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HIV/AIDS

Diagnostics Technology Landscape

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World Health Organization

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Abbreviations

AC	alternating current
ACD	acid citrate dextrose
AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
AZT	zidovudine
BART	Bioluminescent Assay in Real-Time
bDNA	branched chain deoxyribonucleic acid
CDC	Centers for Disease Control and Prevention (United States)
CE	Conformité Européenne (European Conformity)
CHAI	Clinton Health Access Initiative
cm	centimetre
cp	copies
CPA	Cross Priming Amplification
CRF	circulating recombinant forms
CV	coefficient of variation
DBS	dried blood spot
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 (United States)
FSC	forward scatter channel
g	gram
GSM	global system for mobile communications
HIV	human immunodeficiency virus
HSV	herpes simplex virus
Hz	hertz
ID	identification
in/”	inch
iNAAT	isothermal nucleic acid amplification technology
ISO	International Organization for Standardization
IVD	in vitro diagnostic
kg	kilogram
LAMP	loop-mediated amplification
lbs	pounds
LIMS	laboratory information management system
LIS	laboratory information system
LTR	long terminal repeat
MSF	Médicins sans Frontières
MTB	*Mycobacterium tuberculosis*
NASBA	nucleic acid sequence-based amplification
NAT	nucleic acid-based test
NIAID	National Institute of Allergy and Infectious Diseases
NWGHF	Northwestern Global Health Foundation
oz	ounce

PC	personal computer
PCR	polymerase chain reaction
PEPFAR	President's Emergency Plan for AIDS Relief
pg	picogram
PMT	photo-multiplying tube
POC	point of care
QC	quality control
RIF	rifampicin
RNA	ribonucleic acid
RT	reverse transcriptase
RUO	research use only
SAMBA	simple amplification-based assay
SMS	short message service
SSC	side scatter channel
µL	microlitre
UPS	uninterruptible power supply
USAID	United States Agency for International Development
USB	universal serial bus
WBC	white blood cell
W	watt
WHO	World Health Organization

HIV/AIDS DIAGNOSTICS TECHNOLOGY 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 is still 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 site of patient care. This report, therefore, examines the new diagnostic technologies both on the market and in the pipeline – most of which are designed for use at or near the point of patient care – and considers, on the basis of their operational characteristics, at what level of the tiered laboratory structure found in resource-limited settings they are best suited.

With respect to CD4 testing, which is primarily 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 additional high-quality, cost-effective point-of-care (POC) CD4 testing options. Six 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 recommended by the World Health Organization (WHO) as the preferred test for monitoring HIV patients following initiation onto ART, there also are 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 Botswana, Brazil and South Africa. 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 would reduce the need for infrastructure and training, and also could 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 additional platforms and assays in development, several of which may come to market in 2014–2015.

Finally, with respect to testing for infants aged under 18 months, the most widely used test for EID is a DNA polymerase chain reaction (PCR) molecular test, which also is 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 decreased, 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 POC 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 three POC assays being developed specifically for EID, two of which might be launched in 2014.

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 also are becoming available, allowing cost-effective consolidation of testing in high-volume centres (e.g. super-laboratories). The level of CD4, viral load and EID testing required in resource-limited settings over the coming years likely will necessitate scale-up in centralized testing facilities, including, in some cases, super-laboratories. 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 also will 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 POC.

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 point-of-care (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 that 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 also is 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 or near 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 coming years. The optimal approach to ensuring improved access to high-quality diagnostics for HIV/AIDS is still unfolding, however. Improved access likely will be achieved through a mix of diagnostic services in most countries that combines sophisticated, high-volume, low unit-cost laboratories in high-density areas, and lower-volume, simpler, POC or near-POC platforms in less densely populated regions. However, the best technology mix is unclear in most countries and new models for delivery also may 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 early infant diagnosis (EID) (including EID run on viral load platforms) – all of which today are accessed typically through sophisticated laboratory-based testing platforms, even in resource-limited settings. The report describes 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.

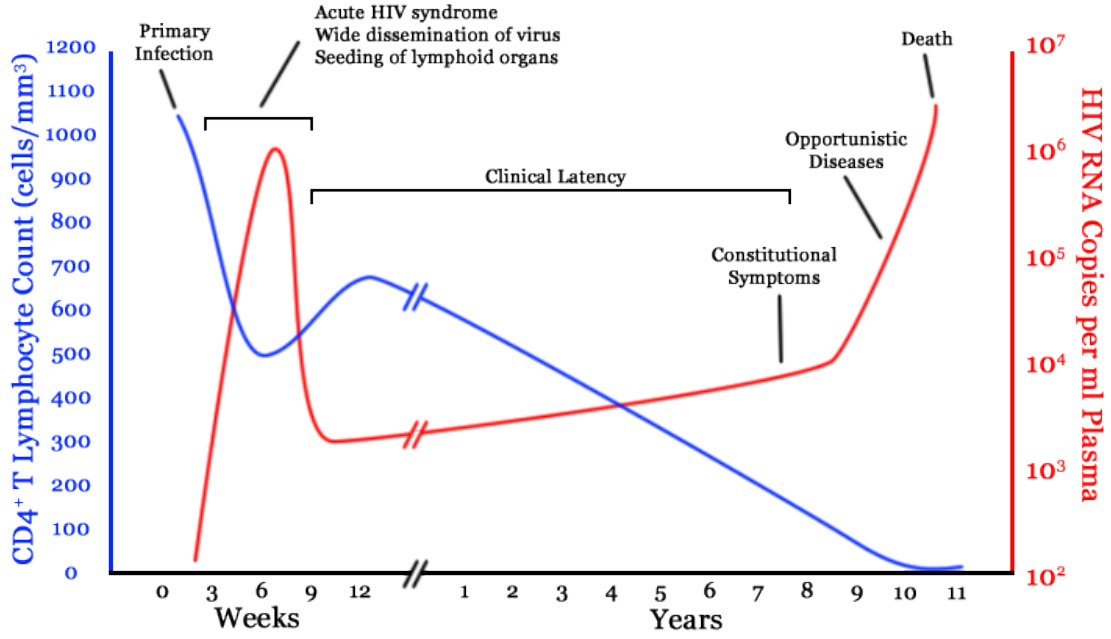
Methodology

The *2014 HIV/AIDS diagnostics technology 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 May 2014.

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 onto 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)*.



Source: Adapted from Pantaleo, Graziosi and Fauci (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)*.

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 for this section are cited below.

The measurement of the number of viral cp/mL 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 polymerase chain reaction (PCR) tests. Of these, HIV antibody tests are most commonly used for routine diagnosis of patients aged more than 18 months because they are inexpensive and accurate when performed correctly. For patients aged more than 18 months, 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 also is 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, then a tie-breaker test is used, which also is usually an HIV rapid disposable.[3]

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, usually can be performed on fingerstick blood or oral fluid, contain built-in quality controls (QCs) and can be administered by technicians and non-technicians alike, including community health workers. Furthermore, 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 markups, ranges from about US$ 0.50 per test to about US$1.60 per test[4] for blood-based tests, but can be as much as US$ 5 per test for saliva-based tests. ELISA testing is laboratory-based and generally costs US$1.50–2 per test, including consumables, but is no longer widely used for HIV screening.

Because of the persistence of maternal antibodies in infants aged under 18 months, the use of antibody tests, such as 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] *(5)*

The most widely used test for EID is the DNA PCR molecular test. It also is 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 centres 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

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. Viet Nam) and, as indicated, some still use an ELISA test for confirmatory testing.

3 Some countries use an ELISA test in the case of discordant results.

4 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 markups, 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 *WHO 2013 Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, recommendations for a public health approach* (WHO 2013 Guidelines): "It is strongly recommended that all HIV-exposed infants have HIV virological testing at 4–6 weeks of age or at the earliest opportunity thereafter *(strong recommendation, high-quality evidence)"*. Furthermore, the WHO 2013 Guidelines also strongly recommend that all infants with unknown or uncertain HIV status being seen in health-care facilities at or around birth or at the first postnatal visit (normally at 4–6 weeks) should have their HIV exposure status determined (*strong recommendation, high-quality evidence*) *(5)*.

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

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; biosafety cabinet; freezer) can range from about US$ 100 000 to more than US$ 200 000, depending on the throughput of the platform. The cost of the test itself ranges from about US$10 per test on low-throughput platforms to about US$ 12–20 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 US$ 1.40 per test to about US$ 2.75 per test depending on bundle configuration. It also does not include more general laboratory consumables (e.g. gloves; pipettes), which cost from about US$ 0.35 per test to US$ 4 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 could 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 aged under 5 years, 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 2013 Guidelines on ART initiation, if the absolute CD4 count of an adult or a child aged 5 years or older is below a defined threshold (currently ≤500 cells/mm³), then ART should be initiated *(5)*.[8] In the absence of CD4 testing, patients aged 5 years and older with WHO clinical stage 3 or 4 disease, also should be initiated onto ART.

Furthermore, the World Health Organization (WHO) now recommends ART initiation regardless of WHO clinical stage or CD4 cell count for all children aged under 5 years,[9] pregnant and breastfeeding women, HIV-positive partners in serodiscordant couples and all individuals with active MTB disease *(5)*.[10] In other words, for these populations, no staging is needed.

Whether in low- or high-throughput settings, CD4 testing is primarily conducted on laboratory-based instruments, although there are six POC CD4 test platforms currently available on the market. In rural and 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 laboratories 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.[11]

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

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 at about the age of 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" *(6)*.

8 The new WHO 2013 Guidelines recommend earlier initiation of ART than the WHO 2010 Guidelines, at CD4 counts of ≤500 cells/mm³, but prioritize treatment initiation at ≤350 cells/mm³ *(5)*.

9 If the recommendation to treat all children aged between 1 and 5 years is not adopted, then the WHO 2013 Guidelines recommend that ART be initiated for such patients with severe or advanced symptomatic disease (WHO clinical stage 3 or 4) or with CD4 count ≤750 cells/mm³ or <25%, whichever is lower, regardless of WHO clinical stage. Furthermore, WHO prioritizes initiating ART for children aged 1–2 years with WHO clinical stage 3 or 4 or with CD4 count ≤750 cells/mm³ or <25%, whichever is lower *(5)*.

10 WHO also recommends that all individuals with hepatitis B co-infection with CD4 ≤500 cells/mm³ and regardless of CD4 cell count in the presence of severe chronic liver disease should be initiated onto ART (5).

11 Recently, the use of DBS as a possible alternative for CD4 testing in resource-limited settings has been investigated *(8)*, 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)*.

varies from a low of about US$ 2 per test to approximately US$ 14 per test, excluding collection and laboratory consumables. The cost of consumables will add between US$ 1 and US$ 2 per test to the cost. Instruments range in price from about US$ 25 000 for low-throughput devices to US$ 90 000 for high-throughput instruments.

The cost of currently available POC CD4 testing ranges from just under US$ 4 per test to about US$ 120 per test for the test reagents alone, with associated sample collection consumables adding approximately US$ 1 per test. The instruments cost from US$ 500 to US$ 25 000 per device. As additional POC CD4 products enter the market, including at least one disposable test, prices likely will fall. It is possible that a disposable CD4 test could ultimately cost between US$ 2 and US$ 3 per test, but early pricing will be higher.

Patient monitoring

Prior to initiation onto ART, the current WHO recommendations are to repeat CD4 testing approximately every 6–12 months *(5)*. WHO guidance indicates that CD4 testing is required to identify whether adult and adolescent patients with HIV who are HIV asymptomatic and WHO clinical stage 1 or 2 disease need to start ART. In recent guidance, WHO has reiterated that "assessment of CD4 cell count is still necessary to guide initiation of ART outside of certain clinical situations" *(9)*. It is worth noting that CD4 testing, along with clinical symptoms, also is being used to diagnose treatment failure in many resource-limited settings.

Chemistry and haematology testing

Clinical chemistry and haematology 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 haematology test recommendations on ART regimens. For example, for zidovudine (AZT)-containing regimens, haemoglobin measurement is recommended before ART initiation, primarily among adults and children with low body weight, low CD4 counts and advanced HIV disease *(5)*, For tenofovir-containing regimens, while laboratory monitoring is not mandatory to initiate treatment, where the creatinine test is routinely available, creatinine clearance calculation is recommended before initiation. Additional tests and test panels are recommended as required depending on patient symptoms.[12]

The technology options available for multiparameter chemistry and haematology 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 US$ 9000 to US$ 32 000 for haematology instruments and from about US$ 3000 to almost US$ 60 000 for chemistry instruments. A number of low-volume, low-cost, robust, automated haematology 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 haematology 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 airconditioning, dedicated uninterruptible power supply (UPS) and well-trained, computer-literate technicians.

The technology options available for POC chemistry or haematology are not widely available in resource-limited settings. Nevertheless, simple handheld 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 US$ 1000–5000, and were designed for doctors' offices, home use or bedside testing in patient wards. There also are a limited number of POC chemistry and haematology platforms that are less mobile, larger in size and capable of running a wider range of tests. With price ranges of approximately US$ 3000–10 000 depending on the features available, these are designed to be placed in a clinical care setting, such as a patient ward, outpatient clinic or doctor's office, and can be operated by non-laboratory health-care workers after minimal training.

12 See the WHO 2013 Guidelines for detailed recommendations with regard to toxicity monitoring, including for children and other key populations.

The average cost of the basic full blood count is approximately US$ 1.15 per test, while consumables average approximately US$ 2 per test. For chemistry testing, the costs vary per test run and on average range from US$ 0.10 per test to US$ 0.45 per test. Consumables average approximately US$ 1.50 per test.

Viral load testing

Finally, post-initiation onto ART, viral load testing ideally should 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.[13] However, due to the cost and complexity of the test, the implementation of viral load testing in resource-poor settings has been relatively limited.

In its 2010 Guidelines on ART for HIV infection in adults and adolescents, WHO did not recommend the routine use of viral load testing to monitor patient response to ART, but rather recommended that viral load testing be used only where it was routinely available *(6)*. However, in its 2013 Guidelines, WHO now recommends routine viral load testing as the preferred monitoring approach to diagnose and confirm ART failure.[14] Furthermore, WHO recommends that HIV viral load testing be done at 6 months after initiating patients onto ART and every 12 months thereafter.[15] Although WHO recommends that viral load be monitored routinely to enable treatment failure to be detected earlier and more accurately, in settings where access to viral load testing is limited, a targeted viral load strategy to confirm failure suspected based on immunological or clinical criteria is recommended in order to avoid unnecessary switching to second-line ART.

In addition, the WHO 2010 Guidelines defined virological failure as persistent viral load readings above 5000 cp/mL. The WHO 2013 Guidelines reduce the threshold to 1000 cp/mL based on two consecutive viral load measurements after three months, with patent adherence support.[16] It should be noted, however, that because of the possibility of reduced sensitivity of DBS for viral load measurement at 1000 cp/mL, WHO suggests that programmes relying on DBS technology for viral load testing may consider retaining a higher threshold (3000–5000 cp/mL) until DBS sensitivity at lower thresholds is established.

At the present time, virtually all viral load testing is laboratory based. Most testing is performed using sophisticated, high-throughput instruments. With the exception of one POC platform available in Malawi and Uganda 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 to date 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 US$ 28–29 per test.[17] To put this in perspective, these costs are roughly four to five times greater than CD4 testing and 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 US$ 100 000 to US$ 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.

13 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 travelling towards a clinical event – such as dying from AIDS – then 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.

14 If viral load testing is not routinely available, WHO still recommends that CD4 count and clinical monitoring should be used to diagnose treatment failure *(5)*. It is well established that viral load detects treatment failure well before CD4 count or clinical signs *(10)*. Recent research in Botswana, Kenya, South Africa and Uganda also has 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 *(11–16)*.

15 In the absence of viral load monitoring, clinical monitoring and CD4 monitoring are recommended (5).

16 WHO emphasizes that patients must be on ART for at least six months before treatment failure can be determined.

17 The US$ 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 US$ 40 per test.

These items add approximately US$ 2.75 per test and US$ 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, low cost and readily available for use at POC. Because chemistry and haematology testing is generally symptom- or regimen-based in HIV care, and because there are already a number of technologies available for use at POC, 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 aged under 18 months.

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 or near-POC platforms currently available, and (iii) the POC technologies in the pipeline for each test category.

Tiered laboratory system

Because the laboratory system in-country importantly influences appropriate diagnostic platform selection and placement, it is worth considering the laboratory system in resource-limited settings before discussing diagnostic platforms in depth. The laboratory system is typically characterized as a tiered system as follows *(17)*.[18]

Laalissa Health Post (Ethiopia)

Wamena Kota Health Centre (Indonesia)

Kandangan Hospital (Indonesia)

Level I – Primary:[19] Health post and health centre laboratories that primarily serve outpatients. Often, health posts have no laboratory capability, but are able to perform some POC testing. Generally, no clinicians are onsite at a health post (above, left). Health centres, however, usually have a simple laboratory, where basic testing can be performed (e.g. POC assays and some microscopy, if a microscopist is available) and clinicians are generally onsite (above, centre).

Level II – District: Laboratories in intermediate referral facilities (e.g. district hospitals). These facilities can perform all of the services provided at Level I and additionally provide a broader menu of tests. They usually have automated equipment for tests such as CD4 count, bacterial culture and blood chemistries. Physicians and other clinicians (e.g. nurses) are commonly available onsite (above, right).

Level III – Regional and provincial: Laboratories in a regional or provincial referral hospital that might be part of a regional or provincial health bureau. These facilities will have still more expansive test menus than those found at Level II facilities. In addition to performing all of the tests and services provided at

18 The Maputo Meeting Report notes that the tiered levels of a laboratory system and the testing performed at each level may vary depending on the population served (e.g. infants' adults), level of service available, physical infrastructure, electricity, water, road conditions and the availability of trained technical personnel in-country.

