focuses on RT-PCR technology products that combine the speed and sensitivity of PCR with the simplicity needed for POC testing. The company develops portable and fully-automated PCR-based diagnostic instruments that were originally targeted to bio-security applications. The company is now expanding its focus to clinical, veterinary disease and homeland security markets. (http://www.enigmadiagnostics.com)

The Enigma system includes: automated sample preparation, real-time PCR instrumentation, unique direct heating thermal cycling, novel real-time PCR chemistries and freeze-dried PCR reagents. Sample analysis consists of three steps: (i) dispensing the sample into a single-use cartridge; (ii) loading the cartridge into the instrument and selecting test (at which point the operator can walk away); and (iii) reading the result.

Although the company indicates that the platform is appropriate for in vitro diagnostics, including for infectious disease testing, there is no indication that a viral load assay is planned in the near future.

BioHelix

BioHelix Corporation is developing assays based on the company's isothermal nucleic acid amplification platform for infectious diseases. BioHelix has developed an "instrument-free" molecular diagnostic platform (the IsoAmp® Molecular Analyzer) for these assays. The platform consists of the company's proprietary Helicase-Dependent Amplification (HDA) technology, which uses a helicase enzyme to unwind double-stranded DNA into single strands, thus eliminating the need for a thermocycler and providing a method for assay development. It also contains an enclosed disposable detection device called the BESt™ (biohelix express strip) Cassette, which minimizes cross-contamination. Workflow on the analyzer consists of simple sample prep, isothermal amplification at 65°C followed by amplicon detection via the BESt Cassette. According to the company, results can be available in as little as 10 minutes. The company is currently developing integrated sample prep, dry reagents and a faster HDA platform. (http://www.biohelix.com/IsoAmp%20Molecular%20Analyzer.asp)

The first assay being developed by BioHelix, with funding from NIH, is for genital herpes. The project is currently in its 3rd year. A clinical trial is underway that is expected to be finished by late spring 2011. Additional assays are in development, one of which is a qualitative test for HIV, which is also funded by NIH. The project involves the development of a primer than can detect different HIV subtypes and, the company notes that, this could be converted into a quantitative assay with the addition of a fluorescence label probe. However, the company has no present plans to develop such a quantitative viral load assay and would only do so with the availability of dedicated funding.

TwistDx

TwistDx (http://www.twistdx.co.uk) has developed a proprietary technology, RPA (Recombinase Polymerase Amplification), for DNA amplification. The company currently produces a line of amplification kits for scientific research, but not yet for *in vitro* diagnostics, using the RPA technology. While the TwistAmp™ kits contain all enzymes and reagents necessary for the amplification of DNA, primers and templates must be supplied by the user. These kits can detect DNA molecules in a mixed sample within 10-15 minutes.

RPA is a nucleic amplification system that uses prokaryotic enzymes (recombinases) to guide synthetic oligonucleotide primers to target sites in sample nucleic acids. Similar to PCR, the process involves exponential amplification of the target by reiteration of oligonucleotide-primed DNA synthesis. But, unlike PCR, RPA does not require a thermocycler. Instead, RPA will operate at low and constant ambient temperatures (from 24°C to as high as 45°C). This means that less power is demanded than with PCR. In addition, RPA begins operating the moment a sample comes into contact with reagents; no melting of DNA or heating of RNA is required first. This cuts the time for amplification.

Although the TwistDx system does not yet include any sort of integrated sample preparation technology, the company has introduced the Twista™ portable real-time fluorometer for monitoring/detection. The Twista contains a heated incubation chamber for a strip of eight reagent tubes. It has a small footprint and can be used with a rechargeable battery pack. Detection data can be analyzed by PC-link or the device can function as a stand-alone unit capable of storing and running user-defined data. The basic kit contains all enzymes and reagents necessary for the amplification of DNA.

Currently, TwistDx is producing two of the building blocks for an integrated, portable system that could be applied to viral load testing, but at this stage, the products are for research use only. Therefore, while the technology is promising, it appears to be years away from being a POC diagnostic platform for *in vitro* use for DNA or RNA detection in resource-limited settings.

Early Infant Diagnosis

As discussed earlier in this report, because of the persistence of maternal antibodies in infants under the age of 18 months, antibody tests, like commercially-available HIV rapid disposable tests, cannot be used to accurately screen infants for HIV/AIDS. Instead, virological testing (either RNA PCR or DNA PCR testing) or ultrasensitive p24 antigen testing should be used to determine the HIV status of infants in that age group [5].[29] Current WHO guidelines call for all HIV-exposed infants to have virological testing at 4 to 6 weeks of age or at the earliest opportunity thereafter [5].

Although it is possible to use viral load testing for initial diagnosis of HIV infection in infants, to date such testing has not been widely used in resource-limited settings. Likewise, p24 antigen testing has been used in very few settings. Instead, the most widely-used test for early infant diagnosis is the DNA PCR molecular test. The qualitative HIV-1 DNA test detects the presence of HIV proviral DNA, a form of the HIV-1 genome produced by the integration of viral DNA into host cell DNA. Unlike the quantitative HIV-1 RNA tests discussed above, the DNA PCR molecular test does not provide a quantitative measure of a patient's viral load but rather provides a "yes" or "no" answer with respect to whether the infant is infected with the HIV virus.

Three HIV-1 DNA assays available in resource-limited settings are used for EID: the Roche AMPLICOR® HIV-1 DNA Test v1.5 (RUO)[30], the Roche COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM) HIV-1 Qualitative Test (RUO) and the Abbott RealTi*m*e RUO Qualitative HIV-1 Test[31]. Like the RNA PCR assays discussed in the previous section of this report, each of these assays must be performed on laboratory-based instruments. In the case of the Roche AMPLICOR qualitative test, the amplification must be done on a thermocycler (e.g., GeneAmp 9700 system) and an ELISA reader/washer, neither of which is supplied by Roche. The Roche COBAS test is designed to be run with the Roche COBAS AmpliPrep and COBAS TaqMan amplification instruments, while the Abbott RealTime assay is designed to be run on the Abbott RealTi*m*e *m*2000rt amplification system, using the *m*2000sp, *m*24sp or manual sample preparation. Technical specifications for each of these assays are set forth in Appendix 3.

The DNA PCR qualitative tests, like the RNA PCR quantitative tests discussed earlier, require sophisticated laboratory infrastructure, including clean rooms and trained laboratory technicians and are subject to some of the same drawbacks and limitations as RNA PCR tests for implementation in resource-limited settings. Nonetheless, the Roche AMPLICOR Qualitative test, which is considered the gold standard for DNA PCR testing, has had considerable uptake in resource-limited settings. One reason is that the cost of the assay is lower than that of quantitative assays and another reason is that the use of DBS with this test is well established and the performance of the test is well-accepted with DBS samples. The ability to use the test with DBS samples, which have greater stability than fresh whole blood or plasma, has made it possible for countries to expand access to testing into peri-urban and rural settings with the use of sample transport networks. In addition, the generic equipment used with the AMPLICOR Qualitative test is quite a bit less expensive (approximately $25,000) than either the TaqMan or the RealTi*m*e amplification platform (the cost of which can range from about $45,000 to $150,000). Therefore, cost has not been as big an issue as it has been for the introduction of viral load testing.

[29] The WHO strongly recommends that in infants and children undergoing virological testing the following assays (and respective specimen types) should be used: (i) HIV DNA on whole blood specimen or dried blood spots (DBS); (ii) HIV RNA on plasma or DBS; or (iii) ultrasensitive p24 antigen (Up24 Ag) on plasma or DBS.

[30] The RUO (Research Use Only) designation is required by the U.S. FDA for non-FDA approved in vitro diagnostic products that are manufactured in the United States and exported for sale and use outside the United States.

[31] Abbott expects to have this test CE-IVD marked in the near future.

New Technologies for EID

Because RNA PCR testing can be used for the detection of HIV in infants under 18 months of age, the new technologies discussed in the previous section on viral load testing, including POC tests from Alere and Liat, should be considered viable options for EID. In addition, the SAMBA system, discussed in some detail earlier in this report, is developing a qualitative assay specifically for EID. Two other potential platforms for EID are discussed below.

NWGHF EID

NWGHF is developing an ultrasensitive p24 antigen rapid lateral flow assay for use at the point of patient care. The technology involves not only a lateral flow strip that detects HIV p24 antigen, but pre-analytical devices for separating plasma from heel-stick blood and disrupting immune complexes that would interfere with immunoassays (pictured below). NWGHF has demonstrated proof of principle of the test.

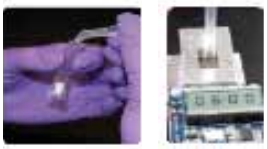
More specifically, the assay procedure involves collecting about 75µL of heel-stick blood from the infant using a capillary tube (the Safe-Tec MICROSAFE® collection and dispensing tube), separating plasma from the sample; adding buffer to the sample and pretreating it with “heat shock” in small, battery-powered processor device; inserting the rapid test strip into the device; waiting approximately 20 minutes to read the result, which will be displayed on a screen on the device (shown above). The total assay duration is about 30 – 35 minutes. See below an illustration of the procedure.

Step 1: Prick infant’s heel and collect blood

Step 2: Collect blood

Step 3: Separate plasma from red blood cells

Step 4: Add buffer and heat



Step 5: Insert strip and wait 20 min

Step 6: Read Test

Note that similar to other rapid tests, if only the top line appears (the control line only), the test is negative and the infant has not been infected with HIV. If both lines appear (the control line and the test line), the test is positive and the infant has been infected with HIV. If the top line does not appear, the test is invalid and must be re-run.

In early testing, the assay has shown about 95% sensitivity and 99% specificity. The price of the processor device is expected to be about $150 and the per-test cost is expected to be about $10. Clinical and field trials on the assay are expected to be conducted in 2011, with availability in late 2011 or early 2012.

Micronics PanNAT™ Diagnostic Platform

Micronics, Inc. has developed the PanNAT system, which is a small, portable microfluidic platform, pictured below, for use near patient in *in vitro* molecular diagnosis of infectious diseases in resource-limited settings. It is a fluorescent-based reader capable of processing individual, disposable, assay-specific cartridges, each of which is designed to perform a single and/or multiplexed nucleic acid assay, and which includes all necessary reagents on board. The system is light weight, portable, battery or mains powered, WiFi-enabled. It can store up to 350 test results before prompting the user to download or delete results, and can provide results within 30 – 40 minutes. (http://www.micronics.net/products/diagnostic-products)

The cartridge incorporates Molecular Beacon probes, primers, enzymes, buffers and controls for sample purification, amplification and detection, and because it is a closed cartridge system, there is no PCR product cross-contamination. Cartridge design permits storage at ambient temperatures for prolonged periods. All waste is captured in the cartridge for safe disposal. Current tests in development include HIV-MTCT and malaria. Clinical validation of the first assay is expected in 2011. Micronics has been funded to develop a qualitative assay for EID but has no current plans for a quantitative viral load assay.

