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Diagnostic Technology Landscape

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This printing (August 2013) includes revisions from an earlier printing of this report (June 2013) as noted on pages 121-126.

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List of Abbreviations

AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
ART	antiretroviral therapy
AZT	zidovudine
BART	Bioluminescent Assay in Real-Time
bDNA	branched chain deoxyribonucleic acid
CAP	COBAS® AmpliPrep
CDC	Centers for Disease Control and Prevention
CRF	circulating recombinant forms
CROI	Conference on Retroviruses and Opportunistic Infections
CTM	COBAS® TaqMan®
DBS	dried blood spot
DDU	diagnostics development unit
DNA	deoxyribonucleic acid
DSP	digital signal processing
EDTA	ethylenediaminetetraacetic acid
EID	early infant diagnosis
ELISA	enzyme-linked immunosorbent assay
EQA	external quality assurance
FDA	Food and Drug Administration
FIND	Foundation for Innovative New Diagnostics
FSC	forward scatter channel
GBS	group B streptococcus
GSK	GlaxoSmithKline
GSM	Global System for Mobile Communications
HBDC	high-burden developing countries
HDA	helicase-dependent amplification
HIV	human immunodeficiency virus
HSV	herpes simplex virus
iNAAT	isothermal nucleic acid amplification test
IVD	in vitro diagnostic
LAMP	loop-mediated amplification
LTR	long terminal repeat
MRSA	methicillin-resistant staphylococcus aureus
MSF	Médicens Sans Frontières
MTB	mycobacterium tuberculosis
NASBA	nucleic acid sequence-based amplification
NAT	nucleic acid-based test
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NWGHF	Northwestern Global Health Foundation
PARC	Palo Alto Research Center
PCR	polymerase chain reaction
PEPFAR	President's Emergency Plan for AIDS Relief
PMT	photo-multiplying tubes
PMTCT	prevention of mother-to-child transmission
POC	point of care
QC	quality control
RIF	resistance to rifampicin
RNA	ribonucleic acid
RPA	recombinase polymerase amplification
RT	reverse transcriptase
RUO	research use only
SAMBA	simple amplification based assay
SBIR	Small Business Innovation Research
SMS	short message service
SSC	side scatter channel
TDF	tenofovir
UPS	uninterruptible power supply
URS	unitized reagent strip
USAID	United States Agency for International Development
USB	universal serial bus
VCT	voluntary counseling and testing
WBC	white blood cell
WHO	World Health Organization

HIV/AIDS DIAGNOSTIC LANDSCAPE

Executive Summary

There is growing demand within the global health community to find ways to simplify and improve the efficiency of diagnostics for HIV/AIDS without diminishing the quality of patient care. At the same time, 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.

Of the various tests required for initial diagnosis, staging, and ongoing monitoring of HIV, those that present the most persistent challenges to improved access and efficiency are CD4, viral load, and early infant diagnosis (EID). This report reviews both current diagnostic platforms and pipeline technologies for these three key tests. For each, the great majority of testing options available today are laboratory-based platforms performed on sophisticated instrumentation requiring dedicated laboratory space and trained laboratory technicians. In many cases, laboratory-based testing is expensive; in almost all cases, it requires sample transport networks to enable access for patients in peri-urban and rural settings.

Given the limitations of laboratory-based testing, it is generally accepted that in order to improve access to, and reduce the cost of, CD4, viral load, and EID testing in resource-limited settings, such testing needs to be brought closer to the point of patient care. This report therefore examines the new diagnostic technologies in the pipeline—most of which are designed for use at or near the point of patient care—and considers to what degree they meet the World Health Organization's (WHO's) "ASSURED" criteria, meaning that they are (or will be): Affordable, Sensitive, Specific, User-friendly, Robust/Rapid, Equipment-free, and Deliverable to those who need the test.

With respect to CD4 testing, which is used for staging and monitoring HIV patients prior to initiation onto antiretroviral therapy (ART), the general conclusion is that currently there are a number of good laboratory-based platforms using proven flow cytometry technology. These tests can be efficient and cost-effective when performed by well-trained laboratory technicians and when combined with good sample transport systems. However, in order to improve access, especially for rural patients, and to reduce patient loss to follow-up, there remains a need for high-quality, cost-effective point of care (POC) CD4 testing options. Three such options are already on the market, and several others are under development with anticipated release over the coming two years. At least one of these will be a disposable POC CD4 test. Assuming that the performance of these POC tests stands up to robust evaluation, the pipeline presents real promise.

With respect to viral load testing, which is primarily used for monitoring HIV patients following initiation onto ART, there are also a good number of sophisticated laboratory-based platforms on the market. However, despite the clinical consensus on the importance of viral load testing for detecting virological failure, access is very limited in resource-limited settings, with a few exceptions, including South Africa and Brazil. 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 viral load testing is considerably higher than CD4. Viral load testing that could be conducted at the point of patient care with assays meeting the ASSURED criteria would reduce the need for infrastructure and training, and could also lower the

cost of testing. Although there is currently only one POC viral load assay on the market in limited release, there are a number of platforms and assays in development, at least one of which may come to market in 2013.

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 performed on viral load platforms. 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, the cost of EID testing has come down, sample transport networks have been developed, and EID training has been implemented with funding from UNITAID and support from its implementing partners. As a result of these improvements and the urgent need for infant testing, there has been considerable uptake of EID. Access is far from universal, however, and the availability of EID at or near the point of care could improve access in harder-to-reach areas, decrease patient loss to follow-up, and bring down the cost of testing. Because viral load platforms can be used for EID, the new technologies in this testing area are viable options as well. In addition, there are at least two POC assays being developed specifically for EID. At least one of these may be launched in 2013.

Advances in access to tests for infant diagnosis, as well as for ART staging and monitoring are needed in resource-limited settings, and new technologies in the pipeline are likely to bring about significant changes in 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 (e.g., super-labs). Innovation is ongoing as well to facilitate the use of open polyvalent platforms, which can be more adapted to medium-size facilities in resource-limited settings.

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. At the same time, increased demand will require 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 and ensure the efficient return of laboratory results back 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.

Determining the optimal mix of centralized, high-volume diagnostics and POC diagnostics based on each country's unique needs is a challenge, but is central to ensuring efficient access to quality HIV diagnostic services in resource-limited settings. Strategic funding from UNITAID and others can help countries meet these challenges and accelerate the introduction of new diagnostic technologies, especially those designed for use at or near the point of care.

Introduction

In the interest of improving the accessibility and affordability of high-quality antiretroviral therapy (ART), there is a growing demand for simple, affordable, reliable, and quality-assured POC diagnostics for use in resource-limited settings. Many contend that POC diagnostics can make ART more scalable and will allow ART service delivery to be significantly decentralized to the community level. At the same time, simplifying diagnostic technologies may reduce the cost of diagnosing and monitoring HIV/AIDS patients without diminishing the quality of care.

In order to understand the benefits POC diagnostics may offer, it is necessary to understand the current diagnostic technology landscape. With an eye to maintaining high standards of patient care, it is also important to consider the future landscape of HIV diagnostics and what efficiencies might be achieved with respect to test algorithms, the cost of testing and decentralized service delivery, especially with respect to the introduction of diagnostics performed 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 access to testing is crucial in facilitating early detection and treatment of HIV/AIDS. This, in turn, will maximize the preventive impact of ART, and will help to ensure an appropriate and rapid response to drug resistance—a problem likely to grow substantially over the com-

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. However, the best technology mix is unclear in most countries and new models for delivery may also emerge. For now, it is essential that stakeholders, including ministries of health, UNITAID and other funders, trying to determine the most appropriate mix of investments to improve access to HIV diagnostics in resource-limited settings understand current diagnostic technologies and the pipeline for new products.

This report reviews the current technology landscape for HIV diagnostics, including (i) the algorithms and tests required in HIV/AIDS care and treatment, both before and after treatment initiation; (ii) the platforms used and price points of that testing; and (iii) the ways in which testing is delivered. With this information as background, the report then reviews the current technologies and diagnostic platforms in three key testing areas: 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 through sophisticated laboratory-based testing platforms, even in resource-limited settings. The report describes the POC and near-POC CD4, viral load, and EID platforms on the market and in the development pipeline, and considers the implications of the landscape, including what efficiencies might be achieved with respect to test algorithms, the cost of testing and decentralized service delivery.

Methodology

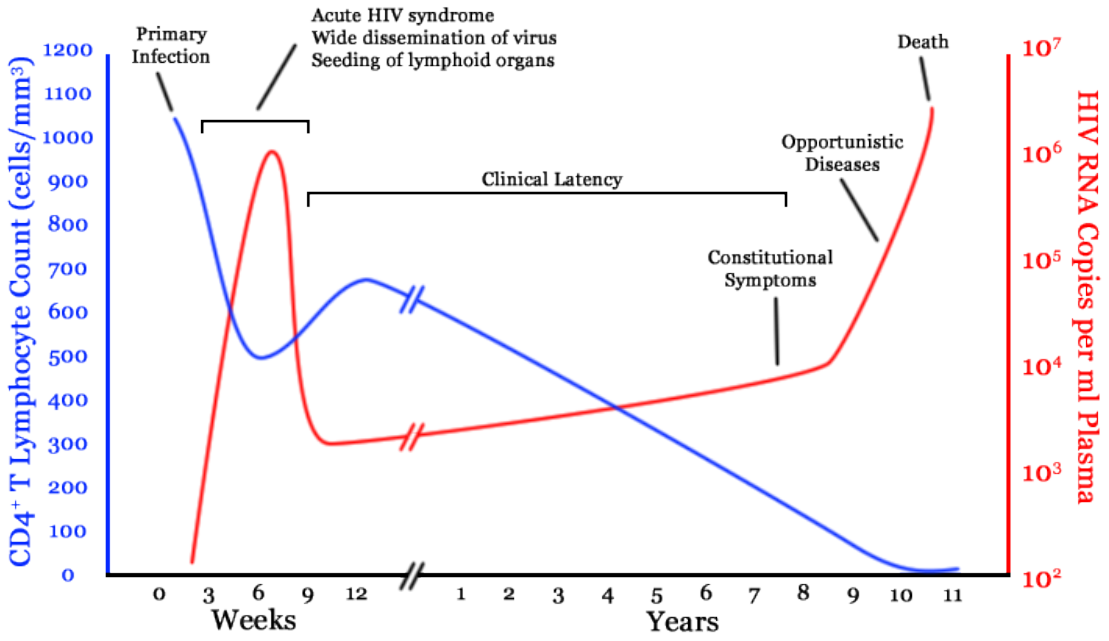
The HIV/AIDS Diagnostic Landscape is compiled by Maurine M. Murtagh with support from UNITAID. The material in this landscape was gathered by the author from publicly available information, published and unpublished reports and prospectuses, and interviews with developers and manufacturers. The prices for diagnostic equipment and reagents cited in this report were obtained directly from manufacturers and are ex works prices, meaning that they are the prices at the manufacturer's factory, and do not include any delivery, distribution or commission charges. The material is current through April 30, 2013.

Overview[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, known as the incubation period, can vary significantly from person to person. It is generally quite long (i.e., a number of years) as compared to the short period (i.e., days or weeks) common to many other viral infections (e.g., the common cold or influenza) [3]. 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 is presented below [4].

1 This section owes much of its content to data originally gathered by the author and the laboratory services team of the Clinton Health Access Initiative (CHAI) in 2010 and subsequently updated by the author. Portions of this overview 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 of information are referenced in the text.



Adapted from Pantaleo G, Graziosi C and Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus 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 initiate 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 ribonucleic acid (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 after initiation of 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.

Initial Diagnosis of HIV

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 when performed correctly. For patients older than 18 months of age, HIV rapid disposable tests, which use blood or saliva, are most commonly used for screening in decentralized settings without laboratory infrastructure. If the patient is positive for HIV/AIDS on the initial test, a second test is used to confirm the diagnosis. Generally speaking, in almost all resource-limited settings, the confirmatory test is also performed 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 usually an HIV rapid disposable.[3]

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.

HIV rapid tests generally come in the form of lateral flow strips or cassettes, which are convenient, self-contained tools for HIV serologic testing. They are relatively 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 - 25 minutes. The cost of these HIV rapid antibody tests in resource-limited settings, excluding any distributor mark-ups, ranges from about $0.50 per test to about $1.60 per test[4] for blood-based tests, but can be as much as $5.00 per test for saliva-based tests. ELISA testing is laboratory-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.[5]

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[6] 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 population, blood collection for the DNA PCR test has been decentralized to clinics, prevention of mother-to-child transmission (PMTCT) centers and the like. The infant's blood is collected on filter paper (known as dried blood spots [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 caregivers. Because this process can sometimes be slow, especially the return of results from laboratories, some countries have introduced short message service (SMS) printers (or other mobile technologies) in order to achieve markedly improved turnaround time for return of results from laboratory to collection sites.

DNA PCR testing can be run on either low-throughput or high-throughput instruments according to the needs in a given setting. The cost of a single instrument platform and related equipment (e.g., centrifuge, bio-safety cabinet, freezer, etc.) can range 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.

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]. 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 such cells. 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.[7] Clinicians therefore seek to routinely test an individual's CD4 count in order to monitor disease progression and to determine when an individual should be initiated onto ART. Per the WHO 2010 Guidelines on ART initiation,[8] if the absolute CD4 count of an adult or a child over 5 years of age is below a defined threshold (currently

4 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. As indicated earlier herein, 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 tests being developed), there is no instrument and the margin is used to cover the costs of importation, storage and handling.

5 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."

6 Viral load testing is discussed in more detail below in connection with monitoring the HIV+ patient who is on treatment.

7 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." [5]

8 It should be noted that the WHO is currently in the process of updating its guidelines on ART initiation and monitoring. The WHO 2013 guidelines are expected to be announced at the meeting of the International AIDS Society in Kuala Lumpur in June 2013.

less than or equal to 350 cells/mm³), ART should be initiated [5].[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/mm³ or at a %CD4 + T-cell ≤25, whichever is lower, irrespective of the clinical stage of HIV infection [5].

Whether in low- or high-throughput settings, CD4 testing is primarily conducted on laboratory-based instruments, although there are three POC CD4 test platforms currently available on 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 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 laboratory-based CD4 testing varies based on testing volumes, reagents used and whether testing is conducted on high- or low-throughput instruments. Generally speaking, the cost of CD4 reagents varies from a low of about $2.00 per test 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 $4.00 per test to about $12.00 per test for the test reagents alone, with associated sample collection consumables adding approximately $1.00 per test. The instruments cost from $6,500 to $25,000 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 onto ART immediately [5]. If a follow-up diagnosis proves negative, ART would be ceased. Countries are currently at various stages of adopting this recommendation.

Patient Monitoring

Prior to initiation onto 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 onto 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 many resource-limited settings.

Chemistry and Hematology Testing

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 of tests are considered essential according to recent WHO Guidelines, which generally base chemistry and hematology test recommendations on ART regimens. For example, for zidovudine (AZT)-containing regimens, haemoglobin measurement is recommended before initiation and at weeks 4, 8 and 12 after initiation, while for tenofovir (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, alanine aminotransferase (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 laboratory-based instruments. The cost of these platforms varies widely, 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

9 It is likely that the WHO 2013 guidelines will recommend earlier initiation of ART, at CD4 counts of ≤500 cells/mm³, but will prioritize treatment initiation at ≤350 cells/mm³. Further, it is likely that the WHO will recommend ART initiation independent of CD4 counts in certain categories of patients, e.g., pregnant women, sero-discordant couples, and children under 5 years of age. In the absence of CD4 testing, the WHO currently 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].

analysers 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 uninterruptible power supply (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 haemoglobin, as well as for fixed ranges of 3 to 6 chemistry parameters. These are mobile units, which cost approximately $1,000 to $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 healthcare 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.

Viral Load Testing

Finally, post-initiation onto ART, viral load testing should ideally be used to monitor patients, especially to detect early signs of virological 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 that recommend, where available, viral load testing be used in a targeted fashion to confirm treatment failure based on immunological and/or clinical criteria [6].[12] The WHO also recommends that, where routinely available, viral load testing should be used every 6 months to detect viral replication (i.e., to detect failure earlier than would be the case if immunological and/or clinical criteria were used) [6].[13] These recommendations stop short of urging all countries to implement viral load testing, and instead encourage its regular use only where such testing is routinely available [6]. It is expected, however, that in its 2013 guidelines, the WHO will recommend viral load testing as the preferred approach to monitor the success of ART and to diagnose ART failure in adults and children.[14]

At the present time, virtually all viral load testing is laboratory-based. Most testing is performed using sophisticated, high-throughput instruments. With the exception one POC platform available in Malawi only, there are no viable POC testing options currently available, although several are under development. Blood samples have to be collected and transported to central laboratories for viral load testing, and although DBS has recently been introduced for several of the viral load platforms, uptake has been limited.

One of the most important barriers to implementing viral load testing in resource-limited settings is the current high cost of testing, with prices for reagents and non-commodity test consumables averaging about $28 to $29 per test.[15] To put this in perspective, these costs are roughly 4 to 5 times greater than CD4 testing and

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 Per the WHO, treatment failure is currently deemed to occur at persistent viral load readings above 5,000 copies per milliliter. 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 [6].

13 It is 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 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].

14 The WHO 2013 guidelines will also likely strongly recommend the use of viral load testing for detection of virologic failure and/or confirmation of treatment failure in those patients with evidence of clinical and/or immunological failure. It is expected that the WHO 2013 guidelines will also modify the threshold for treatment failure with such failure being deemed to occur at persistent plasma viral load readings (2 tests within 3 months) at >1,000 copies per milliliter (and at ≥3,000 copies per milliliter for viral load readings based on DBS). Further, it will likely be recommended that viral load monitoring start at 6 months following ART initiation, with an additional test at the 12-month mark. Subsequent viral load testing will be recommended every 12 months thereafter.

15 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.

do not include the large upfront investment required to establish viral load-ready laboratories and purchase instruments for testing. Instruments themselves generally cost from about $100,000 to $225,000, including installation and training. In addition, collection consumables and laboratory consumables for viral load testing currently are not bundled and must be purchased separately by users. These items add approximately $2.75 per test and $1.50 per test, respectively, to the cost of viral load testing.

Factors to Consider in Diagnostic Platform Selection

As discussed above, rapid assays for detecting the specific HIV antibody are accurate when used correctly, are low cost and are readily available for use at the point of care. Because chemistry and hematology testing is generally symptom- or regimen-based in HIV care, and because there are already a number of technologies available for use at the point of care, these tests do not represent a significant barrier to accessing HIV care and treatment. Of the various tests required for initial diagnosis, staging, and ongoing monitoring of HIV, the tests that present the most persistent access challenges today are CD4, viral load, and EID. Increasing the availability of high-quality POC technologies for these tests has the greatest potential to improve HIV treatment staging and monitoring, as well as disease diagnosis for children under 18 months of age.

This report focuses on CD4, viral load, and EID testing and examines: (i) the underlying technologies used for each test, (ii) the laboratory-based and/or POC platforms currently available, and (iii) the POC technologies in the pipeline for each test category.

Before discussing the diagnostic platforms in depth, it is important to review the operational characteristics of diagnostic platforms/devices that should be considered when choosing platforms appropriate for a given setting [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, the size and weight of the 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 analyser itself (e.g., data station, printer, bar-code scanner);
- Training required;
- Availability of quality control (QC) reagents and compatibility with external quality assurance (EQA) programs; and
- Recommended location for use (e.g., hospitals, clinics, etc.).

These operational characteristics are set out in **Appendix 1** for each of the platforms currently available for CD4, viral load, and EID testing, and, where sufficient information is available from the developer, for each such platform in the pipeline.

In addition to the operational characteristics of the various platforms/devices, it is also important to consider the performance of the platform, i.e., the ability of the technology to give accurate and reproducible results. Both the accuracy and precision of a quantitative test should be evaluated.[16]

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

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 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 is determined by the closeness of results 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+ T-Cell Counting Technologies

CD4 Performance

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

Neither correlation nor Bland-Altman plots alone are sufficient measures of CD4 assay accuracy. Misclassification probabilities provide more clinically relevant information, 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 that would measure above the ART initiation threshold when using the reference test.[17] 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 conducted a systematic review of the available literature on CD4 performance and concluded that it is difficult to draw clinically relevant conclusions 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 downward misclassification, is likely to be underestimated since none of the studies in the literature is restricted to the most clinically relevant range.

The most important considerations for CD4 performance are [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; when available, data are not robust enough to give a clear idea of the comparative merit of different technologies; and

17 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.

- Given the potential for error described above, access to QC reagents and participation in 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.

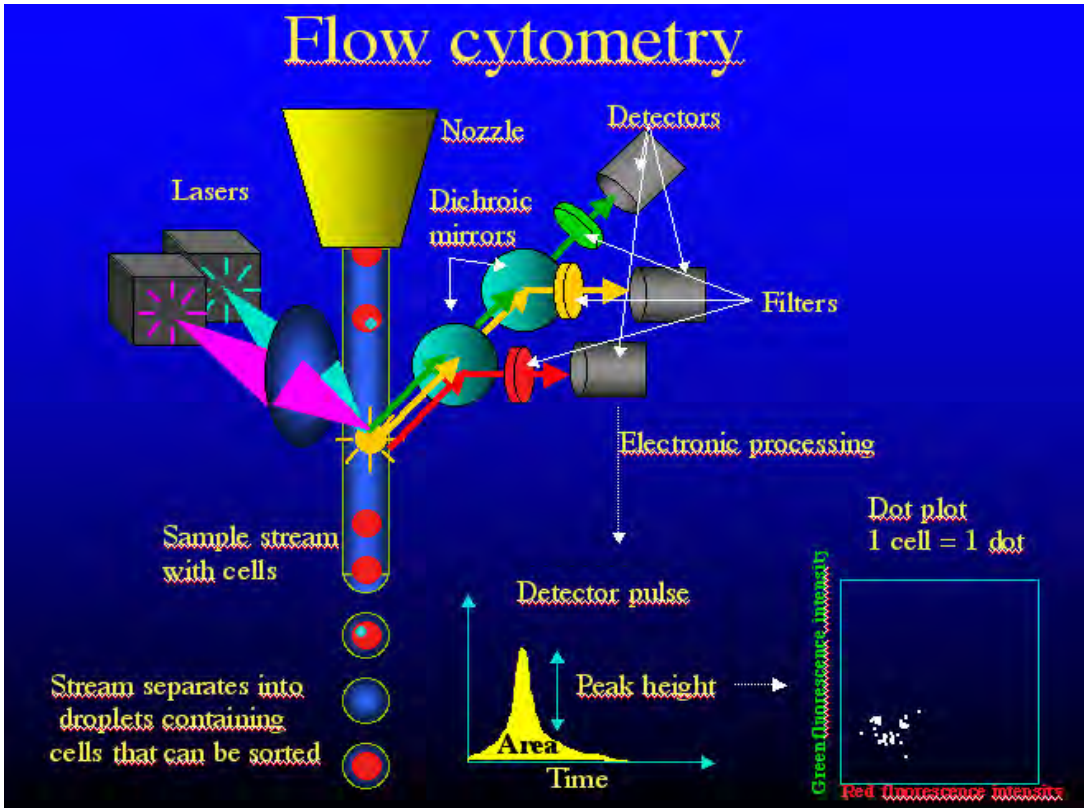
Introduction to Flow Cytometry

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 BD Biosciences, Beckman Coulter, Partec, and Millipore.

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 analysed. 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]. 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 is presented below.