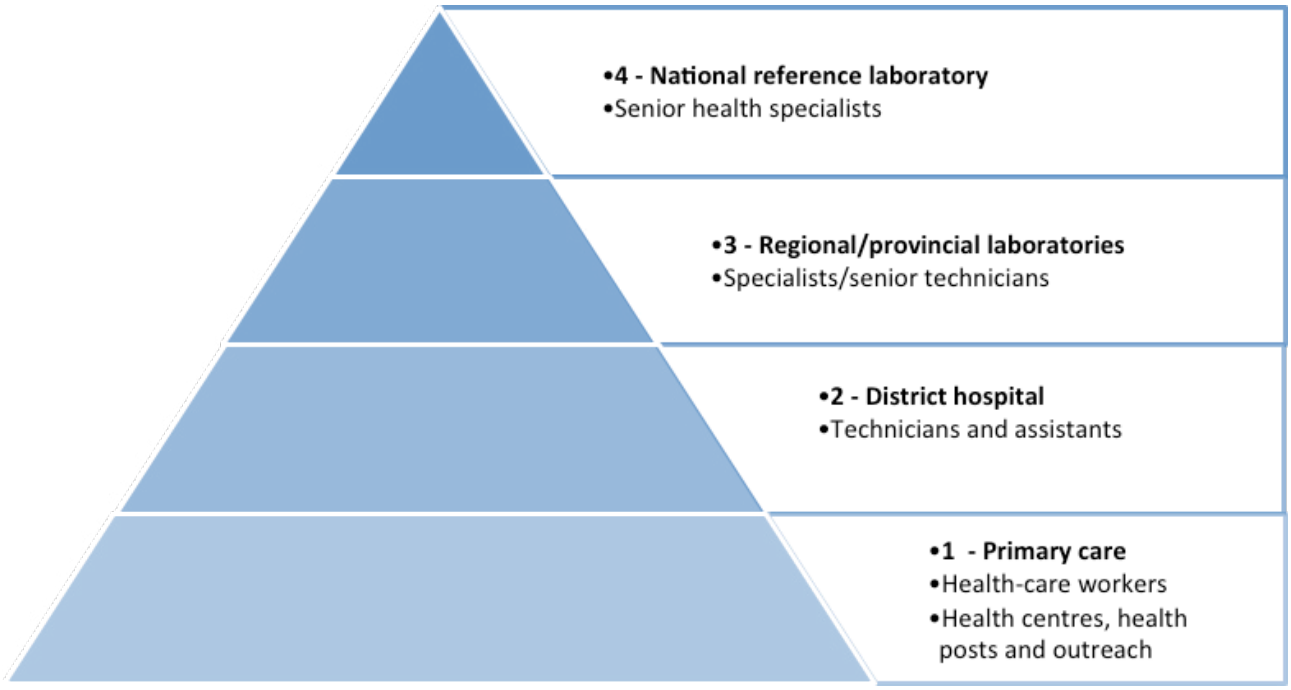
19 The Maputo Meeting Report does not specifically place outreach services in Level I of the tiered laboratory system. Although some experts place outreach activities in Level I, some consider patient outreach to be at a level below Level I and add a fifth tier to the system, referred to as subprimary care or Level 0.

Levels I/II, regional and provincial facilities can usually provide additional testing capabilities such as blood cultures, full chemistry testing, acid-fast bacillus, solid culture and smear. Molecular testing also might be available.

Level IV – National and multicountry reference laboratory: The national reference laboratories are specialized facilities charged with strengthening laboratory capacity for diseases of public health concern. They often provide linkages with research laboratories, academic institutions and other public health laboratories, forming integrated laboratory networks that can provide assistance in clinical trials, evaluation of new technologies and surveillance. In addition, national reference laboratories perform molecular and other sophisticated testing beyond the capabilities of Level III facilities (e.g. nucleic acid assays; HIV drug resistance studies; MTB drug susceptibility testing).

The laboratory system is often depicted as a pyramid, which illustrates that there are generally a large number of Level I facilities in-country and that they serve the most patients directly (Figure 1). In fact, Level I facilities are where most patients initially present for testing, care and treatment. As one migrates up the levels of the system, there are a smaller number of more centralized facilities. In the case of national reference laboratories and some provincial laboratories, they may not serve patients with a broad set of consultative services, but rather are referral centres for quality assurance and training or for conducting complex tests (either using samples drawn at facilities lower in the system and transported or by receiving patients referred directly from other facilities).

Figure 1. Diagram of tiered laboratory system



Testing at POC

The great majority of tests available today in resource-limited settings, other than disposable, rapid tests, were created for developed-country settings, where laboratory-based diagnostics are operated by highly trained technicians on sophisticated instrumentation that is costly, often run in standard 96-well formats (high patient loads) and require dedicated laboratory infrastructure. Also, these instruments rely on a complex medical infrastructure that uses extensive sample transport networks to collect samples from multiple hospitals and clinics and use sophisticated patient tracking mechanisms that enable doctors and hospitals to return results to patients over weeks. These systems are not easily adapted for use in most regions of developing countries or low-resource settings, where access, cost, infrastructure and patient loss

are significant barriers to increasing case detection rates. It is generally believed that the introduction of appropriate, robust POC diagnostics for HIV/AIDS can improve access to testing in developing countries.

Given the robust pipeline of POC diagnostics for CD4, viral load and EID, it is important to consider what is meant by testing at or near the point of patient care. Currently, there is no universally accepted definition of POC testing *(18)*. The College of American Pathologists defines POC tests as "tests designed to be used at or near the site where the patient is located, that do not require permanent dedicated space and that are performed outside the physical facilities of clinical laboratories" *(19)*. But, in resource-limited settings, in particular, there are small, bench-top instruments being used in basic laboratories at Level I facilities, and POC devices being used in Level II and Level III facilities. These testing distinctions and boundaries are somewhat blurred.

It is often suggested that diagnostic tests for use at the point of patient care should meet the ASSURED criteria for the ideal rapid test, which was developed by WHO *(20)*. 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

While the ASSURED criteria provide a useful framework, it is somewhat restrictive in that it demands that tests are disposable and must provide results in less than 30 minutes. As Pai et al. suggest: "the technology as such does not define a POC test nor determine its use at the POC. Rather it is the successful use at the POC that defines a diagnostic process as POC testing" *(18)*. In fact, whatever definition one chooses for POC testing, there are critical features of testing that take place at or near the site of patient care that will determine its effectiveness in resource-limited settings. These include providing both the test and test result to the patient on the same day at a site where linkage to care also is available. In other words, it is not enough to simply offer testing where patients present; rather, it is critically important that test results can be linked to clinical decision-making at the same patient visit. This has important implications for improving the loss of patients from the care and treatment cascade for HIV/AIDS.

In this report, the appropriate target use setting for each of the technologies, both laboratory based and those intended for use at or near the point of patient care, is considered. There are a number of laboratory-based CD4, viral load and EID technologies that are suited only for Level III and Level IV facilities; on the other hand, there are a number of POC technologies that are targeted at Level I and Level II facilities.

It is important that countries review the operational characteristics of diagnostic platforms/devices when selecting which platforms to implement and at which level of the laboratory system to implement them *(1,21)*. These characteristics include the following:

- 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);
- recommended or required instrumentation beyond the analyser itself (e.g. data station; printer; bar code scanner);
- training required;
- availability of QC reagents and compatibility with external quality assurance (EQA) programmes;
- recommended location for use (e.g. hospitals; clinics).

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 also is 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.[20]

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 might be reported by using Bland-Altman analysis, reflects the average/mean difference between the results of the technology under evaluation and the comparator or reference technology *(22)*. Misclassification probabilities, which could 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 cutoff 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" *(7,21)*.

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.[21] As to the precision of CD4 tests (i.e. the reproducibility of results), the %CV can be badly underestimated if it is based on too few replicates; a minimum of eight replicates should be used *(23)*.

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

21 Glover (21) noted 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.

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. Furthermore, the maximum differences could vary at different levels of absolute CD4 count, even within the clinically relevant range *(7,21)*. 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 (*7,21*):

- 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 programmes;
- 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;
- given the potential for error described above, access to QC reagents and participation in EQA programmes 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 *(24,25)* 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, a division of Becton Dickinson (BD), Beckman Coulter Inc (hereafter Beckman Coulter)[22], Partec GmbH (hereafter Partec) and EMD 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.

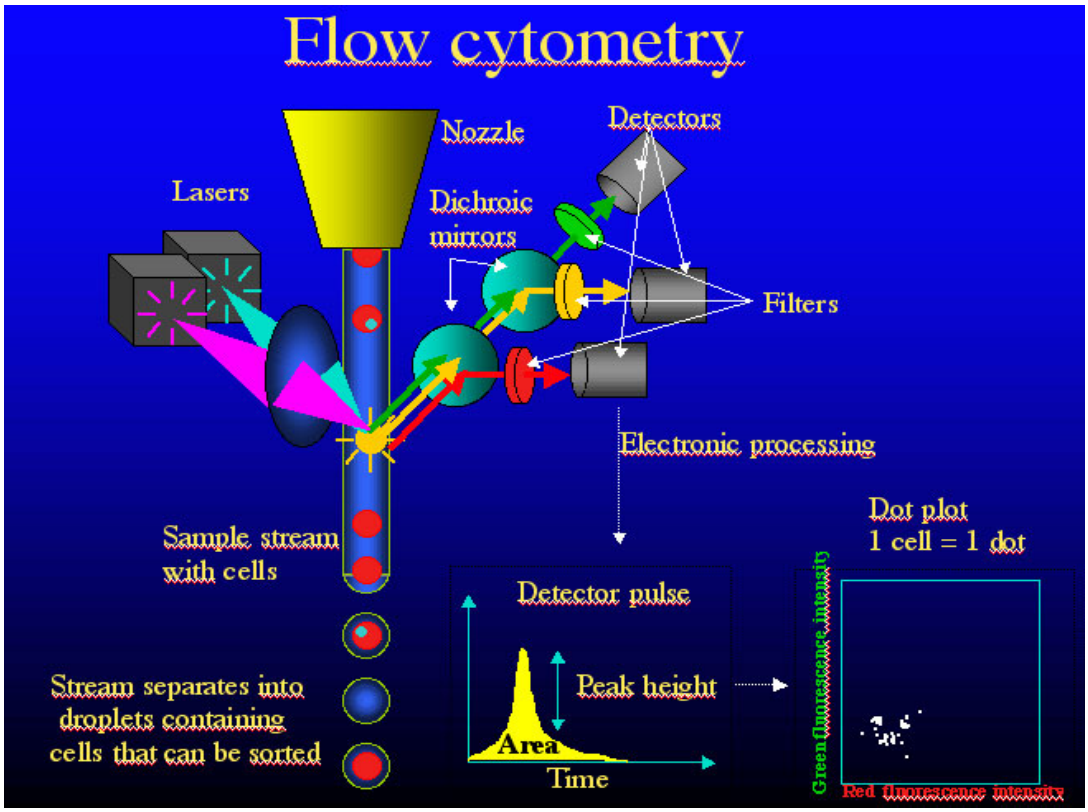
An important requirement of flow cytometry is the need to specifically label cell constituents with fluorescent molecules, which are then used to identify cells carrying this "label". Cell constituents can be made up of a number of cellular components, including DNA, which can be labelled by different dyes/stains. Unique markers or proteins on the cell surface can be labelled 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

22 Beckman Coulter is a registered trademark of Beckman Coulter, Inc.

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) *(26,27)*. 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 *(28)*. Figure 2 gives 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.

Figure 2.Schematic of flow cytometry



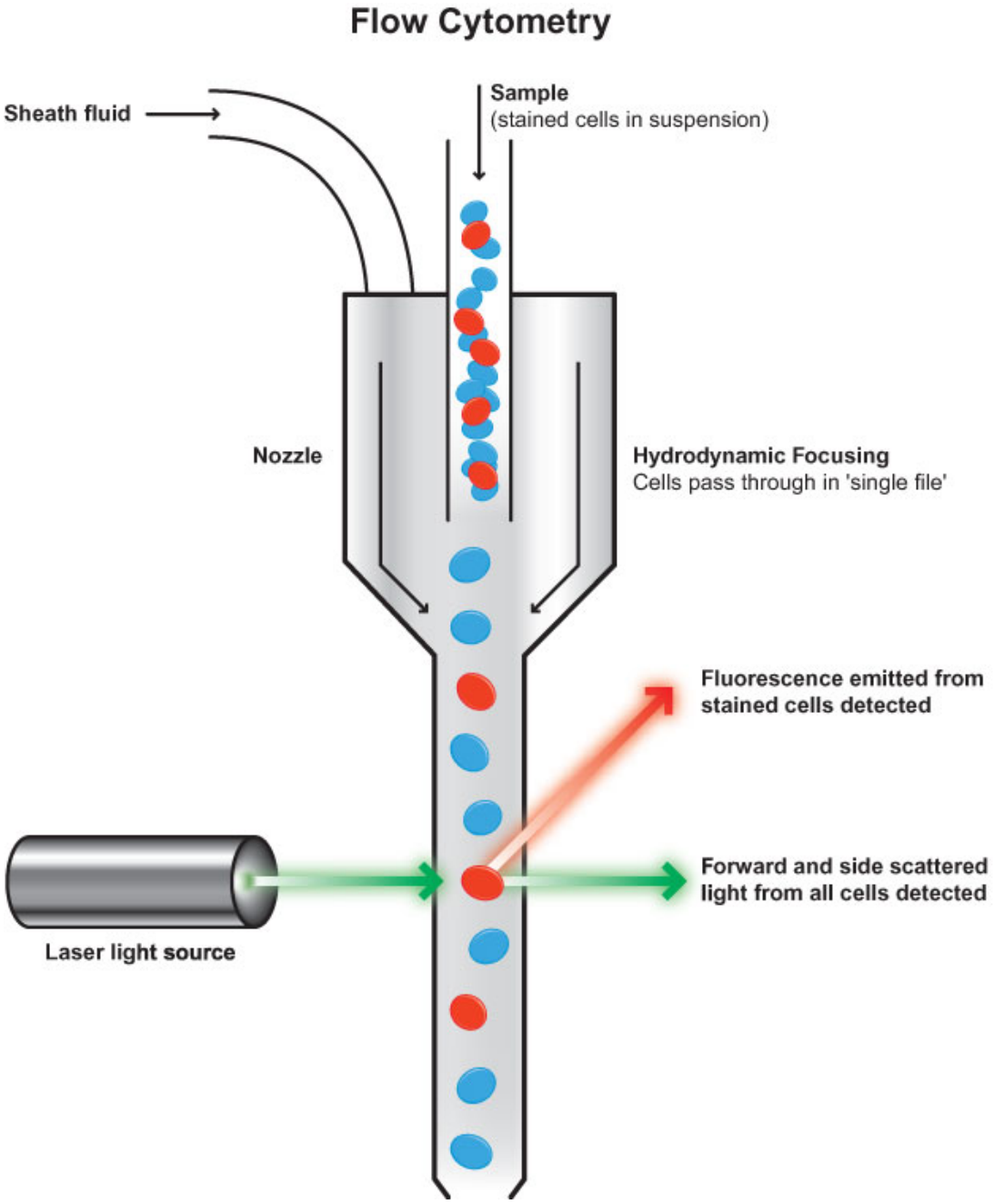
Source: Schematic courtesy of Jan Grawé, BioVis, Uppsala University (http://www.rudbeck.uu.se/node47).

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 (see Figure 3 for an example) 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 centre 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 1000 cells per second *(29)*.

Figure 3. Schematic of fluidics system



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

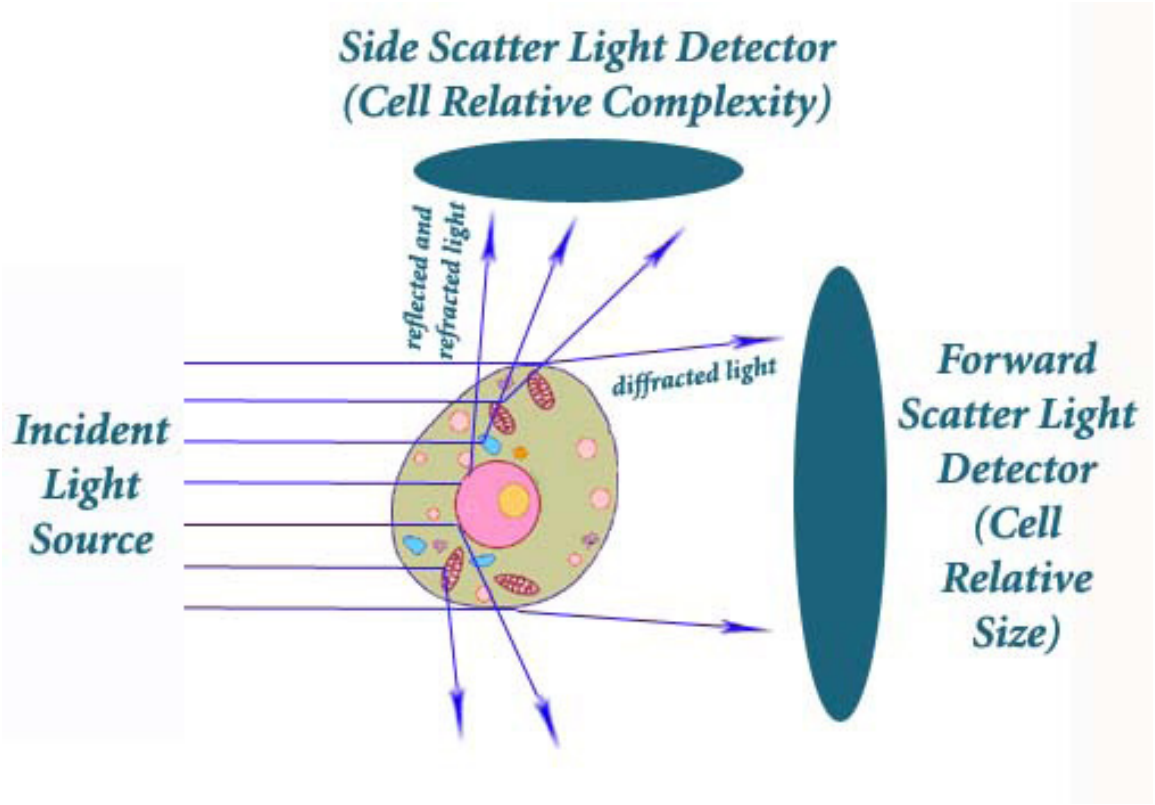
Optics system

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 travelling) 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) (Figure 4). The intensity of FSC depends on the size of the cell and not its refractive index. The intensity of 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.

Figure 4. Schematic of flow cytometry optics system



Source: 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 these 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 channelled 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 photo-multiplying tubes (PMTs).

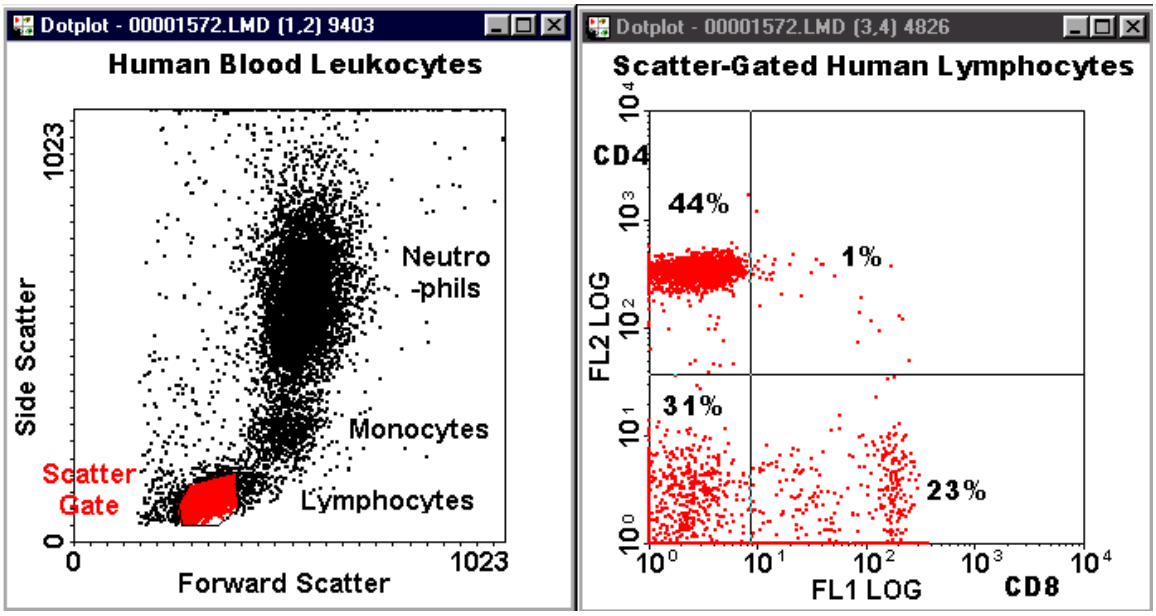
Electronics system

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 can be displayed in a number of formats, including dot plots, contour plots and density plots. Figure 5 shows two examples of dot-plot quadrant analysis for human blood lymphocytes *(30)*.