Conclusions – Viral Load Technologies and Future Directions for Viral Load Testing

The Technologies

For CD4 testing, where even laboratory-based systems have become the norm and are well-established in resource-limited settings, the same cannot be said of viral load testing. The exceptions are South Africa and Brazil, where viral load testing is routinely conducted on a large scale, with more than 1 million viral load tests done in each country annually on systems from Siemens (Brazil) and Roche/Abbott (South Africa). Other countries that have established viral load testing on a relatively large scale include Botswana and Thailand. Beyond that, there is very little viral load testing done in the public health sector in resource-constrained settings. A few countries, including China, Kenya and Lesotho, that are increasingly using viral load, but still on a small scale – e.g., perhaps 30,000 tests annually in Kenya and 5,000 to 10,000 tests per year in Lesotho in 2010. As indicated earlier, the reasons for this low use include cost, infrastructure requirements, the need for trained laboratorians and WHO guidance on the use of viral load testing that stops short of calling for its routine use.

As in the case of CD4 testing, in order to reach patients in peri-urban and rural settings with laboratory-based viral load platforms only, it is necessary to set up sample transport networks to transfer patient blood samples to the reference laboratory for testing and to return results to the patient. Since viral load tests generally require plasma for extraction, there is a requirement to centrifuge the patient's whole blood samples usually within 6 hours of the blood draw. In addition, plasma must be transported and stored under refrigeration. These demands put pressure on the sample transport system and add costs to the process. The introduction of the use of DBS with some of the laboratory-based viral load platforms (Roche Taqman, Abbot RealTi*m*e, Siemens VERSANT kPCR, and bioMérieux EasyQ) and its use for EID testing, help to make the sample transport process more manageable, removing some of the time pressure.

Future Directions for Viral Load Testing and Implications for Viral Load Technologies

Given the growing consensus of the importance of viral load testing for detection of treatment failure for patients on 1st line regimens, it is possible that there will be a movement of testing algorithms towards routine viral load testing. The frequency of testing remains to be determined, but if ease of testing and cost allow, in the future, it might be as frequent as every 1 – 2 months or more often (analogous to glucose testing for diabetics). The purpose of global ART should be the effective, long-term chronic management of patients so as to ensure the successful treatment of as many people as possible for as long as possible. Essential for this goal is (i) early detection of both viral resistance and decreasing treatment efficacy for each patient, followed by (ii) improved adherence in order to preserve the existing treatment regimen, or else (iii) early diagnosis of treatment failure that requires a regimen switch. Patient management algorithms will need to be upgraded to accommodate the effective use of viral load information.

As discussed in connection with the scale-up of CD4 testing, the level of access required for viral load testing will likely necessitate centralized testing facilities, including the so-called super labs, that carry out very high volume testing and, at the same time, a drive towards POC testing. As indicated above, both viral load and EID POC technologies are in development, with possible launch of the first product or products in 2011. It is too early to predict the exact pricing of the POC devices and tests, but it is anticipated that the price per test will be at or below $10.00 per test. Competition among POC and non-POC platforms could eventually lead to pricing similar to CD4 pricing levels.

What Should the HIV Diagnostic Landscape Look Like Going Forward?

This report has detailed the current HIV diagnostic landscape from detection of the virus through staging and monitoring of the disease for the HIV positive patient. Given the current emphasis on ART efficiency and simplification, the main challenge is this; how must the diagnostic landscape adapt and change over the next few years --if the goal is robust, high-quality, efficient, cost-effective and accessible diagnostic services --to achieve necessary complement of testing required to diagnose, stage and monitor the HIV patient effectively?

Arguably, diagnostic services should be delivered strategically, whether centrally or at the point of care, using the most effective, robust and efficient technologies available. A significant increase in the level of access to such robust, high quality diagnostics will play a critical role in: (i) detecting and treating HIV/AIDS early, thereby maximizing the preventive impact of treatment; (ii) detecting drug resistance early, thereby reducing the spread of drug-resistant strains of the virus and (iii) preserving drug regimens, thereby increasing the period of successful treatment for each patient.

While considerable advances have been made in expanding access to tests for initial diagnosis of HIV, the quality of this testing has to be improved and further expansion is needed. Similar advances in access to tests for infant diagnosis, ART staging and monitoring are needed and new technologies in the pipeline are likely to significantly change how these tests are delivered. At the same time, new platforms for high-volume testing are also becoming available, allowing cost-effective consolidation of testing in high volume centers (super-labs). The pace at which countries implement an optimized mix of high-volume centralized and low-volume POC diagnostic services tailored to their individual diagnostic landscapes will be key to the impact on access, efficiency and quality over the next decade.

There are a number of important areas for future work to improve diagnostics for HIV/AIDS. These include:

- Focus on quality improvements at all levels of diagnostic testing for HIV/AIDS;
- Analysis of the optimal mix of monitoring technologies relative to country characteristics;
- Mapping barriers to, and fostering acceleration of, new technology introduction, especially for POC technologies; and
- Improving systems for sample referral and results distribution for central labs.

Strategic funding on the part of UNITAID and other funders could make a difference in a number of these areas, including in the acceleration of new POC diagnostic technology introduction.

APPENDIX 1
Operational Characteristics of CD4 and Viral Load Platforms*

CD4 Systems Operating Characteristics

BD FACSCalibur System

Type of Technology	Large, bench-top, bead-based Flow Cytometer
Output	Absolute and percentage CD4 counts, immunophenotyping, residual white blood cell enumeration, DNA analysis
Turnaround time	60 minutes for 40 tests run on a rack, including incubation time
Capacity	Approximately 200 samples per day
Throughput per technician/ per day	40 per hour, after approximately 30 minute incubation time
Sample needed and stability	At least 100µL whole blood collected in EDTA anticoagulant; staining to take place w/n 72 hours of blood draw; analysis to take place w/n 6 hours of staining.
Sample preparation and protocol complexity	Required. Process: (i) blood is collected and added to tube to which reagent has been added; (ii) sample is vortexed and incubated; (iii) fixative (lyse) is added to the tube, which is vortexed and incubated; and (iv) sample is vortexed and run on the instrument.
Reagent stability	Reagents are shipped to customers with an expiration date of about 6 months; reagents must be stored at 2° to 8° C (36° to 46° F).
Cost/test	Volume based; ranges from approximately $3.00 to $7.00 per test
Cost/instrument	Approximately $75,000 - $100,000
Regulatory Status	FDA approved
Physical dimensions (W x H x D)	Width: 91.4 cm Height: 61,5 cm Depth: 67.3 cm
Weight	109.1 kg (~240 lb)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately $1,500 - $2,500

BD FACSCalibur System (2)

Electric Power Requirements	100 – 240 VAC 50 – 60 HZ
Environmental Requirements	• Temperature: 16 - 19ºC (60 - 85ºF) • Humidity: 10% to 90% relative non-condensing • Maximum altitude: N/A
Data Station	Separate FACSCalibur work station (BD FACStation™); computer and color printer separate from instrument.
Monitor	In work station
Printer	In work station
Bar-code Scanner	Optional
Training	Significant training required for laboratory technicians.
Maintenance	Device is optical with a light source and tubes. Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	BD provides bead-based controls.
External QA	Compatible with CD4 EQA programs.
Infrastructure Requirements	Technology can be used at central/national reference laboratories.

* Per test costs and costs for platforms/devices indicated in this Appendix 1 are approximate and may vary considerably by country, annual volumes and based on negotiations with suppliers.

EPICS XL and XL MCL System

Type of Technology	Large, bench-top, bead-based Flow Cytometer, available with loader (MCL)
Output	Absolute and percentage CD4 counts (CD45, CD3, CD4 and CD8 can be measured), multiparametric DNA analysis, platelet studies, reticulocyte enumeration, cell biology/functional studies and a broad range of research applications
Turnaround time	About 30 minutes, after 20 minute incubation
Capacity	Approximately 150 - 200 samples per day (47 samples per hour, or 375 samples per day, with MCL)
Throughput per technician/ per day	Varies according to test, flow rate and manual versus MCL sampling mode
Sample needed and stability	At least 100 µL whole blood collected in EDTA anticoagulant; white blood count should also be performed to determine whether cell counts are outside the normal range, which may adversely influence CD4 count results.
Sample preparation and protocol complexity	Process: (i) blood is collected and added to tube; (ii) FlowCare reagent is added; (iii) sample is vortexed gently; (iv) sample is incubated at 20 - 25º for 30 minutes; (iv) sample is lysed and run on the instrument.
Reagent stability	Reagents must be stored at 2º to 8º C (36º to 46º F); reagents are shipped with an expiration date of 1 year.
Cost/test	Volume based; ranges from approximately $2.50 to $8.00 per test
Cost/instrument	Approximately $90,000 (with MCL)
Regulatory Status	FDA approved
Physical dimensions (cytometer only) (W x H x D)	Width: 61 cm Height: 50.8 cm Depth: 57.2 cm
Weight	63.5 kg (~140 lb) (cytometer only)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately $1,500 - $2,500

EPICS XL and XL MCL System (2)

Electric Power Requirements	115 – 220 VAC 50 – 60 HZ
Environmental Requirements	• Temperature: 16 - 32ºC (60 - 90ºF) • Humidity: N/A • Maximum altitude: N/A
Data Station	Multimedia workstation, includes computer separate from cytometer
Monitor	In workstation (various LCD displays available)
Printer	Not included
Bar-code Scanner	Optional
Training	Significant training required for laboratory technicians.
Maintenance	Device is optical with a light source and tubes. Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls (normal and low immunotrol) are provided by Coulter.
External QA	Compatible with CD4 EQA programs.
Infrastructure Requirements	Technology can be used at central/national reference laboratories.

Partec CyFlow® Counter

Type of Technology	Desk top, volumetric Flow Cytometer
Output	Absolute and percentage CD4 counts, total lymphocytes and WBC
Turnaround time	Test dependent
Capacity	250 tests/day without loader; 400 tests/day with loader
Throughput per technician/ per day	Depends on tests run
Sample needed and stability	20 µL whole blood collected in EDTA anticoagulant
Sample preparation and protocol complexity	Process for dry reagents only: (i) add 20µL of fresh whole EDTA blood to Partec CD4 tube containing dry reagents; (ii) incubate 10 minutes at room temperature in the dark; (iii) add 820 µL of prefilled buffer to tube; (iv) run sample in CyFlow Counter. For liquid reagents: (i) add 20 µL of fresh whole EDTA blood to a Partec test tube; (ii) add 20 µL of CD4 mAB PE reagent to tube; (iii) incubate 15 minutes at room temperature in the dark; (iv) add 820 µL no lyse buffer and shake or vortex gently; (v) run sample on the Partec device. In either case, the process for CD4% requires the addition of a second buffer.
Reagent stability	Dry reagents may be stored at room temperature and have a maximum shelf life of 12 months; Liquid reagents must be stored at 2º to 8º C (36º to 46º F) in the dark.
Cost/test	$2.40 per test for absolute CD4 and $3.40 for CD4 percentage, regardless of volume
Cost/instrument	Approximately $27,000, but can be higher with the addition of sample preparation and auto-loading systems
Regulatory Status	CE-IVD marked
Physical dimensions (cytometer only) (W x H x D)	Width: 32.5 cm Height: 33.0 cm Depth: 26.5 cm
Weight	9.7 kg (~21.3 lbs) (cytometer only)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately $1,500 - $2,500

Partec CyFlow® Counter (2)

Electric Power Requirements	100 – 240 VAC or 12 V DC/5A power (on car battery or solar panels) 50 – 60 HZ
Environmental Requirements	• Temperature: N/A • Humidity: N/A • Maximum altitude: N/A
Data Station	Dedicated Intel® CPU integrated into instrument
Monitor	8.4” TFT color touch screen integrated into instrument
Printer	Dedicated printer integrated into instrument
Bar-code Scanner	Optional
Training	Moderate level of training is required
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Instrument supports QC (Count Check beads)
External QA	Compatible with CD4 EQA programs.
Infrastructure Requirements	Technology can be used at central, regional, district and mobile labs and some well-developed primary sites with dedicated laboratory facilities and technicians.