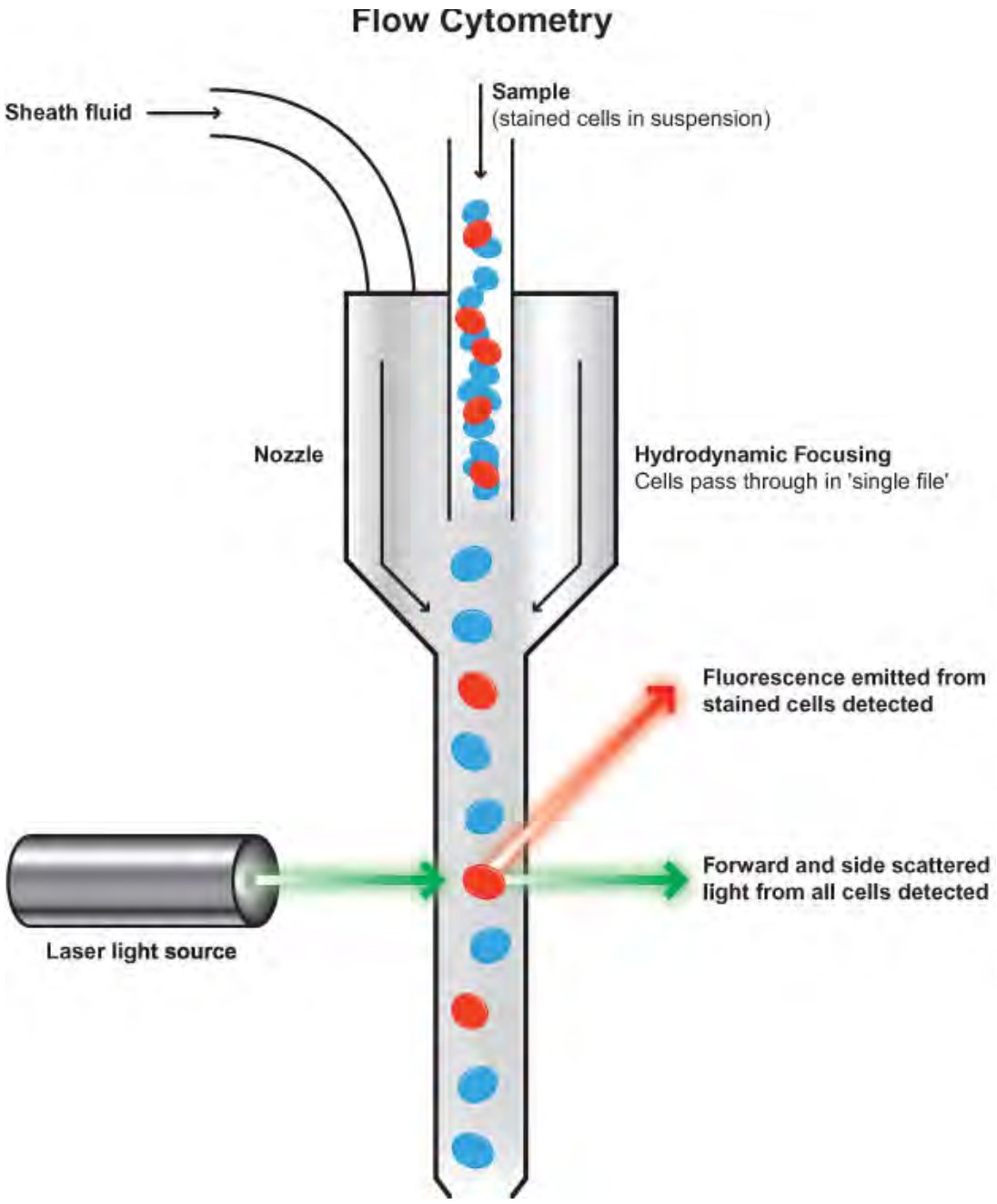


Schematic courtesy of Jan Grawé, BioVis, Uppsala University; http://www.rudbeck.uu.se/node47.

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) 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 then interacts with the cells. Typically, cells are ejected through the flow chamber at a rate of about 1,000 cells per second [24].



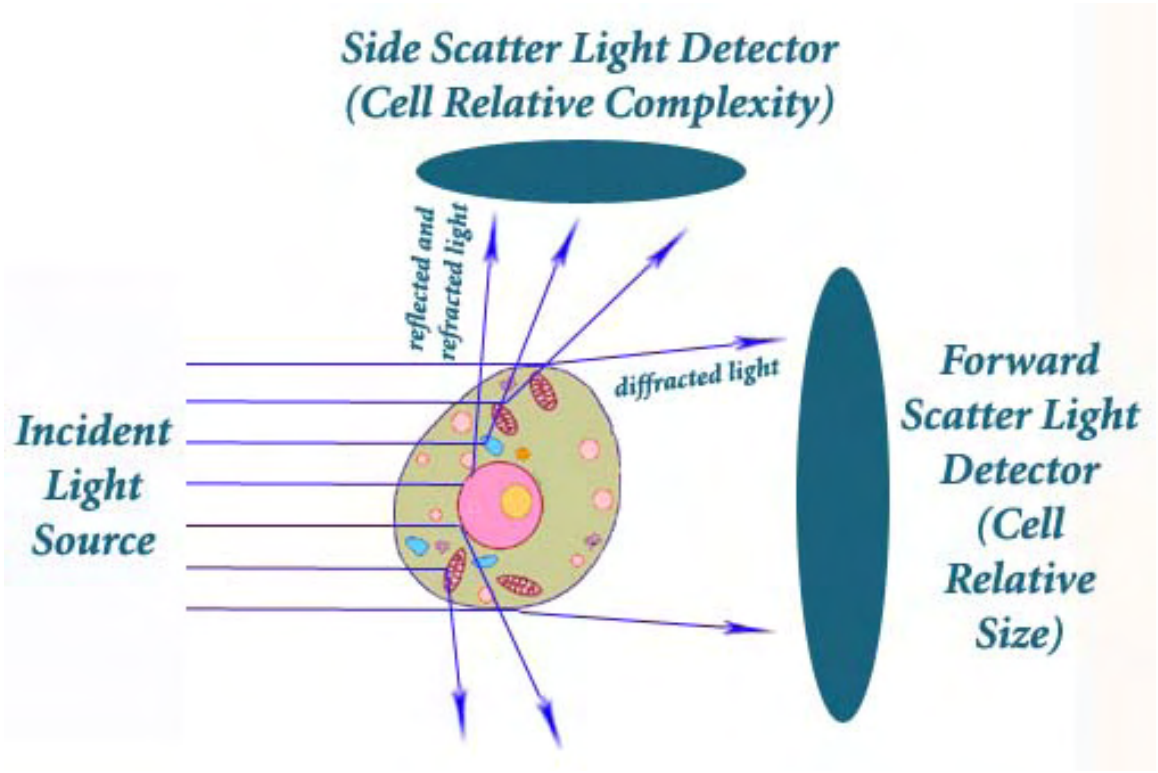
Schematic courtesy of Abcam PLC (http://www.abcam.com).

Optics System

Flow cytometry optics 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 special 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 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).

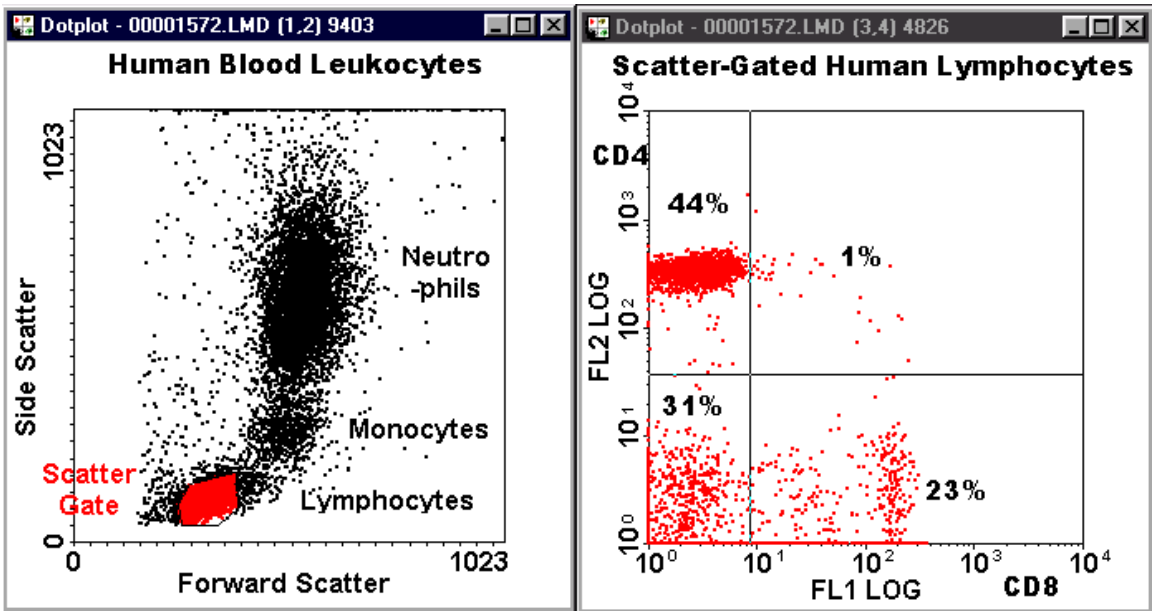
Finally, as the laser interrogates the cell, fluorochromes on or in the cell (either intrinsic or extrinsic) may absorb some of the light and become excited. As those fluorochromes leave their excited state, they release energy in the form of a photon with a specific wavelength, longer than the excitation 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 then processes that light signal and converts 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 [25].



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

Existing CD4 Technologies/Platforms

There are currently a handful of platforms that account for virtually the entire market share for CD4 testing in resource-limited settings. These are laboratory-based single platform systems 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.[18]

However, before considering these platforms in depth, it is important to note that there are other methods of CD4 enumeration available on 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 analyser. 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 analyser. 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 [26]. 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 [27,28,29]. 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 analysers (without the need for a microscope). For example, Dynabeads can be used in conjunction with the POCHi-100

18 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.

hematology analyser 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 analyser 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 lymphocytes 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 and Partec, each of which uses volumetric methods.

Some of these single platform systems, including the BD FACSCalibur and the Coulter Cytomics FC 500, 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 Cytomics platform. Cytognos beads (from Cytognos SL) can be used on Coulter Cytomics FC 500 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 are closed systems, including the FACSCount, Millipore-Guava Auto CD4/CD4% and PointCare NOW platforms. This means that they can only use reagents manufactured by the platform manufacturer; reagents from other manufacturers are not inter-changeable.

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

High-throughput CD4 Systems

Both BD and Coulter manufacture open platform, high-throughput flow cytometry systems: the BD FACSCalibur™ Flow Cytometer and the Coulter Cytomics FC 500™ MCL or Cytomics FC 500™ MPL, 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 Biosciences)*

BD Biosciences 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 [30]. 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 perform a number of routine tasks, including both absolute CD4 counts in cells/µL, which is the international standard for such measurement, and %CD4 counts (using BD TruCount reagents); it can also perform 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.

As discussed earlier, although most experts agree that there is no true "gold standard" for CD4 testing, many consider the FACSCalibur system to be the reference 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 [31]. 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.

Cytomics FC 500™ MCL or Cytomics FC 500 MPL™ System *(Beckman Coulter, Inc.)*

Like the BD FACSCalibur, the Cytomics FC 500 MCL and Cytomics FC 500 MPL Systems[19] (pictured below), manufactured by Beckman Coulter, are large, bench-top flow cytometers. These systems are automated and can simultaneously analyse up to 4 colors of immunofluorescence from a single laser. The Cytomics FC 500 series platform (with either MCL or MPL sample loading capability) is a bead-based system that can perform absolute and percentage CD4 counts (using FlowCARE™ PLG 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.

19 The Epics XL and XL-MCL are being slowly phased out by Beckman Coulter over the next 4-5 years; the company will fully support these platforms during that period. The Cytomics FC 500 MCL (Multi Carriage Loader) replaces the Epics XL-MCL. In addition, the Cytomics FC 500 MPL contains a multi-platform automated loading system, which allows the platform to serve ultra high volume laboratories doing more than 500 samples per day.

The Cytomics FC 500 system automates many of the steps involved in quality control and flow cytometric analysis, which were previously required to be done manually. In addition, the system contains 2 lasers (an air-cooled Argon ion laser and an air-cooled Helium-Neon ion laser) and can measure 5-color antibody combinations from a single or dual laser excitation in a single tube, which enables laboratories using the system to reduce the number of tubes and overall costs. In addition, the system offers state-of-the-art Digital Signal Processing (DSP) for reliable linearity and drift-free amplification and compensation.

Like the FACSCalibur system, the Cytomics FC 500 system is relatively easy to use and provides walk away automation. The MCL system has a carousel that may be loaded with up to 32 tubes, each to be run automatically; while the MPL cytometer loads a 40-tube rack and plate loader (i.e., it has the ability to process samples using either 96-well microtiter plates or tubes, depending on the application or workflow). Like the Epics system, the Cytomics FC 500 system is a high-volume (on average, 47 samples per hour, or about 375 samples per day, with the MCL, and more than 500 samples per day with the MPL and the Coulter CellMek automated preparation system), high-performance system that is geared for use in busy reference laboratories where, in addition to CD4 counting, it can be employed for other analyses, including diagnosis of acute and chronic leukemias, lymphomas and platelet disorders, among others.

Assuming certain test volume commitments, the cost of the Cytomics FC 500 MCL instrument is about $90,000; with the addition of the CellMek system, the cost is about $100,000. For the basic FlowCare PLG 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.

Currently, thirty-five CellMek/Cytomics FC 500 MPL system instruments have been placed in Namibia, Zambia, and South Africa. Although no independent published peer reviewed articles were found evaluating the Cytomics FC 500 system against comparable systems for CD4 testing, there is an article looking at the positive impact of the system as used in a clinical research laboratory in Canada [32].

The CyFlow® Counter *(Partec GmbH)*

The CyFlow® Counter from Partec GmbH is a portable, compact desk-top flow cytomer designed for routine CD4 and %CD4 counting (as well as total lymphocyte and white blood cell [WBC] counting) in a single, dedicated platform (pictured below on the left)[20]. 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 laboratory-based systems like BD FACSCalibur and Coulter Cytomics.

20 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.

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 throughput 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 2012, Partec has made available on the CyFlow Counter a detailed, on-screen video operations manual that covers set-up, instrument operation, instructions on how to perform the CD4 and CD4% assays, basic maintenance instructions, etc.

Because 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 and laboratories at the provincial and district level [33]. 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 company has placed more than 1,800 instruments in-country, which provided more than 3.9 million patient tests (both CD4 and %CD4) in 2012.

The cost of the CyFlow Counter instrument alone is about €16,850 (~$22,220), 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. Absolute CD4 reagents cost approximately €1.75 (~$2.30) per test, while %CD4 reagents for pediatric use cost approximately €2.50 (~$3.30 per test). Discounts on reagent pricing are available with bulk procurement.

Published, peer-reviewed literature is available on performance of CyFlow [34,35].

Medium- to Low-throughput CD4 Systems

BD FACSCount™ System *(BD Biosciences)*

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. 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 assay (pictured below).

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 fewer 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 has 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 of testing per annum per instrument. The 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 up to $10.00 per test for test volumes up to 4,500 tests per instrument per annum.

BD FACSClearCount™ System *(BD Biosciences)*

BD Biosciences is in final development stages for the BD FACSClearCount™ system (pictured below), the next generation CD4 dedicated system, measuring both absolute counts and percentage CD4.

The BD FACSClearCount system includes the instrument, integrated software, a sample prep workstation, dedicated reagents, and whole blood controls. Dedicated reagents are provided in a new dried format, in ready-to-use cartridges. Reagents include both fluorescently-labeled antibodies for the identification of CD4 T-cell populations, and counting beads for simultaneous CD4 T-cell enumeration. The counting beads are also used for daily instrument quality control. Dried-down reagent technology eliminates the need for a cold chain, simplifying storage and reducing costs. The reagents have been designed to meet the requirements of a wide variety of temperature settings.

To simplify the workflow, a carousel holds 20 innovative two-tube reagent cartridges with reagent and beads in one tube and patient sample in the other. In standard operation mode, the instrument automatically prepares and acquires the sample—the precise sample volume is pipetted into the cartridge tube containing the air-dried reagent and beads. Following incubation, the lysing solution is added and the sample is acquired. Manual steps are eliminated to improve workflow. Test results including absolute and percentage CD4 counts, are provided on-screen, and can be printed using the on-board thermal printer. They can also be exported using the front access universal serial bus (USB) port and provided USB flash drive.

The BD FACSClearCount uses an integrated software and touchscreen interface to further simplify use and reduce operator training time. The interface is straightforward—users simply need to touch a button to navigate to and execute a function. All actions are run from the touchscreen. Touchscreen software is available in the following six languages: English, French, Portuguese, Russian, Simplified Chinese and Spanish.

The cost of the FACSClearCount instrument is expected to be about $38,000. Pricing for reagents depends on volume of testing per annum per instrument. The general range of pricing for the reagents alone ranges from approximately $4.50 per test for test volumes of more than 10,000 tests per instrument per annum up to $12.00 per test for test volumes up to 4,500 tests per instrument per annum. The FACSClearCount is now expected to launch in early 2014.

Aquios CL™ *(Beckman Coulter, Inc.)*

In July 2013, Beckman Coulter expects to launch its Aquios CL™ flow cytometry platform (pictured below), with what the company calls Load & Go™ simplicity. The platform is targeted at laboratories that need to automate the most routine, repetitive tests, such as absolute CD4 and %CD4. The system features automated sample preparation with all sample preparation and analysis performed in 96-well mircoplates. In addition, the platform automatically launches each testing protocol, dispenses and mixes applicable reagents and requires no manual pipetting or decapping of primary tubes.

The Aquios CL processes samples continuously; batch processing of samples is not required. The sample loader holds up to 8 cassettes at a time with up to 5 sample tubes each (i.e., total capacity is 40 sample tubes), and allows for continuous loading and unloading. The first test results are available approximately 20 minutes after loading the sample. In addition, the Aquios CL is preloaded with a range of barcoded reagents and consumables and automatically scans barcodes to track reagents, lot numbers, open and closed vial expiration dates, etc. There is continuous tracking of reagent usage by product. This tracking means that there is no need for manual QC or reagent logs, and if QC fails, the operator is notified via text message or email.

The Aquios CL system, which is a bench-top platform with a relatively small footprint, features an all-in-one computer and monitor with touch-screen operation. There is also an alternative keyboard and mouse. Data analysis is performed via advanced automated algorithms with the option of user-adjustable gates and regions.

Future applications that aid in the diagnosis, monitoring and treatment of diseases are pending.

Millipore-Guava® Auto CD4/CD4% System *(Merck)*

The Guava® Auto CD4/CD4% system (pictured below), manufactured by Millipore (a division of Merck), 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 reduce 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 increases 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 up to 100 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. On average, the cost of the Guava instrument is approximately $20,000. The pricing for the reagents (combined CD4 cell count, CD4% and total lymphocyte count) is $2.50 per test (including the distribution margin), regardless of volume.

With respect to the performance of the Guava system, the 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 Cytometer *(Apogee Flow Systems)*

The Apogee Auto40 Flow Cytometer, manufactured by Apogee Flow Systems and pictured below, is a bench top, volumetric flow cytometer 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 90 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 analyse difficult or damaged samples. The cost of the Apogee Auto40 is about $27,000. The pricing for reagents is approximately $2.50 per test for absolute CD4 counts and $3.50 per test for percentage CD4.

Several peer-reviewed studies of the Apogee Auto40 platform have been published [40,41].

Point of Care CD4 Testing Platforms

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 and FACSCount, are used in developed as well as resource-limited 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. 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. Point-of-care CD4 testing is likely to require new, simpler technologies. Both instrument-based and disposable tests are in the CD4 development pipeline. Generally speaking, such POC CD4 tests would preferably meet the ASSURED criteria for the ideal rapid test, which was developed by the WHO [42]. 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. Three of these technologies are already on the market: Point-Care NOW™, the Pima™ CD4 Analyser and the CyFlow™ CD4 miniPOC. The remaining technologies discussed, including those from Daktari, Omega, Zyomyx, MBio and others, are not yet available on the market.

The current CD4 POC pipeline is presented in **Appendix 2**. It is interesting to note that since this report was first published in 2011, the companies in the CD4 POC pipeline have not changed. However, the expected date of market introduction for each of the platforms has been delayed. While in 2011, several platforms were expected to be introduced in 2012, these same platforms are now expected to be launched in mid- to late-2013 and early 2014. These delays generally reflect the technical challenges of developing CD4 platforms for use at the point of care, and in some cases, reflect the difficulty of obtaining funding for developing these products.

PointCare NOW™ *(PointCare Technologies, Inc.)*

The PointCare NOW™ system (pictured below) was developed by PointCare Technologies, Inc. specifically for decentralized and low-resource settings. It is a compact, tabletop system that measures CD4 absolute count and %CD4, WBC count and haemoglobin, 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[21] 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.

21 The label consists of anti-CD4 antibodies coupled with nano-sized gold particles.

PointCare reports that by the summer of 2013 it expects to introduce a validated system of internal QC that entirely does away with the requirement for EQA controls, materials that are both perishable and impractical in remote settings. PointCare also expects to introduce a new version of the PointCare NOW instrument that provides a printed-out warning to clinicians and care-givers when patients have "out-of-range" hematology results and urgent clinical action is required. This upgrade also will provide simplified on-screen instructions to the instrument operator when the sample-run shows abnormalities or when the clinician should be warned about urgent concurrent conditions.

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, which includes PointCare's heat stable Daily Check controls, is approximately $10 per test.

A peer reviewed evaluation of the PointCare NOW™ platform was published in 2012. The review found that the instrument had low sensitivity in adults, misclassifying 53% and 61% of patients at the 350 and 200 cells/μL thresholds, respectively; while sensitivity was better for children, the authors concluded that the sample size was not large enough to draw a conclusion [43].[22] The company concluded a method-comparison with BD's FACSCalibur in March 2011 at the National Microbiology Reference Laboratory (NRL), Harare, Zimbabwe. The results from this evaluation in Zimbabwe as well as the results of an evaluation conducted at military clinics in Uganda are expected to be published in the near future.

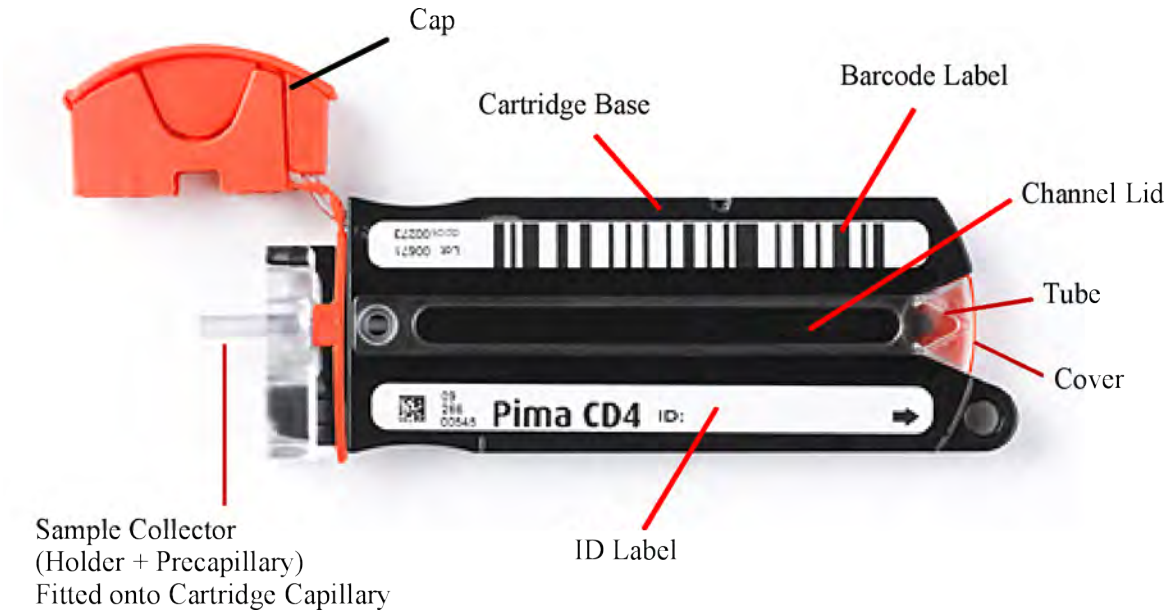
22 Sensitivity to identify children in need of ART using a 25% CD4 threshold was 90% and sensitivity was 100% using a 750 CD4 cells/mm³ threshold.