Figure 5. Examples of dot-plot quadrant analysis for human blood lymphocytes



Source: Diagrams courtesy of Professor Eric Martz, University of Massachusetts, Amherst.

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, Beckman Coulter, Partec, and Apogee. In the developing world, BD Biosciences and Beckman Coulter have the largest CD4 testing market share.[23]

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 haematology 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 haematology 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 *(23)*. However, the PanLeucogating method is producing improved performance over the traditional approach *(31)*. In general, the dual platform method for CD4

23 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.

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 also are manual methods available. These methods involve the use of both a light or fluorescence microscope and a hemocytometer. The Manual CD4 Count Kit from Beckman 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 labelled with beads in a defined area on slides. While such manual bead-based assays have low upfront capital costs, they are quite labour intensive, can be slow and require experienced and capable microscopists to obtain accurate results *(32,33,34)*. These characteristics make manual methods of CD4 cell enumeration less than ideal for resource-limited settings.

Finally, it also is possible to enumerate CD4 cells with reagents designed to be used on haematology analysers (without the need for a microscope). For example, Dynabeads can be used in conjunction with the POCHi-100 haematology 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 haematology 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 *(30)*. There are several single-platform technologies, including the platforms from BD Biosciences and Beckman Coulter, each of which is a bead-based technology, and those from EMD Millipore® and Partec, each of which uses volumetric methods.

Some of these single-platform systems, including the BD FACSCalibur™ and the Beckman Coulter Cytomics FC 500[24], are open platforms. This means that the platforms will accept a variety of reagents. For example, TruCount reagents from BD Biosciences can be used on the Cytomics platform. Cytognos beads (from Cytognos SL) can be used on Beckman Coulter Cytomics FC 500 or BD FACSCalibur™. However, each time different reagents are used on any of these platforms, the instrument must be recalibrated. The remaining single-platform systems commonly used in resource-limited settings are closed systems, including the FACSCount™ and PointCare NOW™ platforms. This means that they can only use reagents manufactured by the platform manufacturer; reagents from other manufacturers are not interchangeable.

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 Biosciences and Beckman Coulter manufacture open platform, high-throughput flow cytometry systems: the BD FACSCalibur™ Flow Cytometer and the Beckman 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 reference laboratories (Level IV facilities). 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 levels (Level II facilities).

BD FACSCalibur™ system (BD Biosciences)

BD Biosciences manufactures the BD FACSCalibur™ system (Figure 6), which is a large, bench-top, automated, multicolour 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

24 FC 500 is a registered trademark of Beckman Coulter, Inc.

fluorescence detection and known concentrations of reference beads in each sample to obtain absolute T-cell concentrations *(35)*. 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 also can perform immunotyping (combined analysis of T-cells, B-cells and NK-cells or blood cell disorders, for example), residual WBC 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.

Figure 6. BD FACSCalibur™ system

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 (IVDs) 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.

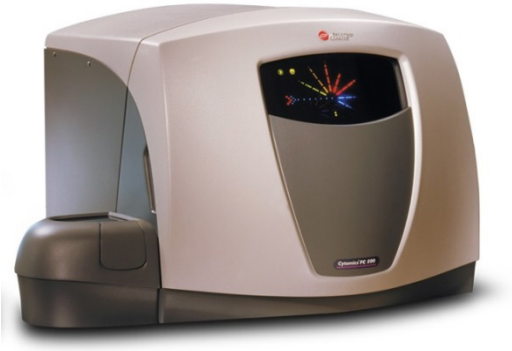
As discussed earlier, although most experts agree that there is no true "gold standard" for CD4 testing, many consider the FACSCalibur™ system to be the reference standard for CD4 counting. It is the platform against which the performance of other CD4 systems is most frequently compared and there is at least one published, peer-reviewed evaluation of the platform using TruCount reagents *(36)*. It is in use in resource-limited settings, but is generally only appropriate for central/national reference laboratories where its high throughput (approximately 200–250 samples per day or 40 samples per hour) and sophisticated capabilities can be used appropriately.

The cost of the FACSCalibur™ instrument is about US$ 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-colour reagent test (TruCount) used by most laboratories in resource-limited settings, the cost of reagents is volume dependent and assay dependent and ranges from about US$ 3 per test at volumes of more than 75 000 tests per instrument per annum to as much as US$ 7 per test at significantly lower annual volumes.

Cytomics FC 500 MCL or Cytomics FC 500 MPL system (Beckman Coulter)

Like the BD FACSCalibur™, the Cytomics FC 500 MCL and Cytomics FC 500 MPL Systems,[25] manufactured by Beckman Coulter, are large, bench-top flow cytometers. These systems are automated and can simultaneously analyse up to four colours of immunofluorescence from a single laser. The Cytomics FC 500 series platform (with either MCL [Multi Carriage Loader] or MPL [Multi-Platform Loader] sample loading capability) is a bead-based system that can perform absolute and percentage CD4 counts (using FlowCARE™ PLG reagents), but also can perform multiparametric 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.

Figure 7. Cytomics FC 500 system



The Cytomics FC 500 system (Figure 7) automates many of the steps involved in QC and flow cytometric analysis that previously needed to be done manually. In addition, the system contains two lasers (an air-cooled argon ion laser and an air-cooled helium-neon ion laser) and can measure five-colour 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 can 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 Beckman 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 leukaemias, lymphomas and platelet disorders, among others.

Assuming certain test volume commitments, the cost of the Cytomics FC 500 MCL instrument is about US$ 90 000; with the addition of the CellMek system, the cost is about US$ 200 000. 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 US$ 2.50 to US$ 4.50 per test at volumes of more than 75 000 tests per instrument per annum from about US$ 5 to US$ 8 per test at volumes under 11 000 tests per instrument per annum.

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

Currently, 45 CellMek/Cytomics FC 500 MPL system instruments have been placed in Namibia, South Africa and Zambia. 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 *(37)*.

The CyFlow® Counter (Partec)

The CyFlow® Counter from Partec, which was recently acquired by Sysmex, is a portable, compact desktop flow cytomer designed for routine absolute CD4 and %CD4 counting (as well as total lymphocyte and WBC counting) in a single, dedicated platform (Figure 8, on the left).[26] As a result of its acquisition, Partec products will now be distributed worldwide under the Sysmex umbrella, increasing its distribution network from 140 to 200 countries worldwide.

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 such as BD FACSCalibur™ and Beckman Coulter Cytomics.

Figure 8. CyFlow® Counter

The CyFlow® Counter can be combined with a CyFlow® sample preparation and auto-loading system (Figure 8, 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. Furthermore, 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, onscreen video operations manual that covers setup, instrument operation, instructions on how to perform the absolute CD4 and %CD4 assays and basic maintenance instructions.

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 levels

26 Note that Partec also manufactures another device, the CyFlow® SL_3, which performs volumetric absolute counting of CD4 and %CD4 for paediatric patients, total lymphocyte count and WBC. The instrument costs about €22 000 (~US$ 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.

(38). The device also is small enough to be used in mobile laboratories. Furthermore, the instrument can be run off of a car battery or solar panels, if needed. The company has placed more than 1900 instruments in-country, which provided more than 3.9 million patient tests (both absolute CD4 and %CD4) in 2012.

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

Published, peer-reviewed literature is available on the performance of CyFlow® *(39,40)*.

Medium- to low-throughput CD4 systems

BD FACSCount™ system (BD Biosciences)

The BD FACSCount™ system (Figure 9) 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.

Figure 9. BD FACSCount™ system

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 aged under 5 years, as discussed earlier in this report) using a single-tube assay (Figure 10).

Figure 10. FACSCount™ single-tube reagent assay

The FACSCount™ system is generally considered to be robust, and due to relatively simplified sample preparation and the degree of automation of the instrument, requires minimal operator training. The system has been used in CD4 monitoring for HIV/AIDS care and treatment programmes in resource-limited settings for more than a decade; its performance is considered to be reliable, and independent performance data are available *(41,42)*. 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 50 samples per day, which is likely to include district hospitals, for example. BD Biosciences has established a comprehensive network of support resources, including service and maintenance resources, for resource-limited settings.

The FACSCount™ platform is a closed system. The cost of the FACSCount™ instrument is about US$ 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 US$ 3.50 per test for test volumes of more than 10 000 tests per instrument per annum up to US$ 10 per test for test volumes up to 4500 tests per instrument per annum.

BD FACSClearCount™ system (BD Biosciences)

BD Biosciences is in final development stages of the BD FACSClearCount™ system (Figure 11), the next generation CD4 dedicated system, measuring both absolute counts and percentage CD4.

Figure 11. BD FACSClearCount™ system

The BD FACSClearCount™ system includes the instrument, integrated software, 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 labelled antibodies for the identification of CD4 T-cell populations and counting beads for simultaneous CD4 T-cell enumeration. The counting beads also are used for daily instrument QC. 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 onscreen, and can be printed using the onboard thermal printer. They also can 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 six languages: English, French, Portuguese, Russian, Simplified Chinese and Spanish.

The cost of the FACSClearCount™ instrument is expected to be less than US$ 40 000. Pricing for reagents depends on volume of testing per annum per instrument. The FACSClearCount™ is not expected to launch before the end of 2015.

Aquios CL™ (Beckman Coulter)

Beckman Coulter expects to launch its Aquios CL™ flow cytometry platform (Figure 12) in 2014. The Aquios CL™, which incorporates a technology called Load & Go™, is equipped with an automatic sample loader that utilizes cassettes to queue samples for preparation and analysis. Each cassette holds up to five specimen tubes, and up to eight cassettes can be loaded at a time for a total of 40 specimens. Cassettes can be continuously loaded and unloaded without interrupting the system's workflow. The first test results are available approximately 20 minutes after loading the sample.

Figure 12. Aquios CL™ flow cytometry platform

In addition, the Aquios CL™ is preloaded with a range of bar coded reagents and consumables and automatically scans bar codes to track reagents, lot numbers and open and closed vial expiration dates, for example. 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, then 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 touchscreen operation. There also is an alternative keyboard and mouse. Data analysis is performed via advanced automated algorithms with the option of user-adjustable gates and regions.

The platform is targeted at laboratories that need to automate the most routine, repetitive tests, such as absolute CD4 and %CD4. Different specimen cassettes will be available to accommodate a variety of blood collection tubes. Beckman Coulter will launch the Aquios CL™ with its Tetra panels in mid- to late-2014, and the company plans to launch the system with PLG (PanLeucogating) in early 2015.

Future applications that aid in the diagnosis, monitoring and treatment of diseases in addition to HIV/AIDS are pending.

Millipore-Guava® Auto CD4/%CD4 system (Merck)

The Guava® Auto CD4/%CD4 system, which was manufactured by EMD Millipore® (a division of Merck), has been discontinued and is no longer available for sale. However, Merck will continue to support the product in the field for the next four years.

Apogee Auto40 Flow Cytometer (Apogee Flow Systems)

The Apogee Auto40 Flow Cytometer, manufactured by Apogee Flow Systems 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 (Figure 13). 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.

Figure 13. Apogee Auto40 Flow Cytometer

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 are 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 US$ 27 000. The pricing for reagents is approximately US$ 2.50 per test for absolute CD4 counts and US$ 3.50 per test for percentage CD4.

Several peer-reviewed studies of the Apogee Auto40 platform have been published *(43,44)*.

POC 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, 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. POC CD4 testing is likely to require new, simpler technologies. Both instrument-based and disposable tests are in the CD4 development pipeline.

Below, POC diagnostics for CD4 testing that are either on the market or in development are discussed in some detail, including technical specifications. Six of these technologies are already on the market: PointCare NOW™, the Pima™ Analyser, the CyFlow® CD4 miniPOC, Daktari™ CD4 Counter, BD FACSPresto™ and the MyT4™ CD4 Test. The remaining technologies discussed, including those from Omega Diagnostics Ltd, MBio Diagnostics Inc and others, are not yet available on the market.

PointCare NOW™ (PointCare Technologies Inc)

The PointCare NOW™ system (Figure 14) was developed by PointCare Technologies Inc (hereafter PointCare) 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 WBCs, and then uses a colloidal gold label[27] to change the natural light scatter characteristics of the CD4 subclass of lymphocytes in order to perform the CD4 enumeration.

Figure 14. PointCare NOW™ system

The PointCare NOW™ instrument is considered to be robust due to its modular injection-molded housings with few moving parts. The system has solid-state electronics and comes precalibrated from the factory, thus eliminating 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 and vortexing. 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 eight minutes.

In September 2013, PointCare announced that it had validated a system of internal QC using its proprietary heat-stable Daily Check controls that eliminate the need for EQA controls. PointCare reports that users find traditional EQA difficult to implement at POC due to the short shelf life and temperature sensitivity of

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

the controls. With a validated stability from 2 °C to 42 °C and a two-year shelf life, Daily Checks offer a practical solution to performing QC in remote settings at no additional cost to the user.

The PointCare NOW™ system is a medium- to low-throughput platform that can handle some 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 US$ 25 000. The pricing for reagents, which includes PointCare's heat-stable Daily Check controls, is approximately US$ 10 per test.

A peer-reviewed evaluation of the PointCare NOW™ platform was published in 2012 based on data collected prior to 2011. 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 *(45)*.[28] A second peer-reviewed evaluation of the PointCare NOW™ was published in November 2013 in the *Journal of AIDS and Clinical Research*. This evaluation was conducted in March 2011 by the National Microbiology Reference Laboratory in Harare, Zimbabwe, and compared the PointCare NOW™ with the BD FACSCalibur™. The results showed no statistically significant difference between the PointCare NOW™ and BD FACSCalibur™ in the classification of patients at the 350 cells/µL threshold and suggested that improvements had been made in the PointCare NOW™ systematic error performance since the earlier studies *(46)*.

Pima™ Analyser (Alere Inc)

The Pima™ Analyser (Figure 15, with printer) is a small, portable bench-top, fixed volume cytometer manufactured by Alere Inc (hereafter Alere) The Pima™ Analyser 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 also is available.

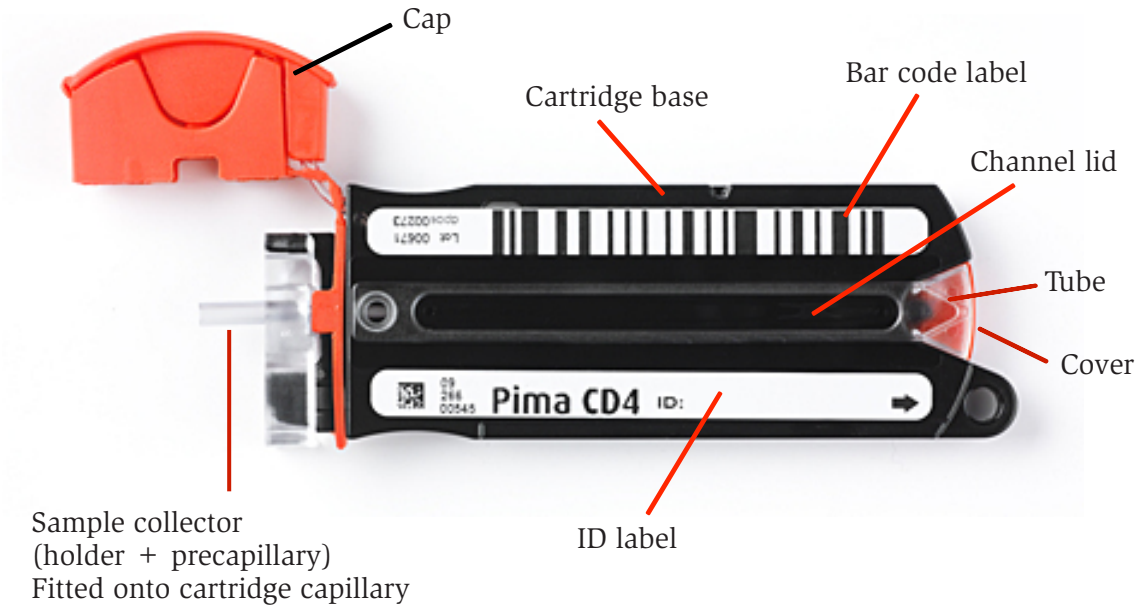
Figure 15. Pima™ Analyser

The Alere Pima™ CD4 system is made up of the Analyser and a disposable CD4 test cartridge (Figure 16) that 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 currently cannot determine %CD4 counts. This capability could be added to the system, along with other cell type counts.

28 Sensitivity to identify children in need of ART using a 25% CD4 threshold was 90% and sensitivity was 100% using a 750 CD4 cells/mm^3 threshold.

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 labelled anti-CD3 and anti-CD4 antibodies, are redissolved 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 minimizing the risk of analyser contamination.

Figure 16. Pima™ CD4 system disposable CD4 test cartridge



The Pima™ Analyser is equipped with miniaturized, multicolour fluorescence imaging optics. Fluorescence images are collected by an onboard camera and analysed using proprietary software algorithms on the embedded computer to derive absolute CD4 counts. Up to 1000 test results are stored in an onboard 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 onboard 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 also has launched an optional USB connectivity module for sending data to central servers via mobile telephone networks. A LAN (local area network) connectivity solution also is available. Alere offers an optional free-of-charge web-interfaced service, that includes Alere Datapoint™ (Figure 17) and Alere SIM cards, providing the user with real-time metrics for inventory planning and control, quality management and technical troubleshooting.[29]

29 To learn more, visit http://alerehiv.com/connectivity.

Figure 17. Alere Datapoint™

A power extender (Figure 18), including an extended-life battery and adaptors for charging sources, including solar panels and mains, has been added to the product family.

Figure 18. Pima™ Analyser power extender

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 health-care 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 Pima™ Analyser has been prequalified by WHO, is CE-IVD marked and has performed well in an evaluation conducted by the United States Centers for Disease Control and Prevention (CDC). Alere is currently in the process of seeking approval for the Pima™. At least 10 peer-reviewed, independent evaluations of the Pima™ system have been published since product launch (see, for example, references *47–51*). In these studies, the Pima™ CD4 system was tested in laboratory as well as non-laboratory settings such as rural voluntary counselling and testing sites and mobile health-care 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 health-care 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 POC on capillary blood obtained by fingerstick, although one study did find that the CVs were slightly larger with fingerstick than venous blood, which the authors noted might have been related to insufficient training of operators *(50)*. One of the published studies demonstrated, for the first time, the positive impact that POC 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 [ART]" *(51)*.

The cost of the Pima™ Analyser varies among countries and regions, with prices ranging from approximately US$ 6500 to US$ 12 000, and the cost per test ranging from approximately US$ 6 to US$ 12. The instrument requires no routine preventive maintenance, and Alere has established a global technical support network to ensure fast, reliable and consistent service.