BD FACSCount System

Type of Technology	Bench-top, bead-based Flow Cytometer
Output	Single tube reagents measure absolute and percentage CD4 (FACSCount CD4 Reagents); Single tube CD4/CD3 reagents measure CD4 and CD3 T-cells; Paired tubes of CD4/CD3 and CD8/CD3 reagents for enumeration of CD4, CD3 and CD8 T-cells
Turnaround time	60 – 90 minute incubation, 2-3 minutes per test
Capacity	Approximately 30 – 80 samples per day
Throughput per technician/ per day	20 per hour, after initial 60 – 90 minute incubation
Sample needed and stability	0.5 – 5 mL whole blood collected in EDTA anticoagulant; staining to take place w/n 24 hours of blood draw; analysis to take place w/n 48 hours of blood draw
Sample preparation and protocol complexity	Required. Process: (i) blood is collected and added to tube; (ii) sample is vortexed and incubated; (iii) fixative is added to the tube, which is vortexed and incubated; and (iv) sample is vortexed and run on the instrument.
Reagent stability	Reagents are shipped to customers with an expiration date of 5 months or longer; reagents must be stored at 2º to 8º C (36º to 46º F)
Cost/test	Volume based; ranges from approximately $3.50 to $10.00 per test
Cost/instrument	Approximately $30,000
Regulatory Status	FACSCount and paired tube reagents, FDA approved and CE-marked; CD4/CD3 reagents neither FDA approved or CE-marked.
Physical dimensions (W x H x D)	Width: 43.2 cm Height: 38.1 cm Depth: 55.9 cm
Weight	25.9 kg (57.1 lb), fluid reservoirs empty
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately $1,500 - $2,500

BD FACSCount System (2)

Electric Power Requirements	100 – 240 VAC 50 – 60 HZ 160 W (maximum rated power)
Environmental Requirements	• Temperature: 10 - 40ºC (50 - 104ºF) • Humidity: N/A • Maximum altitude: N/A
Data Station	Dedicated CPU integrated into instrument
Monitor	Display screen integrated into instrument
Printer	Dedicated printer (thermal paper) integrated into instrument
Bar-code Scanner	Optional
Training	Moderate training required for laboratory technicians. Skills required for phlebotomy, touch-screen data entry. Prompts on the instrument display guide operators through testing. Results are objective, requiring no interpretation or subjective analysis by operators.
Maintenance	Device is optical with a light source and tubes. Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	BD provides bead-based controls
External QA	Compatible with CD4 EQA programs.
Infrastructure Requirements	Technology can be used at central, regional, district labs and some well-developed primary sites with dedicated laboratory facilities and technicians.

Guava® Auto CD4/CD4% System

Type of Technology	Small, bench-top, volumetric Flow Cytometer
Output	Absolute and percentage CD4 counts, total lymphocytes
Turnaround time	2 minutes, after 45 minute incubation
Capacity	Approximately 50 samples per day
Throughput per technician/ per day	50 samples per technician per day
Sample needed and stability	10 µL whole blood collected in EDTA anticoagulant
Sample preparation and protocol complexity	Process: (i) Add 10µL of Guava reagents to tube (ii) add 10 µL of blood from patient; (iii) incubate 30 minutes (iv) add 380 µL of Guava lyse solution; (v) incubate sample 15 minutes in darkness; (iv) sample is run on the instrument.
Reagent stability	Reagents must be stored at 2º to 8º C (36º to 46º F); reagents are shipped with 12 months of shelf life.
Cost/test	$2.00 per test for CD4/CD4%, regardless of volume
Cost/instrument	Approximately $32,500
Regulatory Status	CE-IVD marked
Physical dimensions (cytometer only) (W x H x D)	Width: 32 cm Height: 21.6 cm Depth: 36.3 cm
Weight	11.4 kg (~25 lbs) (cytometer only)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately $1,500 - $2,500

Guava® Auto CD4/CD4% System (2)

Electric Power Requirements	100 – 240 VAC 50 – 60 HZ 80 W
Environmental Requirements	• Temperature: 15 - 35ºC (59 - 95ºF) • Humidity: 10 – 90% • Maximum altitude: N/A
Data Station	Separate laptop supplied with instrument; Height: 11.5”, Width: 12.6”, Depth: 10.4”; Weight: 3.6 kg (8 lbs)
Monitor	Supplied with instrument (in laptop)
Printer	Not included
Bar-code Scanner	Optional
Training	One day of training is required
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Yes; Guava Check beads
External QA	Compatible with CD4 EQA programs.
Infrastructure Requirements	Technology can be used at central, regional, district labs and some well-developed primary sites with dedicated laboratory facilities and technicians.

Apogee Auto40 Flow Cytometer

Type of Technology	Bench-top, volumetric Flow Cytometer
Output	Absolute and percentage CD4 counts, total lymphocytes and additional antigens
Turnaround time	2 minutes, after 35 minute incubation
Capacity	Maximum of 20 samples per hour
Throughput per technician/ per day	Maximum of 160 samples per technician per day
Sample needed and stability	50 µL whole blood collected in EDTA anticoagulant
Sample preparation and protocol complexity	Process: (i) Run control sample of Apogee calibration beads; (ii) add 50µL of blood to tube; (iii) vortex (iv) incubate in dark room for 25 minutes (v) add 450 µL of buffer; (vi) vortex; (vii) choose test type and run sample.
Reagent stability	Reagents must be stored at 2º to 8º C (36º to 46º F); reagents are shipped with 12 months of shelf life.
Cost/test	$2.50 per test for absolute CD4 count; $3.50 per test for % CD4
Cost/instrument	Approximately $30,000
Regulatory Status	CE-IVD marked
Physical dimensions (cytometer only) (W x H x D)	Width: 32 cm Height: 48 cm Depth: 48 cm
Weight	25.0 kg (~55 lbs) (cytometer only)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately $1,500 - $2,500

Apogee Auto40 Flow Cytometer (2)

Electric Power Requirements	100 – 240 VAC (UPS with battery backup included) 50 – 60 HZ 550 W
Environmental Requirements	• Temperature: 5º - 35ºC (41º - 95ºF) • Humidity: < 90% • Maximum altitude: N/A
Data Station	Internal PC running Windows XP
Monitor	Supplied with instrument
Printer	Not included; USB and LAN connections available
Bar-code Scanner	Nor provided
Training	One day of training is required
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Yes; Apogee beads
External QA	Compatible with CD4 EQA programs (manual analysis only)
Infrastructure Requirements	Technology can be used at central, regional, district labs with dedicated laboratory facilities and technicians.

PointCare NOW™

Type of Technology	Desk top, flow cytometer
Output	Absolute and percentage CD4 counts, WBC, hemoglobin concentration, total and percentage lymphocytes, monocyte count and monocyte %, neutrophil count and neutrophil %, eosinophil count and eosinophil %
Turnaround time	8 minutes
Capacity	50 samples per day
Throughput per technician/ per day	~40 - 50 samples per technician per day; no batching capabilities; walk-away operation.
Sample needed and stability	40 µL whole blood collected in 2 mL vacuum K2 EDTA anticoagulant tubes provided by PointCare; system will accept 3 and 4 mL tubes as well. Sample is stable for 8 hours from time of draw.
Sample preparation and protocol complexity	No sample preparation steps. (i) Draw venous blood into Partec-supplied tube; (ii) scan sample ID with barcode reader; (iii) insert unopened sample tube into instrument slot and press "run" button.
Reagent stability	Reagents are stable for 12 months from date of manufacture when stored at 2º to 30º C (36º to 86º F); 6 months from date of manufacture if stored up to 42º C (108º F); transient exposure (shipping delay or temperature excursion) of 10 days at 50ºC (122º F).
Cost/test	About $10.00 per test, not including controls
Cost/instrument	Approximately $25,000
Regulatory Status	FDA cleared (CLIA moderate-complexity rating); CE-IVD marked
Physical dimensions (cytometer only) (W x H x D)	Width: 25 cm Height: 35cm Depth: 34 cm
Weight	12 kg (~26.5 lbs) (cytometer only)
3rd party supplies	All phlebotomy supplies provided in CD4*NOW*™ Reagent Kit 100

PointCare NOW™ (2)

Electric Power Requirements	Uninterruptable Power Supply (UPS) – 110 V or 220 V, 60W; portable battery power system available; solar charge system available
Environmental Requirements	• Temperature: 18º to 34º C (64º to 93º F) • Humidity: <80% • Maximum altitude: N/A
Data Station	Dedicated CPU integrated into instrument; up to 8000 results can be stored on the instrument (unlimited patient records transferable to USB). Menu languages: English, French, Spanish and Portuguese. Indonesian under development.
Monitor	LED color touch screen integrated into instrument
Printer	Separate printer (prints on non-thermal paper)
Bar-code Scanner	Available in Customer Installation Package from Partec
Training	Moderate level of training (2 – 3 days) is required
Maintenance	Instrument is optical with a light source and tubes; should therefore undergo routine preventative maintenance by (i) operator and (ii) vendor technician. In case of breakdown, vendor-trained technician required to repair.
Internal QC	PointCare provides heat stable, synthetic, bead-based reagents (Daily Check™ controls); Controls are stable at 2º - 42º C (36º F to 108º F) for 6 months from date of manufacture.
External QA	Yes
Infrastructure Requirements	Technology can be used at central, regional, district and some well-developed primary sites with dedicated laboratory facilities and technicians.