Pima™ Analyser *(Alere Inc.)*

The Pima™ Analyser (pictured with printer, below) is a small, portable bench-top, fixed volume cytometer manufactured by Alere Inc. The Pima employs the same immunological principles as existing CD4 enumeration systems combined with static image analysis and counting technology, in a compact, portable and robust housing. A separate printer is also available.

The Alere Pima CD4 system is made up of the Analyser and a disposable CD4 test cartridge (pictured below) which contains dried reagents. As such, it is a closed system with no compatible third party reagents available. The system is capable of measuring absolute CD4 counts in whole blood, but it cannot currently determine %CD4 counts. This capability could be added to the system, along with other cell type counts.

Venous blood or capillary blood derived from a fingerprick are both acceptable samples. There is no requirement to measure the volume of blood used in the test; the cartridge is designed to take up 25 µL of blood in a self-regulated manner, eliminating the need for calibrated volumetric pipettes. Once the sample is applied to the cartridge it is irreversibly capped and inserted into the analyser. The dried reagents, including fluorescently labeled anti-CD3 and anti-CD4 antibodies, are re-dissolved in the sample and allowed to incubate before the sample is passed into an optical imaging chamber. Once capped, all test steps are actually performed within the sealed cartridge and no part of the Pima Analyser comes into contact with the blood sample during processing, thus minimising the risk of analyser contamination.



The Analyser is equipped with miniaturized, multi-color fluorescence imaging optics. Fluorescence images are collected by an on-board camera and analysed using proprietary software algorithms on the embedded computer to derive absolute CD4 counts. Up to 1,000 test results are stored in an on-board archive. Operator ID, sample ID, date, time, CD4 count and the outcome of numerous internal controls are stored with every test result. Data can be viewed on the on-board display, printed onto archival thermal paper with the accessory Pima printer, or exported by the operator at any time after the test has been completed. Export can be to a USB memory stick, and Alere has also launched an optional USB connectivity module for sending data to central servers via mobile telephone networks. A LAN connectivity solution is also available. A power extender, including an extended life battery and adaptors for charging sources, including solar panels and mains, has been added to the product family.

The system can perform approximately 20 tests per day (3 tests per hour) with minimal operator interaction – walk away testing. As a simplified, low-throughput POC system, Pima can be used appropriately at all levels of the healthcare system where high throughput is either not required or for use in situations where same-day results are particularly important, even in high-volume settings.

The CD4 Analyser/Pima has been pre-qualified by the WHO, is CE-IVD marked, and has performed well in an evaluation done by the United States Centers for Disease Control and Prevention (CDC). Alere is in the process of submitting a 510k for the product with the FDA and approval is expected in 2013. At least ten peer-reviewed, independent evaluations of the Pima system have been published since product launch (see, for example, references 44-48). In these studies the Pima CD4 system was tested in laboratory as well as non-laboratory settings such as rural voluntary counseling and testing (VCT) sites and mobile healthcare units. The studies also incorporate diverse geographies, including Asia and sub-Saharan Africa, as well as diverse operators, including physicians, laboratory technologists, nurses and lay healthcare workers. Despite the different settings and study objectives, the results all demonstrated very good correlation with the predicate flow cytometry technologies, even when performed at the point of care on capillary blood obtained by fingerstick, although one study did find that the coefficients of variation were slightly larger with fingerstick than venous blood and the authors noted that this may have been related to insufficient training of operators [47]. One of the published studies demonstrated, for the first time, the positive impact point of care CD4 testing can have on patient retention and ART initiation. The study authors concluded that "point of care CD4 testing enabled clinics to stage patients rapidly on-site after enrolment, which reduced opportunities for pretreatment loss to follow-up. As a result, more patients were identified as eligible for and initiated antiretroviral treatment." [48]

The cost of the Pima Analyser varies among countries and regions, with prices ranging from approximately $6,500 to $12,000, and the cost per test ranging from approximately $6.00 to $12.00. The instrument requires

no routine preventive maintenance, and Alere has established a global technical support network to ensure fast, reliable and consistent service.

CyFlow® CD4 miniPOC *(Partec GmbH)*

Partec has introduced a very compact, portable CD4 counter, the CyFlow® CD4 miniPOC (pictured below) that uses the same basic technology as the CyFlow® Counter, i.e., 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 technological range of CD4 absolute counts from 0 CD4 cells/µL to 5,000 CD4 cells/µL and CD4 percentages from 0 to 100%. 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.

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.

Results can be displayed in routine or expert modes (illustrated below on the left and right, respectively). The expert mode features a histogram with the display of cell clusters thus offering an additional built-in quality control.

As it did for the CyFlow Counter, since 2012, Partec has made available on the CD4 miniPOC instrument a detailed, on-screen video operation manual that covers set-up, instrument operation, instructions on how to perform the CD4 and CD4% assays, basic maintenance instructions, etc.

The cost of the CD4 miniPOC instrument is approximately €7,100 (~$9,380). The system uses the same dried reagents as its larger sibling, the CyFlow Counter, but in different packing that includes all required consumables at a total cost of €3.00 (~$3.96) per test kit, which yields both absolute CD4 and %CD4 results. On occasion, the company also offers special point-of-care packages at price savings for the instrument and reagents.

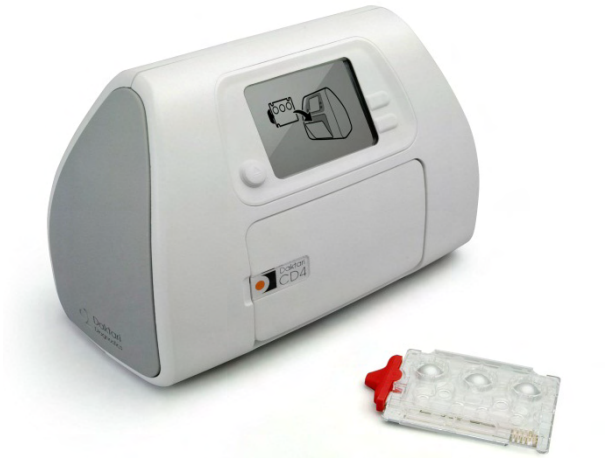
To date, no peer-reviewed, independent performance evaluations of the Partec CD4 miniPOC device were found in a literature review.

CD4 Technologies in the Pipeline

The following CD4 diagnostics are still under development and have not yet been introduced into the market. Expected evaluation and/or launch timelines are provided for each product.

Daktari™ CD4 Counter *(Daktari Diagnostics, Inc.)*

Daktari Diagnostics, Inc. is developing a portable and robust CD4 device, the Daktari™ CD4 Counter (pictured below with its associated cartridge). The Daktari system will be capable of other assays, which may include full blood counts, CD4 percentages, and bacterial and viral diagnostics. The CD4 system has not yet been launched, but performance evaluations are underway, with market launch expected in 2013.



Intended for use at the point of patient care, the Daktari system eliminates sample preparation through the use of a technology known as "microfluidic cell chromatography," which isolates cells and other particles in a miniature sensing chamber. No pipetting, labels or reagents are required; the only user step is to apply a drop of whole blood to the cartridge. 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 10 minutes.

The Daktari CD4 system will include a data management system with a keypad user interface and a back-end data package that will come built into the device.

The anticipated cost of the Daktari CD4 counter is less than $5,000 for the device. Per test cost is anticipated to be approximately $8.00, but volume discounts are expected to drive the price lower. 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 published performance data available for the Daktari CD4 system.

MBio CD4 System *(MBio Diagnostics, Inc.)*

MBio Diagnostics® Inc. is developing a robust and simple diagnostic system for cellular analysis and multiplexed immunoassays in peripheral labs, clinics, and at the point of care. The MBio CD4 System (pictured below) consists of a software-driven reader and single-use disposable cartridges that provide quantitative measurements and "lab-quality" results within the timeframe of a patient visit. The MBio CD4 System (the System) supports multiple assay formats, including whole blood cellular analysis and multiplexed immunoassays, providing flexibility, a product pipeline, and cost profile the company believes is unique in the POC market segment. The System has been designed specifically for applications in resource-limited settings.

System features include:

- Product pipeline: The first cartridge product will deliver absolute CD4 count, with following cartridge releases providing CD4% and haemoglobin on the same system. Pipeline products include immunoassays for HIV and opportunistic infections such as syphilis, viral hepatitis and tuberculosis.
- Sample throughput: Cartridges can be processed in parallel (batch mode) using a separate cartridge rack with automatic timing. One operator with one system can process 10 samples per hour, or approximately 80 samples per day.
- Time-to-result: Turnaround time for a single sample is 20 minutes, with a >1 hour read window.
- Connectivity: The System includes integrated Ethernet and wireless connectivity.
- Sample-to-Answer: Capillary or venous whole blood, serum, or plasma are loaded directly into the cartridge. After a timed incubation, the cartridge is inserted into the reader for automated analysis. There are no additional assay steps, or user interactions.
- Cartridge: All assay reagents are integrated into the cartridge; there are no separate buffers or peripheral bottles to be managed. The disposable cartridge is a simple, robust design with no pumps, valves, or complex fluidic features.
- Storage Stability: Integrated, lyophilized assay reagents and device packaging have been designed to ensure environmental stability (heat and humidity) during transport and storage in resource-limited settings. There is no cold chain storage requirement.
- Internal QC: Every cartridge incorporates multiple internal QC features for every sample run, including sample volume, reagent quality, reader function, and cartridge lot expiration.
- EQA: The System is compatible with internationally accepted EQA materials used for CD4 system proficiency testing.
- Biohazard and safety: Blood and assay fluids stay on the sealed device, minimizing biohazard handling.
- Technology: The Reader is a proprietary two color fluorescence imaging device with results based on immunostaining and image analysis. The novel design capitalized on the robustness and low cost of modern consumer electronic components, such as cell phone cameras and DVD lasers.

The System includes an on-board computer for sample analysis, results management, internal QC and event logs that can be exported in common and viewable file formats for data review. The user interface is an intuitive touch-screen with administrator-configurable settings such as user lockout/validation and QC scheduling. Cartridge

barcodes will be read automatically, and the instrument will have multiple USB ports to support printers, external barcode readers, and other peripherals. The System will include integrated GSM/GPRS connectivity.

Pre-market field evaluations in sub-Saharan Africa were initiated in 2012 and will continue during the first half of 2013. In-country clinical trials are scheduled for the second half of 2013. A CE-IVD mark on the MBio CD4 System is anticipated in 2013 followed by product launch.

BD FACSPresto™ *(BD Biosciences)*

BD Biosciences is developing an image-based counting technology suitable for resource-limited settings that will provide CD4 absolute count, %CD4, and haemoglobin (Hb) all on the same single-use disposable cartridge. The platform will be called the BD FACSPresto™. Features of the automated device (shown below) include touch screen user interface, technician-friendly operation, flexible workflow with high throughput, integrated micro-printer, battery or solar-powered capability, and data archive/transfer capabilities.

BD FACSPresto™ from BD Biosciences. Photo source: BD Biosciences.

The sample is collected from the patient using a fingerstick or an ethylenediaminetetraacetic acid (EDTA) tube. The cartridge is self-contained and is inserted by the operator into the device. After a short incubation period, detection takes place automatically and the result can be read immediately in a single, easy step. The new and innovative cartridge technology contains dried reagents and requires no-cold chain, which enables longer shelf life over a wide range of environmental conditions. Market launch is expected in late 2013.

Zyomyx CD4 Test *(Zyomyx, Inc.)*

Zyomyx, Inc. 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, mixer/spinner (either battery operated or mechanical) 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 and can be read with the help of a lens, which is contained in the mixer/spinner. The Zyomyx system has not yet been launched; market introduction is to take place in 2013.

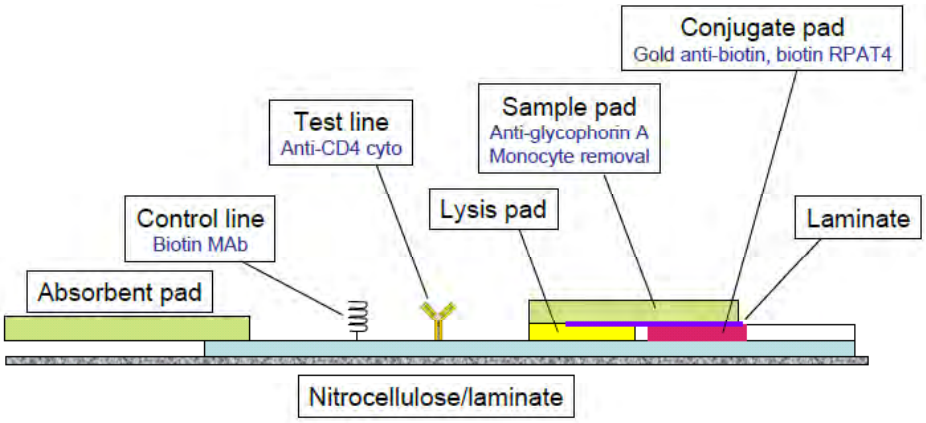
The anticipated cost of the Zyomyx assay is estimated to be less than $8.00 per test. The company expects that, with a minimum purchase volume of cartridges (still be to be determined), there will not be a separate cost for the mixer/spinner. The mixer/spinner is capable of performing a test preparation in less than 10 minutes and will support at least 10,000 tests. If additional throughput is required, it can be increased by adding an additional mixer/spinner at nominal cost.

There is currently no performance data available for the Zyomyx system; clinical trials are in process.

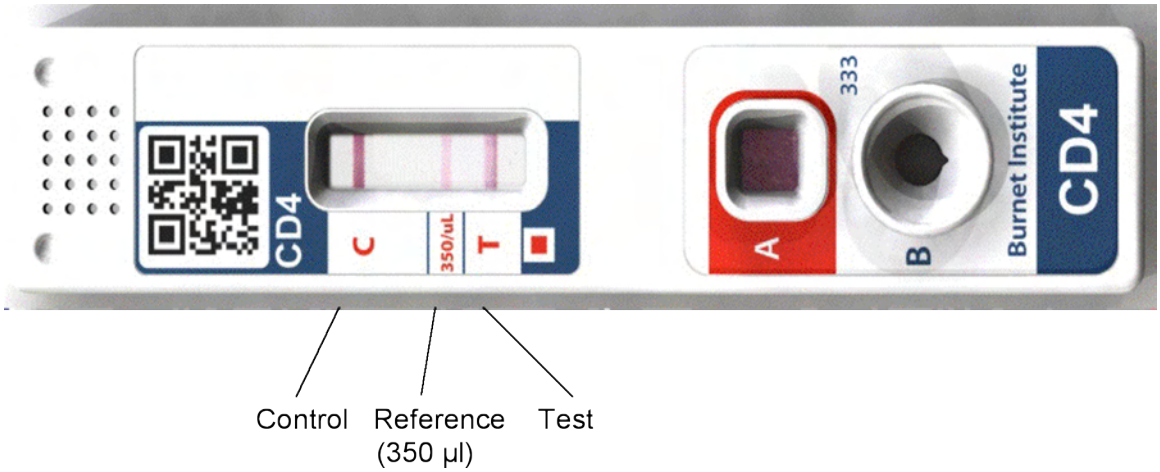
Visitect CD4 *(Burnet Institute and Omega Diagnostics Ltd.)*

Burnet Institute (Burnet) has licensed its semi-quantitative CD4 technology to Omega Diagnostics Ltd. (UK). The platform, which is now called the Visitect CD4, is a rapid, disposable semi-quantitative CD4 test. 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 the CD4 + T-cell count. Burnet used a laboratory-based test (ELISA) as proof of concept, which supported this hypothesis.

Following proof of concept in ELISA format, the Visitect CD4 test has been 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 (illustrated below). The Visitect CD4 is expected to be available for commercial release in the third quarter of 2013. At release, the cost of the test is expected to be about $5.00.



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 (pictured below, top), Burnet developed a reader for the device (pictured below, bottom), which also provides data storage and connectivity options, as well as real-time operating instructions for the test devices. The reader, which has been developed in collaboration with Axxin Ltd. (Australia), is expected initially to cost about $3,000, but may decline to about $2,000 over time. The reader will be provided free of charge dependent on committed volumes.



Evaluation of the prototype version of the test at the 350 CD4/μL cutoff at the Burnet and Alfred Hospital, Melbourne, has shown 97% sensitivity for samples below 350 CD4/μL and 80% specificity for samples above 350 CD4/μL (total n = 126). Clinical validation trials of the Visitect CD4 are planned to follow in the UK, the United States and Southern Africa in the first half of 2013.

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 at The Palo Alto Research Center (PARC) (including Peter Kiesel, Joerg Martini, Markus Beck, Malte Huck, Marshall Bern, and Noble Johnson) 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 [49,50].

The development of this technology is in the research stage and funding is needed to take it further.

Conclusions—CD4 Testing

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, 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, more than one of which are likely to become available in 2013. The current options available for POC CD4 testing are device-based, but disposable CD4 testing is on the near-term horizon. To date, with the exception of extensive evaluations of the Pima Analyser™, the performance data for POC CD4 platforms are limited. It is anticipated that as more POC CD4 testing devices are introduced, the results of independent clinical evaluations by the CDC (United States), 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 Testing and Implications for 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, post-initiation onto ART, 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 still 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 [51]. In this context, since viral load testing is a better indicator of treatment failure, the value of routine CD4 testing drops.

Despite a possible move towards test and treat approaches to HIV care and treatment and 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 onto 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 per 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 infectious 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 or all POC-based.

Viral Load Testing Technologies

Overview

As discussed earlier in this report, viral load testing is the method favored for monitoring HIV patients once they have been initiated onto 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 [52].

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 virological failure in patients. Although still being used, especially in resource-limited 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 percent [53,54,55].

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-limited 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. However, it is expected that in its 2013 guidelines, the WHO will recommend viral load as the preferred approach to monitor treatment success and diagnose ART failure.

Viral Load Testing Complexities

The first molecular assay for quantifying HIV viral RNA was approved by the United States Food and Drug Administration (FDA) in 1999. Since then, a number of assays have been developed and will be considered here in some detail. First it is worth considering some of the complicating factors that characterize viral load assays

and platforms and that should inform the choice of 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 [52]. 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 [56,57]. In an ideal world, viral load assays would detect and quantify all known HIV-1 subtypes (as the Cavidi ExaVir assay can do today), as well as inter-subtype recombinants and emerging variations thereon. But, 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.[23] Each room should have minimal dust and preferably will be temperature controlled (air conditioned in hot climates). 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 to 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 healthcare 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, recently, several studies have demonstrated good correlation between the two using different viral-load methodologies, with sensitivity ranges close to 3 log

23 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.

HIV-RNA copies/mL [58,59].[24] In a review of viral load monitoring technologies, MSF notes that: "given that the DBS technique is currently the only means of sample transport over long distances and without the need for cold storage, it will be important for manufacturers of laboratory-based tests to validate their platforms for use with DBS." [63]

Existing Viral Load Technologies

HIV viral load technologies can be categorized broadly as nucleic acid-based test (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 for laboratory use are detailed below.

Nucleic-Acid Based Technologies	
Type	**Assay Name**
RT-PCR	COBAS® Taqman v 2.0 (Roche Diagnostics)*
	Abbott RealTime HIV-1
	VERSANT® HIV RNA 1.0 (kPCR) (Siemens)
	artus™ HIV-1 QS-RGQ (QIAGEN)
NASBA	NucliSens EasyQ® HIV-1 v2.0 (bioMérieux)
bDNA	VERSANT® HIV-1 RNA v3.0 (Siemens)
Non-Nucleic-Acid Based Technologies	
Type	**Assay Name**
Reverse Transcriptase	ExaVir Load version 3.0 (Cavidi)
p24 Antigen	HIV-1 p24 Ultra ELISA (Perkin Elmer)

* Note that the COBAS® AMPLICOR HIV-1 MONITOR™ v1.5 (the MONITOR assay) from Roche Diagnostics is no longer being sold by Roche except to current customers using the COBAS AMPLICOR Analyser, which is still being supported by Roche, but is no longer available for sale from the company.

NAT-Based Technologies

NAT-based assays have become the core viral load monitoring technology used in both developed countries and resource-limited settings. 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) [52]. Currently, the bulk of commercially available viral load assays are based on target amplification.

24 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 currently considers to be the measure of virological failure. Therefore, for diagnosing virological failure, the poor correlation may not be a problem [60,61]. 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 [62].

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 analysed 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 [52]. 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, Abbott and QIAGEN assays and nucleic acid sequence-based 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) [52]. 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 using these approaches 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-based assays and platforms are discussed below.[25]

Platforms Based on RT-PCR

Currently, there are four commercially available RT-PCR based viral load assays: (i) COBAS® AmpliPrep/COBAS TaqMan v2.0 (Roche Molecular Systems), (ii) RealTime HIV-1 (Abbott), (iii) VERSANT HIV RNA 1.0 (kPCR) (Siemens), and (iv) artus™ HIV-1 QS-RGQ (QIAGEN). There are also a number of in-house procedures and test systems that have good sensitivity and reproducibility and are used in various countries[26], but which will not be described in detail in this report.

Roche COBAS® AmpliPrep/COBAS® TaqMan® System *(Roche Molecular Systems)*

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 Molecular Systems currently manufactures a single real-time PCR assay, the COBAS® AmpliPrep/COBAS® TaqMan® version 2.[27] The assays use the AmpliPrep instrument for automated viral nucleic acid extraction and the COBAS TaqMan analysers (TaqMan 48 or TaqMan 96), both of which are discussed below, for automated amplification and detection of the viral nucleic acid target.

25 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.

26 One example is the Generic HIV Viral Load assay from Bio-Centric (France), which is for research use only. 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 ranges from approximately $10.00 to $20.00.

27 Roche has globally discontinued manufacture of version 1 of the COBAS® AmpliPrep/COBAS® TaqMan® assay.

The COBAS AmpliPrep/COBAS TaqMan version 2 test was designed specifically to address HIV-1 mutations. In order to do this, a dual-target approach is used. The dual-target technology provides additional confidence in results in the event of mutation. The assay is able to co-amplify two target regions of HIV-1 (known as the gag and long terminal repeat [LTR] regions), which were specifically chosen as they are not current HIV drug targets. By targeting both regions of the genome simultaneously, the test increases the probability of detection of virus particles.

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. The assay can be run using DBS in addition to plasma specimens, 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 COBAS AmpliPrep/COBAS TaqMan HIV-1 Test version 2 for TaqMan 48 and TaqMan 96 is prequalified by the WHO. The test is also FDA approved for plasma, but is "research use only" (RUO)[28] for use with DBS. Performance of the test has proven to have good correlation with the AMPLICOR HIV-1 MONITOR™ v1.5 assay (the MONITOR assay), which has generally been considered to be the gold standard [64].

The cost per test for the least developed countries and certain high-burden middle income countries is about $11 to $25. Actual pricing is dependent on variables such as outright instrument purchase, reagent rental and volume-based, tiered pricing arrangements.

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 analysers 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. The run size for the instrument is 24 specimens, but it can process up to 72 samples at any given time. The first 24 samples take 2 hours to process. However, because the instrument allows for parallel processing, subsequent batches of 24 can be completed every hour as one rack of specimens will begin processing before the previous rack processing has been completed. 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 $100,000 (with the lowest pricing reserved for lower income countries).