Daktari™ CD4 Counter (Daktari Diagnostics Inc)

Daktari Diagnostics Inc (hereafter Daktari) has developed a portable and robust CD4 device, the Daktari™ CD4 Counter (Figure 19, with associated cartridge). The Daktari™ system will be capable of other assays, which may include full blood counts, CD4 percentages, and bacterial and viral load diagnostics. The Daktari™ CD4 system launched in February 2014, with customer support from Daktari™ hubs in Johannesburg and Nairobi.

Figure 19. Daktari™ CD4 Counter

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 (or viruses) 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 14 minutes.

The Daktari™ CD4 system includes a data management system with a keypad user interface, wireless data transmission and a back-end data package that can stand alone or can be integrated with customer databases.

The anticipated cost of the Daktari™ CD4 Counter is less than US$ 8000 for the device. Per test cost is anticipated to be approximately US$ 9, but volume discounts are expected to drive the price lower. If the device is damaged, then the low cost and portability of the instrument would allow it to be swapped out with a replacement device rather than being repaired onsite.

Daktari™ reports that independent validation studies on the Daktari™ CD4 system were completed in Kenya and Uganda in 2013. Additional independent studies are expected from several countries in eastern and southern Africa in 2014. The Daktari™ CD4 system is commercially available, and is expected to be CE-IVD marked by mid-2014.

There are currently no published performance data available for the Daktari™ CD4 system.

CyFlow® CD4 miniPOC (Partec)

Partec has introduced a very compact, portable CD4 counter, the CyFlow® CD4 miniPOC (Figure 20) 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 5000 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. Similar to its larger sibling, the Partec CyFlow® Counter, the fact that blood samples can be run one at a time or in batches provides good flexibility for use in multiple settings. The device can run up to 250 CD4 tests per day, but also can be used in small health centres and other sites with a lower daily volume of testing.

Figure 20. CyFlow® CD4 miniPOC

The miniPOC requires only 20 µL 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, and takes place outside of the device.

End user machine interface has been improved with the introduction of new features in the latest version (version 2.8) of the software for the miniPOC. It is now possible to obtain both absolute CD4 and %CD4 results without manually gating the dot plots. This improves the user friendliness of the instrument, making the miniPOC easy to use by less skilled health-care workers. Results are displayed in routine mode, and the option for manually gating the cell clusters offers an additional built-in QC.

Since 2012, Partec has made available on the miniPOC instrument a detailed, onscreen video operation manual that covers setup, instrument operation, instructions on how to perform the absolute CD4 and %CD4 assays and basic maintenance instructions, for example.

The cost of the miniPOC instrument is approximately €8900 (~US$ 11 748). 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 (~US$ 3.96) per test kit, which yields both absolute CD4 and %CD4 results. On occasion, the company also offers special POC 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.

BD FACSPresto™ (BD Biosciences)

BD Biosciences has developed the BD FACSPresto™, an image-based counting technology suitable for resource-limited settings that provides CD4 absolute count, %CD4 and haemoglobin (Hb) all on the same single-use disposable cartridge (Figure 21). Features of the automated device include touch screen user interface, easy to use, intuitive, language-free menu navigation, flexible workflow with high throughput, integrated microprinter, battery or solar-powered capability and data archive/transfer capabilities.

Figure 21. BD FACSPresto™ Platform

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. The product was launched in late March 2014.

MyT4™ CD4 Test (Zyomyx Inc and Mylan Inc)

MyT4™ CD4 is a rapid, POC CD4 test (the Test) providing a quantitative result in 10 minutes. The MyT4™ CD4 Test system has CE-IVD regulatory approval and is available for purchase. It is developed and manufactured by Zyomyx Inc and distributed in developing countries by Mylan Inc. The Test can be used for treatment initiation at any threshold and for monitoring.

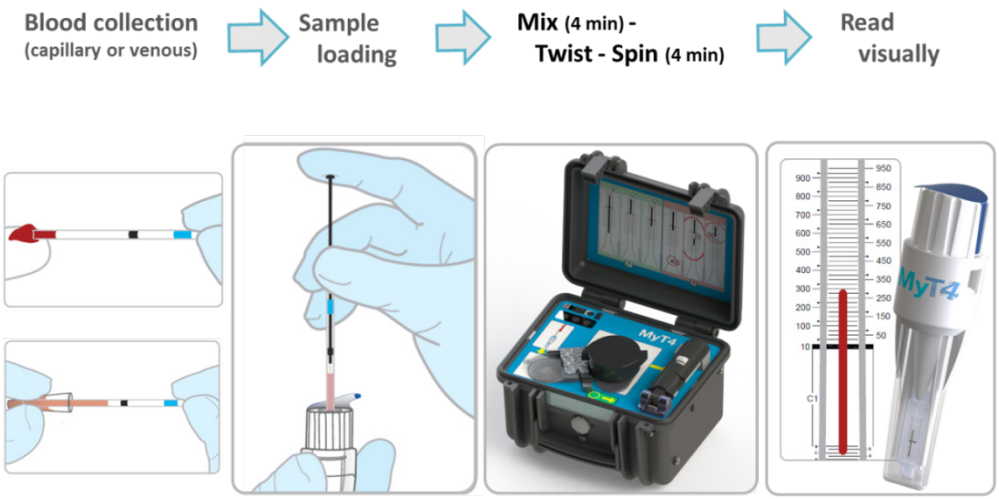
Figure 22. MyT4™ CD4 Test

MyT4™ CD4 (Figure 22), consists of a disposable Test cartridge, a reusable accessory (the Accessory) and a self-metering blood collection tube, and is designed to meet the requirements of resource-limited settings. It has a 10-minute time to result to ensure that health-care workers can act on results during a patient's visit. The Accessory has no complex or fragile components and is expected to be highly durable compared to CD4 instruments containing complex optical sensors and other sensitive parts. The Accessory requires no calibration or ongoing maintenance. As a result, no service contract will be required and no service fees will be incurred; rather, the company will replace any broken Accessory under warranty at no cost. The company will maintain stocks of the Accessory in-country so that replacements can be delivered to health-care facilities within one day to at most a week.

MyT4™ CD4 has been validated against the gold standard of CD4 testing, flow cytometry, as part of CE regulatory qualification by an independent notified body in the European Union. The capital investment is very low, and the components are designed to be easy to use in limited-resource settings, including a robust battery that holds 50 tests per charge.

Inside the Test, CD4 + T-cells are bound to heavy CD4-specific particles through a mixing process that takes place in the Accessory. Figure 23 demonstrates how the Test is subsequently spun slowly in the Accessory, whereby only the conjugated cells penetrate into a high-density medium, forming a cell stack in a microcapillary. The CD4 + T-cell count is a function of the height of this cell stack and is read visually.

Figure 23. MyT4™ CD4 Test procedure



MyT4™ CD4 has a 10-minute procedure that is easy to use, with a fully quantitative readout.

The company highlights the following features of the MyT4™ CD4 Test and Accessory, which include:

- Rapid: MyT4™ CD4 takes less than 10 minutes to deliver results, making it the fastest time-to-result of any POC CD4 currently on the market.
- No service fees: the MyT4™ CD4 Accessory does not require calibration, maintenance or service contracts. Rather, the Accessory will be replaced as needed, with no swap-in of the broken item required.
- Daily throughput: 10 minutes per test facilitates a daily throughput of about 40 tests per operator. If additional throughput is desired, then it can be increased by adding an additional Accessory at nominal cost.
- Power: the Accessory can be operated using mains or battery power. The battery holds 50 tests per charge and has been specifically selected to remain robust despite power peaks and dips and frequent partial recharging and discharging.
- Results: results are fully quantitative, with an intuitive visual readout. MyT4™ CD4 can be used for initiation at any threshold and for monitoring.
- Accurate: MyT4™ provides fully quantitative results which, based on studies conducted by the company in Africa, demonstrated strong correlation with the predicate laboratory-based flow cytometry technologies, the gold-standard of CD4 testing.
- Stability: the MyT4™ CD4 Test is stable at room temperature, with a shelf life of one year at 2 °C–30 °C. During storage and transport, it can withstand fluctuations up to 40 °C for two weeks, and up to 50 °C for 48 hours.
- Samples: venous or capillary whole blood. No sample preprocessing is required.
- Quality assurance: internal QCs are built into all components. Three internal QC indicators in the MyT4™ Test show whether the Test has run to completion or whether an error has occurred. The indicators in the MyT4™ Accessory check the calibration of the key parameters, time and speed for every test run. Indicator lights inform the operator of any errors.
- Reagents: all reagents and internal QC are fully contained in the MyT4™ CD4 Test. No reagent preprocessing is required.
- Easy to use: designed to have a simple user interface with a single button on the Accessory. There are no complex features on the Test or Accessory such as optics, valves or separate chemicals.
- Portable: The MyT4™ CD4 Accessory is designed to be durable and is housed in a protective carrying case, for which there is no additional charge.

The anticipated cost of the MyT4™ CD4 Test is less than US$ 8. The accompanying Accessory is anticipated to cost less US$ 500.

Field and laboratory performance of MyT4™ CD4 was confirmed in South Africa in 2013 to support the CE mark, the results of which have not yet been published.

CD4 technologies in the pipeline

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

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 laboratories, clinics and at POC. The MBio CD4 System (Figure 24) consists of a software-driven reader (the 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 that the company believes is unique in the POC market segment. The System has been designed specifically for applications in resource-limited settings.

Figure 24. MBio CD4 System

System features include:

- Product pipeline: the first product will deliver absolute CD4 count, with following releases delivering cartridges for immunoassays such as HIV, 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 10–15 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 in the MBio Rack, 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, dried 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 type, 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-colour 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 peripheral MBio Rack provides incubation timing and visual indicators to guide users running multiple cartridges in batch mode.

The System includes an onboard 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 touchscreen with administrator-configurable settings such as user lockout/validation and QC scheduling. Cartridge bar codes are read automatically and the instrument will have multiple USB ports to support printers, external bar code readers and other peripherals. The System will include integrated GSM/GPRS connectivity.

The MBio CD4 System has demonstrated clinical performance in low CD4 count patient populations with excellent correlation to flow cytometry. The MBio Diagnostics Inc product launch schedule will be determined by the company's commercialization priorities and funding availability.

EMD Millipore® Muse™ (Merck)

EMD Millipore® is poised to introduce a new platform, the Muse™ cell analyser (Figure 25), for its CD4/%CD4 assay. The Muse™ cell analyser uses patent-pending, miniaturized fluorescent detection and microcapillary technology to provide accurate, precise and quantitative cell analysis. The microcapillary and miniaturized options of the system take up about one tenth of the space of typical cytometers, and the laser-based fluorescence detection can evaluate up to three cellular parameters, as compared to two parameters for imaging-based systems.

Figure 25. Muse™ cell analyser

The Muse™ cell analyser is easy to use; with the primary skills required being pipetting and operating the software on the analyser. The Muse™ requires only 10 µL of patient sample. Sample preparation requires two simple dilutions and two 15-minute incubations. The operator loads the CD4/%CD4 reagents on the Muse Auto CD4/%CD4 system and then follows easy guided menus on the Muse™ touchscreen. Results, which are displayed in both graphical and statistical formats, are provided from two to four minutes.

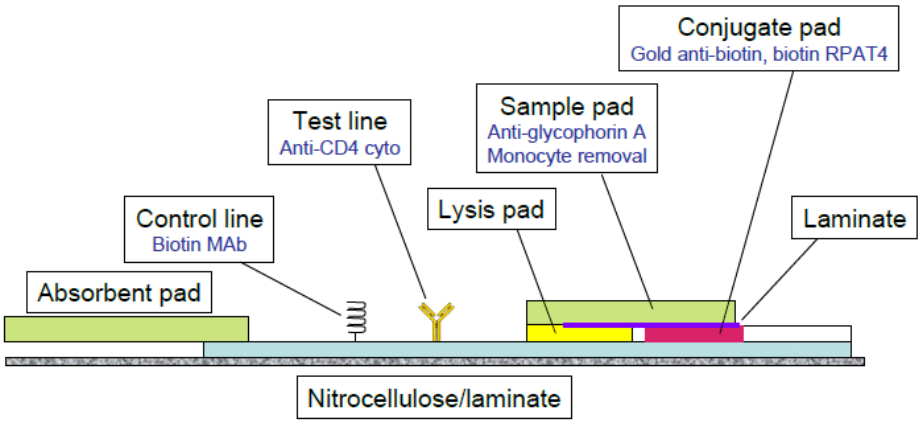
The Muse™ Auto CD4/%CD4 system will have two power sources. In the clinic laboratory, the Muse™ system can be plugged into a UPS. However, for portability, the Muse™ system offers an optional battery pack that will provide hours of operation.

The Muse™ Auto CD4/%CD4 system is in final clinical trials and is expected to be released in July/August 2014. When released, the cost of the system is expected to be approximately €10 000 (~US$ 13 700), and the price per test is expected to be €2 (~US$ 2.75). EMD Millipore® will obtain CE-IVD marking for the Muse™ system.

Visitect® CD4 (Burnet Institute and Omega Diagnostics Ltd)

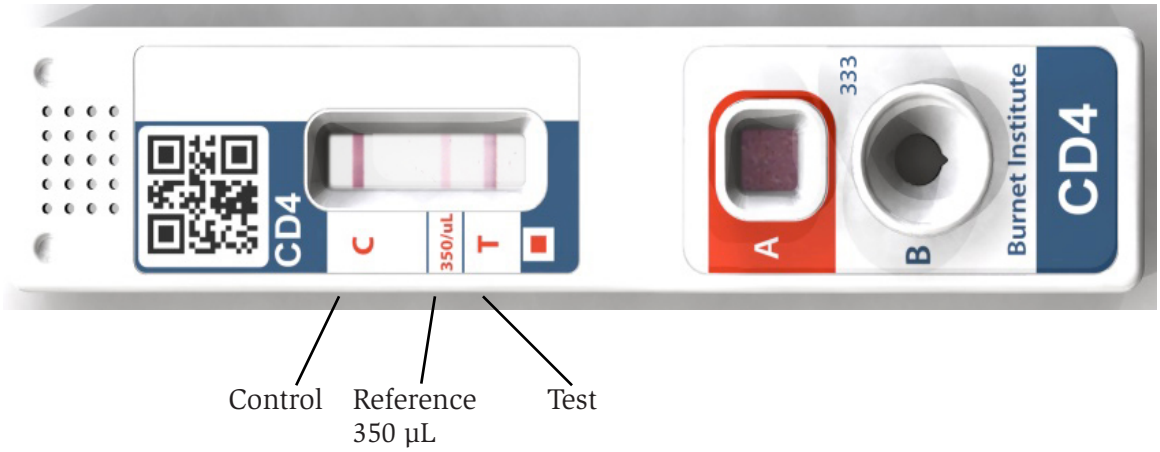
The Burnet Institute has licensed its semi-quantitative CD4 technology to Omega Diagnostics Ltd (United Kingdom). The platform, which is now called the Visitect® CD4, is a rapid, disposable semi-quantitative CD4 test. The approach of the test is to measure CD4 protein on T-cells, rather than to directly 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. The Burnet Institute used a laboratory-based test (ELISA) as proof of concept, which supported this hypothesis. Subsequently, the Visitect® CD4 test was 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 (Figure 26).

Figure 26. Visitect® CD4 lateral flow strip



As an aid to 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 (Figure 27, top), Omega Diagnostics Ltd has developed a smartphone application for the Visitect® CD4 assay. The application uses the camera in an Android smartphone to read the test result, and a software application provides interpretation and interface to an external laboratory information management system or cloud database. In addition, the Burnet Institute developed a reader for the Visitect® CD4 device (Figure 27, 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 US$ 3000, but may decline to about US$ 2000 over time. The reader will be provided free of charge dependent on committed volumes.

Figure 27. Visitect® CD4 reader



Evaluation of the prototype version of the test at the 350 CD4/µL cutoff at the Burnet Institute 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 have now begun in India and Africa with further trials to follow in Southern Africa, the United Kingdom and the United States in the first half of 2014.

Omega Diagnostics Ltd has applied for CE-IVD marking for the Visitect® CD4, and commercial launch will follow this. The per test cost of the assay at release is expected to be about US$ 5.

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.

ChipCare-CD4 (ChipCare Corporation)

ChipCare Corporation is developing a mobile, easy-to-use, laboratory-quality blood testing platform for CD4 testing. The platform uses disposable cartridges that leverage recent advances in microfluidic and biomarker technologies to provide cell surface and blood analyte tests. From a 10 µL sample of blood,

health centre and community-level health-care workers will be able to rapidly and accurately perform tests to diagnose or monitor a range of infectious diseases.

ChipCare Corporation's initial test – absolute/percentage CD4 count – will stage HIV-positive patients for treatment. Research on serum analyte tests related to kidney and liver function, diabetes and the systemic inflammation pathway is ongoing.

Designed for community-level health-care workers in remote or rural settings, the ChipCare-CD4 will weigh less than 1 kg and will be small and rugged enough to be carried in a small backpack. Time to result for the CD4 test is expected to be less than 15 minutes, with throughput of about 24 tests per 8-hour day. The platform does not require sophisticated laboratory infrastructure, trained laboratory technicians, continuous power or running water. Users will be able to charge the platform's lithium ion battery via AC mains socket, car battery or solar panel. Cloud connectivity, which can facilitate electronic medical record data aggregation, also will enable the review of test results by a clinician in a central facility for purposes of QC and clinical decision-making.

The price for the ChipCare-CD4 platform device is expected to be less than US$ 1200 per unit, and test cartridges are expected to cost US$ 6–8 per test. Market launch of the platform and CD4 cartridges likely will take place in 2016.

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. A number of such options are already on the market, and others are under development, at least one of which is likely to become available in 2014. 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 these 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. Per WHO, "assessment of CD4 cell count is still necessary to guide initiation of ART outside of certain clinical situations". 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, which is likely given the WHO recommendation that viral load testing is the preferred method for monitoring patients on ART, then there might be a movement away from six-monthly CD4 count testing. This transition would still require broader international consensus. Once patients have stabilized, generally after one year on ART, CD4 testing does not demonstrate important, decision-driving changes, except in a small percentage of failing patients *(52)*. 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 US$ 3 per test without the need for investment in instruments/devices.

However, the level of CD4 testing access required in resource-limited settings likely will necessitate both a scale-up in centralized testing facilities 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 such as diabetes glucose monitoring) and could 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 laboratories 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 highly centralized laboratories and POC facilities could be relatively small, but, in any event, the landscape will neither be all laboratory based nor all POC based.

Viral load testing technologies

Overview

As discussed earlier in this report, viral load testing is the method strongly favoured 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 *(53)*.

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 might be relatively disease- and symptom-free, there is still low level, active viral replication. Over a period of time, however, HIV's 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 millimetre) 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% *(54,55,56)*.

Identifying treatment failure early enables patient adherence counselling and may enable patients to stay on first-line ART longer than otherwise, thereby avoiding unnecessary switches to more expensive second-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 second-line therapies, and it also supports migration to second-line treatment in a timely manner, thus saving patients' lives. It also should 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 also can 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 previously mentioned, 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 and a high degree of training. In addition, in order to provide centralized viral load testing to patients outside of urban centers, supply chains capable of handling labile reagents and effective sample transport systems are also required. Until recently, another deterrent to scale-up was that WHO guidance had counselled caution in the deployment of viral load testing in resource-limited settings, at least partially on the basis of cost. However, in its 2013 Guidelines, WHO now recommends viral load testing as the preferred approach to monitor treatment success and diagnose ART failure. It is expected that this recommendation will cause more countries to implement viral load testing over the next few years.