Alere Pima™ Analyzer and Alere CD4 Test

Type of Technology	Portable bench-top, fixed volume cytometer
Output	Absolute CD4 counts only
Turnaround time	18-20 minutes
Capacity	Maximum of ~20 samples per day
Throughput per technician/ per day	~20 samples per technician per day; no batching capabilities; walk-away operation.
Sample needed and stability	25 µL of capillary (fingerstick) blood wicked directly into the sample collector contained in the Pima cartridge or 25 µL of venous blood collected in EDTA anti-coagulant tube. Cartridge must be inserted and tested within 5 minutes of sample application. When using venous blood, sample is stable for 36 hours from time of draw.
Sample preparation and protocol complexity	No sample preparation required. For capillary blood: (i) lancet finger; (ii) wipe away first drops and apply following blood drops to cartridge; (iii) close cartridge; (iv) insert cartridge into analyzer; (v) analysis starts automatically; (vi) enter patient ID data; (vii) read result from LED screen; (viii) print result
Reagent stability	Freeze-dried reagents require no refrigeration. Stable for 12 months at 2º to 30°C
Cost/test	~ $6 per test
Cost/instrument	~ $5,500
Regulatory Status	CE-IVD marked, FDA-approval expected in 2011
Physical dimensions (cytometer only) (L x H x D)	Length: 22 cm (8.7”) Height: 16 cm (6.3”) Depth: 13 cm (5.1”)
Weight	2.54 kg (~5.6 lbs) (instrument only)
3rd party supplies	For venous samples: volumetric or transfer pipette For capillary samples: sterile lancets, alcohol swabs, dry swabs (also available from Alere)

Alere Pima™ Analyzer and Alere CD4 Test (2)

Electric Power Requirements	100 to 240 V (A/C) at 47 – 63 Hz mains power Analyzer contains on-board rechargeable battery.
Environmental Requirements	• Operating Temperature: 10º to 40º C (50º to 104º F) • Humidity: N/A; no direct sunlight; keep dry • Maximum altitude: N/A
Data Station	Dedicated CPU integrated into instrument; approximately 1,000 test results can be stored on the instrument archive; results can be downloaded via USB. Potential to install an SMS chip to transmit results or internal calibration data.
Monitor	LED mono-color screen integrated into instrument
Printer	Separate printer (prints on thermal paper); battery powered L 95mm x W 93mm x H 66mm, weight: ~350 grams, including paper roll.
Bar-code Scanner	Integrated into instrument for test cartridges only
Training	Minimal training required. Lay person can be trained in less than half a day. Primary skill required is for correct lancet blood draw.
Maintenance	Analyzer contains an integrated camera and computer that might be susceptible to damage if dropped. If damaged, low cost and portability of device allows for direct swap-out replacement rather than on-site repair.
Internal QC	Extensive internal controls: sample volume control; reagent control; automatic control of cartridge expiry date; internal process controls; automatic test identification
External QA	TBD whether compatible with CD4 EQA programs; cartridge cannot be retested to confirm results.
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	16 button keypad

Partec CyFlow® miniPOC

Type of Technology	Portable and compact flow cytometer
Output	Absolute and percentage CD4 counts
Turnaround time	Set up time <5 minutes; Blood preparation time: 15 minutes; Run time: 70 seconds
Capacity	Up to 250 tests/day
Throughput per technician/ per day	N/A
Sample needed and stability	20 µL whole blood collected in EDTA anticoagulant
Sample preparation and protocol complexity	Process for dry reagents only: (i) add 20µL of fresh whole EDTA blood to Partec CD4 tube containing dry reagents; (ii) incubate 10 minutes at room temperature in the dark; (iii) add 820 µL of prefilled buffer from syringe to tube; (iv) shake tube; (v) refill volume from sample tube into syringe; (vi) attach syringe to CyFlow mini POC.
Reagent stability	Dry reagents may be stored at room temperature and have a maximum shelf life of 12 months.
Cost/test	€1.75 (~$2.40) per test for absolute CD4 and €2.50 (~$3.40) for CD4 percentage, regardless of volume
Cost/instrument	Approximately $12, 790
Regulatory Status	CE-IVD marked
Physical dimensions (cytometer only) (W x H x D)	Width: 36.8 cm Height: 24.3 cm Depth: 18.6 cm
Weight	< 5 kg (~ 11 lbs)
3rd party supplies	Syringe, sterile lancets, alcohol swabs, dry swabs, gauze, bandage

Partec CyFlow® miniPOC (2)

Electric Power Requirements	100 – 240 VAC or 12 V DC power (car battery) 50 – 60 HZ
Environmental Requirements	• Temperature: N/A • Humidity: N/A • Maximum altitude: N/A
Data Station	Dedicated Intel® Atom™ CPU integrated into instrument; Windows™-based analysis software
Monitor	5.7” color touch screen integrated into instrument
Printer	Dedicated thermal printer integrated into instrument
Bar-code Scanner	No
Training	Moderate level of training is required given sample handling requirements
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Supports internal QC
External QA	Compatible with CD4 EQA programs
Infrastructure Requirements	Technology is suited for use at primary health centers and remote areas, but can be used at central, regional, district and mobile labs.

Daktari™ CD4 Counter

Type of Technology	Small, portable device that uses cartridge microfluidic-based system to selectively capture CD4 cells in whole blood and to count them by measurement of electrical sensing.
Output	Absolute CD4 counts only
Turnaround time	8 minutes
Capacity	~40 – 50 samples per day
Throughput per technician/ per day	~40 to 50 samples per technician per day; no batching capabilities; walk-away operation.
Sample needed and stability	16 µL of capillary (fingerstick) blood applied to Daktari cartridge.
Sample preparation and protocol complexity	No manual sample preparation required. Protocol: (i) lancet finger; (ii) apply blood drop to cartridge; (iii) insert into CD4 counter; (iv) press "start"; (v) read result from LCD screen.
Reagent stability	Dried reagents require no refrigeration.
Cost/test	$8 per test (estimated)
Cost/instrument	$800 (estimated)
Regulatory Status	TBD
Physical dimensions (cytometer only) (W x H x D)	Width: 22.9 cm Height: 17.8 cm Depth: 12. 7 cm
Weight	2.5 kg (~5.5 lbs)
3rd party supplies	Sterile lancets (for capillary blood samples), alcohol swabs, dry swabs, gauze, bandaid

Daktari™ CD4 Counter (2)

Electric Power Requirements	Regular AC with long-life rechargeable battery self-contained in device
Environmental Requirements	• Operating Temperature: TBD • Humidity: TBD • Maximum altitude: TBD
Data Station	Daktari is developing a model that will include a data management system and will contain a keypad user interface. It will also have a back-end data package built into the device.
Monitor	LCD screen integrated into instrument. Results stored on instrument and can be downloaded, if needed, and can be automatically uploaded to a remote server for analysis.
Printer	None
Bar-code Scanner	No
Training	Minimal training required. Lay person can be trained in less than 90 minutes. Primary skill required is for correct lancet blood draw.
Maintenance	Device uses lasers rather than optics and may be less prone to damage. If damaged, depending on the cost of the instrument, it might be possible to swap-out replacement device rather than repair on-site.
Internal QC	TBD
External QA	TBD whether compatible with CD4 EQA programs; cartridge cannot be retested to confirm results.
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.

MBio CD4 system

Type of Technology	Small, portable instrument that is a two-color fluorescence imaging cytometer that uses immunostaining and direct cell counting; system uses disposable, self-contained fluidic cartridges.
Output	Absolute CD4 counts only; could be configured for other stain combinations
Turnaround time	20 minutes (15 minutes in cartridge and 5 minute instrument processing/read). Cartridges can be processed in parallel.
Capacity	8 to 10 tests per hour – running cartridges in parallel.
Throughput per technician/ per day	~60 to 80 samples per technician per 8 hour day.
Sample needed and stability	10 µL of capillary (fingerstick) blood; can also use venipuncture
Sample preparation and protocol complexity	Product protocol development target is single blood transfer to device.
Reagent stability	N/A; company has not released reagent stability data.
Cost/test	TBD
Cost/instrument	Not yet available from company
Regulatory Status	TBD
Physical dimensions (cytometer only) (LxWxH)	Length: 38.0 cm (15”) Width: 19.0 cm (7.5”) Height: 15.0 cm (5.9”)
Weight	3.0 kg (~6.6 lbs)
3rd party supplies	Sterile lancets (for capillary blood samples), alcohol swabs, dry swabs, gauze, bandaid

Mbio CD4 System (2)

Electric Power Requirements	Battery powered
Environmental Requirements	• Operating Temperature: TBD • Humidity: TBD • Maximum altitude: TBD
Data Station	TBD
Monitor	LCD screen integrated into instrument.
Printer	None
Bar-code Scanner	No
Training	Minimal training required. Lay person can be trained in less than 90 minutes. Primary skill required is for correct lancet blood draw.
Maintenance	Device is non-optical and should be less prone to damage. If damaged, low cost and portability of the instrument may allow direct swap-out replacement rather than on-site repair.
Internal QC	TBD
External QA	TBD whether compatible with CD4 EQA programs; cartridge cannot be retested to confirm results.
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.

Zyomyx CD4 Counter

Type of Technology	Disposable cartridge used with a mechanical mixing / spinning tool. CD4 cells bind to heavy, anti CD4 antibody coated particles inside the cartridge. The cartridge is subsequently spun slowly whereby only the conjugated cells penetrate into a high density medium, forming a cell stackwidth in a small micro-capillary. The CD4+ T-cell count is proportional to the stacking height of the cells in that capillary. Result can be visually read without the need for an electronic reader.
Output	Absolute CD4 counts only
Turnaround time	~10 minutes
Capacity	~40 samples per day
Throughput per technician/ per day	~40 samples per technician per day; batch processing TBD
Sample needed and stability	100 µL of finger-prick blood
Sample preparation and protocol complexity	Protocol: (i) Uptake 100 µL of finger-stick blood into pick-up capillary tube; (ii) transfer blood into Zyomyx cartridge; (iii) place cartridge in to mixer/spinner tool and mix; (iv) twist top portion of cartridge; (v) spin cartridge in mixer/spinner tool (vi) read results.
Reagent stability	6 months at room temperature up to 40°C
Cost/test	$6-7 per test (estimated)
Cost/instrument	$100 (mechanical mixer/spinner for short mixing and spinning)
Regulatory Status	TBD
Physical dimensions of cartridge (W x H x D)	Width: 0.5 inch Height: 2.5 inch Depth: 0.5 inch
Weight	0.4 ounces
3rd party supplies	Sterile lancets (for capillary blood samples), blood pick-up capillary tube, alcohol swabs, dry swabs, gauze, bandage

Zyomyx CD4 Counter (2)

Electric Power Requirements	None; field version of mixer/spinner is purely mechanical.
Environmental Requirements	• Operating Temperature: TBD • Humidity: TBD • Maximum altitude: TBD
Data Station	None
Monitor	None
Printer	None
Bar-code Scanner	No
Training	Minimal training required. Lay person can be trained in less than 30 minutes. Primary skill required is for correct lancet blood draw.
Maintenance	Test is disposable and does not require service/maintenance.
Internal QC	Several control windows integrated to alert of potential device failures modes
External QA	TBD
Infrastructure Requirements	Can be used at all levels of health facility, including health centers, in mobile facilities or in the field. Small, portable tool not requiring electric power / batteries.