Roche TaqMan Analysers—Roche manufactures two versions of its TaqMan Analyser, the COBAS® TaqMan 48 Analyser and the COBAS® TaqMan 96 Analyser. Each of the analysers 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.

28 The RUO (Research Use Only) designation is required by the 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.

The cost of the COBAS TaqMan 48 Analyser is approximately $40,000 to $50,000.

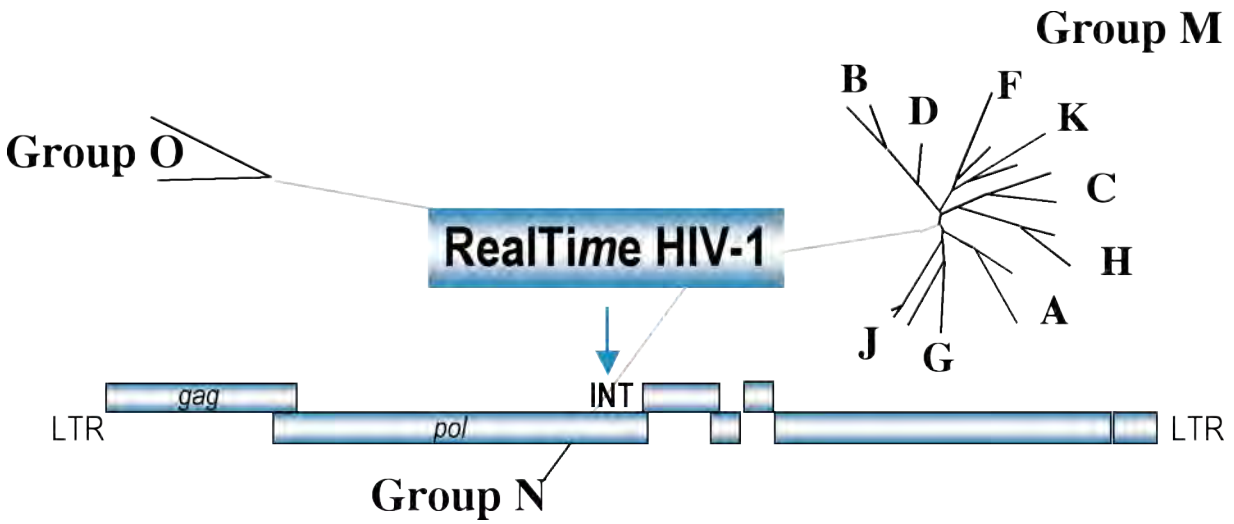
In contrast to its smaller sibling the TaqMan 48, the COBAS TaqMan 96, pictured below, is a large instrument, weighing about 450 pounds.[29] It also has higher capacity and can run up to 96 samples at a time in a run time of approximately 180 minutes with automated transfer from the COBAS AmpliPrep via a docking station.

The cost of the COBAS TaqMan 96 Analyser is approximately $100,000 to $110,000. This price includes a docking station.

Abbott *m*2000 System *(Abbott Molecular)*

Abbott Molecular manufactures the Abbott RealTi*m*e 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.

29 In addition, Roche provides the COBAS p630 instrument for use with the COBAS AmpliPrep/COBAS TaqMan System, which provides a fully automated pre-analytical solution for primary tube handling. The instrument will de-cap and cap sample tubes, pipette Roche controls from control tubes to sample tubes, and pipette samples from primary tubes to sample tubes. The COBAS p630 also provides sample traceability (using bar-code tracking from primary tube to result) and process surveillance (through liquid handling monitoring). In addition, the device transfers samples, controls and order information to AMPLILINK Software.



The Abbott RealTi*m*e assay can be automated using the Abbott *m*2000sp (or *m*24sp) for sample preparation and the *m*2000rt 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 RealTi*m*e 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 0.6mL input and 150 copies/mL for 0.2mL input. Performance has been assessed with good results [65]. 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 Abbott RealTi*m*e HIV-1 assay has been pre-qualified by the WHO. The price per test of the assay ranges from $25 to $40 and is dependent on volumes as well as any negotiations with Abbott.

Sample Preparation with the *m*2000 System—The Abbott RealTi*m*e 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 that 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 *m*24sp is approximately $90,000.

The *m*2000sp—The *m*2000sp by Abbott (pictured in the center of the image, below), is a larger and more automated sample preparation device than its sibling, the *m*24sp. 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 instruments, 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.

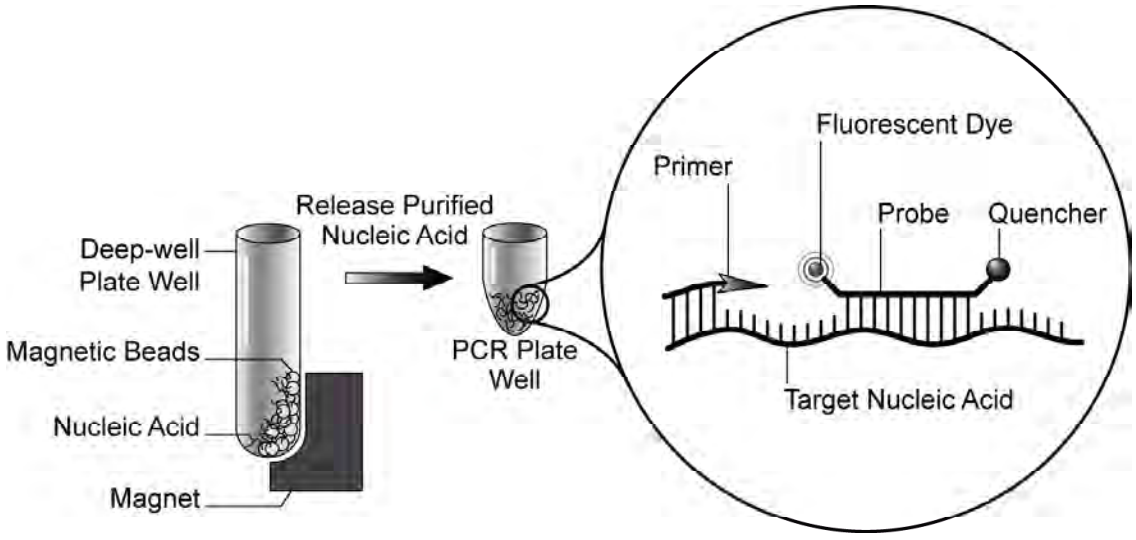
The cost of the *m*2000rt is approximately $38,000 when purchased with the *m*24sp or *m*2000sp, but about $44,000 if manual extraction is used.

VERSANT™ kPCR Molecular System *(Siemens Healthcare Diagnostics, Inc.)*

The VERSANT™ kPCR Molecular System and the VERSANT® HIV RNA 1.0 Assay (kPCR) are manufactured by Siemens Healthcare Diagnostics, Inc. Because they are CE-IVD marked, but not FDA approved, they are only available outside of the United States. The Siemens HIV assay 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 a "one-room" technology with no need for clean room operations due to closed-tube processing.

Photo courtesy of Siemens Healthcare Diagnostics.

The Sample Preparation module along with the VERSANT Sample Preparation 1.0 Reagents Kit are used to extract RNA from plasma. The reagents kit includes proprietary magnetic silica beads that provide for efficient and high-quality extraction of nucleic acids. 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 this 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 highly conserved region of the pol integrase gene. A schematic representation of the assay principle is shown below.



Schematic courtesy of Siemens Healthcare Diagnostics.

The VERSANT kPCR Molecular System processes samples in batch mode in a 96-well format. The HIV assay provides patient results for up to 89 samples 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 [66]. Performance of the assay is comparable to its competitors [67].

The VERSANT HIV-1 RNA 1.0 assay has been pre-qualified by the WHO.

The artus™ HIV-1 RG/QS-RGQ RT-PCR System *(QIAGEN N.V.)*

QIAGEN has recently introduced a real-time RT-PCR-based assay for HIV, the artus™ HIV-1 RG/QS-RGQ RT PCR kit. The assay is CE-IVD marked and targets the LTR (long terminal repeat) region of the genome. The kits can be used in combination with either a manual (artus™ HIV-1 RG RT-PCR kits) extraction and sample preparation system (QIAamp® DSP Virus Kit) or an automated (artus™ HIV-1 QS-RGQ RT-PCR kits) extraction and sample preparation system (QIAsymphony™ SP/AS). The assay must then be run on one of the QIAGEN Rotor-Gene Q thermocyclers for amplification and detection. An example of a complete QIAsymphony RGQ system is pictured below.

The artus HIV-1 QS-RGQ assay has a linear range of 45 HIV-1 RNA copies/mL to 45 million copies/mL (using automated extraction) and can detect HIV-1 group M (subtypes A-H) down to a limit of detection of approximately 35 copies/mL. The time to result is about 5 to 6 hours for 24 samples. Performance of the artus assay has been evaluated and is comparable to that of the Abbott RealTi*m*e system [68].

QIAsymphony® SP/AS

Sample preparation for the artus HIV assay may be conducted manually using the CE-IVD marked QIAGEN QIAamp® DSP Virus Kit, which provides silica-membrane-based RNA purification using a vacuum process. Fully-integrated automated sample preparation and assay setup is also available using the QIAsymphony SP/AS instruments. The QIAsymphony SP can process 1 to 96 samples (in batches of 24) with sample volumes up to 1 mL. It is a ready-to-run instrument that requires minimal installation. The SP can be combined with the

QIAsymphony AS device in a fully-integrated system that can automate the entire workflow. To reduce manual handling and minimize the risk of sample contamination, samples processed on the SP can be transferred automatically to the AS, or the two instruments can be operated independently.

The SP/AS system includes touchscreen controls, bar code-labeled sample tubes containing pre-filled reagents, and allows for continuous loading in batches of up to 24 samples plus internal controls. The QIAsymphony SP/AS instruments can also be integrated in laboratory information management systems. In addition to HIV, the artus panels for QIAsymphony Rotor-Gene Q (RGQ) include assays for the hepatitis B and C viruses, plus a transplantation/immunosuppressed panel, with assays for detection and quantification of cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1 and 2, varicella-zoster virus, and BK virus.

The Rotor-Gene Thermocycler

The artus HIV assay can be run on the real-time PCR thermocycler RGQ. The RGQ has a unique centrifugal rotary design in which each sample tube spins in a chamber of moving air, which keeps all samples at precisely the same temperature. As each tube aligns with the detection optics in the device, the sample is illuminated and a fluorescent signal is quickly collected. QIAGEN indicates that this results in sensitive, precise and fast real-time PCR analysis and eliminates sample-to-sample variations and edge effects, which are unavoidable in traditional block-based instruments. The Rotor-Gene Q can be ordered with the Rotor-Gene AssayManager software for molecular diagnostics that automatically analyzes real time PCR data of artus assays.

NASBA Platform

NucliSENS HIV Solution (*bioMérieux*)

The NucliSENS HIV solution is manufactured by bioMérieux. The NucliSENS EasyQ® HIV-1 v 2.0 assay targets a well-conserved region of the gag gene and is based on NASBA®. Following sample extraction with proprietary magnetic BOOM® technology, the highly efficient real-time NASBA® amplification reaction ensures very sensitive test results in only one hour.

NASBA is an isothermal transcription-based amplification method which amplifies RNA from an RNA target. The amplicons produced through this process are detected in real time by molecular beacons, which are hairpin-shaped molecules with an internally quenched fluorophore whose fluorescence is restored upon binding to a target nucleic acid [69]. Kinetic analysis of the fluorescent signals reveals the transcription rates of both the HIV RNA target and a calibrator RNA added during the extraction step. This transcription rate is used to determine the quantity of HIV-1 RNA in the original specimen.

The linear range of the EasyQ HIV assay v 2.0 is from 10 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 [70,71].

The average price of the EasyQ HIV assay v 2.0, including extraction and amplification/detection, is about €18.00 (~$23.75) per test, and the assay for use on either the semi-automated or automated systems described below is pre-qualified by the WHO.

NucliSENS® miniMAG® and NucliSENS® easyMAG® Extraction Systems—The NucliSENS® miniMAG® and NucliSENS® easyMAG® extraction instruments make up part of the NucliSENS HIV solution, but can be used for any other molecular diagnostic assay requiring the purification of nucleic acids from clinical samples.

The miniMAG (pictured below) is a small, semi-automatic extraction device for both DNA and RNA in various specimens. It uses proprietary magnetic silica based beads Boom® technology. (See easyMAG for the principle of the Boom® technology).

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 is about €6,800 (~$9,000).

For higher throughput needs, the easyMAG is an automated benchtop nucleic acid extraction device that is able to perform 24 extractions in as little as 40 minutes (and offers the possibility to extract different samples types, to be used in several applications, in the same run). The instrument (pictured below) 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 uses magnetic silica based beads and is based on Boom® technology. The average price of the easyMAG instrument is approximately €72,000 (~$95,000).

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; the instrument is pictured below. 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 analyser 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 require only 60 minutes.

The average price of the analyser is approximately €37,100 (~$49,000).

NucliSENS Connectivity—bioMérieux also provides NucliSENtral™, which is an integrated software system that can be used to link NucliSENS® easyMAG® and NucliSENS EasyQ® with a Laboratory Information System.

bDNA Technology

Versant™ 440 Molecular System *(Siemens Healthcare Diagnostics, Inc.)*

Siemens Healthcare Diagnostics, Inc. 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 is 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 analyser and has a linear range of 50 to 500,000 copies/mL; it can detect HIV-1 Group M (subtypes A through G). The performance of the assay correlates well with that of the Roche AMPLICOR assay [72, 73].

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. Like the VERSANT kPCR Molecular System, this 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 analyser has a relatively compact footprint.

Photo courtesy of Siemens Healthcare Diagnostics.

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.

ExaVir™ Load *(Cavidi AB)*

Cavidi manufactures the ExaVir™ (Version 3), which is a quantitative HIV-RT test that is designed to measure viral-bound 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 and washed 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. It is a manual assay performed with standard ELISA equipment as well as the ExaVir Separation equipment. The latter is provided by the manufacturer (pictured below).

The ExaVir Load assay is more manual than most of the other viral load assays described herein, but it is generally less expensive than other current molecular detection methods. Samples are processed in batches of 30. A total of 180 samples can be run during a five-day week. The total time to result for 30 tests is 48 hours, which

includes 5 hours of hands-on time for the operator. The remaining time is used for incubations. The hands-on time per test is comparable to running some of the automated NAT-technologies.

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 to 3,000 femtograms/mL). There is performance data available on the ExaVir Load showing good correlation with the AMPLICOR assay [74,75].

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 analyse results, the ExaVir Load Analyser software is required (supplied with the first order) as well as a computer with Microsoft Excel® and Adobe® Reader®.

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 not gained 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 antigens 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 [52]. 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 [76]. 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 [76]. 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.

Viral Load Technologies in the Pipeline

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.

Currently there is only one POC viral load assay in limited use on the market. However, there are a number of additional platforms/assays in development, more than one of which may be launched in 2013. Described below are new viral load assays in the pipeline.

The current viral load POC pipeline is presented in **Appendix 2**. Since this report was first published in 2011, additional platforms for POC viral load have been added to the pipeline. However, like the CD4 POC pipeline, there have been delays in the introduction of POC viral load platforms. In 2011, it was expected that at least two POC viral load platforms would be introduced into the market that year, but in fact, only one platform has been introduced to date. It is now anticipated that at least 1 to 2 additional products will be launched in 2013. Similar to POC CD4 platforms, these delays can be attributed primarily to the technical challenges of product development, and in some cases, difficulty in obtaining sufficient funding to complete such development.

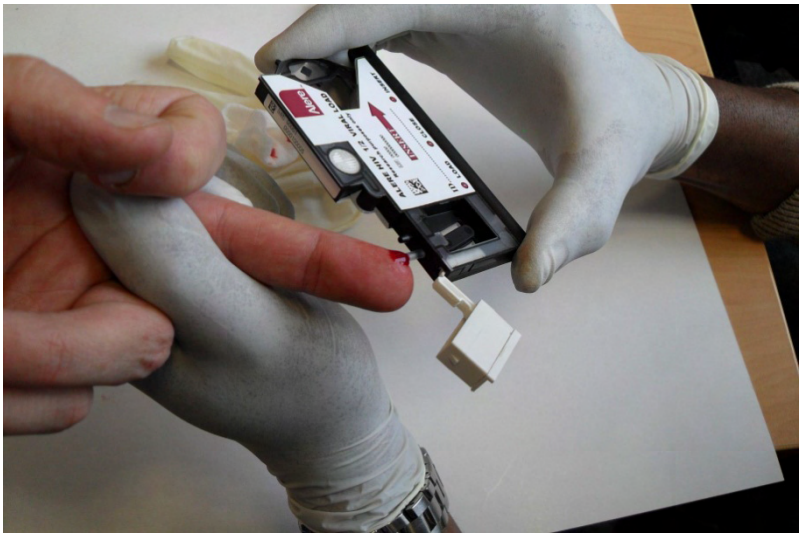
Alere Q *(Alere Inc.)*

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-1 and HIV-2 viral load from approximately 25µL of whole blood. The device on which the assay is run (a prototype of which is pictured below) has a small footprint, is portable, contains an integrated UPS, can be run either on mains power or from a dedicated battery pack, and is ruggedized to withstand harsh environments.

The Alere Q HIV viral load test is comprised of a disposable cartridge that contains all reagents required for the assay in a stabilized form. The cartridge provides for sample collection, cell lysis, amplification target capture, reverse transcription, PCR amplification and real time fluorescence detection based on competitive reporter probe hybridization on an integrated micro probe array. The company expects sensitivity and specificity will be comparable to current virological testing reference technologies (e.g., COBAS® AmpliPrep/COBAS® TaqMan). The system detects HIV-1 Groups M, N, and O, and HIV-2.

The Alere Q platform is designed to require no manual sample preparation or pre-treatment. The required 25 µL of blood can be collected via fingerstick, heelprick or venipuncture. In the case of either fingerstick or heelstick, blood is applied directly into the test cartridge's sample collection capillary as shown below. When using venous blood, the sample is transferred to the cartridge capillary with a transfer pipette. Although a volumetric pipette can also be used, it is not necessary as there is no need to apply a precise volume of blood to the cartridge. The disposable assay cartridge is fully self-contained, and once capped, cannot be reopened; the cartridge remains completely sealed. At no time does the sample or the reagent actually come into contact with the analyser, thus greatly reducing any possibility for cross contamination. The actual hands-on time for the device is expected to

be less than 3 minutes (i.e., sample collection and loading of the cartridge onto the analyser and subsequent reading of result and cartridge disposal).



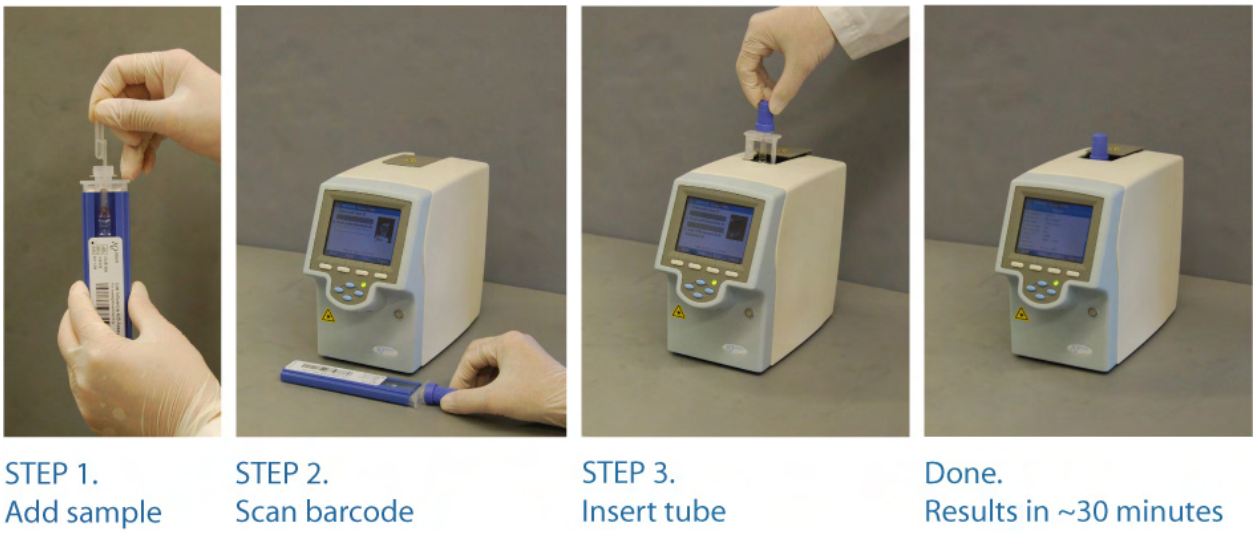
Test workflow for the operator is straightforward and consists of: (i) lancing the patient's finger/heel (or collecting blood via venipuncture) and wicking whole blood directly into the cartridge sample collection capillary; (ii) manually capping the cartridge; (iii) inserting the cartridge into the analyser; (iv) entering the operator and sample IDs on the analyser; and (v) selecting "run." When the assay is complete, audible and visual prompts alert the operator to remove the cartridge from the instrument and the viral load results are displayed on a built-in screen. The result can be printed immediately, but results are also stored in an on-board archive and can be viewed and printed at a later date, exported to a USB memory stick or exported to a remote server via the use of an optional USB connectivity package that makes use of GSM mobile telephone network infrastructure. Additionally, the system will be fully compatible with existing EQA programs.

It is anticipated that the product will be commercially available in 2013. The company will be seeking CE-IVD marking of the Alere Q system in the EU and FDA clearance throughout 2013. Pricing for the instrument and disposable test cartridges has not yet been determined.

Liat™ Analyser *(IQuum, Inc.)*

The Liat™ Analyser, manufactured by IQuum, Inc., is an automated sample-to-result NAT platform that performs sample nucleic acid extraction, purification, reverse transcription, PCR amplification, and real-time detection to detect and/or quantify pathogens. IQuum currently has assays clinically validated and FDA-cleared for the detection of influenza H1N1 virus, as well as influenza A and B strains. An assay for dengue virus is under development, and the platform can also accommodate TB, and other disease categories. Liat assays for HIV viral load testing and diagnostics have also been developed and independently evaluated by third party laboratories.

As illustrated below, the test procedure is straightforward, with no sample manipulation or reagent loading steps other than inputting the plasma or whole blood samples directly into the Liat Tube. The Liat system is a closed system, thus minimizing cross-contamination and biohazard risks, and allowing testing to be performed in non-laboratory or near patient facilities.



To aid the operator and provide reliable results, the Liat Analyser incorporates a variety of intelligent and advanced features: barcode data entry avoids errors in sample or assay coding and on-screen prompts provide easy-to-follow directions to guide the operator through sample loading and tube insertion. Sample metering capabilities ensure that the correct volume of sample is used for the test, or outputs a warning if the sample volume is insufficient. A comprehensive set of sensors further monitors system operations in real time and automatically recovers from errors or aborts the assay to prevent incorrect results from being reported. An internal control contained in each Liat tube is processed and detected with the sample to ensure the proper function of each step of the assay process. PCR curve pattern recognition and automated data interpretation provide results in plain English. The developer states that, collectively, these sophisticated features ensure the quality of results when testing is performed by minimally-trained operators.

The analyser 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 required 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 Liat Analyser has an internal optical system that provides six independent optical detection channels for real-time monitoring and quantification, allowing for the detection of multiple targets in each test and providing future expandability for detection of multiple diseases at lower per test cost. It can be powered by AC mains or by battery, allowing mobile use.