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 are considered here in some detail. First, it is worth considering some of the complicating factors that characterize viral load assays and platforms, which 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 *(53)*. Further complicating matters, HIV-1 is divided into four groups, designated M, N, O and P, the main group of which is group M. In addition, there are multiple clades, and within each clade, there are subclusters 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 (CRFs) 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 *(57,58)*. 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 climate control/airconditioning. 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.[30] Each room should have minimal dust and preferably would be temperature controlled (airconditioned 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 biosafety 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, workflow must proceed from Room 1 to Room 2 to Room 3. Each room needs to have 3–4 metres (approximately 10–13 feet) of bench space. Furthermore, test reagents generally will have to be stored between 4 °C and 8 °C. And, as mentioned above, steady current is required so that the electrical test equipment is not damaged.

Sample transport

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

Therefore, the ability to use DBS samples for viral load is an important consideration in the implementation of the testing because it greatly simplifies the transport of samples, providing enhanced stability and ease of use for health-care workers. The use of DBS also is cost effective. However, there has been some concern about the correlation of viral load measures using DBS as opposed to plasma. Although several studies have demonstrated good correlation between the two using different viral-load methodologies, with sensitivity ranges close to 3 log HIV-RNA cp/mL *(59,60,61)*; other studies have found that the correlation between plasma and DBS viral load falls away at low cp/mL because of interference from non-plasma-associated virus *(62,63,64)*. As a result, because of the possibility of reduced sensitivity of DBS for viral load measurement at 1000 cp/mL, the WHO 2013 Guidelines suggest that programmes relying on DBS technology for viral load testing may consider retaining a higher threshold (3000–5000 cp/mL) until DBS sensitivity at lower thresholds is established *(5)*. In a review of viral load monitoring technologies, Médicens sans Frontières (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" *(65)*.

30 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.

Existing viral load technologies

HIV viral load technologies can be categorized broadly as nucleic acid-based test (NAT) and non-NAT-based technologies (Figure 28). 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.

Figure 28. Currently available NAT-based and non-NAT-based viral load technologies for laboratory use

NAT-based technologies[1]	
Type	**Assay name**
RT-PCR	COBAS® Taqman v 2.0 (Roche Molecular Systems)[2]
	Abbott RealTi*m*e HIV-1
	VERSANT® HIV RNA 1.0 (kPCR) (Siemens Healthcare Diagnostics)
	artus™ HIV-1 QS-RGQ (QIAGEN N.V.)
NASBA	NucliSENS EasyQ® HIV-1 v2.0 (bioMérieux)
bDNA	VERSANT® HIV-1 RNA v3.0 (Siemens Healthcare Diagnostics)
Non-NAT-based technologies	
Type	**Assay name**
RT	ExaVir™ Load version 3.0 (Cavidi AB)
p24 Antigen	HIV-1 p24 Ultra ELISA (PerkinElmer) (RUO)

1 In addition to these assays, Hologic expects to introduce its RT-TMA Technology (Real-Time Transcription Mediated Amplification) for the Panther® System in 2015.
2 Note that the COBAS® AMPLICOR HIV-1 MONITOR™ v1.5 (the MONITOR assay) from Roche 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 developed countries as well as resource-limited settings. The NAT-based systems manufactured by Abbott Molecular (hereafter Abbott), bioMérieux, Roche Molecular Systems (hereafter Roche), and Siemens HealthCare Diagnostics Inc (hereafter 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) *(53)*. Currently, the bulk of commercially available viral load assays are based on target amplification.

Whether an assay is based on target amplification or signal amplification, the assay will consist of the following common steps: (i) sample preparation and/or viral nucleic acid extraction; (ii) the actual amplification step that is either target amplification based 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 *(53)*. Although HIV nucleic acids are relatively stable, molecular detection methods require prompt processing of samples (generally within six 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 (RT)-PCR used in the Roche, Abbott and QIAGEN N.V.(hereafter QIAGEN) assays and the 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; thus, 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) *(53)*. Detection can be achieved using any one of a number of reagents, for example, colourimetric, radioactive or 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 labelled 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.[31]

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; (ii) RealTime HIV-1 (Abbott); (iii) VERSANT® HIV RNA 1.0 (kPCR) (Siemens); and (iv) artus™ HIV-1 QS-RGQ (QIAGEN). There also are a number of in-house procedures and test systems that have good sensitivity and reproducibility that are used in various countries,[32] but which will not be described in detail in this report.

Roche COBAS® AmpliPrep/COBAS® TaqMan® System (Roche)

Real-time PCR technology options are increasingly being used in resource-limited settings because they are faster, have higher throughput, larger dynamic ranges and automate all extraction steps. Roche currently manufactures a single real-time PCR assay, the COBAS® AmpliPrep/COBAS® TaqMan® version 2.[33] The assay uses 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.

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

31 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.
32 One example is the Generic HIV Viral Load assay from Bio-Centric (France), which is for RUO. This assay can be run on a real-time thermocycler and requires other basic consumables that would cost about US$ 40 000. Time to result is about four hours, including RNA isolation. The cost per test range is approximately US$ 10–20.
33 Roche has globally discontinued manufacture of version 1 of the COBAS® AmpliPrep/COBAS® TaqMan® assay.

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 cp/mL. At the other end of the spectrum, it also can quantify the amount of HIV-1 in a patient sample up to 10 million cp/mL.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test version 2 for TaqMan® 48 and TaqMan® 96 is prequalified by WHO. The test also is FDA approved for plasma, but is "research use only" (RUO)[34] for use with DBS. Performance of the test has proven to have good correlation with the AMPLICOR HIV-1 MONITOR™ v1.5 assay (hereafter MONITOR assay), which has generally been considered to be the gold standard (66).

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

COBAS® AmpliPrep System. The COBAS® AmpliPrep instrument is an automated sample preparation technology (Figure 29) 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.

Figure 29. COBAS® AmpliPrep system

The AmpliPrep is large, weighing over 680 lbs. 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 two 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 US$ 80 000–100 000 (with the lowest pricing reserved for lower-income countries).

34 The RUO designation is required by the FDA for non-FDA approved IVD products that are manufactured in the United States and exported for sale and use outside the United States.

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 (Figure 30) 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 90–120 minutes.

Figure 30. COBAS® TaqMan® 48

The cost of the COBAS TaqMan® 48 Analyser is approximately US$ 40 000–50 000.

In contrast to its smaller sibling, the TaqMan® 48, the COBAS TaqMan® 96 (Figure 31) is a large instrument, weighing about 450 lbs.[35] 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.

Figure 31. COBAS® TaqMan® 96

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

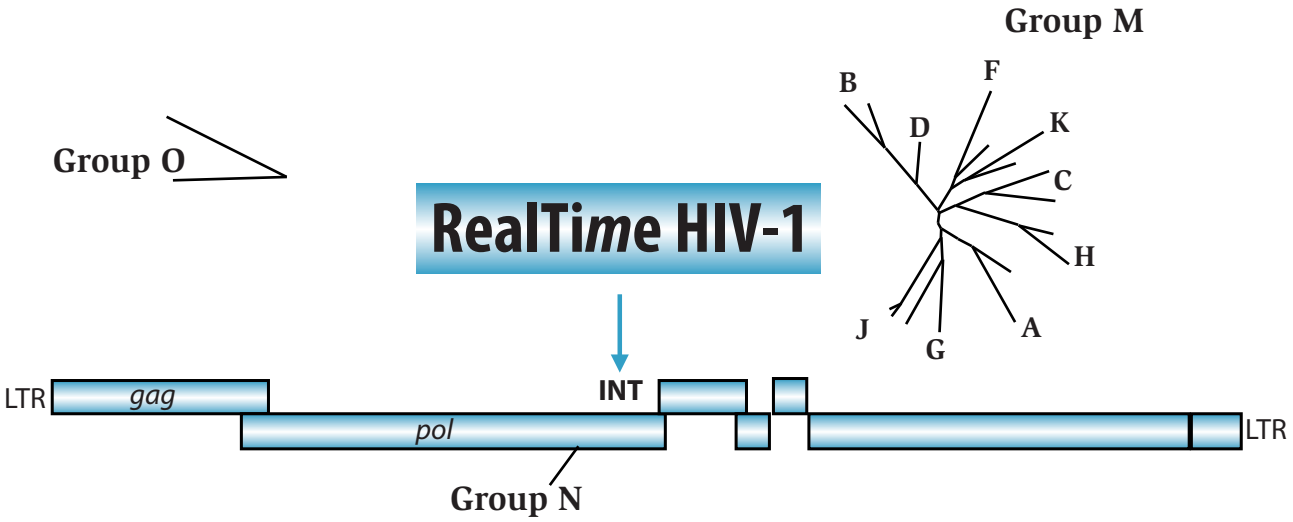
35 In addition, Roche provides the COBAS® p630 instrument for use with the COBAS® AmpliPrep/COBAS® TaqMan® System, which provides a fully automated pre-analytical solution for primary tube handling. The instrument will de-cap and cap sample tubes, pipette Roche controls from control tubes to sample tubes 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.

Abbott *m*2000 System (Abbott)

Abbott manufactures the Abbott RealTi*m*e HIV-1 assay, which is an RT-PCR assay for the quantification of HIV-1 on its automated *m*2000 and *m*24 systems and the Abbott RealTi*m*e HIV-1 qualitative assay (*m*2000system) for qualitative detection of HIV-1 in plasma and DBS used as an aid in the diagnosis of HIV-1 infection in paediatric and adult subjects.

The primers and probes of the assays are targeted to the conserved integrase region of the polymerase (or pol) gene (Figure 32), as opposed to the gag region targeted by the Roche assays, with the aim of minimizing inefficient binding due to sequence mismatch at the probe binding site. The combination with the unique probe design (a partially double-stranded probe) and cycling conditions ensures a high mismatch tolerance to detect HIV-1 groups and subtypes. The Abbott RealTi*m*e HIV-1 assay uses an external calibration strategy to allow high precision at the clinical decision point.

Figure 32. Target regions of HIV-1 RNA



The Abbott RealTi*m*e HIV-1 assay can be automated using the Abbott *m*2000*sp* (or *m*24*sp*) for sample preparation and the *m*2000*rt* 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-labelled oligonucleotide probes on the *m*2000*rt* 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*2000*rt* is proportional to the log of the HIV-1 RNA concentration present in the original sample.

The RealTi*m*e HIV-1 assay has a linear range of 40 cp/mL to 10 million cp/mL and can detect HIV-1 group M (subtypes A through H, including recombinant forms), group O and group N. The sensitivity of the assay is dependent on specimen volume. The limit of detection is 40 cp/mL for 0.6 mL input and 150 cp/mL for 0.2 mL input. Performance has been assessed with good results *(67)*. As with 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 prequalified by WHO. The price per test of the assay ranges from US$ 25 to US$ 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*24*sp* instrument (for laboratories with low- to medium-throughput requirements); or (iii) the *m*2000*sp* instrument (for laboratories with medium- to high-throughput requirements).

The *m*24*sp* (Figure 33) 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 1.5 mL tubes) with ready-to-use and reusable reagents as well as flexible batch size capabilities.

Figure 33. *m*24*sp* instrument

The cost of the *m*24sp is approximately US$ 80 000.

***m*2000*sp*.** The *m*2000*sp* by Abbott (pictured in the centre of Figure 34) is a larger and more automated sample preparation device than its sibling, the *m*24*sp*. 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*2000*rt*, the amplification and detection instrument, the system can provide automation from bar coded laboratory tube through patient result.

The cost of the *m*2000*sp* is approximately US$ 162 000.

Figure 34. *m*2000*sp* instrument

***m*2000*rt*.** The Abbott *m*2000*rt* is the amplification and detection platform for use with manual extraction, the *m*24*sp* and the *m*2000*sp* instruments, as described above. It is a high-performance system, but is relatively compact, weighing just over 75 lbs. The *m*2000*rt* (Figure 35) can run 96 samples at a time in about three hours of cycling time (not including time for sample preparation). The system will run both quantitative and qualitative analyses and offers key validity parameters such as maxRatio. 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.

Figure 35. *m*2000*rt* instrument

The cost of the *m*2000*rt* is approximately US$ 45 000.

VERSANT® kPCR Molecular System (Siemens)

The VERSANT® kPCR Molecular System and the VERSANT® HIV RNA 1.0 Assay (kPCR) are manufactured by Siemens (Tarrytown, NY, USA). 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 real-time PCR technology. The system (Figure 36) consists of two modules: the Sample Preparation Module used to extract nucleic acids from plasma as well as a wide variety of other 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 and other physical and chemical contamination controls.

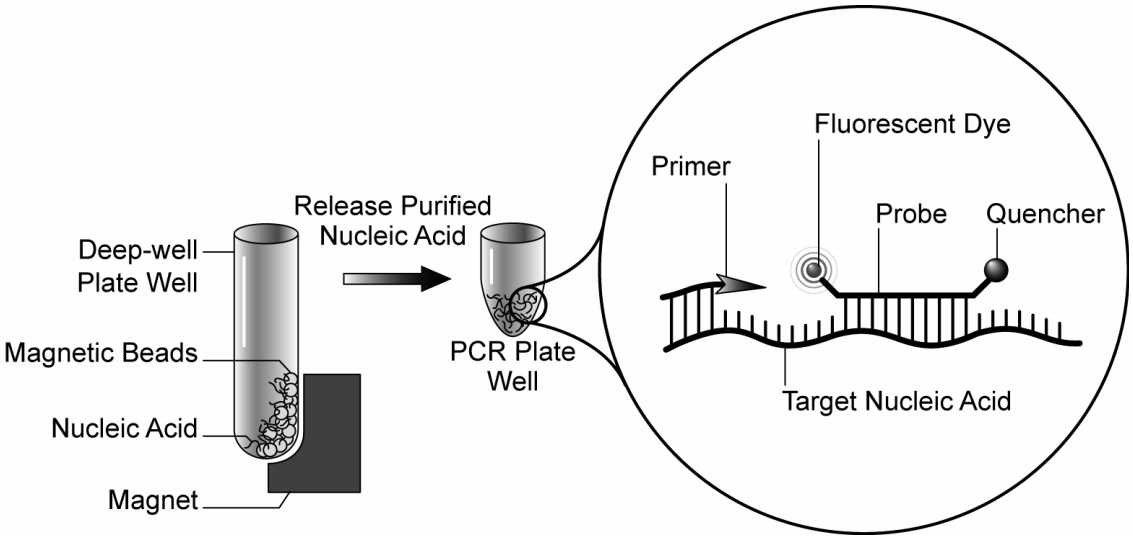
Figure 36. VERSANT® kPCR Molecular System

Photo source: courtesy of Siemens Healthcare Diagnostics Inc,© 2011 Siemens Healthcare Diagnostics Inc.

The VERSANT® kPCR 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, and several washes to remove non-nucleic acid components of the sample and elution. The VERSANT® kPCR Sample Preparation module also pipettes the purified RNA to a PCR plate containing an HIV-1 primer/probe mix and the HIV-1 enzyme mix. The wells are then sealed and transferred to the Amplification Detection module where the HIV and internal control RNA molecules are reverse transcribed to make complementary DNA (known as 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. Figure 37 shows a schematic representation of the assay principle.

Figure 37. Schematic of VERSANT® HIV RNA 1.0 Assay principle



Source: Schematic courtesy of Siemens Healthcare Diagnostics Inc, © 2011 Siemens Healthcare Diagnostics Inc.

The VERSANT® kPCR Molecular System provides the flexibility to process samples in batch sizes of 1–96 tests per run. The HIV assay provides patient results for up to 89 samples per run with a total time to result of less than six hours. The linear range of the assay is between 37 HIV-RNA cp/mL and 11 million cp/mL. The assay can detect HIV-1 Group M (subtypes A through G) and Group O variants. Performance of the assay is comparable to assays from other manufacturers (68).

The VERSANT® HIV-1 RNA 1.0 assay has been prequalified by WHO.

artus™ HIV-1 RG/QS-RGQ RT-PCR System (QIAGEN)

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 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 shown in Figure 38.

Figure 38. Example of a complete QIAsymphony RGQ system

The artus HIV-1 QS-RGQ assay has a linear range of 45 HIV-1 RNA cp/mL to 45 million cp/mL (using automated extraction) and can detect HIV-1 group M (subtypes A through H) down to a limit of detection of approximately 35 cp/mL. The time to result is from about five to six hours for 24 samples. Performance of the artus assay has been evaluated and is comparable to that of the Abbott RealTi*m*e system *(69)*.

QIAsymphony® SP/AS - Sample preparation for the artus HIV assay can 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 also is available using the QIAsymphony® SP/AS instruments. The QIAsymphony® SP can process 1–96 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-labelled sample tubes containing prefilled reagents, and allows for continuous loading in batches of up to 24 samples plus internal controls. The QIAsymphony® SP/AS instruments also can 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 virus (HBV) and hepatitis C virus (HCV), plus a transplantation/immunosuppressed panel, with assays for detection and quantification of cytomegalovirus, Epstein-Barr virus, herpes simplex virus (HSV) 1 and 2, varicella-zoster virus and BK virus.

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 analyses 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 *(70)*. 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 million cp/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 *(71,72)*.

The average price of the EasyQ® HIV assay v 2.0, including extraction and amplification/detection, is about €18 (~US$ 23.75) per test, and the assay for use on either the semi-automated or automated systems described below is prequalified by WHO.

NucliSENS® miniMAG® and NucliSENS® easyMAG® extraction systems. These 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® (Figure 39) 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).

Figure 39. NucliSENS® miniMAG® extraction system

Despite its relatively small size, the miniMAG® has reasonably high throughput – with 12 extractions in 45 minutes (using one miniMAG® system) and 24 extractions in 60 minutes (using two 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 €6800 (~US$ 9000).

For higher throughput needs, the easyMAG® is an automated bench-top 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 (Figure 40) has one generic extraction protocol (DNA/RNA) and one set of reagents for all applications, which together with touchscreen technology, makes the process relatively simple.

Figure 40. NucliSENS® easyMAG® extraction system

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 (~US$ 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 (Figure 41). 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.

Figure 41. NucliSENS EasyQ® Amplification and Detection system

The EasyQ® analyser is compact, weighing only about 45 lbs, and can fit easily onto the average laboratory workbench. Furthermore, amplification and real-time detection of 48 samples require only 60 minutes.

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

NucliSENS Connectivity. bioMérieux also provides NucliSENtral™ (Figure 42), which is an integrated software system that can be used to link NucliSENS® easyMAG® and NucliSENS EasyQ® with a laboratory information system (LIS).