Burnet Institute CD4 Counter

Type of Technology	Disposable cartridge containing test strip (lateral flow) that measures CD4 proteins on T cells
Output	Absolute CD4 counts only
Turnaround time	~40 minutes, including incubation
Capacity	
Throughput per technician/ per day	~120 samples per technician per day; batching capabilities (up to ≈10/technician).
Sample needed and stability	40 µL of capillary (fingerstick) blood, or peripheral blood into EDTA anticoagulant
Sample preparation and protocol complexity	Protocol: (i) lancet finger; (ii) add whole blood to Well A of test strip using MicroSafe pipette; (iii) add 1 drop of supplied buffer to Well A and allow sample to run for 18 minutes; (iv) add 3 drops of buffer to Well B of test strip; (v) wait for 20 minutes; (vi) read results.
Reagent stability	> 6 months at 40°C
Cost/test	$2 per test (estimated)
Cost/reader	$1,200 for reader (eventual price estimated $400). Note that tests can also be read by eye.
Regulatory Status	TBD
Physical dimensions of reader (W x H x D)	Width: 12 cm (4.7”) Height: 8.5 cm (3.3”) Depth: 7.7 cm (3”)
Weight of reader	390 g (~14 oz)
3rd party supplies	Sterile lancets (for capillary blood samples), alcohol swabs, dry swabs, gauze, bandage

Burnet Institute CD4 Counter (2)

Electric Power Requirements	None for cartridge; reader 12V DC via adapter (110-240V), optional battery pack.
Environmental Requirements	• Operating Temperature: TBD • Humidity: TBD • Maximum altitude: TBD
Data Station	None (reader stores most recent 1,000 tests; downloadable via USB/ethernet)
Monitor	None (reader 2.4 inch color touch screen)
Printer	None (reader can support printing)
Bar-code Scanner	Yes (optional on reader)
Training	Minimal training required. Lay person can be trained in less than 120 minutes. Primary skill required is for correct lancet blood draw, and for visual test reading (automated with reader). Reader provides on-board training instructions (can be used in instruction/assay run mode, or read-only for batched tests).
Maintenance	Test is disposable and does not require service/maintenance; reader is expected to be robust and will be swapped out if it fails.
Internal QC	None (Reader has internal QC)
External QA	TBD
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.

Viral Load and EID Platform Operating Characteristics

RT-PCR: Roche COBAS® AmpliPrep® System Automated extraction instrument

Type of Technology	Automated extraction and sample preparation
Output	Samples ready for amplification and detection on COBAS AMPLICOR or COBAS TaqMan Analyzer
Turnaround time	Three racks of 24 specimens in approximately 5 hours; 216 seconds processing time per specimen
Capacity (per run)	72 samples per run (maximum)
Throughput per technician/ per day	Up to 144 specimens per day, based on testing combinations and laboratory workflow.
Sample needed and stability	250 – 1,100 µL whole blood collected via venipuncture to obtain 70 µL plasma for Standard procedure; 500 µL for UltraSensitive Procedure; 200 and 500 µL for TaqMan analyzers . Can also use 60 – 70 µL DBS for Taqman. Whole blood must be transported at 2 - 25º C and processed within 6 hours of collection; plasma may be transported/stored at 2 - 8º C for 5 days or frozen at - 70º C.
Specimen preparation and protocol complexity	Plasma transferred to a properly identified, sterile screw-cap, polypropylene tube after centrifugation. Requires test-specific, bar-coded, ready-to-use COBAS AmpliPrep Kits. Reagents are all liquid and ready to use, but require mixing and other procedures before ready for processing.
Reagent stability	Varies by reagent, but most must be stored at 2º to 8º C (36º to 46º F); all reagents are stable until expiration date.
Cost/test	N/A
Cost/instrument	Approximately $80,000 to $150,000
Regulatory Status of Assays	FDA approved; CE-IVD marked (DBS is RUO only)
Physical dimensions (cytometer only) (W x D x H)	Width: 165 cm (65”) Depth: 73.7 cm (29”) Height: 94 cm (37”)
Weight	310 kg (683 lbs)
3rd party supplies	Input and output tubes, pipettors, vortex mixer, refrigerator, gloves and other lab consumables

RT-PCR: Roche COBAS® AmpliPrep® System Automated extraction instrument (2)

Electric Power Requirements	100 – 125 VAC and 200 -240 VAC (+10, -15%) 50 – 60 HZ
Environmental Requirements	• Temperature: 15ºC to 32º C (59ºF to 89ºF) • Humidity: <80% (for temperatures up to 32ºC) • Maximum altitude: 2,000 meters (6,500 feet)
Data Station	Custom-built PC (included) with Microsoft® Windows® (NT v. 4.0 and higher) and AMPLILINK® Software to control COBAS AmpliPrep System
Monitor	Monitor VGA 14"
Printer	Printer HP 1320; printer interface: LPT interface via parallel port
Bar-code Scanner	Supplied with instrument On COBAS AmpliPrep: on-board bar-code scanner for reagent racks, reagent cassettes and specimen clips. On AMPLILINK Data Station: handheld bar-code scanner for original specimen/specimen clip
Training	Fully-trained lab tech required; dedicated training on instrument, which requires strong computer skills
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	An Internal Control/Quantitation Standard (IC/QS) can be incorporated into each individual sample and is carried through the sample preparation. Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays.
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Roche COBAS® AMPLICOR Analyzer Automated amplification/detection instrument

Type of Technology	Automated end-point amplification and detection
Output	RNA HIV-1 quantification; DNA qualitative measure
Turnaround time	Depending on assay, 5 to 6 hours; 20 minutes hands-on time
Capacity (per run)	24 samples (21 patient samples + 3 controls); in batches of 12
Throughput per technician/ per day	48 samples on DP or parallel mode
Sample needed and stability	PCR-ready set-up samples from AmpliPrep; processed specimens and controls should not be exposed to light after completion of specimen and control preparation.
Specimen preparation and protocol complexity	Once removed from the COBAS AmpliPrep Instrument, processed specimens and processed controls may be stored in the output tubes at 2-8ºC for up to one day (24 hours). Preparation of reagent cassettes for amplification and extraction is moderately complex.
Reagent stability	Varies by reagent, but most must be stored at 2º to 8º C (36º to 46º F); all reagents are stable until expiration date.
Cost/test	AMPLICOR Test: $17 - $25 per test (least developed countries); $35 - $90 elsewhere
Cost/instrument	Approximately $15,000 to $20,000
Regulatory Status of Assays	MONITOR test is FDA approved; CE-IVD marked
Physical dimensions (cytometer only) (W x D x H)	Width: 86 cm (33.9”) Depth: 57 cm (22.4”) Height: 41 cm (41”); Height with cover up: 90 cm (35.4”)
Weight	75 kg (165 lbs)
3rd party supplies	Centrifuge, refrigerator, laboratory freezer and various additional laboratory consumables

RT-PCR: Roche COBAS® AMPLICOR Analyzer (2) Automated amplification/detection instrument

Electric Power Requirements	100 – 125 VAC and 200 -240 VAC (autoranging) 50 – 60 HZ
Environmental Requirements	• Temperature: 15ºC to 32º C (59ºF to 89ºF) • Humidity: <80% (at 32ºC) • Maximum altitude: 3,000 meters (9,800 feet)
Peripherals/Supporting Instrumentation	Hardware and monitor: optional, but not required AMPLILINK software if connected to COBAS® AmpliPrep
Bar-code Scanner	Handheld unit can be used instead of keypad for analyzer commands or order entry On AMPLILINK handheld bar-code scanner for original specimen/specimen clip
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays.
External QA	Amenable to EQA
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Roche COBAS® TaqMan® 48 Automated amplification/detection instrument

Type of Technology	Fully automated real-time amplification and detection
Output	HIV-1 RNA quantification; DNA qualitative measure
Turnaround time	Amplification and detection cycle takes 3 hours, 5 minutes
Capacity (per run)	2 independent segments of 24 samples each up to 2 different tests on board simultaneously; each thermal cycler can run individual PCR profiles.
Throughput per technician/ per day	Including processing time on AmpliPrep, 48 samples (on an 8 hour shift)
Sample needed and stability	PCR-ready set-up samples from AmpliPrep; processed specimens and controls should not be exposed to light after completion of specimen and control preparation.
Sample preparation and protocol complexity	Once removed from the COBAS AmpliPrep instrument, processed specimens and processed controls may be stored in the output tubes at 2-8ºC for up to one day (24 hours). Preparation of reagent cassettes for amplification and extraction is moderately complex.
Reagent stability	Varies by reagent, but most must be stored at 2º to 8º C (36º to 46º F); all reagents are stable until expiration date.
Cost/test	TaqMan HIV-1 Monitor Test v2.0: $20 - $30 per test (least developed countries); $35 - $90 per test elsewhere
Cost/instrument	$45,000 to $100,000
Regulatory Status	COBAS® TaqMan® HIV-1 Test, v2.0 is FDA approved and CE-IVD Marked
Physical dimensions (W x D x H)	18" x 30" x 20“ 45.7 x 76.2 x 50.8 cm
Weight	121 lbs (55 kg)
Third party supplies	Microtiter plate centrifuge (not supplied by Roche)

RT-PCR: Roche COBAS® TaqMan® 48 (2) Automated amplification/detection instrument

Electric Power Requirements	120 or 240 VAC 50 – 60 HZ
Environmental Requirements	• Temperature: 15ºC to 32ºC (59ºF to 89ºF) • Humidity: <80% (for temperatures up to 32ºC) • Maximum altitude: 2,000 meters (6,500 feet)
Peripherals/Supporting Instrumentation	Custom-built PC supplied with the analyzer; data station runs Microsoft® Windows XP Professional operating system and AMPLILINK Software AMPLILINK software is a Windows-based, LIS-compatible user interface that manages up to 3 COBAS® TaqMan® 48 Analyzers
Bar-code Scanner	On AMPLILINK handheld bar-code scanner for original specimen/specimen clip
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays.
External QA	Amenable to EQA
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Roche COBAS® TaqMan® 96 Automated amplification/detection instrument

Type of Technology	Fully automated real-time amplification and detection
Output	HIV-1 RNA quantification; DNA qualitative measure
Turnaround time	Amplification and detection cycle takes 3 hours, 5 minutes
Capacity (per run)	24 samples per K-carrier. Up to 4 K-carriers can be amplified and detected at one time. Up to 8 K-carriers can be present on the instrument.
Throughput per technician/ per day	Including processing time on AmpliPrep, 96 samples (on an 8 hour shift)
Sample needed and stability	PCR-ready set-up samples from AmpliPrep; processed specimens and controls should not be exposed to light after completion of specimen and control preparation.
Sample preparation and protocol complexity	Once removed from the COBAS AmpliPrep instrument, processed specimens and processed controls may be stored in the output tubes at 2-8ºC for up to one day (24 hours). Preparation of reagent cassettes for amplification and extraction is moderately complex.
Reagent stability	No onboard reagents are required on the Analyzer. All reagent addition is performed during the sample preparation process.
Cost/test	TaqMan HIV-1 Monitor Test v2.0: $20 - $30 per test (least developed countries); $35 - $90 per test elsewhere
Cost/instrument	$80,000 to $150,000
Regulatory Status	COBAS® TaqMan® HIV-1 Test, v2.0 is FDA approved and CE-IVD Marked
Physical dimensions (W x D x H)	Analyzer: 45" x 30" x 37" (114.3 x 76.2 x 94 cm) Table: 45" x 30" x 20" (114.3 x 76.2 x 50.8 cm) PC: 8" x 20" x 18" (20.3 x 50.8 x 45.7 cm) Monitor: 20" x 20" x 12" (50.8 x 50.8 x 30.5 cm) Computer Table: 32" x 32" x 31" (81.3 x 81.3 x 78.7 cm)
Weight	485 lbs (220 kg)