The company expects that the list price for the Liat Analyser, which is currently $25,000, may decrease for resource-limited settings. Susan A. Fiscus at the University of North Carolina at Chapel Hill has completed an evaluation similar to that previously conducted by Robert Coombs at the University of Washington, comparing the Liat Analyser's viral load detection capabilities against the Roche COBAS® and the Abbott *m*2000 system. In both evaluations, the performance of the Liat device compared favorably to the predicate devices. The HIV viral load assay for the Liat platform is ready for market launch, but actual launch will depend on the availability of financing for in-country clinical evaluations and implementation.

EOSCAPE-HIV™ HIV Rapid RNA Assay System *(Wave 80 Biosciences)*

Based on its EOSCAPE technology, Wave 80 Biosciences is developing a rapid HIV NAT-based POC viral load assay designed for use in resource-limited settings. Further expansion of the EOSCAPE assay platform includes development of assays for TB, hepatitis, and other infectious and non-infectious diseases. The company describes the assay technology as incorporating iNAAT and proprietary bipartite photonic detection for detection and quantitation, fingerstick whole blood processing within a single-use, enclosed cartridge. The cartridge contains all reagents necessary to run the test and does not require cold chain transport.

The system (pictured below) has three components: (i) the disposable cartridge, which contains integrated sample preparation and assay modules; (ii) a small, low cost, battery-powered processing unit; and (iii) a small, portable reader, with touchscreen display, that can run on a rechargeable 8 hour battery or mains power. The system is easy to use and will require at most 1-day training for operators.

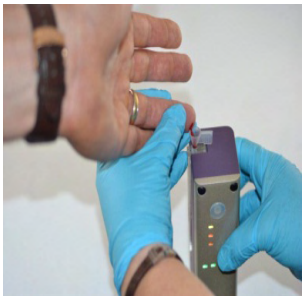
EOSCAPE System:

EOSCAPE Cartridge

single-use, all reagents on-board

EOSCAPE Processing Unit

Deposit fingerstick blood into cartridge, 45 minute runtime



EOSCAPE Analyser

Scan results in 2 minutes; intuitive touchscreen with data connectivity

The testing process is straightforward. The operator inserts a disposable cartridge into the small processing unit. Using a fingerstick lancet, 50 µL of whole blood is applied directly into the cartridge; no external sample preparation is required. The sample is automatically processed in 45 minutes; the operator then inserts the processing unit into the reader for a quick 2 minute scan. Equipped with a simple touchscreen interface, the reader is capable of transmitting test results through wired and wireless connectivity. For higher patient loads, multiple processing units can be used for parallel processing, ~50 samples per day per analyser.

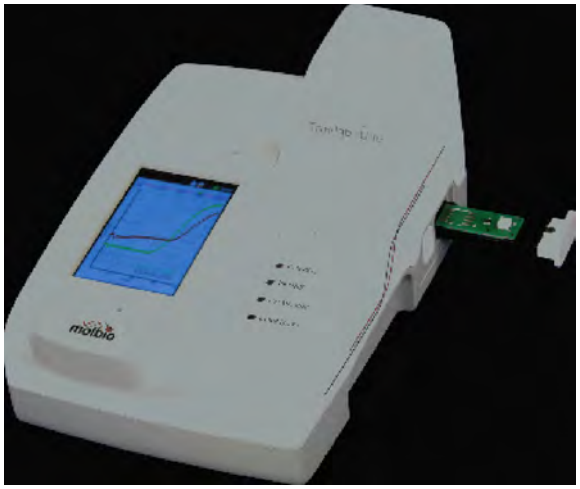
Two HIV NAT cartridge models will be available as part of the EOSCAPE-HIV system. The EOSCAPE-HIV-D cartridge will provide a qualitative HIV-1 RNA test result with a threshold above 1,000 copies/mL. The EOSCAPE-HIV-Q cartridge will return a fully quantitative viral load result. Full scale validation and clinical testing of the Wave 80 EOSCAPE HIV-1 RNA rapid test is expected to begin in late 2013, followed by in-country testing for market launch.

Truelab™ Real Time micro PCR System *(Molbio Diagnostics Pvt. Ltd. [A Tulip Group – Bigtec labs partnership])*

Molbio Diagnostics has developed a comprehensive, rapid, near-patient RT PCR platform, called the Truelab™ Real Time micro PCR System. The system is portable and includes all instrumentation, reagents and essential accessories that are required for the operator to conduct a real time, quantitative PCR assay, from sample preparation through to final result reporting, all within one hour. A Truelab™ micro PCR printer is also available. The system works on ready-to-use Truenat™ disease-specific assays that are stable at room temperature. Assays for MTB, HBV, dengue fever, Chikungunya, H1N1 and malaria (both p. falciparum and p. vivax) are currently available, and assays for HIV viral load, among others, are in development.

The testing process begins with sample collection (blood, serum or plasma) followed by extraction, which uses the Trueprep™ MAG Sample Prep Device and Trueprep Mag sample prep kits. The extraction process takes about 20 to 25 minutes per sample. From there, 6 µL of the extracted nucleic acid is dispensed into the reaction well of the disease-specific Truenat™ micro PCR chip. The chip, which contains all of the chemistry required to complete an assay, is then inserted into the Truelab™ Uno Real Time micro PCR Analyser, pictured below. Thermal cycling takes place automatically within the analyser.

During amplification, the Truenat micro PCR chip exponentially releases flurophores. These signals are captured by sensors and are displayed as an amplification curve on the Truelab screen. Test results are compared to lot-specific standard values preset into the Truenat chip, which enables quantitative estimation of the test analyte and display as RT PCR results in approximately 30 minutes. An internal control is provided from the extraction stage for a complete validation of the test results.

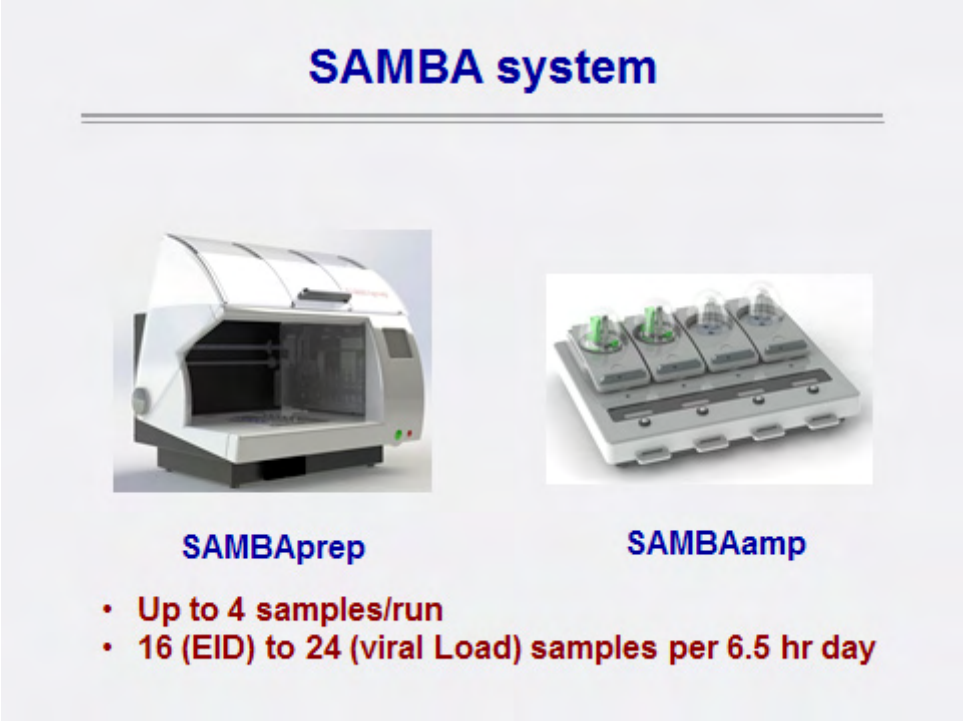


Test results are automatically stored in the analyser memory (up to 5,000 results), can be printed, transported wirelessly to any server/compatible device by Wi-Fi, GPRS, Bluetooth or even SMS.

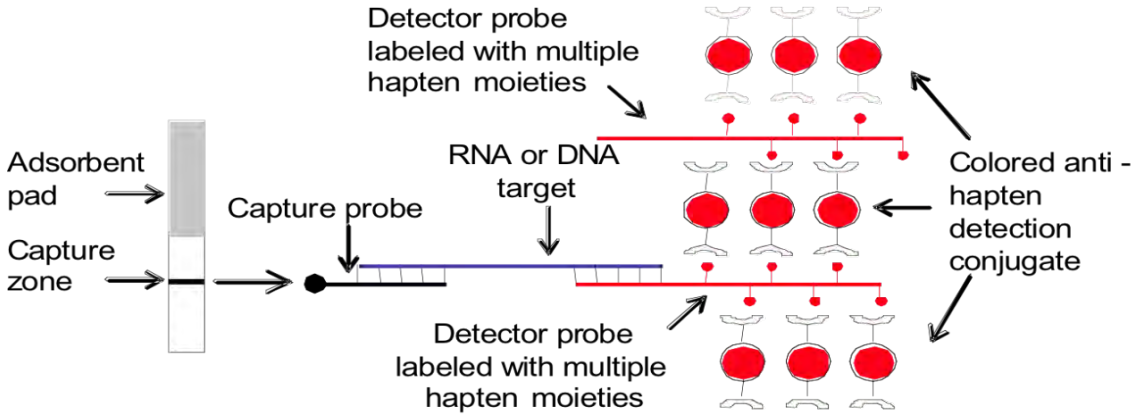
The HIV viral load assay is expected to launch in the third quarter of 2013.

SAMBA *(Diagnostics for the Real World, Ltd.)*

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. Three NAT-based HIV assays are being developed: (i) a semi-quantitative test with a cutoff of 1,000 cp/mL for monitoring of ART (ii) a qualitative test based on plasma for detection of acute HIV infection during the window period before the appearance of antibodies, and (iii) an EID test based on whole blood. The first SAMBA HIV assay to be launched will be the semi-quantitative viral load assay. The SAMBA system, pictured below, automates extraction and integrates amplification and detection into a bench-top analyser with amplification and detection taking place in a closed cartridge.



The SAMBA HIV test uses 200 µL of plasma for the semi-quantitative viral load assay, 500 µL of plasma for the qualitative acute infection assay and 100µL of whole blood for the EID assay. 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 visually read off of a test strip within 25 minutes. The test strip is based on a nitrocellulose membrane in a lateral flow format.

Based on an assessment with the WHO international standard HIV RNA genotype panel containing 400 cp/mL, the SAMBA assay was able to detect all HIV-1 subtypes. Several evaluations have taken place:

- SAMBA semi-quantitative viral load test: evaluated in clinical samples from St. Thomas Hospital, Royal London Hospital and two MSF sites (Chiradzulu, Malawi and Arua, Uganda).

- SAMBA EID assay: evaluated in HIV-positive or negative adult whole blood clinical samples in comparison with DBS testing using the Roche AMPLICOR assay and the COBAS AmpliPrep/COBAS TaqMan carried out by laboratories in Zambia and Uganda.
- Field evaluation in infant blood will take place in Uganda and Malawi during second quarter of 2013 upon receiving ethical approvals.

Currently, the total assay time is 2 hours for the SAMBA EID and 90 minutes for the semi-quantitative viral load assay. SAMBA is suitable for use at the district hospital level or in large clinics in sub-Saharan Africa where electricity is available.

Diagnostics for the Real World, Ltd, the spinout company of DDU located in California, is the manufacturer of the SAMBA system. The SAMBA viral load assay was released in Malawi in the second quarter of 2013 and is expected to be released shortly in Uganda. The expected market release date for the EID assay is the fourth quarter of 2013. There is currently no pricing information available from the company.

Additional Viral Load 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, there are other diagnostics in the pipeline that are not quite as far along in the development of viral load assays. Some of these are discussed briefly below.[30]

GeneXpert® System *(Cepheid)*

The Cepheid GeneXpert® System, which is a fully-automated and integrated system for PCR-based nucleic acid testing, currently has 11 FDA-cleared and 14 CE-IVD assays, including tests for enteroviral meningitis, methicillin-resistant staphylococcus aureus (MRSA), C. difficile, influenzas A and B, mycobacterium tuberculosis detection and resistance to rifampicin (MTB/RIF), and group B strep, among others. In 2012, a multiplex PCR test for the sexually transmitted diseases C. trachomatis/N. gonorrhea was released, and multiplex tests for respiratory and gastrointestinal infections are in development. Release of the HIV viral load assay on the GeneXpert is expected in early 2014.

Although it is not currently known what the price per cartridge will be for the viral load assay, the Foundation for Innovative New Diagnostics (FIND)-negotiated price of the GeneXpert® System (with four modules, pictured below) for high-burden developing countries (HBDC) is approximately $17,000; and, as a result of an agreement among the U.S. President's Emergency Place for AIDS Relief (PEPFAR), USAID, UNITAID and the Bill & Melinda Gates Foundation, the current price per cartridge for MTB/RIF is about $9.98 in HBDCs. Uptake of the program via USAID, PEPFAR and other agencies has been escalating rapidly; as of December 31, 2012, a total of 966 GeneXpert instruments (comprising more than 5,017 modules) and more than 1,891,970 Xpert MTB/RIF cartridges have been shipped to 77 HBDC countries for TB testing. All GeneXpert tests, including HIV viral load, can be run on the systems placed initially for TB testing.

30 This is a non-exclusive list of potential viral load assays.

The GeneXpert® System integrates and automates sample preparation, amplification, and detection in a single-use, self-contained cartridge, pictured below. Most liquids and dry reagents along with enzymes are prefilled so that pre-analytical steps are minimized, greatly reducing opportunities for sample mix-ups and operational errors. GeneXpert cartridges can handle a variety of sample volumes (milliliter range) within macrofluidic chambers and then concentrate the target material down to microfluidic volumes, which can increase the sensitivity of the assays, if needed. Currently, the HIV viral load assay involves a single transfer of 1 mL of plasma directly into the open sample port of the cartridge by using a single disposable Pasteur transfer pipette. By carrying out all dilution and extraction steps inside the chambers of the cartridge, plastic disposables are kept to a minimum.

Further, the GeneXpert® System is modular. Individual modules contain solid state circuitry that control temperature, pressure, rotation of the valve that moves the liquid between reservoirs, and the detection software. These individual modules are packaged in units of 1, 2, 4, 16, 48, or 80, and the latter two systems are fully automated, walk-away robotic instruments developed for high-throughput laboratory applications. Additionally, the modules can be removed and replaced individually so that the entire system is not incapacitated if one module fails.

The GeneXpert® System is sufficiently simple that training can usually be completed within half a day. Further, although the system was designed to use AC power, its low wattage requirements allow it to be powered by a 12VDC/120VAC voltage converter in mobile laboratories, and it has also been installed in remote clinic sites powered by solar panels. The GeneXpert software comes pre-installed on a desktop or laptop computer and results can be displayed for each module in real time or uploaded via an Internet connection to a central database. Wireless data connections via satellite phone networks are in development, as is a cloud-based system for remote access, online system calibration, and interfacing with laboratory information systems.

NWGHF LYNX Viral Load Test and Platform *(Northwestern Global Health Foundation)*

The Northwestern Global Health Foundation (NWGHF) 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 process multiple samples at a time with test results in 60 to 90 minutes. The proposed viral load assay will achieve a limit of detection of 1,000 copies/mL of plasma, using ~150 µL of whole blood that is converted into plasma with simple sample preparation materials provided by NWGHF.

The processor is powered by an external power transformer that connects to either an AC or DC power cable that, in turn, connects to an AC or DC power socket in the clinic or laboratory. A fully-charged battery will complete the cartridges in the processor.

NWGHF/Quidel expect to launch this product in late 2014 or early 2015.

Viral Load Assay using BART Technology (*Lumora Ltd.*)

Lumora Ltd. has developed a simple and robust HIV viral load assay that can utilize plasma as its sample type. The company is also developing a test that can utilize a small volume of whole blood, but needs to fully understand the clinical implications of measuring viral load directly from this matrix. The Lumora assay system incorporates proprietary chemistries in sample preparation, amplification and detection. Lumora's novel approach to primer design through its STEM technology has enabled the development of a fully-inclusive HIV viral load test utilizing isothermal LAMP (loop-mediated amplification)-like amplification methodology. Lumora's overall approach to viral load testing has been to produce an assay system, from extraction to result, that will enable a minimally-trained user to perform the assay in a non-specialist laboratory or clinic setting. The market launch of the Lumora HIV viral load assay is dependent on the company finding a suitable commercialization partner.

Lumora develops assay systems that incorporate its proprietary chemistries, including STEM primers and BART (Bioluminescent Assay in Real-Time), and these technologies allow real-time, closed-tube (requiring minimal electrical input and temperature regulation), quantitative detection of amplification. BART is a bioluminescent reporter system for molecular diagnostics that enables extraordinarily simple and robust readers and therefore opens up new applications for diagnostics and disease monitoring in low-resource settings. Requiring only a single-temperature heating block and a photo-diode light detection system, BART is designed for use with isothermal nucleic acid amplification technologies (iNAAT), such as LAMP. Lumora's assay systems and technology have been shown to be more tolerant of less processed samples or samples that have inherent fluorescence or are turbid. The robust nature of the chemistries has enabled the development of simpler sample processing protocols, as well as the ability to provide reagents that are stable at ambient temperature and do not require cold chain shipping or storage.

LAMP has been used as an alternative to PCR, but its widespread adoption has been limited due to challenges associated with primer design. Lumora has developed and patented a different type of primer, known as a stem primer that allows greater flexibility in primer design and also increases the speed of LAMP assays. This increased flexibility has enabled Lumora to cover the large sequence diversity of HIV genomes.

The unique nature of the BART signal makes it possible to determine when a result has completed without the need for complex or sensitive light detectors. The time taken to reach the peak light signal reflects the amount of target nucleic acid in the samples, and BART can quantify the target in a time similar to fast PCR systems. These features make Lumora technology ideally suited to use in settings with limited laboratory infrastructure and adverse operating conditions, such as for viral load monitoring in sub-Saharan Africa.

Lumora's assay system and technology is flexible enough to be utilized in high-throughput applications as well as in highly decentralized settings. The hardware (pictured below) is also portable and powered by mains or a battery, culminating in a low-cost unit with a small footprint that can be used in challenging environments, including non-laboratory settings.

The successful launch of a food pathogen test in sixty countries that includes Lumora's technology demonstrates that BART is a proven technology for low resource settings because it is easy-to-use and relatively low cost. Wider adoption of this technology could be expected in laboratories where currently available methodologies are not being used, due to ongoing high costs or the practical limitations created by the accessibility of consumables, power, and a laboratory environment that is suitable for highly sensitive preparation and testing procedures. Lumora believes that BART will offer a simple and effective method for monitoring viral load in developing countries and could support current efforts to increase the effectiveness of, and adherence to, ART regimens.

RT CPA HIV-1 Viral Load Test *(Ustar Biotechnologies)*

Ustar Biotechnologies has developed Cross Priming Amplification (CPA), a novel isothermal nucleic acid amplification technology with multiple iterative designs that can address a wide variety of key obstacles to traditional amplification technologies such as PCR. By using multiple crossing primers and probes, target DNA sequences can be rapidly and precisely amplified at a uniform temperature (typically 63°C) in an easy-to-use protocol with high sensitivity and specificity. By utilizing its CPA technology, Ustar is now developing assays for HIV, hepatitis C virus (HCV), chlamydia/gonorrhea and polio virus (the latter two together with PATH).

Recent work at Ustar and the University of Victoria has shown that RT CPA can effectively amplify an RNA template with similar performance to existing DNA-based assays. After extensive testing, results indicate that the use of an RNA template does not alter the overall performance in CPA (e.g., sensitivity or specificity) compared to the use of a DNA template. Additionally, using novel enzymes together with inherent RT activity, as little as 0.1 pg of RNA can be detected in less than 30 minutes. Therefore, the company believes that RT CPA is an excellent candidate for the development of a new HIV viral load diagnostic test. Finally, Ustar also possesses a proprietary glassification process that stabilizes enzymes for ambient temperature transport and storage.

Ustar's goal is to develop a quantitative RT CPA HIV-VL test in conjunction with a robust and user-friendly real time isothermal amplification/detection instrument. For this purpose, Ustar plans to use the commercially-

available Genie®, a portable instrument developed by OptiGene Ltd. In order to accomplish this, Ustar plans to: (i) develop a modified CPA reaction for amplification from direct plasma or minimally-processed whole blood sample (i.e., diluted/buffered); (ii) develop a quantitative RT CPA viral load assay with internal control using a novel RT-active DNA polymerase; and (iii) develop new user interface software for automatic viral load calculations.

The final Ustar diagnostic test kit is expected to comprise only reagents and a portable device for amplification and detection. Reagents will consist of glassified enzymes for ambient temperature transport and storage with a reconstitution buffer.

The testing process will require the user to: (i) take a finger/heel prick and place a drop of blood directly onto a plasma separating filter; (ii) add the reconstitution buffer using a preloaded pipette to the glassified enzyme contained in the amplification tube; (iii) punch a fixed volume disk out of the plasma containing a portion of the filter, and place it directly into the amplification tube; and (iv) close the tube and place it in the Genie instrument for amplification and detection.

A fully-quantitative viral load measure will be available in as little as 20 minutes (depending on the limit of detection required), and the sample can be run for 45 minutes to ensure a viral load measure of < 1,000 cp/mL. On-board software will calculate an offset value based on any delay in the amplification of the internal control caused by inhibition and a simple readout – "number of RNA cp/mL", "not detectable", or "invalid" – will be available to the user and will be automatically uploaded to an external server (e.g., a national HIV program), along with detailed information regarding each run.

The development of the Ustar viral load assay is in an early stage, and its completion is dependent on funding. No market launch date has yet been determined.

Gene-RADAR® Platform *(Nanobiosym® Diagnostics)*

Nanobiosym® Diagnostics has developed a portable nanotechnology platform called the Gene-RADAR®. This chip-based system, which is about the size of a laptop computer, uses approximately one drop of specimen (e.g., blood or saliva) to recognize the genetic "fingerprint" (DNA or RNA signature) of a disease. The Gene-RADAR® system is easy to use. About 10 to 20µl of sample is collected and transferred to a disposable chip, which is inserted into the platform. The Gene-RADAR® then extracts DNA/RNA present in the sample and determines whether it matches the DNA/RNA of a particular pathogen. It has the potential to be utilized for a variety of applications, including *in vitro* diagnostics, water testing, food and beverage safety, and agricultural and biofuel applications. The Gene-RADAR® platform does not require sophisticated laboratory infrastructure, trained laboratory technicians, continuous power or running water.

Nanobiosym® Diagnostics has developed a viral load assay for the Gene-RADAR® v 1.0 platform, which can give a fully-quantitative viral load measure down to 500 copies/mL in less than 60 minutes. No specific cost data is currently available, and the company does not yet have an anticipated launch date for its viral load assay. As Gene-RADAR® is a flexible and reconfigurable platform, the company is continuously incorporating its pipeline of innovations to optimize its performance metrics, further reducing the sample to answer time, cost, and the size of the device.

Cavidi AMP *(Cavidi)*

Cavidi is developing a new automated platform for near-patient viral load monitoring, a prototype of which is pictured below.

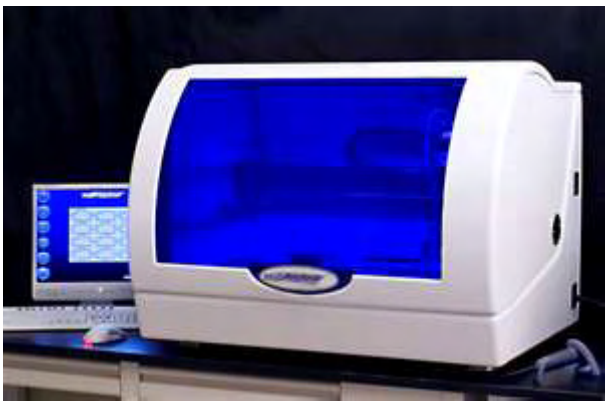
The system will combine the strengths of RT technology with the advantages of an automated walk-away platform. This should provide fast and robust viral load monitoring for all HIV types and subtypes. The company's planned launch date for the AMP system is the end 2014.