Figure 42. NucliSENtral™software system

bDNA technology

VERSANT® 440 Molecular system (Siemens)

Siemens manufactures the VERSANT® HIV-1 RNA 3.0 Assay, which is a bDNA sandwich nucleic acid hybridization method that targets a well-conserved region of the gag gene. Plasma HIV-1 is quantified by amplifying the signal rather than the target RNA. A phosphorescent chemical that binds to the HIV RNA is added to the sample. The amount of light is measured and converted into a viral count. This assay does not require viral RNA purification/extraction or PCR amplification steps. The bDNA assay is performed on the VERSANT® 440 analyser and has a linear range of 50–500 000 cp/mL. The VERSANT® HIV-1 RNA 3.0 Assay can detect HIV-1 Group M (subtypes A through G). The performance of the assay correlates well with that of the Roche AMPLICOR assay *(73,74)*.

The Siemens VERSANT® 440 Molecular system (Figure 43) and bDNA technology eliminate 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 also is 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. This technology is particularly well-suited for resource-constrained environments due to the lack of need for amplification of the virus and separate clean areas.

Figure 43. VERSANT® 440 Molecular system

Photo source: courtesy of Siemens Healthcare Diagnostics Inc, © 2011 Siemens Healthcare Diagnostics Inc.

Real-time transcription mediated amplification platform

RT-TMA technology (real-time transcription mediated amplification) for the Panther® system (Hologic Gen-Probe Incorporated)

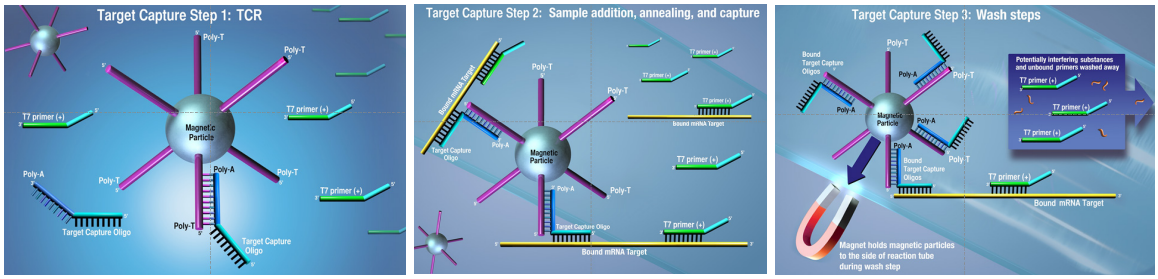
Hologic Gen-Probe Incorporated (hereafter Hologic) has introduced the Panther® system, a molecular diagnostic platform with true random access testing capability on a fully integrated and automated NAT system (Figure 44). The platform brings the flexibility of clinical chemistry instrumentation to molecular diagnostics.

Figure 44. Panther® system

The company has developed a fully quantitative viral load assay, the Aptima® HIV-1 Quant Dx Assay (Aptima assay), for the Panther® system. The Aptima® assay involves three main steps (Figure 45), all of which take place in a single tube on the Panther® system: target capture; target amplification by transcription-mediated amplification (TMA); and detection of the amplification products (amplicon) by the fluorescent labelled probes (torches).

Target capture. During target capture, viral RNA is isolated from samples. The sample is treated with a detergent to release viral genomic RNA. Oligonucleotides capture and hybridize to highly conserved regions of HIV-1 RNA, if present, in the sample. The hybridized target is then captured onto magnetic microparticles that are separated from the sample in a magnetic field. Finally, wash steps remove extraneous components from the reaction tube.

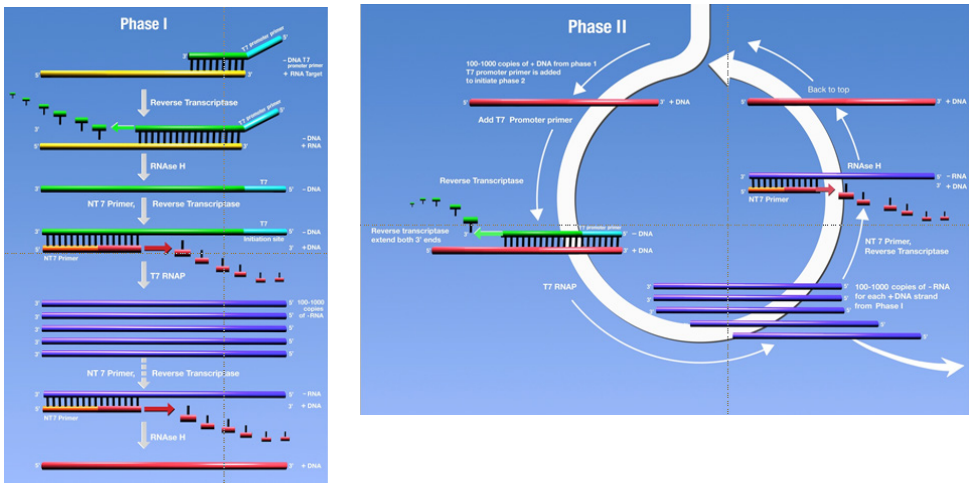
Figure 45. Aptima® HIV-1 Quant Dx Assay steps



Target amplification. TMA is a transcription-based nucleic acid amplification method that utilizes two enzymes, RT and T7 RNA polymerase (Figure 46). The RT is used to generate a DNA copy (containing a

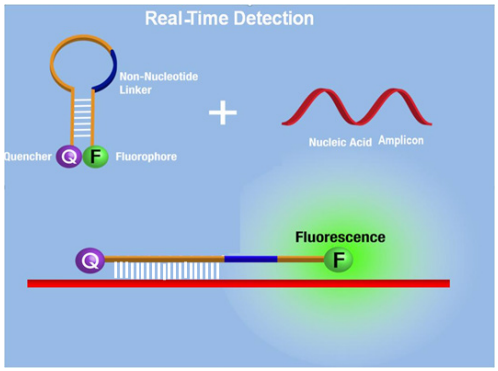
promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Aptima assay utilizes the TMA method to amplify two regions of HIV-1 RNA (Pol and LTR). Amplification of these specific regions is achieved using specific primers that are designed to amplify HIV-1 groups M, N, O and P. The primer design and the dual target approach ensure accurate detection and quantitation of HIV-1.

Figure 46. TMA transcription-based nucleic acid amplification method



Detection of amplicon. Detection is achieved using single-stranded fluorescent probes (torches) that are present during the amplification of the target and hybridize specifically to the amplicon in real time (Figure 47). The torches consist of a fluorophore and a quencher. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and it will emit fluorescence at a specific wavelength. As more torched hybridize to more amplicon, more fluorescence is generated. The time taken for the fluorescent signal to reach a threshold is proportional to the starting HIV-1 concentration. Each reaction has an internal calibrator/internal control that controls for variations in sample processing, amplification and detection. The concentration of a sample is determined automatically by the Panther® system software using the HIV-1 and internal control signals for each reaction and comparing them to stored calibration information.

Figure 47. Real-time detection of amplicon



Within the Panther®, all nucleic acid testing steps, from primary sample tube to results, are fully automated in one system with first reportable results within three hours after loading samples, and five results every five minutes thereafter. Samples can be continuously loaded with up to 120 samples at a time (Figure 48). Reagent controls and calibration are valid for 24 hours. At least 275 samples can be run within an 8-hour shift or 500 in a 12-hour period (an additional 225 samples can be run without operator attendance). Four reagent lanes allow up to four kits of the Aptima test kits to be onboard and randomly accessed at

any time: this could be four kits of the Aptima® HIV-1 Quant Dx Assay or any combination of the other molecular diagnostic assays available on the Panther®, including CT/GC, Trichomonas Vaginalis, HPV, and HPV genotyping, HCV Quant Dx, HBV Quant, and HSV1/2 assays.[36]

Figure 48. Panther® continuous loading system

The Panther®'s intuitive task-driven software with touchscreen (Figure 49) interface simplifies setup, adding or removing reagents or samples, and onboard inventory management of reagents and consumables; bi-directional laboratory information system (LIS) interface capability can automate test requests for samples placed on the instrument and automated release of results as configured by the operator. Low-volume dilution option allows quantitative results to be obtained on as little as 240 µL of plasma, with the software calculation to adjust for the dilution and report actual concentration. The system can be programmed to perform automated maintenance outside of laboratory hours. Reagent management is simplified with 48-hour onboard stability or 30 days' refrigeration for assay reagents. Up to 2000 tests of common system fluids are managed by radio frequency identification tags and Panther software.

36 Each of the HCV Quant Dx, HBV Quant and HSV1/2 assays is still in development.

Figure 49. Panther touchscreen

Hologic is pursuing CE–IVD marking, FDA approval and WHO prequalification for Aptima® HIV-1 QuantDx Assay on the Panther® System. DBS also is being validated for use with the system. The assay is expected to be available with CE–IVD approval in 2015. Pricing will be variable and dependent on variables such as instrument purchase, reagent rental and volume-based pricing.

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 RT activity and assays that measure the concentration of circulating p24 protein.

RT 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" (RT). Without RT, the viral genome could not become incorporated into the host cell and could not reproduce: 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 (hereafter Cavidi).

ExaVir™ Load (Cavidi)

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 antiretroviral 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 (Figure 50).

Figure 50. ExaVir™ Separation equipment

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 200–600 000 cp/mL (or 1–3000 femtograms/mL). There are performance data available for the ExaVir™ Load showing good correlation with the AMPLICOR assay *(75,76)*.

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 laboratory commodities. Furthermore, 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 US$ 9000–10 000, and the cost per test, which varies according to volume, ranges from about US$ 13 to US$ 15. Although the assay is manual, it is reasonably priced and easy to implement, especially in Level II settings.

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 antigenaemia, 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 antigenaemia is the core protein, p24, the major internal structural protein of HIV-1. The p24 appears within two weeks after infection as a result of the initial increase in viral replication and is associated with the period of antigenaemia 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 *(53)*. In particular, the HIV-1 p24 ELISA assay from PerkinElmer (an ultrasensitive, heat denatured p24 antigen quantification assay), described below, has been used for this purpose.

HIV-1 p24 ELISA kit (PerkinElmer)

The PerkinElmer HIV-1 p24 ELISA is a sensitive enzyme immunoassay kit for the detection of p24 antigen in human serum or plasma and cell culture supernatants. The protocol includes an immune complex dissociation step for serum and plasma samples, thus increasing the sensitivity of the method for detecting low levels of the antigen in the presence of p24 antibodies. The analytical sensitivity for serum and plasma assay is about 26 pg/mL (or about 430 000 cp/mL), and for cell culture supernatants 4.3 pg/mL (or about 71 000 cp/mL). By combining the p24 ELISA assay with PerkinElmer ELAST Amplification System, the sensitivity of the method can be increased by about 25 fold, thus enabling measurement of femtogram levels of p24 or well below 10 000 cp/mL.[37]

A protocol for analysing DBS samples with the p24 ELISA kit also is available. The method includes an elution and immune complex dissociation step before the actual p24 assay, and an amplification step using the ELAST Amplification System kit. Several studies have been published where the DBS protocol has been used successfully for EID testing and for viral load assays. Both the HIV-1 p24 ELISA kit and the ELAST Amplification System kit are currently available for RUO purposes and not for patient results.

POC viral load testing platforms

To date, only one viral load assay, a semi-quantitative assay for use on the simple amplification-based assay (SAMBA) system, has been launched. It is described below.

SAMBA (Diagnostics for the Real World Ltd)

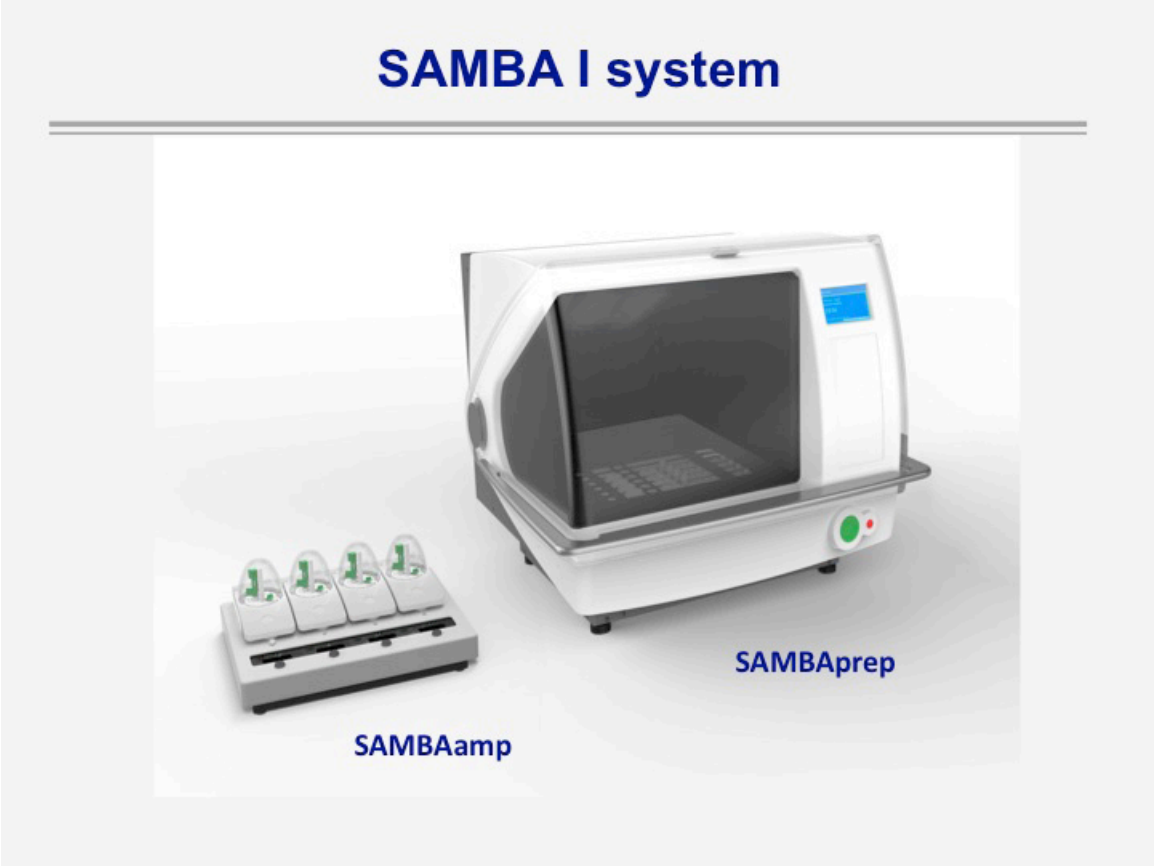
SAMBA is being developed by a team led by Dr Helen Lee, Director of the Diagnostics Development Unit (DDU) at the University of Cambridge. Four NAT-based HIV assays have been developed: (i) a semi-quantitative test with a cutoff of 1000 cp/mL for monitoring ART using plasma; (ii) a semi-quantitative test with a cutoff of 1000 cp/mL for monitoring ART using whole blood; (iii) a qualitative test based on plasma or whole blood for the detection of acute HIV infection during the window period before the appearance of antibodies; and (iv) an EID test based on whole blood.

There are two SAMBA systems:

SAMBA I (Figure 51) is for semi-automated batch testing with a throughput of 30–42 samples per day. It automates extraction (SAMBAPrep) and integrates amplification and detection (SAMBAamp) into a benchtop analyser with amplification and detection taking place in a hermetically sealed cartridge.

37 Note that unless the p24 antigen test is optimized using the ELAST System or otherwise, the assay will be of limited utility in detecting early treatment failure and would not be useful in patients with low viral replication because of its relatively low sensitivity (77).

Figure 51. Samba I system

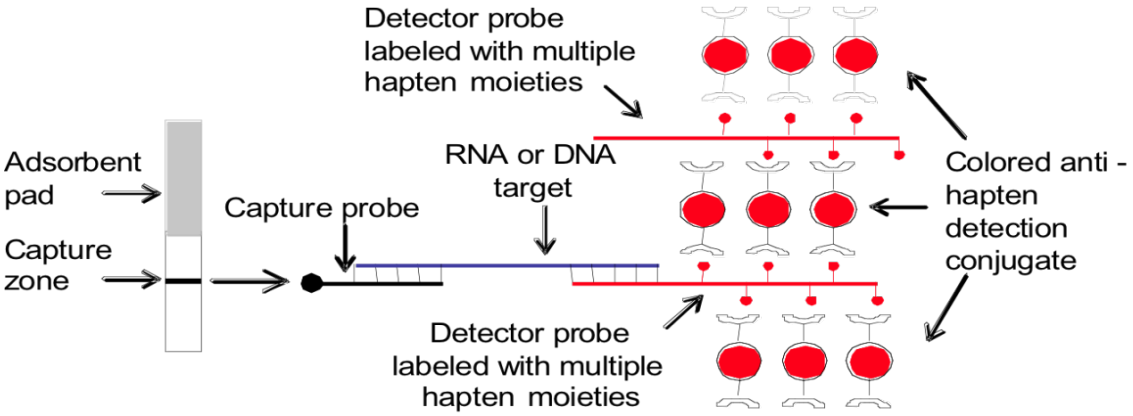


SAMBA II (Figure 52) is a fully automated "sample-in, result-out" system. It is suitable for both low- and high-volume testing sites with the throughput of 4–64 samples per day. A display unit controls the assay module and each display unit can control up to 16 assay units. It is a modular, random access system that allows the throughput to be adjusted as per the requirements of the site.

Figure 52. Samba II system

The SAMBA HIV test uses 200 µL of plasma or 120 µL of whole blood for the semi-quantitative viral load assay, 500 µL of plasma for the qualitative acute infection assay and 100 µL of whole blood for the EID assay. 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 (Figure 53).

Figure 53.SAMBA amplification process showing lattice structures



A capture probe is used to capture the target sequence, and a detector 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 (Figure 53) 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 semi-quantitative viral load test for whole blood: evaluated in clinical samples from KEMRI/CDC, Kisumu, Kenya;
- 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 Uganda and Zambia ;
- Field evaluation using infant blood is currently ongoing in MU-JHU, Uganda, and KEMRI/CDC, Kisumu, Kenya.

Currently, the total assay time is 2 hours for the SAMBA EID and 90 minutes for the semi-quantitative viral load assay. The SAMBA II system is best suited for use at Level II facilities or in large clinics (Level I facilities) in sub-Saharan Africa where laboratory technicians and electricity are available.

Diagnostics for the Real World Ltd the spinout company of DDU located in California, is the manufacturer of the SAMBA system. Following launch and implementation of the SAMBA viral load platform in two clinics in Malawi in early 2013, MSF also implemented the test in the Arua district hospital in Uganda in September 2013. Pricing information is available from the company and is volume dependent.

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 two days to obtain a result; to date, p24 antigen testing has been of limited value in patient monitoring due to its low sensitivity. Viral load testing that could be conducted at the point of patient care 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 could minimize loss to follow-up.

As indicated above, there is only one POC viral load assay in limited release on the market. However, there are a number of additional platforms/assays in development, more than one of which likely will be launched in 2014. 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, 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 one or two additional products will be launched in 2014. 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)

The Alere q system (Figure 54) is a generic platform for the implementation of nucleic acid testing. The first test to be commercialized will be an integrated test for the qualitative detection of HIV-1 and HIV-2 simultaneously from 25 ml of whole blood. This will be followed by the release of a test for the quanti-

tative measurement of HIV-1 and HIV-2 viral load from 500 µL of plasma and a test for the quantitative detection of HIV-1 and HIV-2 simultaneously from 25 ml of whole blood. Additional tests in development include those for the detection of *Mycobacterium tuberculosis* (MTB) in sputum as well as a series of drug susceptibility tests for MTB. The device on which the assay is run (Figure 54) 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 rugged enough to withstand harsh environments.