RT-PCR: Roche COBAS® TaqMan® 96 (2) Automated amplification/detection instrument

Electric Power Requirements	Analyzer: 100-125 and 200-240 VAC (+10%; -15%); 50 or 60 Hz (± 2 Hz) Data station: 100-125 and 200-240 VAC (+10%; -15%); 47 - 63 Hz (± 2 Hz)
Environmental Requirements	• Temperature: 15ºC to 32ºC (59ºF to 89ºF) • Humidity: <80% (for temperatures up to 32ºC) • Maximum altitude: 2,000 meters (6,500 feet)
Peripherals/Supporting Instrumentation	Custom-built PC supplied with the analyzer. Data station runs Microsoft ® Windows® NT operating system and TaqLink® Software to control the COBAS® TaqMan® Analyzer
Bar-code Scanner	Handheld bar-code scanner for original specimen/specimen clip
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays.
External QA	Amenable to EQA
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Abbott *m*24sp Automated extraction instrument

Type of Technology	Automated extraction and sample preparation
Output	Quantification HIV-1 RNA levels; DNA qualitative measure
Turnaround time (full run)	HIV VL = 400 min / 6h 40 min (total TAT incl *m*2000rt); HIV VL extraction time (incl. Loading of instrument) = 210 min / 3h 30 min
Capacity (per run)	1 minimum – 24 maximum
Throughput per technician/ per day	Within 8 hour shift: 2 full runs = 48 samples
Sample needed and stability	Freshly drawn whole blood may be held at 15 - 30º C for up to 6 hours or at 2 - 8ºC for up to 24 hours prior to centrifugation. After centrifugation, plasma, which is required for the Abbott RealTi*m*e HIV-1 assay, may be stored at 15 - 30º C for up to 24 hours or at 2 - 8º C for up to 5 days. If longer storage is required, may be stored at -70º C.
Sample preparation and protocol complexity	Moderately complex. Steps include vortexing (assay calibrators, each control and specimens, pipetting, centrifuge, etc.).
Reagent stability	Reagents (liquid), as well as controls and calibrators, must be stored at -10º C or colder when not in use and must be shipped on dry ice. All reagents may be reused up to three times within two weeks. Extraction reagents are ready-to-use and can be stored in the refrigerator (+4°C).
Cost/test	N/A
Cost/instrument	$90,000
Regulatory Status	CE-IVD marked
Physical dimensions (W x D x H)	Width: 88.1 cm (34.7 in.) Height: 75.9 cm (29.9 in) Depth: 69.6 cm (27.4 in.)
Weight	185 lbs (84 kg)
3rd Party Supplies	Pipettes, vortex mixer and refrigerator

RT-PCR: Abbott *m*24sp (2) Automated extraction instrument

Electric Power Requirements	100 – 240 V
Environmental Requirements	• Temperature: 15 to 35º C (59º to 95ºF) • Humidity: 5% to 80% relative (non condensing) at 30º C (86º F) or below • Maximum altitude: Up to 2,000 m/6,600 ft
Peripherals/Supporting Instrumentation	Data station, monitor and printer are supplied with the instrument.
Bar-code Scanner	Hand-held barcode scanner is supplied with the instrument
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays.
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Abbott *m*2000sp Automated extraction instrument

Type of Technology	Automated extraction and sample preparation
Output	Quantification HIV-1 RNA levels; DNA qualitative measure
Turnaround time	Extractions: depends on number of samples from 2h30' for 24 samples to 5h30' for 96 samples; Amplification and detection: 3 hours per run (up to 96 samples)
Capacity (per run)	93 patient samples + 3 controls
Throughput per technician/ per day	192 samples (2 batches of 96 samples) per 8 hour day
Sample needed and stability	Freshly drawn whole blood may be held at 15 - 30º C for up to 6 hours or at 2 - 8ºC for up to 24 hours prior to centrifugation. After centrifugation, plasma, which is required for the Abbott RealTi*m*e HIV-1 assay, may be stored at 15 - 30º C for up to 24 hours or at 2 - 8º C for up to 5 days. If longer storage is required, may be stored at -70º C.
Sample preparation and protocol complexity	Moderately complex. Steps include vortexing (assay calibrators, each control and specimens), pipetting, centrifuge, etc). Once 96 well plate is loaded and placed in *m*2000rt, process is walk away.
Reagent stability	Reagents (liquid), as well as controls and calibrators, must be stored at -10º C or colder when not in use and must be shipped on dry ice.
Cost/test	N/A
Cost/instrument	$120,000 USD
Regulatory Status	CE-IVD marked, FDA approved
Physical dimensions (W x D x H)	Width: 298.5 cm (117.5 in.) Height: 203 cm (80 in) Depth: 196.2 cm (77.3 in.)
Weight	621 lbs (281.7 kg)
3rd Party Supplies	Pipettes, vortex mixer and refrigerator

RT-PCR: Abbott *m*2000sp (2) Automated extraction instrument

Electric Power Requirements	100 – 240 V
Environmental Requirements	• Temperature: 15 to 30°C/59 to 86°F • Humidity: 30% to 80% relative (non condensing) at 30°C/86°F or below • Maximum altitude: Up to 2,000 m/6,600 ft
Peripherals/Supporting Instrumentation	Data station, monitor and printer are supplied with the instrument.
Bar-code Scanner	Supplied with instrument (integrated on workdesk)
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays.
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Abbott *m*2000rt Automated amplification/detection instrument

Type of Technology	Fully automated real-time amplification and detection
Output	HIV-1 RNA quantification; DNA qualitative measure
Turnaround time	Amplification and detection cycle takes: 3 hours
Capacity (per run)	Up to 93 patient samples + 3 controls
Throughput per technician/ per day	288 samples per day; sample preparation and extraction can be the limiting factor
Sample needed and stability	PCR-ready samples from manual, *m*24sp or *m*2000sp sample preparation/extraction protocol.
Sample preparation and protocol complexity	Freshly drawn whole blood may be held at 15 - 30º C for up to 6 hours or at 2 - 8ºC for up to 24 hours prior to centrifugation. After centrifugation, plasma, which is required for the Abbott RealTi*m*e HIV-1 assay, may be stored at 15 - 30º C for up to 24 hours or at 2 - 8º C for up to 5 days. If longer storage is required, may be stored at -70º C.
Reagent stability	No onboard reagents are required on the instrument. All reagent addition is performed during the sample preparation process.
Cost/test	$25 - $40 per test
Cost/instrument	$38,000 USD (with *m*24sp or *m*2000sp) – Add $6,000 USD for all manual extraction items
Regulatory Status	CE-IVD marked
Physical dimensions (W x D x H)	34 cm (13.4 ins) 48 cm (17.8 ins) 49 cm (19.3 ins)
Weight	75.2 lbs (34.1 kg)

RT-PCR: Abbott *m*2000rt (2) Automated amplification/detection instrument

Electric Power Requirements	100 – 240 V
Environmental Requirements	• Temperature: 15 to 30°C (59 to 86°F) • Humidity: 30% to 80% relative humidity, noncondensing • Maximum altitude: not exceeding 3,000 m (9,800 ft) above sea level
Peripherals/Supporting Instrumentation	Data station, monitor and printer are supplied with the instrument.
Bar-code Scanner	Supplied with the instrument.
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays.
External QA	Amenable to external EQA
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

bDNA: VERSANT® kPCR Molecular System Automated Sample Preparation and Amplification/Detection Modules

Type of Technology	Automated real-time extraction, amplification and detection (kPCR technique)	
Output	HIV-1 RNA quantification	
Turnaround time	Sample preparation system set-up <10 minutes; sample extraction <3 hours; amplification detection <3 hours	
Capacity (per run)	96 samples per run (89 clinical samples and 4 calibrators and 3 controls) run in 6 hours	
Throughput per technician/ per day	Up to 178 patient results per shift	
Sample needed and stability	Up to 500 µL input volume or 1 DBS (50 - 100µL); whole blood collected in EDTA tubes can be stored for 6 hours at room temperature or for up to 24 hours at 2º - 8ºC before centrifugation; plasma may be stored for up to 24 hours at room temperature or for up to 5 days at 2º - 8ºC.	
Sample preparation and protocol complexity	(i) load the dedicated sample preparation reagents into a trough; (ii) place them on the module; (iii) load plasma samples onto the sample carrier; and (iv) place the sample carriers on the auto load tray of the VERSANT Sample Prep module. From that point, sample prep module is fully automated.	
Reagent stability	Reagents must be frozen prior to use (-30º to -10ºC)	
Cost/test	~ €31.25 – 41.65 ($43.25 - $57.70) per test for reagents; €7.80 – 10.41 ($10.80 - $14.40) per test for sample preparation materials	
Cost/instrument	~ €120,000 – 160,000 ($166,200 - $221,600)	
Regulatory Status	CE-IVD marked	
Physical dimensions SP module; AD module (W x D x H)	112.4 cm (44 ins) 100.6 cm (39.5 ins) 90.5 cm (35.5 ins)	36.8 cm (14.5 ins) 53.4 cm (21 ins) 45.7 cm (18 ins)
Weight	320 lbs (145 kg)	55 lbs (25 kg)

bDNA: VERSANT® kPCR Molecular System Automated Sample Preparation and Amplification/Detection Modules (2)

Electric Power Requirements	100 – 240 V; 50 or 60 Hertz
Environmental Requirements	• Temperature: 18º - 30º C • Humidity: 30% - 80% non-condensing • Maximum altitude: 0 to 2,000 meters (to 6,560 feet)
Peripherals/Supporting Instrumentation	Computer supplied. Dimensions: 38.1 cm × 14.0 cm × 33.0 cm (15 in × 5.5 in × 13 in). Weight: 12 kgs (26 lbs); 17 in screen and separate keyboard. Printer optional
Bar-code Scanner	Supplied with the instrument.
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Controls are included as part of reagent kit and required for each preparation run.
External QA	Amenable to EQA
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

NASBA: bioMérieux NucliSens® miniMAG® Semi-automated extraction instrument

Type of Technology	Semi-automated extraction instrument
Output	Samples ready for amplification and detection on NucliSENS EasyQ analyzer
Turnaround time	12 samples: 45 minutes (1 miniMAG system) 24 samples: 60 minutes (2 miniMAG systems)
Capacity (per run)	12 patient samples (no controls)
Throughput per technician/ per day	Up to 144 specimens per day (6 runs of 24 – 2 miniMAGs at the same time)
Sample needed and stability	100 – 1,000 µL plasma (LOD is higher with larger sample). For transport, reagents must be refrigerated (some with dry ice). DBS possible.
Specimen preparation and protocol complexity	
Reagent stability	Varies by reagent, but most must be stored at 2º to 8º C (36º to 46º F); some must be stored at -20º C; all reagents are stable until expiration date
Cost/test	N/A
Cost/instrument	Approximately $12,900
Regulatory Status of Assays	CE-IVD marked
Physical dimensions (cytometer only) (W x D x H)	Width: 43.8 cm (17.2”) Depth: 11.4 cm (4.5”) Height: 15.3 cm (6”)
Weight	<5 kg (<11 lbs)
3rd party supplies	Pipettes, vortex mixer and refrigerator; strip centrifuge

NASBA: bioMérieux NucliSens® miniMAG® Semi-automated extraction instrument (2)

Electric Power Requirements	100 – 240 VAC 50 – 60 HZ
Environmental Requirements	• Temperature: • Humidity: • Maximum altitude:
Data Station	None
Monitor	None
Printer	None
Bar-code Scanner	N/A
Training	Fully-trained lab tech required; dedicated training on instrument, which requires strong computer skills
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	
Infrastructure Requirements	