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 *(Becton Dickinson)*

The BD MAX™ System (previously known as the HandyLab Jaguar System) 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.

The Enigma™ ML System *(Enigma Diagnostics Limited)*

The Enigma™ ML (mini laboratory) System (pictured below), developed by Enigma Diagnostics Limited, is a fully-automated, molecular test platform for *in vitro* use that is suitable for use both in the clinical laboratory and at the point of patient care. The ML system can deliver diagnostic test results in less than 60 minutes. The company indicates that its PCR technology can be applied to a wide range of samples. The initial focus of assay development will be for a range of respiratory tests.

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 Enigma has plans to develop assays across blood-borne infectious diseases, at this point in time, there is no indication that a viral load assay is planned for the ML platform in the near future.

BioHelix Corporation

BioHelix Corporation, which was recently acquired by Quidel Corporation, is developing assays based on the company's iNAAT platform for infectious diseases. BioHelix has developed an "instrument-free" molecular diagnostic platform (the IsoAmp® Molecular Analyser) 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) and an enclosed disposable detection device called the BESt™ (biohelix express strip) Cassette, which minimizes cross-contamination. Workflow on the analyser consists of simple sample prep, isothermal amplification at 65°C followed by amplicon detection via the BESt Cassette. Per the company, the test results on the strip can be available in as little as 10 minutes. The company is currently developing integrated sample prep, dry reagents and a faster HDA platform.

The first assay being developed by BioHelix, with funding from the National Institutes of Health (NIH), is for genital herpes. The company recently received FDA 510(k) clearance on the IsoAmp HSV Assay for detecting genital and oral herpes. 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, per the company, this could be converted into a quantitative assay with the addition of a fluorescence label probe.

BioHelix has received a two-year SBIR grant from the NIAID to develop a quantitative HIV RNA assay based on its isothermal HDA amplification technology and a portable fluorescence analyser that is capable of monitoring fluorescent signals in real time. Pursuant to the SBIR grant, BioHelix will collaborate with Dr. Jeanne Jordan at George Washington University to develop a low-cost HIV viral load test for use at the POC in resource-limited settings.

TwistDx *(Alere Inc.)*

TwistDx has developed a proprietary technology, recombinase polymerase amplification (RPA), 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 to 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.

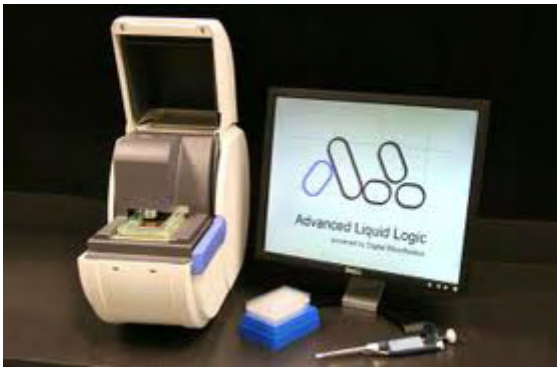
Although the TwistDx system does not yet include any sort of integrated sample preparation technology, the company has introduced the Twista™ portable real-time fluorometer for monitoring/detection. The Twista contains a heated incubation chamber for a strip of eight reagent tubes. It has a small footprint and can be used with a rechargeable battery pack. Detection data can be analysed 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, any HIV assay using this chemistry is only in very early stage development.

Advanced Liquid Logic[31]

Advanced Liquid Logic, Inc. (ALL) 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 analyser (pictured below) that is currently being evaluated. The company indicates that it is evaluating various assay formats as well as the portable analyser.



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.

Genedrive™ *(Epistem, Ltd.)*

Epistem, Ltd., a biotechnology company headquartered in the UK, has developed a new molecular diagnostic platform called the Genedrive™ (pictured below), which uses end-point PCR-based detection. The Genedrive is a highly portable, POC platform weighing about 550 grams (1.2 lbs.) and is approximately the size of an iPad mini. The platform accommodates both electric (110-240V AC) and battery (12V DC) power.

31 Note that this summary of the ALL platform has not been updated by the company since September 2012.

To date, Epistem has developed a TB assay, which is CE-IVD marked, for the Genedrive. In addition to detecting TB, the platform also detects Rifampicin resistance mutations. Test results are available in less than 60 minutes. The Genedrive platform is integrated with a simple extraction cartridge based on composite paper that allows extraction in a single step. The sample is manually transferred with one pipetting step into the Genedrive reaction cartridge. Analysis and diagnosis are integrated. Epistem expects to launch the TB assay in India in 2013 and has entered into an agreement with BD for the supply and distribution of the platform in the rest of the world (excluding India and the Indian sub-continent).

Epistem also plans to develop Genedrive as a diagnostic platform for a number of additional infectious diseases, including HCV and HIV (viral panels).

Early Infant Diagnosis

As discussed earlier in this report, 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. 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]. 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 EID 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.

There are currently three HIV-1 DNA assays available in resource-limited settings and that are used for EID: the Roche AMPLICOR® HIV-1 DNA Test v1.5 (RUO),[32] the Roche COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM) HIV-1 Qualitative Test (RUO) and the Abbott RealTi*m*e Qualitative HIV-1 Test, which has CE-IVD marking. 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 RealTi*m*e 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**.

In addition, Cavidi is currently considering re-labelling its viral load test, ExaVir Load, for use in EID. Because studies have shown valuable benefits of using RT enzyme activity measurement for EID [77,78,79], this would

32 The RUO (Research Use Only) designation is required by the 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.

bring the advantages of RT technology to EID, like subtype independence, cost-efficiency and accessibility, while producing results that should be at least as sensitive and specific as DNA PCR testing.

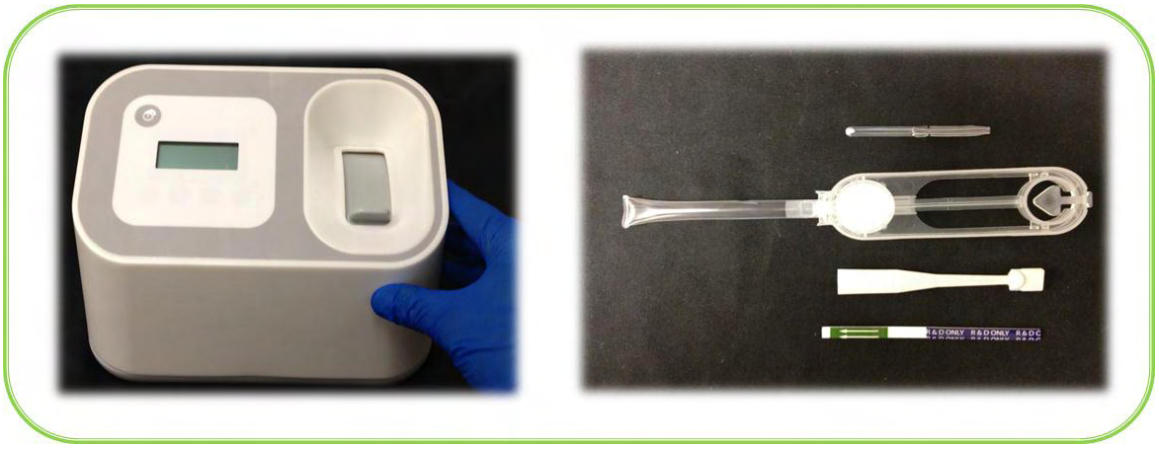
The DNA PCR qualitative tests, like the RNA PCR quantitative tests discussed earlier, require sophisticated laboratory infrastructure, including clean rooms and trained laboratory technicians, and are subject to some of the same drawbacks and limitations as RNA PCR tests for implementation in resource-limited 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 for this 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.

New Technologies for EID in the Pipeline

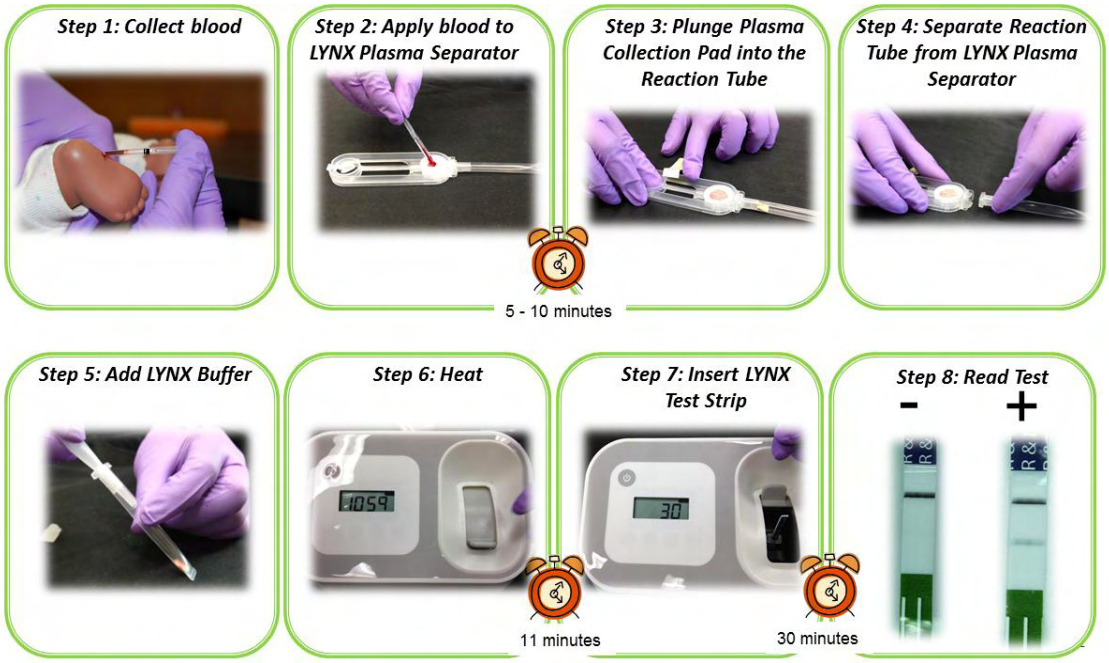
Because RNA PCR testing can be used for the detection of HIV in infants under 18 months of age, the new technologies discussed in the previous section on viral load testing, including POC tests from Alere and Liat, should be considered viable options for EID. In addition, the SAMBA system, discussed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

LYNX HIV p24 Antigen Assay *(NWGHF)*

NWGHF is developing an ultrasensitive p24 antigen rapid lateral flow assay for use at the point of patient care. The technology (pictured below), LYNX, involves a lateral flow strip that detects HIV p24 antigen, and pre-analytical devices for separating plasma from heel-stick blood and disrupting immune complexes that would interfere with immunoassays. NWGHF has demonstrated proof of principle of the test.



The assay procedure involves collecting about 80µL of heel-stick blood from the infant using a blood collection tube; separating plasma from the sample; adding buffer to the sample and "heat shocking" it in a small, battery-powered processor device; inserting the rapid test strip into the device; and waiting approximately 30 to 40 minutes to read the result. The total assay duration is about 45 to 50 minutes. The procedure is illustrated below.

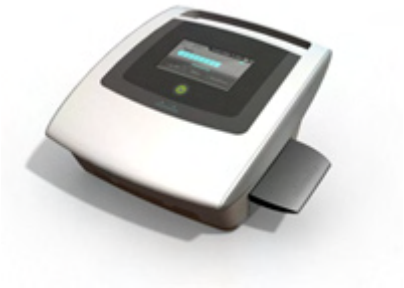


Note that, similar to other rapid tests, if only the top line appears (the control line only), the test is negative and the infant has not been infected with HIV. If both lines appear (the control line and the test line), the test is positive and the infant has been infected with HIV. If the top (control) 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 between $700 and $900, and the per-test cost is expected to range from $7 to $15. Clinical and field trials on the assay commenced in 2012, with availability expected in 2013.

PanNAT® Platform – *(Micronics, Inc.)*

Micronics, Inc., a subsidiary of Sony Corporation of America, has developed the PanNAT system (pictured below), which is a small, portable microfluidic platform 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. The cartridge includes all necessary reagents on board. The system is lightweight, mains-powered, can store up to 350 test results before prompting the user to download or delete results, and can provide results within 30 to 40 minutes, depending upon assay parameters. A battery-operated/WiFi-enabled option is planned.



The cartridge incorporate 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.

Micronics has a number of tests in development, including an assay for Shiga toxin-producing E-coli, as well as other infectious disease diagnostics. Commercial launch for a first test and system is targeted for 2013. Micronics has also been funded to develop qualitative assays for each of HIV, HBV and HCV; however, the company has no current plans for a quantitative viral load assay.

Viral Load Technologies and Future Directions for Viral Load Testing

The Technologies

Unlike 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. With the exception of 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-limited settings. There are a few countries, including China, Kenya and Lesotho, that are increasingly using viral load, but still on a small scale As indicated earlier, the reasons for this include cost, infrastructure requirements, the need for trained laboratory technicians and WHO guidance on the use of viral load testing that stops short of calling for its routine use.

Analogous to 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 whole blood samples from patients, 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, 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 virological failure for patients on ART, 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 to 2 months or more often (analogous to glucose testing for diabetics). The purpose of global ART should be the effective, long-term management of chronic patients so as to ensure the successful treatment of as many people for as long as possible. Early detection of viral resistance and reductions in treatment efficacy on an individual basis, followed by improvements in adherence to save the existing treatment regimen or early diagnosis of treatment failure requiring a switch, is essential for this goal. 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, there has been limited launch of the SAMBA viral load technology in 2013 and additional viral load and EID POC technologies are in development, with possible launch of additional products in 2013. 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 emphasis of Treatment 2.0 on ART efficiency and simplification, consideration should be given to how the diagnostic landscape must adapt and change over the next few years in order to achieve robust, high-quality, efficient, cost-effective and accessible diagnostic services for the 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, similar advances in access to tests for infant diagnosis and ART staging and monitoring are needed, and new technologies in the pipeline 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. The pace at which countries implement an optimized mix of high-volume centralized and low-volume POC diagnostic services tailored to suit their individual needs will determine the impact these improved technologies have 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. Recently, UNITAID committed to funding several projects to facilitate and support the commercialization of POC diagnostics, including a project that will fund procurement of quality POC diagnostics and a project that will fund pre-market evaluation of new POC diagnostics.

"The author notes no conflicts of interest."

APPENDIX 1:
Operational Characteristics of Diagnostic 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 either 2mL or 4 mL K2 EDTA anticoagulant tubes; 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 stable for 12 months from date of manufacture when stored at 2° – 30° C; transient exposure (shipping delay or temperature incursion) of 10 days at 50° C (122° F).
Cost/test	Volume based; ranges from approximately $3.00 – $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 – 19°C (60 – 85°F) • Humidity: 10% – 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.

CYTOMICS FC 500™ MCL and MPL™ Systems	
Type of Technology	Large, bench-top, bead-based Flow Cytometer; two different loaders (MCL and MPL)
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 375 samples per day (47 samples per hour) with the MCL; or more than 500 samples per day, with the MPL)
Throughput per technician/ per day	Varies according to test, flow rate and MCL versus MPL 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 (v) the test is run on the instrument.
Reagent stability	Reagents must be stored at 2º – 8º C (36º – 46º F); reagents are shipped with an expiration date of 1 year.
Cost/test	Volume based; ranges from approximately $2.50 – $8.00 per test
Cost/instrument	Approximately $90,000; approximately $100,000 with CellMek
Regulatory Status	FDA approved
Physical dimensions (cytometer only; computer/monitor and power supply are separate)) (W x H x D)	Width: 90 cm (35.5 in.); with MPL (97.8 cm, 38.5 in.) Height: 61 cm (24 in.); with MPL (61 cm, 24 in.) Depth: 73.7 cm (29 in.); with MPL (88.9 cm, (35 in.)
Weight	84.8 kg (~187 lb) (cytometer with MPL; computer/monitor and power supply are separate)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately $1,500 – $2,500

CYTOMICS FC 500™ MCL and MPL™ Systems (2)	
Electric Power Requirements	115 – 220 VAC Four 50 – 60 HZ lines required Power Supply weighs 54.4 kg (120 lbs)
Environmental Requirements	• Operating Temperature: 16 – 32°C (60 – 90°F) • Humidity: N/A • Maximum altitude: N/A
Data Station	Operating System: Micrsoft® Windows™ 2000
Monitor	External monitor with 17″ flat screen display
Printer	Included
Bar-code Scanner	Included
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 – 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, CD3 and CD8 optional
Turnaround time	After 15 minutes incubation, 40 – 70 seconds per test
Capacity	250 tests/day without loader; 400 tests/day with loader
Throughput per technician/ per day	Maximum of 250 samples
Sample needed and stability	20 µL whole blood collected in EDTA anticoagulant; unstained anti-coagulated blood can be stored at room temperature (18° – 25°C) for up to 48 hours; alternatively anti-coagulated blood can be refrigerated at 2° – 8°C for up to 7 days prior to sample processing. CD4 mAB-stained blood samples can be stored at room temperature (18° – 25°) for up to 24 hours or alternatively refrigerated at 2° – 8° for at least 72 hours.
Sample preparation and protocol complexity	Process for dry reagents only: (i) add blood to Partec CD4 tube containing dry mAB reagent; (ii) incubate 15 minutes at room temperature in the dark; (iii) add prefilled buffer to tube; (iv) run sample in CyFlow Counter. For liquid reagents: (i) add blood to a test tube; (ii) add 20 µL of liquid mAB reagent to tube; (iii) incubate 15 minutes at room temperature in the dark; (iv) add 820 µL no lyse buffer and shake 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 – 8º C (36º – 46º F) in the dark.
Cost/test	€1.75 (~$2.30 per test for absolute CD4 and €2.50 (~$3.30) for CD4 absolute and percentage, high volume discounts available
Cost/instrument	Approximately $22,220; higher with the addition of auto-preparation and auto-loading unit
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	11.5 kg (~25.3 lbs) (cytometer only)
3rd party supplies	Refrigerator (only required when using liquid mAB reagents; cost ~$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: 10° – 40°C (50° – 104°F) • Humidity: <95% non-condensing • Maximum altitude: 3,000m (9,843 feet)
Data Storage and Data Transfer	Dedicated Intel® CPU integrated into instrument; data storage of approximately 20,000 data sets; USB port
Monitor	8.4" TFT color touch screen integrated into instrument; option to connect other printers
Printer	Built-in thermal printer integrated into instrument; connection system via GSM module will be available for data transfer from Q2 2013
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 (generally available locally). Alternatively, instrument replacement possible.
Internal QC	Instrument supports QC (Partec CountCheck beads as non-biological controls and Partec ControlBlood – dry as biological controls)
External QA	Compatible with CD4 EQA programs.
Infrastructure Requirements	Technology can be used at all levels of the health system, including 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 2° – 8° C (36° – 46° F)
Cost/test	Volume based; ranges from approximately $3.50 – $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 – 40°C (50 – 104°F) • 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.

BD FACSClearCount™ System	
Type of Technology	Bench-top, bead-based Flow Cytometer
Output	Single tube reagents measure absolute and percentage CD4 (BD CD4 Assay Kit)
Turnaround time	Standard sample preparation mode: 30 – 180 minutes, including incubation and run (time dependent on number of samples loaded on carousel). Manual sample preparation mode: 30 – 40 minute incubation time; 1 – 2 minutes per test
Capacity	Standard sample preparation mode: approximately 30 – 60 samples per day Manual sample prep mode: approximately 60 – 120 samples per day
Throughput per technician/ per day	Standard sample preparation mode: 30 – 180 minutes depending on number of samples loaded on carousel. Manual sample preparation mode: 30 per hour, after initial 30 – 40 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. Standard sample prep process: (i) blood is collected and added to tube; (ii) instrument performs remainder of sample preparation Manual sample prep 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 4º – 35º C (39º – 95º F) in sealed pouches
Cost/test	Volume based; ranges from approximately $4.50 – $12.00 per test
Cost/instrument	Approximately $38,000
Regulatory Status	FACSClearCount Systems and BD CD4 Assay Kit, FDA approved and CE-marked
Physical dimensions (W x H x D)	Width: 59.44 cm Height: 57.24 cm Depth: 60.78 cm

BD FACSClearCount™ System (2)	
Weight	43.1 kg (95 lb), fluid reservoirs empty
3rd party supplies	For manual sample preparation only: vortex and pipettor; cost: approximately $1,000 – $1,500
Electric Power Requirements	100 – 240 VAC 50 – 60 HZ 240 W (maximum rated power)
Environmental Requirements	• Temperature: 10° – 40°C (50° – 104°F) • 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.

Millipore-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 – 8º C (36º – 46º F); reagents are shipped with 12 months of shelf life.
Cost/test	$2.50 per test for CD4/CD4% (including the distribution margin), regardless of volume
Cost/instrument	Approximately $20,000
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

Millipore-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 25 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 are stable for 9 months when stored at 3° – 30° C (37.4° – 86° F); no refrigeration is required.
Cost/test	$2.50 per test for absolute CD4 count; $3.50 per test for % CD4
Cost/instrument	Approximately $27,000
Regulatory Status	CE-IVD marked
Physical dimensions (cytometer only) (W x H x D)	Width: 32 cm (12.6″) Height: 48 cm (18.9″) Depth: 48 cm (18.9″)
Weight	25.0 kg (~55 lbs) (cytometer only)
3rd party supplies	Vortex and pipettor; cost: approximately $400

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	Not 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 or 4 mL vacuum K2 EDTA anticoagulant tubes provided by PointCare. Sample is stable for 8 hours from time of draw.
Sample preparation and protocol complexity	No sample preparation steps. (i) Draw venous blood into PointCare-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 2° – 30° C (36° – 86° F); transient exposure (shipping delay or temperature excursion) of 10 days at 50°C (122° F).
Cost/test	About $10.00 per test, including Daily Check™ 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 CD4NOW™ 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 – 34º C (64º – 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 PointCare
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 – 108º F) for 2 years from date of manufacture.
External QA	Yes; currently uses quality control materials from Streck Laboratories. PointCare expects to eliminate the need for EQA controls by summer 2013.
Infrastructure Requirements	Technology can be used at central, regional, district and some well-developed primary sites with dedicated laboratory facilities and technicians.