Figure 54. Alere q system

The Alere q tests are disposable cartridges that contain all reagents required for the assay in a stabilized form. The HIV Detect and HIV viral load (whole blood) cartridges provide for sample collection, cell lysis, target capture, reverse transcription, RT PCR amplification and real-time fluorescence detection based on competitive reporter probe hybridization on an integrated micro array. The HIV viral load (plasma) cartridge uses the same principles and chemistry as the whole blood cartridges, but requires a separate plasma preparation step. 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 whole blood tests are designed to require no manual sample preparation or pretreatment. 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 in Figure 55. When using venous blood, the sample is transferred to the cartridge capillary with a transfer capillary; a volumetric pipette also can be used. 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 three minutes (i.e. sample collection and loading of the cartridge onto the analyser).

Figure 55. Alere q test cartridge

Test workflow for the operator is straightforward and consists of: (i) lancing the patient's finger/heel (or collecting blood via venipuncture) and transferring whole blood directly into the cartridge sample collection capillary; (ii) manually capping the cartridge; (iii) inserting the cartridge into the analyser; and (iv) entering the operator and sample IDs on the analyser. When the assay is complete, audible and visual prompts alert the operator to remove the cartridge from the instrument and the results are displayed on a built-in screen. The result can be printed immediately, but results also are stored in an onboard 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.

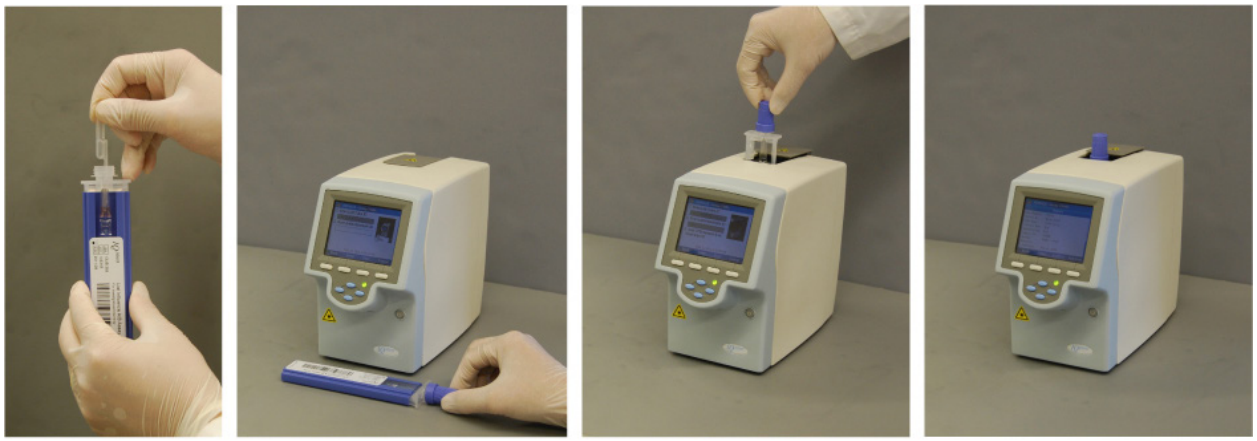
CE-IVD marking of the Alere q system in the European Union and the first commercial sales are expected in 2014. Pricing for the instrument and disposable test cartridges has not yet been determined.

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 A and B strains. An assay for dengue virus is under development, and the platform also can accommodate MTB, HCV and other disease categories. Liat™ assays for HIV viral load testing and diagnostics also have been developed and independently evaluated by third- party institutions.

As illustrated in Figure 56, 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.

Figure 56. Liat™ Analyser and test procedure



STEP 1.
Add sample

STEP 2.
Scan barcode

STEP 3.
Insert tube

Done.
Results in ~30 minutes

To aid the operator and provide reliable results, the Liat™ Analyser incorporates a variety of intelligent and advanced features: bar code data entry avoids errors in sample or assay coding and onscreen 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 Liat™ Analyser is small and portable and executes all required assay steps and reports a qualitative or quantitative test result within 15–35 minutes, depending on the test. For example, if the user wants to measure viral load from a plasma sample down to 80 cp/mL, then the device takes about 30 minutes to produce a result; if the user wants to measure viral load from a whole blood sample down to 1000 cp/mL, then the device will take about 35 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 US$ 25 000, may decrease for resource-limited settings. Dr Robert Coombs at the University of Washington, Dr James Bremer at Rush University, Dr Susan A. Fiscus at the University of North Carolina at Chapel Hill, Dr Wendy Stevens and Dr Lesley Scott at the University of the Witwatersrand and National Health Laboratory Service, Johannesburg, have completed multiple evaluations comparing the Liat™ Analyser's viral load detection capabilities against the Roche COBAS® and the Abbott *m*2000 system. In all of these evaluations, the performance of the Liat™ device compared favourably to the predicate devices. The HIV viral load assay for the Liat™ platform is complete, but its actual market 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 liquid micropiston technology, Wave 80 Biosciences (hereafter Wave 80) is developing the EOSCAPE-HIV™, a rapid HIV NAT-based POC viral load test designed for use in resource-limited settings. The company describes the cartridge as incorporating automated sample metering with filtration for removal

of pro-viral DNA, a no-spin, cartridge-integrated nucleic acid extraction process, and tuned iNAAT coupled with a proprietary ultrasensitive bipartite luminescent signaling/detection system. The system processes fingerstick whole blood 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 (Figure 57) 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 a 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 one day of training for operators.

Figure 57. EOSCAPE-HIV™ system and test procedure

EOSCAPE Cartridge
Single use, all reagents onboard

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. Processing within the cartridge takes approximately 65 minutes. At the conclusion of the primary processing step, the operator inserts the processing unit into the reader for a scan; scan time, including associated data entry, is less than five minutes. Equipped with an easy-to-use touchscreen interface, the analyser has full LIS capabilities, including transmitting test results through wired and wireless connectivity. For higher patient loads, multiple processing units can be used for parallel processing, with ~50 samples per day per analyser.

Wave 80 recently added radio frequency identification communication among the three EOSCAPE system components to ensure integrity of patient data. Internal assay and amplification controls enable thresholding at 1000 cp/mL and quantitation over the range from 1000 to 50 000 cp/mL without the need for external calibration. A later version of the system will detect lower copy counts and quantitate across an even wider dynamic range.

Beyond the EOSCAPE-HIV™, assays in development for the base of the EOSCAPE system include a single-plex assay for diagnosing active MTB infection, a 2-plex assay for chlamydia and gonorrhoea and a 2-plex assay for acute respiratory infection. A variant of the system with enhanced multiplexing capability also is in development, with assays including a MTB drug resistance test and a 20-plex acute respiratory infection test as well as other infectious and non-infectious diseases.

Release of the EOSCAPE-HIV™ has been delayed to implement certain reagent formulation improvements and enhancements of in-cartridge storage to ensure that the product's stability during transportation and storage are optimally matched to the requirements of high-disease-burden countries. Finalization of the product's design is now planned for the end of 2014. Commercial availability is still planned for 2015, pending securing sufficient funding for clinical trials, and Wave 80 is currently putting its ISO production facilities into place to support the product launch.

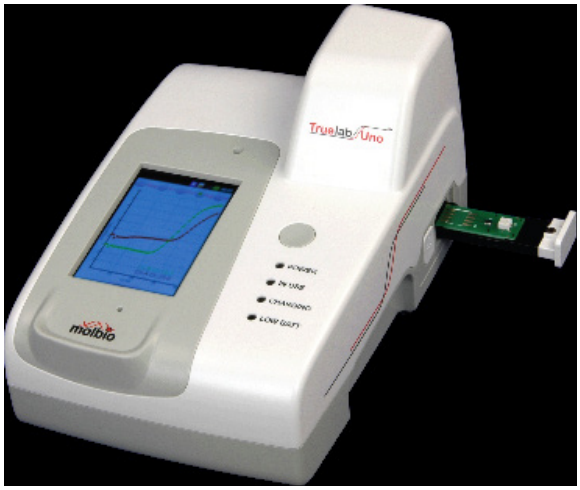
Truelab™ Real Time micro PCR System (Molbio Diagnostics Pvt Ltd – a Tulip Group–Bigtec laboratories partnership)

Molbio Diagnostics Pvt Ltd 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 to final result reporting, all within one hour. A Truelab™ micro PCR printer also is 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 *Plasmodium falciparum* and *Plasmodium 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 20–25 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 (Figure 58). 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.

Figure 58. Truelab™ Uno Real Time micro PCR System



Test results are automatically stored in the analyser memory (up to 5000 results) and can be printed and transported wirelessly to any server/compatible device by Wi-Fi, GPRS (a mobile data service), Bluetooth or even SMS.

The HIV viral load assay is expected to launch in the third quarter of 2014. The assay is currently undergoing laboratory-based trials in India.

GeneXpert® System (Cepheid)

The Cepheid GeneXpert® System, which is a fully automated and integrated system for PCR-based nucleic acid testing, currently has 14 FDA-cleared and 14 CE-IVD-approved assays, including tests for enteroviral meningitis, methicillin-resistant *Staphylococcus aureus*, *C. difficile*, influenzas A and B, MTB detection and with simultaneous detection of resistance to rifampicin (RIF), *C. trachomatis/N. gonorrhoea* and group B streptococcus, among others. In addition to the tests listed, Cepheid has 14 tests in active development, including tests for human papillomavirus (simultaneous detection and typing), Qualitative and Quantitative HIV, Quantitative HCV, *Trichomonas vaginalis* and Carba-R (Carbapenemase Resistance). Any of these tests can be run on virtually all of the more than 5500 GeneXpert®s placed worldwide.

The GeneXpert® HIV viral load assays are on track to be launched commercially around late 2014. The quantitative HIV-1 assay using plasma and the qualitative HIV-1 assay using either whole blood or DBS are expected to CE-IVD marked at launch. The assay targets one genomic region of HIV-1 that is proven both in silico and in vitro to detect the vast majority of all HIV-1 strains independent of group and subtype. The forward and reverse primer and the TaqMan probe are located in the most conserved region of the LTR. To be able to detect Group O HIV with equal efficiency to Groups M and N, an additional TaqMan probe was designed. The HIV genome target forward primer and the two probes included in the assay incorporate Cepheid's proprietary special chemistry to maximize inclusivity and exclusivity at the sequence level. The assay detects all strains of HIV-1, including HIV Group M subtypes A, B, C, D, F, G, H, J, K, AB, AE, AG and Group N and Group O.

The quantitative assay has a limit of detection of approximately 20 cp/mL and a limit of quantitation of approximately 40 cp/mL with a 1 mL plasma sample input volume. The quantitative assay includes two internal quantification standards, high and low, to provide the accuracy and precision in quantitation. The qualitative assay has a limit of detection of approximately 200 cp/mL for 100 µL of whole blood sample input volume. No special instrumentation or handling is required for either assay.

The workflow for the quantitative assay is as simple as: (i) collecting whole blood in acid citrate dextrose (ACD) or EDTA tube; (ii) centrifuging the tube; (iii) transferring 1 mL (pipette provided) directly into the

GeneXpert® cartridge; (iv) scanning the cartridge bar code; and (v) loading the cartridge into the GeneXpert® module and closing the door with an approximate 95-minute time to result.

The workflow for the qualitative assay is as easy as: (i) collecting whole blood or DBS with whole blood spot; (ii) transferring the whole blood or DBS to a diluent reagent (provided) and mixing; (iii) transferring the 1 mL mixture (pipette provided) directly into the cartridge; (iv) scanning the cartridge bar code; and (v) loading the cartridge into the GeneXpert® module and closing the door with an approximate 95-minute time to result. An early termination step is included to shorten the time to a positive result.

Although it is not currently known what the price per cartridge will be for the viral load assay, the cost negotiated by the Foundation for Innovative New Diagnostics of the GeneXpert® System (Figure 59, with four modules pictured on the left) for high-burden developing countries is approximately US$ 17 000; and, as a result of an agreement between the United States President's Emergency Plan for AIDS Relief (PEPFAR), United States Agency for International Development (USAID), UNITAID and the Bill & Melinda Gates Foundation, the current price per cartridge for MTB/RIF is about US$ 9.98 in high-burden developing countries. Uptake of the programme via USAID, PEPFAR and other agencies has been escalating rapidly; as of 31 December 2013, a total of 2021 GeneXpert® instruments (comprising more than 10 561 modules) and more than 5 219 960 GeneXpert® MTB/RIF cartridges have been procured in the public sector in 98 of the 145 countries eligible for concessional pricing. All GeneXpert® tests, including the quantitative and qualitative HIV tests, can be run on the systems placed initially for TB testing.

Figure 59. GeneXpert® System (left) and cartridge (right)

The GeneXpert® System integrates and automates sample preparation, amplification and detection in a single-use, self-contained cartridge (Figure 59, pictured on the right). Most liquids and dry reagents, along with enzymes, are prefilled so that pre-analytical steps are minimized, greatly reducing opportunities for sample mixups and operational errors. GeneXpert® cartridges can handle a variety of sample volumes (millilitre range) within macrofluidic chambers and then concentrate the target material down to microfluidic volumes, which can increase the sensitivity of the assays, if needed.

Furthermore, 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 so that training usually can be completed within half a day. Furthermore, although the system was designed to use AC mains power, its low wattage requirements allow it to be powered by a 12 V DC/120 V AC voltage converter in mobile laboratories; it also has 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 LISs.

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.[38]

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

NWGHF, in collaboration with Quidel Corporation, is developing a POC rapid RT-PCR testing platform, Savanna, which will be both easy to use and low cost. The product (Figure 60) can accommodate 13 tests in an 8-hour day. The proposed viral load assay will achieve a limit of detection of 1000 cp/mL of plasma, using ~150 µL of whole blood that is converted into plasma with simple sample preparation materials provided by NWGHF.

Figure 60. Savanna Viral Load Platform

The processor is powered by an external power transformer that connects to either an AC mains or DC power cable that, in turn, connects to an AC mains or DC power socket in the clinic or laboratory. The system is expected to cost US$ 12 000, with a cartridge cost of US$ 10 per test.

NWGHF/Quidel Corporation expect to launch the Savanna viral load test and platform in 2015.

Viral load assay using BART (Bioluminescent Assay in Real-Time) technology (Lumora Ltd)

Lumora Ltd (hereafter Lumora) has introduced the BART, a platform for performing molecular diagnostics that allows real-time closed-tube quantitative detection of amplification by using a hardware system that can generate and store objective test results. BART is a bioluminescent reporter system for molecular diagnostics that can reduce instrument costs and open up new applications for diagnostics and disease monitoring in resource-constrained settings.

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

Lumora is now seeking partners to commercialize both its viral load and the EID assays. The company has developed an assay system, from extraction to result, that will enable a minimally trained user to perform viral load assays in a non-specialist laboratory or clinic setting.

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 (iNAATs). It combines simple and robust chemistry and technology in real-time, closed-tube analysis (requiring minimal electrical input and temperature regulation) and less demanding sample preparation.

Lumora has introduced technology to simplify viral load measurement in low-resource settings in each of the key stages involved:

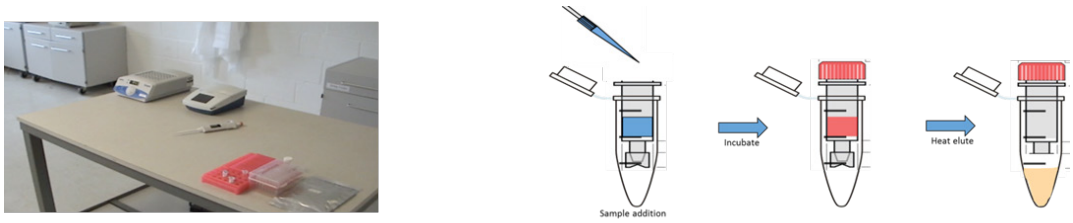
- sample preparation
- amplification
- detection and instrumentation.

Sample preparation. The company believes that ease of sample preparation and processing is key to the success of any assay system, and Lumora's pursuit of simplicity and robustness has led to the development of two novel and proprietary approaches:

- heat elution for whole blood and DBS;
- bead-based viral extraction from whole blood or plasma.

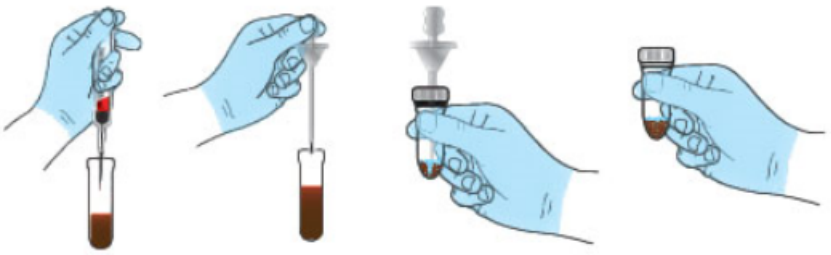
Whole blood and DBS. Through the utilization of Lumora's proprietary heat elution sample preparation technology (Figure 61) it is possible to extract a sample from whole blood or DBS in 10 minutes using only a heating block and Lumora sample preparation kits. The processed sample can then be added directly to freeze-dried amplification reagents.

Figure 61. BART heat elution sample preparation technology



Viral extraction kit for whole blood or plasma. Lumora's novel manual three-step viral extraction technology (Figure 62) allows nucleic acid to be extracted from whole blood or plasma in 20 minutes. The processed sample can then be added directly to freeze-dried amplification reagents.

Figure 62. BART manual three-step viral extraction technology



Three step sample extractions:

- binding and lysis
- wash
- elute

Amplification. Lumora has developed and patented improvements to loop-mediated amplification (LAMP), (an iNAAT), technology, not only enhancing performance for viral detection and difficult bacterial targets, but also for speed and ease of development. Lumora's stem primer technology has facilitated the development of a fully inclusive HIV viral load test utilizing isothermal LAMP technology. In recent years, LAMP has been used as an alternative to PCR. However, its widespread adoption has been limited due to challenges associated with designing appropriate primers. Lumora has developed and patented a technique that uses a different type of primer, known as a stem primer, to increase the speed of LAMP assays. The stem primers are preferable because, among other advantages, they can be multiplexed. In addition, the stem primer technology offers additional exciting strategies for dealing with the large sequence diversity of HIV genomes.

Detection. The iNAATs generally used in molecular diagnostics produce amplicons at an exponential rate. As a result, pyrophosphate, a by-product of amplicon production, also is generated exponentially. BART employs pyrophosphate as the start of a reaction driven by firefly luciferase enzymes to generate light and signal the presence of a specific target. Because BART follows the rate of change, it is robust to different sample types and tolerant of contaminating substances. The enzymes are modified to be thermally stable, meaning the reaction can occur at 60 °C, well above environmental temperatures even in hot climates. 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 similar time to fast PCR systems.