NASBA: bioMérieux NucliSens® easyMAG® Automated extraction instrument

Type of Technology	Automated extraction instrument
Output	Samples ready for amplification and detection on NucliSENS EasyQ analyzer
Turnaround time	24 samples, lysis on board: 60minutes 24 samples, lysis off board: 40minutes
Capacity (per run)	1 to 24 patient samples per run
Throughput per technician/ per day	Up to 168 extractions per shift – lysis on board workflow Up to 240 extractions – lysis in tube workflow
Sample needed and stability	10 – 1,000 µL plasma (LOD is higher with larger sample). For transport, reagents must be refrigerated (some with dry ice). DBS possible.
Specimen preparation and protocol complexity	Entire extraction process takes place in a single sample compartment, which minimizes potential sample loss and cross contamination
Reagent stability	Varies by reagent, but most must be stored at 2º to 8º C (36º to 46º F); some must be stored at -20º C; minimum shelf life: 60 days; maximum: 15 to 24 months, varies by reagent
Cost/test	N/A
Cost/instrument	Approximately $114,100
Regulatory Status of Assays	CE-IVD marked
Physical dimensions (cytometer only) (W x D x H)	Width: 100 cm (39.4”) Depth: 65 cm (25.6”) Height: 53 cm (20.9”)
Weight	125 kg (275.6 lbs)
3rd party supplies	Pipettes, vortex mixer and refrigerator; strip centrifuge

NASBA: bioMérieux NucliSens® easyMAG® Automated extraction instrument (2)

Electric Power Requirements	100 – 240 VAC 50 – 60 HZ
Environmental Requirements	• Temperature: • Humidity: • Maximum altitude:
Data Station	Yes
Monitor	On-board Monitor
Printer	None supplied
Bar-code Scanner	None supplied
Training	Fully-trained lab tech required; dedicated training on instrument, which requires strong computer skills
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Yes
Infrastructure Requirements	Technology can be used at regional/central or national reference laboratories

NASBA: bioMérieux NucliSens EasyQ® Automated amplification/detection instrument

Type of Technology	Automated, real-time NASBA amplification and detection
Output	HIV-1 RNA quantification; DNA qualitative measure
Turnaround time	<2 hours for 48 samples (at 60 minutes amplification time)
Capacity (per run)	Up to 48 patient samples (minimum is 8 patient samples)
Throughput per technician/ per day	192 samples (4 runs of 24)
Sample needed and stability	PCR-ready samples from manual, miniMAG or easyMAG
Sample preparation and protocol complexity	
Reagent stability	Varies by reagent, but most must be stored at 2º to 8º C (36º to 46º F); some must be stored at -20º C; minimum shelf life: 30 days; maximum shelf life: 24 months
Cost/test	From approximately $14.60 per test to $27.20 per test
Cost/instrument	Approximately $57,400
Regulatory Status	CE-IVD marked
Physical dimensions (W x D x H)	42 cm (16.5 ins) 42 cm (16.5 ins) 22 cm (8.7 ins)
Weight	~44 lbs (20 kg)

NASBA: bioMérieux NucliSens EasyQ® Automated amplification/detection instrument (2)

Electric Power Requirements	100 – 240 V
Environmental Requirements	• Temperature: +15 to +30 ºC • Humidity: no greater than 80% • Maximum altitude: 2000 m above sea level
Peripherals/Supporting Instrumentation	Data station and monitor are supplied with the instrument. Printer not supplied with instrument.
Bar-code Scanner	Not supplied with instrument.
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Yes
External QA	Amenable to EQA
Infrastructure Requirements	Technology can be used at central/national reference (or comparable) laboratories

bDNA: VERSANT™ 440 Molecular System Automated amplification/detection instrument

Type of Technology	Automated signal amplification and detection based on branched DNA technology
Output	HIV-1 RNA quantification
Turnaround time	~24 hours (HIV-1 assay), including ~2.5 hours hands-on time
Capacity (per run)	12 - 168 patient samples from 2, 96 well plates (each of which contains 84 patient samples and 12 calibrators and controls)
Throughput per technician/ per day	Up to 168 patient samples per day
Sample needed and stability	200 – 1,000 µL plasma
Sample preparation and protocol complexity	
Reagent stability	
Cost/test	~ €26.00 (RUO assay) – €52.00 (FDA IVD/CE marked assay) per test; ($36 - $72)
Cost/instrument	~ €40,000 ($55,400)
Regulatory Status	FDA approved, CE-IVD marked
Physical dimensions (W x D x H)	59.7" x 30.6" x 24.5" 152 x 78 x 62 cm
Weight	~ 350 lbs. (159 kg)
3rd Party Supplies	Centrifuge, heat block, water bath, vacuum system; pipettes, vortex mixer and refrigerator

bDNA: VERSANT™ 440 Molecular System (2) Automated amplification/detection instrument

Electric Power Requirements	100-120 VAC±10%; 200-240 VAC±10%; 50/60 Hz; 500 VA maximum
Environmental Requirements	• Temperature: 18-30°C • Humidity: 24-80%, non-condensing • Maximum altitude:0 - 2000 m above sea level
Peripherals/Supporting Instrumentation	On board computer; user interface is Windows®XP operating system; software supplied with instrument; monitor supplied with instrument; printer not supplied with instrument.
Bar-code Scanner	Supplied with instrument.
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	Yes
External QA	Amenable to EQA
Infrastructure Requirements	Technology can be used at central/national reference (or comparable) laboratories

Reverse Transcriptase: ExaVir™ Load Separation and RT Assay

Type of Technology	Manual measurement of RT activity
Output	Determination of the activity of the RT enzyme as a marker of retroviral replication
Turnaround time	48 hours for 30 tests
Capacity (per run)	30 tests
Throughput per technician/ per day	Sample run of 30 tests requires 48 hours; 180 samples per week
Sample needed and stability	1 mL plasma; Plasma must be prepared from EDTA anti-coagulated whole blood. Plasma should be separated within 4 hours of the blood collection. Plasma samples must be frozen once before being analyzed and should be frozen at or below -20º C.
Sample preparation and protocol complexity	Sample preparation requires about 20 steps over 2 days; it is therefore complex.
Reagent stability	Reagent kits must be stored at -14ºC to -25ºC; reagent kits are stable >12 months at -20ºC. If stored between 4º and 8º C, must be used within one week.
Cost/test	Approximately $13 - $15
Cost/instrument	Approximately $9,000 - $10,000 (start-up pack includes other necessary equipment and 3 kits).
Regulatory Status	CE-IVD marked
Physical dimensions	N/A
Weight	N/A
3rd Party Supplies	ELISA plate reader with A405 filter; incubator set at 33ºC; freezer set at -14ºC to -25ºC; end-over-end mixing table; vortex; computer

Reverse Transcriptase: ExaVir™ Load (2) Separation and RT Assay

Electric Power Requirements	Not required for basic set-up
Environmental Requirements	• Temperature: • Humidity: • Maximum altitude:
Peripherals/Supporting Instrumentation	ExaVir Viral Load Analyzer software for processing results; computer required, but not supplied; no printer supplied
Bar-code Scanner	None
Training	
Maintenance	Routine preventative maintenance required.
Internal QC	Yes
External QA	No
Infrastructure Requirements	Technology can be used

Alere NAT System

Type of Technology	Portable bench-top, NAT-based purification, amplification and detection system for total HIV RNA
Output	Quantitative HIV-1 and HIV-2 RNA viral load measurement
Turnaround time	About 30 – 60 minutes
Capacity	Maximum of ~10 samples per day
Throughput per technician/ per day	~10 samples per technician per day; no batching capabilities; walk-away operation.
Sample needed and stability	25 µL of capillary (fingerstick) blood wicked directly into the sample collector contained in the dedicated cartridge. Cartridge containing sample can be stored and shipped if needed as sample is expected to be stable for weeks.
Sample preparation and protocol complexity	No sample preparation required. For capillary blood: (i) lancet finger; (ii) wick whole blood directly into cartridge; (iii) close cartridge; (iv) insert cartridge into analyzer; (v) enter operator and sample ID; (vi) analysis starts automatically; (vii) remove cartridge from analyzer and dispose of it; and (viii) read result from screen.
Reagent stability	Freeze-dried reagents require no refrigeration. Stable for 12 months at 2 to 30°C
Cost/test	TBD
Cost/instrument	TBD
Regulatory Status	TBD
Physical dimensions (analyzer only) (L x H x D)	Length: 28 cm (11”) Height: 17 cm (6.7”) Depth: 17 cm (6.7”)
Weight	<5 kg (< 11 lbs)
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs (also available from Alere)

Alere NAT System (2)

Electric Power Requirements	Analyzer contains on-board rechargeable battery that provides a full work day (at least 8 hours) of operation.
Environmental Requirements	• Operating Temperature: 15º to 40º C (59º to 104º F) • Humidity: < 90% relative humidity • Maximum altitude: N/A (permissible atmospheric pressure: 850 to 1100 hPa)
Data Station	Dedicated CPU integrated into instrument; approximately 5,000 test results can be stored on the instrument archive; results can be downloaded via USB. Potential to install an SMS chip to transmit results or internal calibration data.
Monitor	Color touch screen integrated into instrument
Printer	Separate printer (prints on thermal paper); battery powered L 95mm x W 93mm x H 66mm, weight: ~350 grams, including paper roll.
Bar-code Scanner	Integrated into instrument for test cartridges only
Training	Minimal training required. Lay person can be trained in less than half a day. Primary skill required is for correct lancet blood draw.
Maintenance	If damaged, low cost and portability of device allows for direct swap-out replacement rather than on-site repair.
Internal QC	Yes
External QA	TBD whether compatible with EQA programs; cartridge cannot be retested to confirm results.
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	Touch-screen color display to enter patient information, view results, adjust settings, download results and navigate system software

Liat™ Analyzer

Type of Technology	Portable bench-top, real time PCR
Output	Viral Load (limit of detection ~50 cp/mL)
Turnaround time	30 - 55 minutes depending on limit of detection set (30 minutes for 500/1000 cp/mL)
Capacity	~8 - 15 samples per day, depending on limit of detection
Throughput per technician/ per day	~8 - 15 samples per technician per day; no batching capabilities on device.
Sample needed and stability	200 µL of plasma or 10 – 50 µL of finger-stick blood wicked directly into Liat tube.
Sample preparation and protocol complexity	No sample preparation required if using capillary blood. For capillary blood: (i) lancet finger; (ii) apply blood drops to Liat tube; (iii) scan the tube's bar code on the device; (iv) insert tube into Liat analyzer; (v) start device.
Reagent stability	Reagents expected to be shipped with an expiration date of at least 6 months; reagents must be stored at approximately 4º C (39.2º F)
Cost/test	TBD
Cost/instrument	TBD
Regulatory Status	TBD
Physical dimensions (W x H x D)	Width: 4 inches Height: 7 inches Depth: 7 inches
Weight	3.64 kg (~8 lbs)
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs

Liat™ Analyzer (2)