Pima™ Analyser	
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 analyser; (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 – 30°C
Cost/test	Between $6 and $12 per test
Cost/instrument	From $6,500 – $12,000
Regulatory Status	CE-IVD marked; WHO pre-qualified
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)

Pima™ Analyser	
Electric Power Requirements	100 – 240 V (A/C) at 47 – 63 Hz mains power Analyser contains on-board rechargeable battery with sufficient capacity to run approximately 17 tests (actual duration will depend on conditions of use). Power extender is available (module with an extended battery life and adaptors for charging sources, including solar panels, car batteries, mains power).
Environmental Requirements	• Operating Temperature: 10° – 40° C (50° – 104° F) • Humidity: 10% – 95%; no direct sunlight; keep dry • Maximum altitude: Tested to 2,000 meters (~6,500 feet); actual maximum operating altitude not evaluated.
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. Supports wired connectivity via LAN and wireless connectivity via an optional UBS GPRS modem to connect to remote servers over mobile telephone networks.
Monitor	LED mono-color screen integrated into instrument
Printer	Separate printer (prints on thermal paper); powered by the instrument (with rechargeable batteries on board) 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	Analyser 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	Known to be compatible with Pima: QASI and UK-NEQAS
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, total lymphocytes and WBC, CD3 and CD8 optional
Turnaround time	15 minutes incubation; 40 – 70 seconds per test
Capacity	Up to 250 tests/day
Throughput per technician/ per day	Maximum of 250 samples
Sample needed and stability	20 µL whole blood collected in EDTA anticoagulant; analysis within 48 hours when stored at room temperature; unstained anti-coagulated blood can be stored at room temperature (18° – 25°C) for up to 48 hours; alternatively anti-coagulated blood can be refrigerated at 2° – 8°C for up to 7 days prior to sample processing. CD4 mAB-stained blood samples can be stored at room temperature (18° – 25°) for up to 24 hours or alternatively refrigerated at 2° – 8° for at least 72 hours.
Sample preparation and protocol complexity	Process for dry reagents only: (i) add 20µL blood to Partec CD4 tube containing dry mAb reagents; (ii) incubate 15 minutes at room temperature in the dark; (iii) add prefilled buffer to tube; (iv) after gently shaking the tube, refill volume from sample tube into syringe; (v) 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	€3.00 (~$3.96) per test for absolute CD4 and CD4 percentage combined, high volume discounts available
Cost/instrument	~€7,100 (~$9,380). Partec offers a "point-of-care" package (including, instrument, reagents for 5,000 tests, 36-month instrument warranty) with an effective instrument price of ~$4,000.
Regulatory Status	CE-IVD marked
Physical dimensions (cytometer only) (W x H x D)	Width: 26.8 cm (10.6") Height: 24.3 cm (9.6") Depth: 18.6 cm (7.3")
Weight	6.2 kg (~13.7 lbs)
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs. For venous samples: micropipette included in the CyFlow® instrument starter kit

Partec CyFlow® miniPOC (2)	
Electric Power Requirements	100 – 240 VAC or 12 V DC power (car battery) 50 – 60 HZ
Environmental Requirements	• Temperature: 10° – 40°C (50° – 104°F) • Humidity: <95% noncondensing • Maximum altitude: 3,000 m (9,843 ft)
Data Storage and Data Transfer	Dedicated Intel® Atom™ CPU integrated into instrument; Windows™-based analysis software; data storage of approximately 20,000 data sets; USB port
Monitor	5.7" color touch screen integrated into instrument
Printer	Built-in thermal printer integrated into instrument; option to connect other printers; connection system via GSM module will be available for data transfer from Q2 2013
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 (generally available locally). Alternatively, instrument replacement possible.
Internal QC	Supports internal QC (Partec CountCheck beads as non-biological controls and Partec ControlBlood – dry as biological controls).
External QA	Compatible with CD4 EQA programs
Infrastructure Requirements	Technology can be used at all levels of the healthcare system, including central, regional, district and mobiles labs and some well-developed primary sites with dedicated laboratory facilities and technicians.

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 electrical sensing.
Output	Absolute CD4 counts only
Turnaround time	10 minutes
Capacity	40 – 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	16 µL of capillary (fingerstick) or venous 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 or printout. Venipuncture blood may also be used via capillary tube transfer.
Reagent stability	Dried reagents require no refrigeration. Stable to 50° C in preliminary studies
Cost/test	$8 per test (estimated), but may be lower with volume discounts
Cost/instrument	<$5,000 (estimated)
Regulatory Status	TBD
Physical dimensions (cytometer only) (W x H x D)	Width: 22.9 cm (9.0") Height: 17.8 cm (7.0") Depth: 12. 7 cm (5.0")
Weight	2.5 kg (~5.5 lbs)
3rd party supplies	Sterile lancets (for capillary blood samples), alcohol swabs, dry swabs, gauze, bandage

Daktari™ CD4 Counter (2)	
Electric Power Requirements	Regular AC. Long-life rechargeable battery self-contained in device that can operate for up to 3 days on a single battery charge.
Environmental Requirements	• Operating Temperature: TBD • Humidity: TBD • Maximum altitude: Up to 3,280 m (10,000 feet)
Data Station	Daktari instruments will include a data management system with a back-end data package built into the device. Instruments will also contain a keypad user interface.
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	Daktari will offer a USB printer accessory for printed results.
Bar-code Scanner	TBD
Training	Minimal training required. Lay person can be trained in less than 90 minutes. Primary skill required is for correct lancet blood draw.
Maintenance	The device does not use lasers, but rather employs an electronic measurement system similar to a glucose meter and may be less prone to damage. If damaged, the company plans to swap-out the device rather than repair it on-site.
Internal QC	Internal QC of instrument performed with each assay run; internal QC of cartridge with each run includes checks on sensors, assay protocol and key reagents. No calibration required. Instrument will also perform QC of capillary blood draw and inform user if fingerstick is inadequate prior to running assay.
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 with disposable, self-contained sample cartridges; measurement is by fluorescence imaging cytometry with on-board immunostaining and direct cell counting
Output	First release product will deliver absolute CD4 count. Future releases will provide CD4%, hemoglobin, and a panel of immunoassay results (e.g., HIV/syphilis).
Turnaround time	~20 minutes. The reader is only occupied for a short time (~3 minutes); multiple cartridges can be processed off-reader in batch mode.
Capacity	~10 tests per hour
Throughput per technician/ per day	~80 samples per technician per 8 hour day.
Sample needed and stability	10 µL of capillary (fingerstick) or venous whole blood
Sample preparation and protocol complexity	One-step whole blood transfer to cartridge. Cartridge incubation on rack with automatic timing. Manual cartridge insertion into reader.
Reagent stability	Capable of being stored in package between 2° and 40° for 12 months at 70% relative humidity.
Cost/test	$6 per test (estimated); volume discounts
Cost/instrument	<$5,000 per system; reagent rental or leasing plans are available.
Regulatory Status	CE-IVD mark anticipated in late 2013; country approvals as necessary.
Physical dimensions (cytometer only) (LxWxH)	Length: 25.0 cm (~10″) Width: 15.0 cm (~6″) Height: 17.0 cm (~7″)
Weight	3.0 kg (~6.6 lbs)
3rd party supplies	Sterile lancets (for capillary blood samples), alcohol swabs, dry swabs, gauze, bandage.

Mbio CD4 System (2)	
Electric Power Requirements	Rechargeable battery operation (8 hours) or plug-in to electrical supply (100 – 220 VAC)
Environmental Requirements	• Operating Temperature: 15° – 35°C • Humidity: 5% – 95%, non-condensing • Maximum altitude: 2,000 meters (~6,500 feet)
Data Station	On-board computer for sample analysis, results management and event logs. Instrument will have a built-in Ethernet connection and multiple USB ports to support printers, external barcode readers and wireless adapters. Data reports, including QC, are available for off-line analysis or transfer.
Monitor	Integrated touchscreen interface with administrator-configurable settings such as user lockout/validation and QC scheduling. Predominantly icon-driven.
Printer	External USB printer
Bar-code Scanner	Internal barcode reader captures cartridge information. Capable of supporting an eternal barcode reader.
Training	Minimal training required: less than one day's training, minimally skilled staff. Primary skill required is for correct fingerstick blood draw.
Maintenance	No routine maintenance or service; system replacement via depot/distributor swap-out
Internal QC	Internal QC on every cartridge for multiple parameters, incuding sample addition, reagent quality, lot expiration, etc.
External QA	Compatible with pre-identified, third-party external QA materials
Infrastructure Requirements	Minimal infrastructure – no water requirements; access to power for battery recharge required. Target settings are health centers/health posts and peripheral labs.

BD FACSPresto™	
Type of Technology	Small, bench-top, fixed volume cytometer
Output	Absolute CD4, CD4% and Hb
Turnaround time	2 – 5 minutes reading; plus incubation of cartridge (20 minutes)
Capacity	Maximum of ~40 – 50 samples per day
Throughput per technician/ per day	~40 – 50 samples per technician per day; [batching] capabilities; walk-away operation.
Sample needed and stability	~ 20 µL of capillary (fingerstick) blood wicked directly into BD cartridge or ~ 20µL of venous blood collected in EDTA anti-coagulant tube. Cartridge must be inserted and tested within a few hours of sample application.
Sample preparation and protocol complexity	No sample preparation required. For capillary blood: (i) lancet finger; (ii) apply blood drops to cartridge; (iii) close cartridge; (iv) incubate cartridge; (v) insert cartridge into analyser; (vi) press "start"; (vii) read result from LED screen; (viii) print result
Reagent stability	Dried reagents require no refrigeration. Stable for 12 months at 10° – 40°C
Cost/test	TBD
Cost/instrument	TBD
Regulatory Status	Will be CE-IVD marked, FDA-approval will follow.
Physical dimensions (cytometer only) (L x H x D)	Length: ~ 26 cm (10.2") Height: ~ 28.5 cm (11.2") Depth: ~ 25 cm (9.8")
Weight	~ 5 kg (~ 11lbs) (instrument only)
3rd party supplies	For venous samples: volumetric or transfer pipette For capillary samples: sterile lancets, alcohol swabs, cotton gauze, Band Aid

BD FACSPresto™ (2)	
Electric Power Requirements	100 – 240 V (A/C) at 45 – 65 Hz mains power Analyser contains on-board rechargeable battery. Can be charged with cigarette lighter.
Environmental Requirements	• Operating Temperature: 10º – 40º C (50º – 104º F) (ongoing validation) • Humidity: 5% – 95% (ongoing validation) • Maximum altitude: 2500 meters (8200 feet) (ongoing validation)
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. The USB port also can be used to support an external blue tooth or GPRS/GSM module to communicate with SMS printer or the port would be developed but not enabled, providing an option for wireless to be enabled post launch. Potential to install an SMS chip to transmit results or internal calibration data.
Monitor	LED multi-color screen integrated into instrument
Printer	On board printer (prints on thermal paper);
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	Analyser contains an integrated camera and microscope 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	Yes. Instrument will check itself each day and each cartridge will have onboard QC.
External QA	Will e compatible with CD4 EQA programs (ongoing validation)
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	Touch screen keyboard on the device

Zyomyx CD4 Test	
Type of Technology	Disposable cartridge used with a 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.
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 via lens on mixer/spinner.
Reagent stability	12 months at 2°C – 30°C, 70% humidity, including transport stress of 2 weeks with fluctuations up to 50°C (targeted)
Cost/test	< $8.00 per test (estimated)
Cost/instrument	$600 (mixer/spinner); cost is expected to be included in cost per test with purchase of a still to be determined volume of test cartridges
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, cartridge only; mixer/spinner, less than 5 kg (11 lbs)
3rd party supplies	Sterile lancets (for capillary blood samples), blood collection tube, alcohol swabs, dry swabs, gauze, bandage

Zyomyx CD4 Test (2)	
Electric Power Requirements	110 – 220V AC current and/or DC power with rechargeable battery for mixer/spinner; mechanical version (no electric power required) in development
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	Positive controls internalized in cartridge and visual checks to confirm.
External QA	TBD
Infrastructure Requirements	Can be used at all levels of health facility, including health centers, in mobile facilities or in the field.

Visitect CD4	
Type of Technology	Disposable cartridge containing test strip (lateral flow) that measures CD4 proteins on T cells qualitatively (above and below 350 cells/µL).
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	30 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) wait 3 minutes; (iv) add 1 drop of supplied buffer to Well A and allow sample to run for 17 minutes; (v) add 3 drops of buffer to Well B of test strip; (vi) wait for 20 minutes; (vii) read results.
Reagent stability	> 6 months at 40°C
Cost/test	$5 per test (estimated)
Cost/reader	$3,000 for reader (eventual price estimated to be $2,000). Reader will be provided free of charge dependent on committed volumes. 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 (3.0″)
Weight of reader	390 g (~14 oz)
3rd party supplies	None required. Sterile lancets (for capillary blood samples) and alcohol swabs are provided in the test kit.

Visitect CD4 (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 TaqMan Analyser
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), which can be analysed simultaneously. Batch size is 24 specimens per run.
Throughput per technician/ per day	Up to 168 specimens per 8 hour shift, based on testing combinations and laboratory workflow.
Sample needed and stability	1,000 µL of plasma or 70 µL DBS. Plasma may be transported/stored at 2 – 8° C for 5 days or frozen at -70° C; DBS can be stored up to 12 weeks at 30°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 specimens require mixing to HIV-1 RNA uniformity prior to testing.
Reagent stability	Varies by reagent, but most must be stored at 2° – 8° C (36° – 46° F); all reagents are stable until expiration date.
Cost/test	N/A
Cost/instrument	Approximately $80,000 – $100,000
Regulatory Status of Assays	FDA approved; WHO prequalified; CE-IVD marked (DBS is RUO only)
Physical dimensions (cytometer only) (W x D x H)	Width: 165 cm (65″) Depth: 75 cm (29.5″) Height: 95 cm (37.4″) Trolley Table: 167 cm (65.7″) x 76 cm (29.9″) x 55 cm (21.7″)
Weight	373 kg (822 lbs)
3rd party supplies	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 – 32º C (59ºF – 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® XP 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
Maintenance	Routine preventative maintenance required. In case of breakdown, vendor-trained technician required to repair.
Internal QC	An Internal Control/Quantitation Standard (IC/QS) is 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® 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 – 8º C (36º – 46º F); all reagents are stable until expiration date.
Cost/test	TaqMan HIV-1 Test v2.0: $11 – $25 in resource-limited settings; range is dependent on instrument purchase, reagent rental and volume-based tiered pricing.
Cost/instrument	$40,000 – $50,000
Regulatory Status	COBAS® TaqMan® HIV-1 Test, v2.0 is FDA approved, WHO prequalified, CE-IVD Marked
Physical dimensions (W x D x H)	19.7" x 31.1" x 22.8" 50 x 79 x 58 cm
Weight	121 lbs (55 kg)
Third party supplies	Microtiter plate centrifuge (not supplied by Roche) and other general supplies

RT-PCR: Roche COBAS® TaqMan® 48 (2) Automated amplification/detection instrument	
Electric Power Requirements	120 or 240 VAC 50 – 60 HZ
Environmental Requirements	• Temperature: 15°C – 32°C (59°F – 89°F) • Humidity: <80% (for temperatures up – 32°C) • Maximum altitude: 2,000 meters (6,500 feet)
Peripherals/Supporting Instrumentation	Custom-built PC supplied with the analyser; 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 Analysers
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, including automated transfer from the COBAS Ampliprep to a docking station
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 Analyser. All reagent addition is performed during the sample preparation process.
Cost/test	TaqMan HIV-1 Test v2.0: $20 – $30 per test (least developed countries); $35 – $90 per test elsewhere
Cost/instrument	$100,000 – $110,000, including dockng station
Regulatory Status	COBAS® TaqMan® HIV-1 Test, v2.0 is FDA approved, WHO prequalified, CE-IVD Marked
Physical dimensions (W x D x H)	Analyser: 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	448 lbs (203 kg)

RT-PCR: Roche COBAS® TaqMan® 96 (2) Automated amplification/detection instrument	
Electric Power Requirements	Analyser: 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: 15°C – 32°C (59°F – 89°F) • Humidity: <80% (for temperatures up – 32°C) • Maximum altitude: 2,000 meters (6,500 feet)
Peripherals/Supporting Instrumentation	Custom-built PC supplied with the analyser. Data station runs Microsoft ® Windows® XP operating system.
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	Abbott RealTi*m*e HIV-1 is WHO prequalified and 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 – 35° C (59° – 95°F) • Humidity: 5% – 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	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 – 30° C for up to 6 hours or at 2 – 8°C 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 – 30° C for up to 24 hours or at 2 – 8° C for up to 5 days. If longer storage is required, may be stored at -70° 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 -10° 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	Abbott RealTi*m*e HIV-1 is WHO prequalified, CE-IVD marked and FDA approved
Physical dimensions (W x D x H)	Width: 145 cm (57.1 in.) Height: 174.5 cm (68.7 in) Depth: 78 cm (30.7 in.)
Weight	211 kg (465 lbs)
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 – 30°C/59 – 86°F • Humidity: 30% – 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 – 30° C for up to 6 hours or at 2 – 8°C 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 – 30° C for up to 24 hours or at 2 – 8° C for up to 5 days. If longer storage is required, may be stored at -70° 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, dependent on volumes and subject to negotiations with Abbott
Cost/instrument	$38,000 USD (with *m*24sp or *m*2000sp) – Add $6,000 USD for all manual extraction items
Regulatory Status	Abbott RealTi*m*e HIV-1 is WHO prequalified and 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 – 30°C (59 – 86°F) • Humidity: 30% – 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

RT-PCR: 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	
Cost/instrument	
Regulatory Status	VERSANT® HIV-1 RNA 1.0 Assay (kPCR) is WHO prequalified and CE-IVD marked
Physical dimensions SP module; AD module (W x D x H)	112.4 cm (44 ins) 36.8 cm (14.5 ins) 100.6 cm (39.5 ins) 53.4 cm (21 ins) 90.5 cm (35.5 ins) 45.7 cm (18 ins)
Weight	320 lbs (145 kg) 55 lbs (25 kg)

RT-PCR: VERSANT™ kPCR Molecular System (2) Automated Sample Preparation and Amplification/Detection Modules	
Electric Power Requirements	100 – 240 V; 50 or 60 Hertz
Environmental Requirements	• Temperature: 18º – 30º C • Humidity: 30% – 80% non-condensing • Maximum altitude: 0 – 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

RT-PCR: QIAsymphony® SP/AS: Sample Preparation and Assay Set-up Instruments	
Type of Technology	Automated sample preparation and assay set up
Output	Samples ready for amplification and detection on Rotor-Gene Q
Turnaround time	Approx. 3 h for extraction and assay set up
Capacity (per run)	1 – 96 samples per run with continuous loading in batches of 24 samples plus internal controls
Throughput per technician/ per day	Up to three specimens per 8 hour shift, based on testing combinations and laboratory workflow.
Sample needed and stability	Up to 1,000 µL of plasma. [Plasma may be transported/stored at 2 – 8° C for 5 days or frozen at - 70° C.]
Specimen preparation and protocol complexity	The QIAsymphony SP accepts a wide variety of primary tubes for process safety and reduced hands-on time. The instrument offers over 17 different sample purification kits with over 45 standard protocols. Customized protocols are available on request.
Reagent stability	Varies by reagent, but most must be stored at 2° – 8° C (36° – 46° F); Shelf life of the purification kit is 12 months
Cost/test	N/A
Cost/instrument	N/A
Regulatory Status of Assays	The entire automated workflow from sample to result, including the assay, is CE-IVD marked
Physical dimensions (W x D x H)	QIAsymphony SP: 130 x 75 x 103 cm (51.2 in x 29.5 in x 40.6 in) QIAsymphony AS: 59 x 103 x 73 cm (23.2 in x 29.5 in x 28.7 in) Integratedt: 185 x 103 x 73 cm (72.8 in x 29.5 in x 28.7 in)
Weight	QIAsymphony SP: 175 kg (385.8 lbs); QIAsymhony AS: 90 kg (198.4 lbs); Integrated: 265 kg (584.2 lbs)
3rd party supplies	Vortex, refrigerator, gloves and other lab consumables

RT-PCR: QIAsymphony® SP/AS: Sample Preparation and Assay Set-up Instruments (2)	
Electric Power Requirements	100 – 240 VAC, 50 – 60 HZ
Environmental Requirements	• Temperature: 15°C – 32° C (59°F – 89°F) • Humidity: 15% – 75% (for temperatures up to 31°C, decreasing linearly to 50% humidity at 32°C) (noncondensing) • Maximum altitude: 2,000 meters (6,500 feet)
Data Station	
Monitor	
Printer	
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	
Infrastructure Requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Rotor-Gene Q Automated amplification/detection instrument	
Type of Technology	Automated amplification and detection
Output	RNA HIV-1 quantification
Turnaround time	Including sample preparation, 5 – 6 hours per 24 reactions
Capacity (per run)	67 samples
Throughput per technician/ per day	
Sample needed and stability	PCR-ready set-up samples from QIAsymphony AS or QIAamp DSP Virus Kit
Specimen preparation and protocol complexity	
Reagent stability	Varies by reagent, but most must be stored at 2º – 8º C (36º – 46º F)
Cost/test	
Cost/instrument	
Regulatory Status of Assays	CE-IVD marked
Physical dimensions (cytometer only) (W x D x H)	Width: 37 cm (14.6") Depth: 42 cm (16.5"); door open: 56 cm (22.0") Height: 27.5 cm (41")
Weight	14 kg (31 lbs)
3rd party supplies	Centrifuge, refrigerator, laboratory freezer and various additional laboratory consumables

RT-PCR: Rotor-Gene Q (2) Automated amplification/detection instrument	
Electric Power Requirements	100 – 240 VAC and 200 – 240 VAC, 50 – 60 HZ; 560 VA (peak)
Environmental Requirements	• Temperature: 15°C – 32° C (59°F – 89°F) • Humidity: 15% – 75% (for temperatures up to 31°C, decreasing linearly to 50% humidity at 32°C) (noncondensing) • Maximum altitude: 2,000 meters (6,500 feet)
Peripherals/Supporting Instrumentation	
Bar-code Scanner	
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	
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	Purified nucleic acids (RNA and DNA)
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 for NucliSENS EasyQ HIV assay (sensitivity is higher with larger sample). DBS protocol available (CE-marked protocol on EDTA whole blood on capillary whole blood).
Specimen preparation and protocol complexity	Plasma or DBS are transferred to a lysis tube. After addition of silica, washing steps are performed on the miniMAG system. Reagents are then ready to use.
Reagent stability	Reagents must be stored at 2º – 8º C (36º – 46º F); all reagents are stable until expitation date
Cost/test	N/A
Cost/instrument	Approximately €6,800 ($9,000)
Regulatory Status of Assays	NUCLISENS EasyQ® HIV-1 2.0 is WHO prequalified and 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	3.6 kg (8 lbs)
3rd party supplies	Pipettes, vortex mixer and refrigerator; benchtop centrifuge (1.5 ml tubes), thermoshaker, centrifuge, (2 ml lysis tubes)

*Note: some details in above table revised August 2013 following first printing of this report (June 2013)

NASBA: bioMérieux NucliSENS® miniMAG® Semi-automated extraction instrument (2)	
Electric Power Requirements	100 – 240 VAC 50 – 60 HZ
Environmental Requirements	• Operating temperature: 4º C – 45º C • Humidity: Maximum of 90% relative humidity • Maximum altitude: 2,000 meters (6,500 feet)
Data Station	None
Monitor	None
Printer	None
Bar-code Scanner	N/A
Training	Fully-trained lab tech required; dedicated training on instrument
Maintenance	Routine preventative maintenance required.
Internal QC	Yes, a synthetic calibrator added in a known concentration at the extraction stage, functions as an internal control for the isolation, amplification and detection procedure.
Infrastructure Requirements	Technology can be used at the regional/central level or national reference (or comparable) laboratories. Access to decentralized settings via DBS.

*Note: some details in above table revised August 2013 following first printing of this report (June 2013)

NASBA: bioMérieux NucliSENS® easyMAG® Automated extraction instrument	
Type of Technology	Automated extraction instrument
Output	Purified nucleic acids (RNA and DNA)
Turnaround time	24 samples, lysis on board: 60minutes 24 samples, lysis off board: 40minutes
Capacity (per run)	1 – 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	100 – 1,000 µL plasma for NucliSENS EasyQ HIV assay (LOD is better with larger sample). DBS protocol available (CE-marked protocol 100µL EDTA whole blood and on 100µL capillary whole blood).
Specimen preparation and protocol complexity	Entire extraction process takes place in a single sample compartment, which minimizes potential sample loss and cross contamination. Reagents are ready-to-use.
Reagent stability	Reagents must be stored at 2º – 8º C (36º – 46º F), except the third buffer and silica must be stored at 4º C. All reagents are stable until expiration date.
Cost/test	N/A
Cost/instrument	Approximately €72,000 ($95,000)
Regulatory Status of Assays	NUCLISENS EasyQ® HIV-1 2.0 is WHO prequalified and 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	106 kg (233.7 lbs); PC monitor and keyboard: 8 kg (17.6 lbs)
3rd party supplies	Pipettes, vortex mixer and refrigerator; strip centrifuge

*Note: some details in above table revised August 2013 following first printing of this report (June 2013)

NASBA: bioMérieux NucliSENS® easyMAG® Automated extraction instrument (2)	
Electric Power Requirements	100 – 240 VAC 50 – 60 HZ
Environmental Requirements	• Operating temperature: 15° C – 30° C • Humidity: Maximum relative humidity: 80%, non-condensing at 30° C DB • Maximum altitude: 2,500 meters (8,202 feet)
Data Station	Yes. May be linked with LIS using NucliSENtral™ software.
Monitor	On-board Monitor
Printer	None supplied
Bar-code Scanner	Supplied with the system
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, a synthetic calibrator added in a known concentration at the extraction stage, functions as an internal control for the isolation, amplification and detection procedure.
Infrastructure Requirements	Technology can be used at the regional/central level or national reference (or comparable) laboratories. Access to decentralized settings via DBS.