Electric Power Requirements	AC or battery powered
Environmental Requirements	• Operating Temperature: 15º to 30º C (59º to 86º F) • Humidity: N/A • Maximum altitude: N/A
Data Station	Dedicated CPU integrated into instrument; approximately 10,000 test results can be stored on the instrument archive; results can be downloaded via USB.
Monitor	LED color screen integrated into instrument
Printer	No printer provided
Bar-code Scanner	Integrated into instrument for tubes only
Training	Minimal training required. Lay person can be trained in about an hour. Primary skill required is for correct lancet blood draw.
Maintenance	Analyzer contains an internal optical system and controls that could be damaged. On site service/maintenance required.
Internal QC	Extensive internal controls: sample volume control, internal process controls, and more.
External QA	TBD whether compatible with EQA programs; cartridge cannot be retested to confirm results.
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	7 button keypad

SAMBA

Type of Technology	Isothermal target/signal amplification and visual detection; integrated extraction
Output	Qualitative for EID and semi-quantitative for viral load
Turnaround time	About 60 minutes
Capacity	4 samples per run anticipated
Throughput per technician/ per day	TBD
Sample needed and stability	200µL (plasma) or 100µL of blood
Sample preparation and protocol complexity	TBD
Reagent stability	SAMBA cartridge contains all required reagents/components for NAT and dipstick detection and may be stored at room temperature.
Cost/test	TBD
Cost/instrument	~$2,500 to $5,000
Regulatory Status	TBD
Physical dimensions (W x H x D)	Width: Height: Depth:
Weight	__ kg (~ lbs)
3rd party supplies	TBD

SAMBA (2)

Electric Power Requirements	AC powered; can be battery powered
Environmental Requirements	• Operating Temperature: • Humidity: N/A • Maximum altitude: N/A
Data Station	None
Monitor	None
Printer	No printer provided
Bar-code Scanner	None
Training	Minimal training required
Maintenance	
Internal QC	Synthetic non-target nucleic acid internal controls
External QA	TBD whether compatible with EQA programs; cartridge cannot be retested to confirm results.
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	

NWGHF P24 Antigen Rapid Lateral Flow Assay (EID)

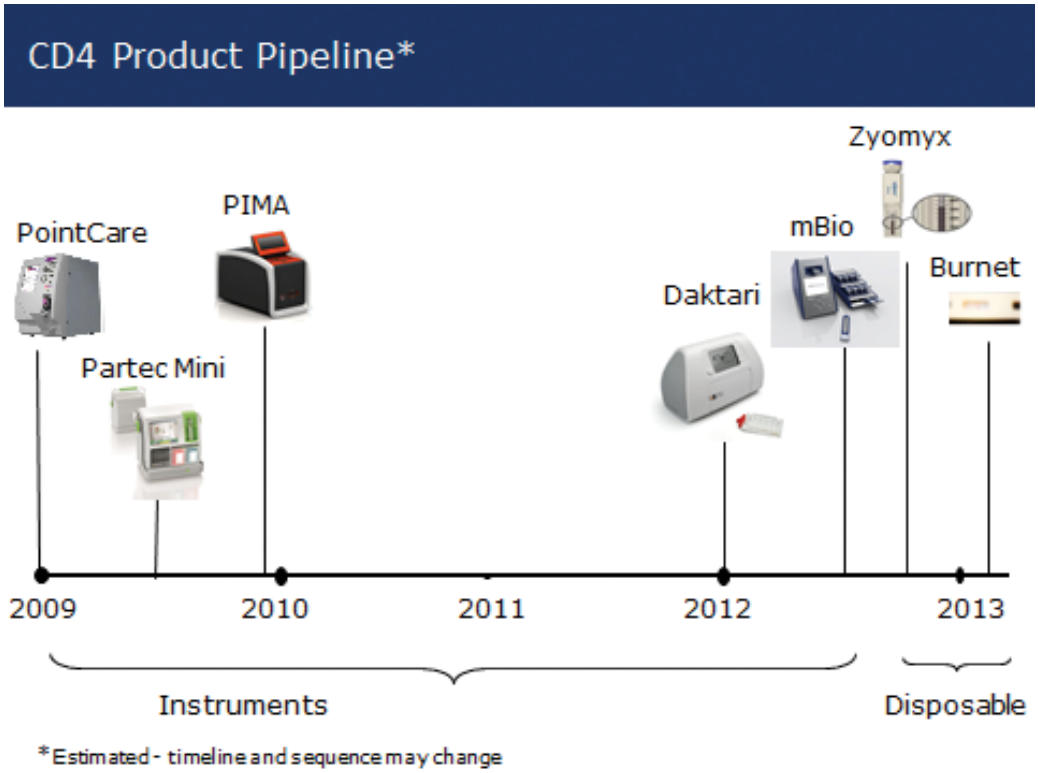
Type of Technology	P24 Antigen Assay for EID
Output	Detection of HIV infection
Turnaround time	30 minutes, including blood draw and sample preparation
Capacity	1 sample tested sequentially
Throughput per technician/ per day	~16 samples per day
Sample needed and stability	3 drops of blood from the infant's heel (~75 µL)
Sample preparation and protocol complexity	(i) Prick infant's heel and collect blood; (ii) separate plasma from red blood cells; (iii) add buffer and heat; (iv) insert test strip into heat block and wait 20 minutes; (v) read test.
Reagent stability	Reagents should be stored from 2º - 37º C; reagent kit shelf life is likely to be 12 months at launch, but will try to improve this to 18 or 24 months)
Cost/test	Estimated to be: $10 per test
Cost/instrument	~$150 for device
Regulatory Status	TBD
Physical dimensions (W x H x D)	Width: Height: Depth:
Weight	__ kg (~ lbs)
3rd party supplies	Sterile lancets (for blood samples); alcohol swabs, dry swabs, gauze, bandage

NWGHF P24 Antigen Rapid Lateral Flow Assay (EID) (2)

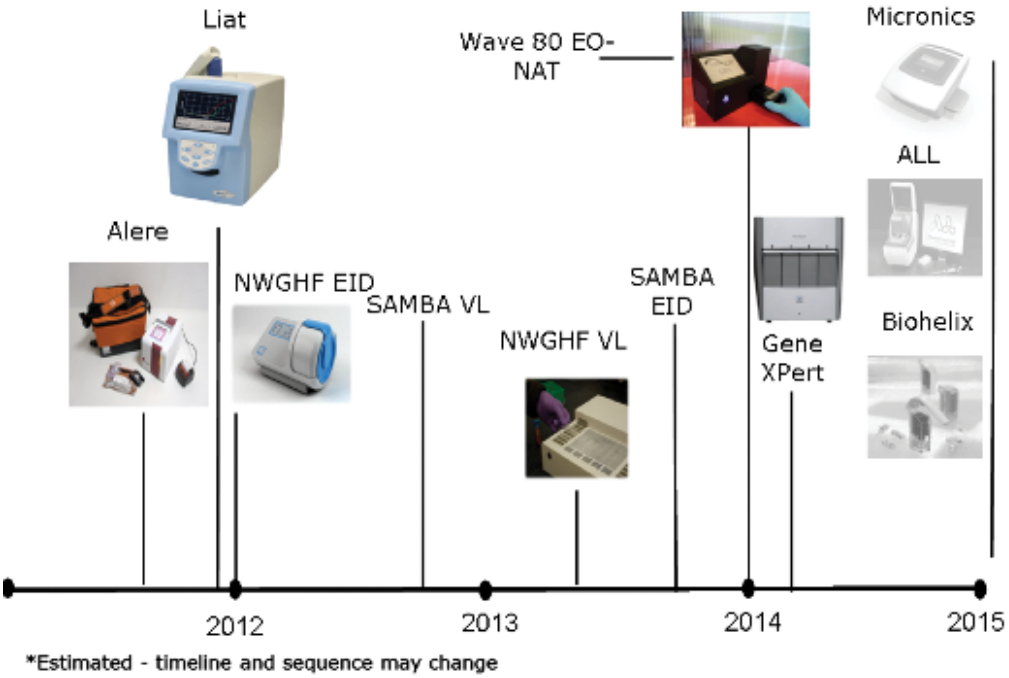
Electric Power Requirements	Heat block is battery powered
Environmental Requirements	• Operating Temperature: TBD • Humidity: TBD • Maximum altitude: TBD
Data Station	None
Monitor	None
Printer	No printer provided
Bar-code Scanner	None
Training	Minimal training required; primary skill required is for correct lancet blood draw.
Maintenance	Test is disposable; heat block is expected to last 2 years with original battery; life can be extended to 5 years if battery is swapped out.
Internal QC	Yes
External QA	TBD whether compatible with EQA programs; cartridge cannot be retested to confirm results.
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	

APPENDIX 2
CD4 and Viral Load Technology Pipelines

CD4 Platforms



Viral Load and EID Platforms



APPENDIX 3
Technical Specifications for HIV Qualitative Assays

Summary Main Characteristics of DNA PCR Qualitative Assays

Company	Roche	Roche	Abbott
Assay Name	AMPLICOR® HIV-1 DNA Test v1.5 (RUO)	COBAS® AmpliPrep / COBAS® TaqMan® (CAP/CTM) HIV-1 Qualitative (RUO)	Abbott RealTi*m*e RUO Qualitative HIV-1
Type of assay	PCR, Qualitative	Real Time PCR, Qualitative	Real-time PCR, Qualitative
Dynamic Range (copies/ml)	N/A	N/A	N/A
Contamination Control	Amperase	Amperase	Not reported
Controls	Run-in (neg.,pos) Internal Control	Run-in (neg.,pos) Internal Control	Not reported
Specimen Type	Whole Blood, Dried Blood Spot	Whole Blood / Dried Blood Spot	EDTA and ACD plasma
Specimen volume	100 µl whole blood (Infants) 500 µl whole blood (adults) 60 – 70 µl for DBS	100 µl Whole Blood 60-70 µl Dried Blood Spot	200µL plasma or DBS
Area of HIV genome amplified	Gag	Gag	Pol/INT
HIV-1 subtypes amplified	Group M, subtypes A-H	Group M, subtypes A-H	Group M, subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, and H), Group O and Group N
Time for result	7-8 hours	5-6 hours	5 hours
Cost/test[32]	$10 - $15 per test in resource-limited settings; $15 - $30 per test elsewhere	$12 - $16 per test in resource-limited settings; $16 - $30 per test elsewhere	$15 - $20 per test
Number of samples/run	9-21	22-66 Batch Loading (176 / 8 hour day Continuous Loading)	21-93 patient samples (+3external controls)
Equipment required[33]	Thermal cycler, ELISA Reader / Washer, Microcentrifuge Not supplied by Roche	COBAS® AmpliPrep with COBAS® TaqMan® 96 COBAS® TaqMan® 48	*m*2000rt; *m*2000sp, *m*24sp or manual simple preparation
Equipment Cost ($US)	~$25,000	COBAS TaqMan 48: $45,000 - $100,000 COBAS TaqMan 96: $80,000 - $150,000 COBAS AmpliPrep: $80,000 - $150,000	*m*24sp: $90,000, *m*2000sp: $120,000; or manual (magnetic racks, plate cooler): $500 and *m*2000rt: $38,000

The author notes no conflicts of interest.

[32] Prices will vary considerably depending on quantities, infrastructure and support required plus special negotiations.
[33] All assays require pipettes, vortex mixers (& refrigerator for all but Primagen).

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