*Note: some details in above table revised August 2013 following first printing of this report (June 2013)

NASBA: bioMérieux NucliSENS EasyQ® Automated amplification/detection instrument	
Type of Technology	Automated, real-time NASBA amplification and detection
Output	Qualitative or quantitative results for DNA or RNA targets Quantitative results for NucliSENS EasyQ HIV-1 assay v 2.0
Turnaround time	~1.5 hours for 48 samples; 1 hour for NucliSENS EasyQ HIV-1 assay v 2.0
Capacity (per run)	Up to 48 patient samples (minimum is 8 patient samples)
Throughput per technician/ per day	192 samples (4 runs of 48)
Sample needed and stability	Eluates extracted with miniMAG or easyMAG. Can be stored at 2º C – 8º C; all reagents are stable until expiration date.
Sample preparation and protocol complexity	Moderate complexity. Dehydrated reagents are quickly reconstituted.
Reagent stability	NucliSENS EasyQ HIV-1 v 2.0 assay storage at 2º C – 8º C; all reagents are stable until expiration date.
Cost/test	The average price per test of EasyQ HIV assay v 2.0, including extraction and detection/ amplification is about €18.00 ($23.75).
Cost/instrument	Approximately €37,100 ($49,000)
Regulatory Status	NUCLISENS EasyQ® HIV-1 v 2.0 is WHO prequalified and 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	20.5 kg (45 lbs)

*Note: some details in above table revised August 2013 following first printing of this report (June 2013)

NASBA: bioMérieux NucliSENS EasyQ® Automated amplification/detection instrument (2)	
Electric Power Requirements	100 – 240 V
Environmental Requirements	• Operating temperature: 15° C – 30° C • Humidity: no greater than 80% relative humidity • Maximum altitude: 2,000 meters (6,500 feet)
Peripherals/Supporting Instrumentation	Data station and monitor are supplied with the instrument. Printer not supplied with instrument. May be linked with LIS using NucliSENtral™ software.
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, a synthetic calibrator added in a known concentration at the extraction stage, functions as an internal control for the isolation, amplification and detection procedure
Infrastructure Requirements	Technology can be used at central/national reference (or comparable) laboratories. Access to decentralized settings via DBS.

*Note: some details in above table revised August 2013 following first printing of this report (June 2013)

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	
Cost/instrument	
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	ELISA-based manual measurement of RT activity
Output	Quantification of HIV-1 RT enzyme activity
Turnaround time	48 hours for 30 tests, including ~5 hours hands-on time
Capacity (per run)	30 tests
Throughput per technician/ per day	Up to 60 samples (two batches of 30). Maximum 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 analysed and should be frozen at or below -20° 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 -14°C to -25°C; reagent kits are stable >12 months at -20°C. If stored between 4° and 8° 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 33°C; freezer set at -14°C to -25°C; end-over-end mixing table; vortex; computer

Reverse Transcriptase: ExaVir™ Load (2) Separation and RT Assay	
Electric Power Requirements	100 – 240V; 50 – 60Hz
Environmental Requirements	• Temperature: 16° – 33°C • Humidity: N/A • Maximum altitude: N/A
Peripherals/Supporting Instrumentation	ExaVir Viral Load Analyser software for processing results; computer required, but not supplied; no printer supplied
Bar-code Scanner	None
Training	Four days of training required
Maintenance	Routine preventative maintenance required.
Internal QC	Yes
External QA	Available in some regions
Infrastructure Requirements	Technology can be used at central, regional, district and some well-developed primary sites with dedicated laboratory facilities and technicians.

Alere Q	
Type of Technology	Portable bench-top, NAT-based purification, amplification and detection system for total HIV RNA
Output	Quantitative HIV-1, Groups M, N and O, 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. Alternatively, 25 µL of heelprick or venous blood can be used in the device. For venous blood, no metering is required onto the 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 analyser; (v) enter operator and sample ID; (vi) analysis starts automatically; (vii) remove cartridge from analyser and dispose of it; and (viii) read result from screen. Hands-on time expected to be ~10 minutes.
Reagent stability	Freeze-dried reagents require no refrigeration. Stable for 12 months at 2 – 30°C
Cost/test	TBD
Cost/instrument	TBD
Regulatory Status	Alere will seek regulatory approval for CE-IVD marking and FDA approval in 2012 and 2013.
Physical dimensions (analyser 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 Q (2)	
Electric Power Requirements	Analyser contains on-board rechargeable battery that provides a full work day (at least 8 hours) of operation.
Environmental Requirements	• Operating Temperature: 15º – 40º C (59º – 104º F) • Humidity: < 90% relative humidity • Maximum altitude: N/A (permissible atmospheric pressure: 850 – 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. Will support wireless connectivity and device can be attached to a USB port for sample tracking, if desired by user.
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	Will be fully-compatible with existing EQA programs.
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™ Analyser	
Type of Technology	Portable bench-top, sample preparation and real time PCR
Output	Qualitative or quantitative (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 8 hour day, depending on limit of detection
Throughput per technician/ per day	~8 – 15 samples per technician per 8 hour 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 manual sample preparation required, even using capillary blood. Operation only requires: (i) apply blood drops from finger lancet or plasma to Liat tube; (ii) scan the tube's bar code on the device; (iii) insert tube into Liat analyser; the analyser will start assay and the result will be reported in ~30 minutes automatically.
Reagent stability	Reagents expected to be shipped with an expiration date of at least 6 months; reagents should be stored at approximately 4°C (39.2°F); but 37°C (98.6°F) storage allowed for 3 weeks.
Cost/test	TBD
Cost/instrument	~$25,000, but may be priced lower for resource-limited settings
Regulatory Status	US FDA 510 (k) clearance for Liat Influenza A/B Assay, TBD for Liat HIV Quant Assay
Physical dimensions (W x H x D)	Width: 4.5 inches Height: 7.5 inches Depth: 9.5 inches
Weight	3.75 kg (~8.3 lbs)
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs

Liat™ Analyser (2)	
Electric Power Requirements	AC or battery powered
Environmental Requirements	• Operating Temperature: 15° – 32° C (59° – 89.6° F) • Humidity: 15% – 80% (non-condensation) • Maximum altitude: 2,000 m (6,500 ft.) above sea level (expanding operation conditions is possible but requires further validation)
Data Station	Dedicated CPU integrated into instrument; approximately 20,000 test results can be stored on the instrument archive; results can be downloaded via USB or Ethernet.
Monitor	LED color screen integrated into instrument
Printer	No printer provided; can be connected via USB or Ethernet (optional).
Bar-code Scanner	Integrated into instrument for operator barcode, patient barcode and Liat tube barcode
Training	Minimal training required. Lay person can be trained in less than 30 minutes. Primary skill required is for correct lancet blood draw.
Maintenance	• No operator troubleshooting, calibration or service required; self-diagnostics during power-on start-up and advanced error diagnostics during assay run alerts the operator in the event of malfunction or error. • Remote system monitoring/diagnosis performed via the Liat Analyzser's built-in network connectivity interface. • If damaged, portability of analyser allows for swap-out of device and shipment for depot repair.
Internal QC	Extensive internal controls: sample volume control, internal process controls, and more.
External QA	TBD whether compatible with EQA programs
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	LCD touch screen with 4 hard-keys and 4 arrow buttons

EOSCAPE-HIV™ HIV Rapid RNA Assay System	
Type of Technology	Nucleic acid test
Output	HIV-1 RNA level (quantitative/qualitative)
Turnaround time	50 minutes
Capacity	1 cartridge per processing unit; multiple processing units can be run in parallel with single analyser unit
Throughput per technician/ per day	>50 (with 6 – 8 processing units and a single analyser)
Sample needed and stability	Fresh fingerstick whole blood, 50 µL. Also accepts plasma samples.
Sample preparation and protocol complexity	Low complexity: no external sample preparation or other manipulation of samples or reagents by the user. All assay processes take place within single-use disposable cartridges. Acquiring 50 µL sample from fingerstick requires specialized technique (e.g., massaging of finger, positioning of hand below heart).
Reagent stability	Cartridges are shelf-stable for 1 year at 37° C
Cost/test	<$12 per test (semi-quantitative >1,000 cp/mL; EID) <$20 per test (fully quantitative with internal calibrants; viral load)
Cost/instrument	<$10,000 for one analyser unit with two processing units (typical)
Regulatory Status	CE-IVD mark expected
Physical dimensions (W x H x D)	Width: 250mm (9.8") Height: 90mm (3.5") Depth: 150mm (5.9")
Weight	1.2 kg (~2. 7 lbs)
3rd party supplies	Lancet, alcohol swab (supplied in kit)

EOSCAPE-HIV™ HIV Rapid RNA Assay System (2)	
Electric Power Requirements	Mains power with 8 hour rechargeable battery backup. Solar charging capable.
Environmental Requirements	• Operating Temperature: < 40° C • Humidity: N/A • Maximum altitude: N/A
Data Station	Standard laboratory information management systems; USB; integrated wireless connectivity
Monitor	Integrated 7" touchscreen
Printer	External
Bar-code Scanner	Integrated
Training	8 hours training for U.S. high school education level; 1 hour training for medical professionals
Maintenance	Wipe-down with diluted bleach solution; replace rechargeable batteries after extended cycling
Internal QC	Internal amplification/process control (IAC)
External QA	Separate cartridges required to run external positive/negative controls; separate cartridges required to run external calibration process (to operate in quantitative mode)
Infrastructure Requirements	Biohazard disposal; cartridge storage
User interface	Touchscreen interface; power on/off switches on processing unit and analyser unit

SAMBA	
Type of Technology	Isothermal target/signal amplification and visual detection; separate extraction
Output	Qualitative for EID and semi-quantitative for viral load (limit of detection ~400 cp/mL RNA with 100µL of whole blood, also detects DNA); detection of acute infection (limit of detection ~100 cp/mL with 500µL of plasma); and semi-quantitative viral load test for monitoring of patients on ART (1,000 cp/mL cutoff with 200µL of plasma).
Turnaround time	90 – 120 minutes, depending on the assay
Capacity	6 samples per run
Throughput per technician/ per day	24 samples per day for EID or acute infection tests; 36 viral lad tests, assuming a 6.5 hour work day.
Sample needed and stability	200µL (plasma) for viral load test; 500µL (plasma) for acute infection test; 100µL (whole blood) for EID test. Sample is stable at room temperature for 6 – 8 hours.
Sample preparation and protocol complexity	Simple, pre-loaded, disposable cartridges containing all required liquid or dry reagents
Reagent stability	Transport stability up to 55°C for one month. Reagents do not require cold-chain storage and are stable up to 37° for 1 year.
Cost/test	TBD
Cost/instrument	TBD
Regulatory Status	Regulatory approval obtained in Malawi for viral load assay
Physical dimensions (W x H x D)	Width: TBD Height: TBD Depth: TBD
Weight	TBD
3rd party supplies	

SAMBA (2)	
Electric Power Requirements	AC powered (100 – 240V); can be battery powered to compare the run
Environmental Requirements	• Operating Temperature: 10° – 37°C • Humidity: up to 95% • Maximum altitude: N/A
Data Station	None
Monitor	Small screen integrated into instrument
Printer	No printer provided
Bar-code Scanner	None
Training	Minimal training required
Maintenance	No maintenance required; swap-out of instrument if needed.
Internal QC	Synthetic, non-target nucleic acid internal controls
External QA	Freeze-dried EQA panel provided consisting of a negative sample and a range of positive samples.
Infrastructure Requirements	Can be used at various levels of health facility, including health centers or in mobile facilities. Electricity is required.
User interface	Touch screen

GeneXpert® System	
Type of Technology	PCR-based NAAT test
Output	Quantitative HIV-1 (viral load) and Qualitative HIV-1
Turnaround time	< 90 min
Capacity	Dependent on GeneXpert system and number of modules ranging from 1 – 80 per system, Comparable to GeneXpert MTB
Throughput per technician/ per day	Dependent on GeneXpert system and number of modules. For example, 397 results per 8 hour shift with an Infinity-80 *(80 modules)
Sample needed and stability	1 ml plasma for Quantitative HIV-1; Qualitative whole blood and DBS under development.
Sample preparation and protocol complexity	Automated within cartridge
Reagent stability	No refrigeration required. In development.
Cost/test	TBD
Cost/instrument	Comparable to GeneXpert MTB
Regulatory Status	Regulatory submissions for CE IVD and FDA expected in 2014.
Physical dimensions (W x H x D)	Please see www.cepheid.com for brochure on systems available. Below are specifications for a GX-IV Processing Unit Length: 11.00″ Height: 12.00″ Depth: 13.25″
Weight	For GX-IV Processing Unit: 25 lbs

GeneXpert® System (2)	
Electric Power Requirements	Mains power required: 100 – 240 V
Environmental Requirements	Operating Temperature: 15°– 30° C Relative Humidity: 10% – 95%, non-condensing
Bar-code scanner	Included with system
Training	Minimal training required. Lay person can be trained in less than half a day. Primary skill required is for correct blood draw.
Maintenance	Remote calibration kit for onsite user calibration. If damaged, modules are exchangeable.
Internal QC	Internal to the cartridge
External QA	Will be fully-compatible with existing EQA programs
Infrastructure Requirements	Can be used at all levels of health facility that have electricity, including health centers or in mobile facilities.

LYNX HIV Viral Load Test and Platform	
Type of Technology	A bench-top automated cartridge-based system that extracts, amplifies and detects nucleic acid targets for IVD applications.
Output	Quantitative HIV-1
Turnaround time	~60 – 90 minutes
Capacity	The Processor can be configured to accommodate throughput of 8 – 96 tests per 8-hour work day.
Throughput per technician/ per day	~8 – 96 samples per day, depending on Processor configuration
Sample needed and stability	To achieve 1,000 copies/mL of plasma, ~150 µL of whole blood will be converted into plasma with simple sample preparation materials provided by NWGHF.
Sample preparation and protocol complexity	(i) Add sample to cartridge (ii) Close sample port and cap to seal cartridge iii) Place the cartridge into the loading/unloading position on the system iv) Read the results on the screen
Reagent stability	The shelf life of the assay kit is expected to be 12 – 18 months at 30°C – 40°C, 70% – 90% humidity
Cost/test	<$10 per test
Cost/instrument	Varies based on configuration. Smallest configuration is <$12,000
Regulatory Status	TBD
Physical dimensions (W x H x D)	TBD
Weight	TBD
3rd party supplies	Blood collection supplies

LYNX HIV Viral Load Test and Platform (2)	
Electric Power Requirements	The Processor is powered by an external power transformer that connects to either an AC or DC power cable that connects to an AC or DC power socket in the clinic or laboratory. A fully charged battery will complete the cartridges in the processor.
Environmental Requirements	No cold chain or humidity control is required for shipping and transport.
Data Station	Internal EDGE/3G modem provided upon request
Monitor	Integrated into the instrument
Printer	Optional
Bar-code Scanner	Optional
Training	Minimal training required; primary skill required is for correct lancet blood draw.
Maintenance	Minimal maintenance
Internal QC	Yes
External QA	Will be fully-compatible with existing EQA programs
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	Onboard display

Viral Load Assay using BART Technology	
Type of Technology	iNAAT platform
Output	HIV-1 RNA level (quantitative)
Turnaround time	1 hour
Capacity	8 – 16 samples per run per device
Throughput per technician/ per day	32 – 64 samples per technician per device per day
Sample needed and stability	Whole blood/Plasma; up to 1 ml
Sample preparation and protocol complexity	Bead-based approach. Moderate complexity.
Reagent stability	12 months at 37˚C
Cost/test	$10.00
Cost/instrument	$1,000
Regulatory Status	Company will apply for CE-IVD marking and WHO prequalification
Physical dimensions (analyser) (W x H x D)	Width: 20 cm (7.9”) Height: 20 cm (7.9”) Depth: 25 cm (9.8”)
Weight (analyser)	1 kg (~2.2 lbs)
3rd party supplies	Powder-free disposal gloves, waste disposal container with lid, sterile lancets, alcohol swabs, dry swabs

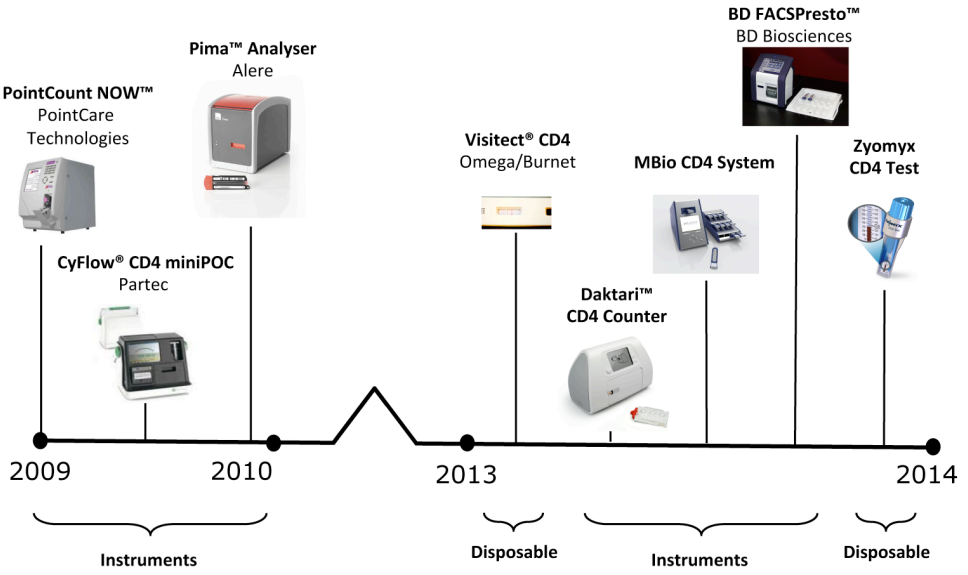
Viral Load Assay using BART Technology (2)	
Electric Power Requirements	Mains power for laboratories or car battery for field use
Environmental Requirements	• Operating Temperature: 4°to 40˚ C • Humidity: up to 100% • Maximum altitude: 2500m (~8,200 feet)
Data Station	Stand-alone machine. Data retrieved via USB stick/data cable
Monitor	Built-in touch screen
Printer	Device can be attached via USB cable to a computer.
Bar-code Scanner	Can be added to the system.
Training	½ day of training for healthcare worker
Maintenance	Minimal maintenance: Routine bleaching of unit. If damaged, the company plans to swap-out the device rather than repair it on-site.
Internal QC	Calibration algorithms to check light detection systems, temperature and power failures
External QA	System is expected to be compatible with most available EQA systems
Infrastructure Requirements	Indoors or a covered area (e.g., sheltered area in the back of a truck).
User interface	Simple "load and play" with automated diagnostic algorithms for result calling.

LYNX HIV p24 Antigen Assay (EID)	
Type of Technology	p24 Antigen Assay for EID
Output	Qualitative detection of HIV infection
Turnaround time	40 – 50 minutes, including blood draw and sample preparation (30 minutes for readout only)
Capacity	1 sample tested sequentially
Throughput per technician/ per day	~12 samples per day
Sample needed and stability	~80 µL of blood from the infant's heel
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 sample processor and wait 30 – 40 minutes; (v) read test.
Reagent stability	TBD
Cost/test	Estimated to be: $7 – $15 per test
Cost/instrument	~$700 – $900 for sample processor
Regulatory Status	TBD
Physical dimensions (W x H x D)	Width: 202mm (~8 inches) Height: 156mm (6.1 inches) Depth: 134mm (5.3 inches)
Weight	1.7 kg (~3.7 lbs)
3rd party supplies	None

LYNX HIV p24 Antigen Assay (EID) (2)	
Electric Power Requirements	Sample processor is battery powered with 12 VDC (e.g., solar or car battery) or 100 – 240 VAC recharging
Environmental Requirements	TBD
Data Station	Internal EDGE/3G modem provided upon request
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; sample processor is expected to last 3 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
Infrastructure Requirements	Can be used at all levels of health facility, including health centers or in mobile facilities.
User interface	Display with timer and battery indicator

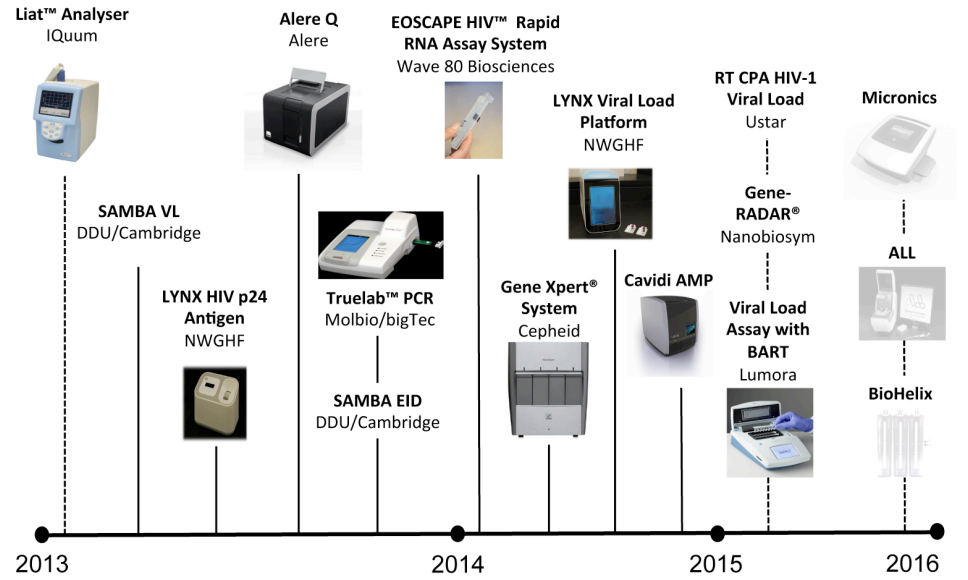
APPENDIX 2:
Pipeline for POC Diagnostics

CD4 Product Pipeline*



*Estimated as of March 2013 - timeline and sequence may change.

Viral Load and EID Product Pipeline*



*Estimated as of March 2013 - timeline and sequence may change. ----No market launch date set by company.

APPENDIX 3:
Technical Specifications for HIV 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, DBS	Whole blood / EDTA plasma / DBS	EDTA and ACD plasma
Specimen volume	100 µl whole blood (Infants) 500 µl whole blood (adults) 60-70 µl DBS	100 µl whole blood 60-70 µl DBS	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[1]	$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 – $20 per test
Number of samples/ run	9-21	22-66 batch loading (176/8 hour day continuous loading)	21-93 patient samples (+3 external controls)
Equipment required[2]	Thermocycler, ELISA reader / washer, microcentrifuge not supplied by Roche	COBAS® AmpliPrep with COBAS® TaqMan® 96 COBAS® TaqMan® 48	*m*2000rt; *m*2000sp, *m*24sp or manual sample preparation
Equipment Cost ($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
Regulatory Status			WHO Prequalified

1 Prices will vary considerably depending on quantities, infrastructure and support required plus special negotiations.

2 All assays require pipettes, vortex mixers, and a refrigerator.

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