The unique nature of the BART biochemistry means that test results can be detected using a simple light detection system (e.g. a photodiode), facilitating early detection and decision-making with no need for an expensive, highly sensitive light detection apparatus. The BART technology has been shown to be more tolerant of less processed samples, due to the fact that the DNA polymerase used in iNAATs is less affected by common inhibitors. Similarly, it is possible to analyse samples that either have inherent fluorescence or are turbid, which is an issue for other techniques. These properties enable simpler protocols to be established for a particular test as a whole. For example, where magnetic particles have been used to capture specific bacteria, it is not necessary to elute DNA from the beads before attempting amplification as with PCR. Rather, the beads can be added directly to the BART reagent, which can tolerate the presence of magnetic beads. This enhanced sample tolerance means that sample preparation can be simplified, an advantage in resource-limited settings and challenging physical environments. BART is well suited to high-throughput applications, making it equally useful in both highly decentralized settings and centralized laboratories requiring high-throughput technologies. The hardware also is 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.

All of these features make BART ideally suited to use in settings with limited laboratory infrastructure and adverse operating conditions, such as for viral load monitoring in low-resource settings. The first-generation assays with either heat elution or manual sample extraction are now ready to license and Lumora, with the help of a commercial partnership, anticipates that the assay can be in the market within six months.

Fully integrated device for low volume POC testing. The simplicity and robustness of the manual viral sample extraction chemistry plus the simplicity of BART enabled the development of a second-generation assay (Figure 63) that is fully integrated (i.e. "sample in, result out"). The second-generation assays with viral extraction technology are focusing on the technical challenges of low-volume quantification for POC applications.

Figure 63. BART second-generation integrated assay

True quantification from volumes such as 50 µL of blood is being developed, and Lumora is seeking partners to commercialize this product as well. As this development continues, it will not be limited to HIV viral load, but also will have access to the menu of tests that Lumora is developing.

The fact that BART and the associated technologies from Lumora are easy to use and relatively low cost means that wider adoption of this technology could be expected in laboratories where currently available methodologies are not being used, whether 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 (hereafter Ustar) has developed Cross Priming Amplification (CPA), a novel iNAAT 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, HCV, chlamydia/gonorrhoea 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, by 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 viral load assay and test cartridge in conjunction with a robust and user-friendly portable instrument that will provide viral load measurements from fingerstick

whole blood. For this purpose, Ustar plans to modify the commercially available Genie®, a portable instrument developed by OptiGene Ltd. Ustar will: (i) develop an automated sample preparation instrument and method for the extraction of viral RNA from whole blood; (ii) develop an RT CPA assay for the detection and quantitation of all major HIV-1 subtypes; and (iii) integrate the automated sample preparation instrument with the existing Genie® instrument for a fully automated sample-in, answer-out system.

The final Ustar diagnostic test kit is expected to be comprised of a reagent-containing cartridge and a portable device for sample preparation, amplification and detection. Reagents will consist of glassified enzymes for ambient temperature transport and storage, a reconstitution buffer and sample preparation buffers, all housed in the cartridge.

The testing process will require the user to: (i) take a fingerprick or heelprick and place a drop (100 µL) of blood directly onto a plasma separating filter for RNA concentrating; (ii) invert the filter over the cartridge and punch out the RNA containing filter into the processing chamber; and (iii) close the cartridge and place it into the instrument for automated sample preparation, 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 < 1000 cp/mL. Onboard 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 programme), along with detailed information regarding each run.

Ustar is now actively working on the development of its viral load assay with completion and launch expected in 2016–2017.

Gene-RADAR® Platform (Nanobiosym® Diagnostics)

Nanobiosym® Diagnostics (hereafter Nanobiosym) has developed a portable nanotechnology platform called the Gene-RADAR® (Figure 64). 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 20–100 µL 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 IVDs, 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.

Figure 64. Gene-RADAR® Platform

Nanobiosym has developed a viral load assay for the Gene-RADAR® v 1.0 platform, which can give a fully quantitative viral load measure in real time. No specific cost data are 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.

Most recently, Nanobiosym received the prestigious XCHALLENGE Grand Prize for Gene-RADAR® in a US$ 2.25 million mobile diagnostic competition sponsored by XPRIZE and Nokia. The company was selected from a pool of 26 competing teams from around the world for the award. Teams were evaluated for their distinction in the areas of accuracy and consistency, demonstration quality, technical innovation, human factors, market opportunity, originality and user experience.

In addition, Nanobiosym has been awarded grants from USAID and Grand Challenges Canada under the programme Saving Lives at Birth: A Grand Challenge for Development. The grants are for the design and implementation of a pilot trial in Rwanda for viral load testing using the Gene-RADAR® platform.

ZIVA™ (Cavidi)

Cavidi is developing an easy-to-use, bench-top platform, the ZIVA™, for near-patient HIV monitoring, a prototype is pictured in Figure 65. The ZIVA™ is targeted at district hospitals (Level II) and large clinics to provide high-quality viral load test results using existing facilities and staff in decentralized settings.

Figure 65. ZIVA™ platform

ZIVA™ uses a proven technology as it builds upon the existing Cavidi platform, ExaVir™ Load, which is a fully 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 assay principle is based on three principle steps:

- isolation of HIV from plasma and preparation of RT-lysate;
- RT reaction;
- detection and quantification of RT-reaction product by chemiluminescence immunoassay (CLIA).

The viral load assay is a straightforward, fully automated procedure with minimal hands-on time. Once plasma has been separated, only the ZIVA™ instrument is needed. The operator loads patient plasma samples together with reagents and consumables (provided by Cavidi), starts the assay run and leaves the machine. The operator only needs to return after the run for the results and to empty waste.

The measuring range of the viral load assay is the equivalent of about 200–500 000 cp/mL in standard mode and 50–200 000 cp/mL in high-sensitivity mode. Sample volume is 500 µl of plasma and the system will offer two kits, one for 20 and one for 48 patient samples.

The ZIVA™ system will combine the strengths of RT technology with the advantages of an automated walk-away platform. The system will be easy to integrate with a laboratory information management system (LIMS and will include UPS and battery backup to ensure reliable testing results even when power is lost during a run. This will provide reliable and robust viral load monitoring for all HIV types and subtypes.

The company's planned launch date for the viral load assay on the ZIVA™ system is set for mid-2015. Additional HIV tests such as EID, CD4 and drug resistance testing are planned to be added to the ZIVA™ system as well as other types of virus and bacteria diagnostics.

Genedrive™ (Epistem Ltd)

Epistem Ltd (hereafter Epistem), a biotechnology company headquartered in the United Kingdom, has developed a new molecular diagnostic platform called the Genedrive™ (Figure 66), which uses end-point PCR-based detection. The Genedrive™ is a highly portable, POC platform weighing about 550 g (1.2 lbs) and is approximately the size of an iPad mini. The platform accommodates both electric mains (110–240 V AC) and battery (12V DC) power.

Figure 66. Genedrive™ platform

The first major test developed for the Genedrive® platform is for MTB and mutations associated with resistance to the front line antibiotic rifampicin. Test results are available in less than 60 minutes. The Genedrive® platform is integrated with a simple extraction process based on an advanced composite paper technology that allows extraction and decontamination in a single step and is suitable for use in low-resource settings. The sample is manually transferred with one pipetting step into the Genedrive® reaction cartridge. Epistem expects to launch the MTB assay in India in 2014.

Epistem is developing diagnostic tests for a number of additional infectious diseases, including the RNA viruses HCV and HIV from plasma and whole blood using the same integrated process. Epistem is involved in collaborations with the Pasteur Institute (for HCV) and the United States Department of Defense (Biosurveillance).

EID

As discussed earlier in this report, because of the persistence of maternal antibodies in infants aged under 18 months, the use of antibody tests, such as 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 4–6 weeks of age or at the earliest opportunity thereafter[39] *(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.

39 It has been suggested by programmes and policy-makers that virological testing at birth as an additional test to virological testing at 4–6 weeks of age in the diagnostic algorithm may improve testing uptake and ART initiation and may accelerate the testing cascade. However, WHO has pointed out programmatic barriers to birth testing in resource-limited settings and the relatively low sensitivity for detection of HIV at birth. Nonetheless, WHO encourages countries to consider pilot assessments and consideration of whether testing infants at birth could be implemented in the future *(9)*.

There are currently two HIV-1 DNA assays available in resource-limited settings and that are used for EID: the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test and the Abbott RealTi*m*e Qualitative HIV-1 Test, both of which have 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. 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 these assays are set forth in Appendix 3.

In addition, after the launch of its ZIVA™ platform, Cavidi is planning to add to its ExaVir™ Load platform an assay for use in EID. Because studies have shown the benefits of using RT enzyme activity measurement for EID *(78,79,80)*, this would bring the advantages of RT technology to EID, such as 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, such as 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, DNA PCR testing has had considerable uptake in resource-limited settings. One reason for this is that the cost of the assays is lower than that of quantitative assays; another reason is that the use of DBS with these tests is well established and the performance of the tests is well accepted with DBS samples. The ability to use these tests 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.

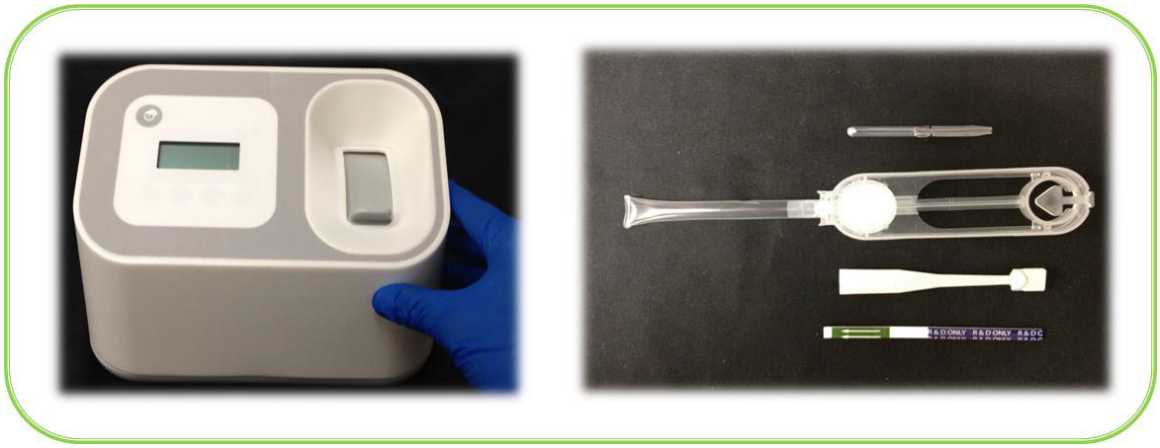
New technologies for EID in the pipeline

Because RNA PCR testing can be used for the detection of HIV in infants aged under 18 months, the new technologies discussed in the previous section on viral load testing, including POC tests from Liat™, should be considered viable options for EID. In addition, the SAMBA system, the Alere q, the GeneXpert®, and the ZIVA™ platform discussed in some detail earlier in this report, will have qualitative assays specifically for EID. Two other potential platforms for EID are discussed below.

LYNX HIV p24 Antigen Assay (NWGHF)

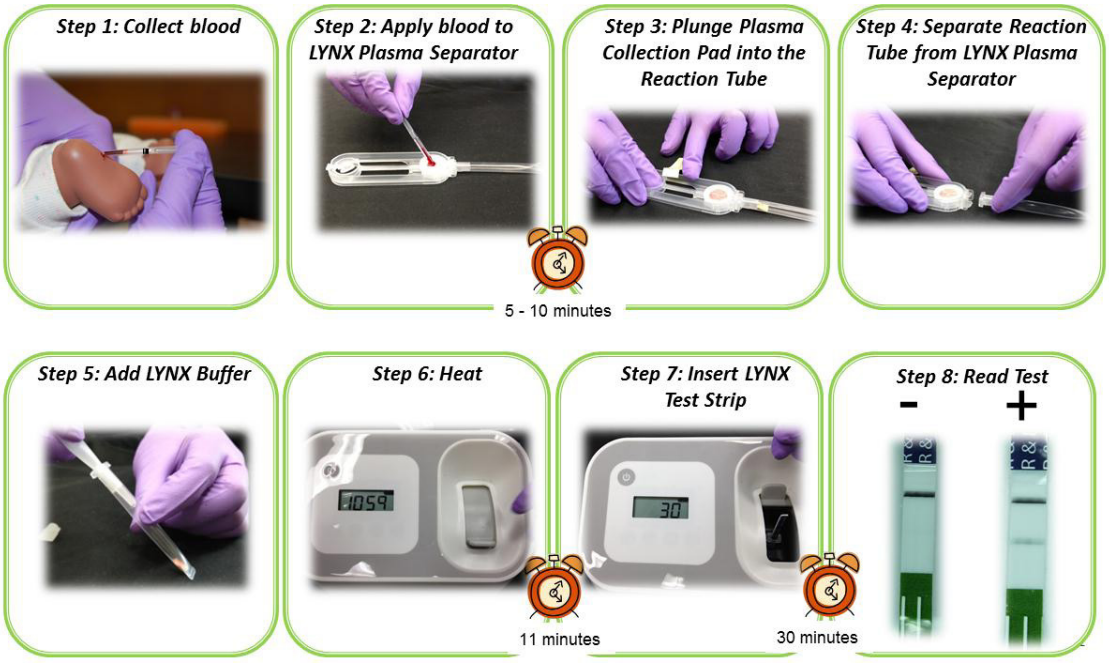
NWGHF is developing an ultrasensitive p24 antigen rapid lateral flow assay for use at the point of patient care. The technology (Figure 67), LYNX, involves a lateral flow strip that detects HIV p24 antigen, and pre-analytical devices for separating plasma from heelstick blood and disrupting immune complexes that would interfere with immunoassays. NWGHF has demonstrated proof of principle of the test.

Figure 67. LYNX HIV p24 AntigenAssay and processor



The assay procedure involves collecting about 80 µL of heelstick 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 30–40 minutes to read the result. The total assay duration is about 45–50 minutes. The procedure is illustrated in Figure 68.

Figure 68. LYNX HIV p24 Antigen Assay procedure



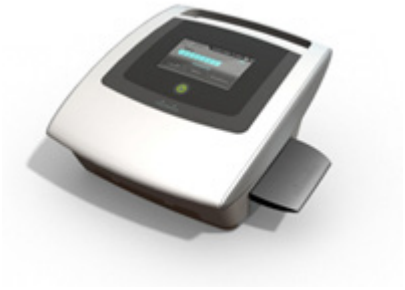
Note that, similar to other rapid tests, if only the top line appears (the control line only), then the test is negative and the infant has not been infected with HIV. If both lines appear (the control line and the test line), then the test is positive and the infant has been infected with HIV. If the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

In initial laboratory testing, the assay has shown about 95% sensitivity and 99% specificity. The price of the processor device is expected to be between US$ 700 and US$ 2000 depending on volume, and the per test cost of the assay is expected to range from US$ 7 to US$ 15, and also will be dependent on volume. Clinical and field trials on the assay commenced in 2013, with availability expected in 2014.

PanNAT® Platform (Micronics Inc)

Micronics Inc (hereafter Micronics), a subsidiary of Sony Corporation of America, has developed the PanNAT® system (Figure 69), which is a small, portable microfluidic platform for use near patient for 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 30–40 minutes, depending upon assay parameters. A battery-operated/WiFi-enabled option is planned.

Figure 69. PanNAT® Platform



The cartridge incorporates 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 2015. Micronics has 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

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 Brazil and South Africa, where viral load testing is routinely conducted on a large scale, with more than 1 million viral load tests done annually in Brazil and with more than 2 million viral load tests done annually in 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 increasingly are using viral load, but still on a small scale. As indicated earlier, the reasons for this include cost, infrastructure requirements and the need for trained laboratory technicians. It is expected, however, that since the WHO 2013 Guidelines recommend routine viral load testing as the preferred monitoring approach to diagnose and confirm ART failure, more countries will begin to scale up viral load testing.

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 six 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, Abbott 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 likely 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 few 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, are essential to reach 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 likely will necessitate centralized testing facilities and, at the same time, a drive towards both the use of DBS and 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 2014 and beyond. It is too early to predict the exact pricing of the POC devices and tests, but it is hoped that the price per test will be at or below US$ 15 per test. Over time, 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 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 POC, 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 and improved automated platforms for high-volume testing also are becoming available, allowing cost-effective consolidation of testing in high-volume centres. 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 of barriers to, and fostering acceleration of, new technology introduction, especially for POC technologies;
- improvement of systems for sample referral and results distribution for central laboratories.

Strategic funding on the part of UNITAID and other funders could make a difference in a number of these areas, including in accelerating the introduction of new POC diagnostic technologies. UNITAID has committed to funding several projects to facilitate and support the commercialization of high-quality POC diagnostics, including projects to facilitate and accelerate their evaluation, regulatory approval, adoption and procurement in order to promote the widespread uptake of new diagnostic technologies.

"The author notes no conflicts of interest."

Appendix 1: Operational characteristics of diagnostic platforms

I. CD4 systems operating characteristics	
BD FACSCalibur™ System	
Type of technology	Large, bench-top, bead-based flow cytometer
Output	Absolute and percentage CD4 counts, immunophenotyping (including combined analysis of T-cells, B-cells and NK-cells), residual WBC enumeration, DNA analysis, leukaemia and lymphoma immunophenotyping (4-colour)
Turnaround time	60 minutes for 40 tests run on a rack, including incubation time
Capacity	Approximately 200–250 samples per day
Throughput per technician/ per day	40 per hour, after approximately 30-minute incubation time
Sample needed and stability	At least 100 µL whole blood collected in either 2 mL or 4 mL K2 EDTA anticoagulant tubes; staining to take place within 72 hours of blood draw; analysis to take place within 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- and assay-based; ranges from approximately US$ 3–7 per test
Cost/instrument	Approximately US$ 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 lbs)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately US$ 1500–2500
Electric power requirements	100–240 V AC mains 50–60 Hz
Environmental requirements	Temperature: 16–29 °C (60–85 °F) Humidity: 10–90% relative non-condensing Maximum altitude: Not available
Data station	Separate FACSCalibur workstation (BD FACStation™); computer and colour printer separate from instrument
Monitor	In workstation
Printer	In workstation
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
EQA	Compatible with CD4 EQA programmes
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 1 mL (100 µL used) whole blood collected in EDTA anticoagulant; white blood count also should be performed to determine whether cell counts are outside the normal range, which could 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 20–25 °C for 30 minutes; (iv) sample is lysed; (v) the test is run on the instrument Alternatively, a TQPrep for automated sample preparation can be used
Reagent stability	Reagents must be stored at 2–8 °C (36–46 °F); reagents are shipped with an expiration date of 1 year
Cost/test	Volume based; ranges from approximately US$ 2.50–8 per test
Cost/instrument	Approximately US$ 90 000; approximately US$ 200 000 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 lbs) (cytometer with MPL; computer/monitor and power supply are separate)
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately US$ 1500–2500
Electric power requirements	115–220 V AC mains 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: Not available Maximum altitude: Not available
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 to repair
Internal QC	Controls (normal and low immunotrol) are provided by Coulter
EQA	Compatible with CD4 EQA programmes
Infrastructure requirements	Technology can be used at central/national reference laboratories

Partec CyFlow® Counter	
Type of technology	Desktop, volumetric flow cytometer
Output	Absolute and percentage CD4 counts, total lymphocytes and WBC, CD3 and CD8 optional
Turnaround time	After 15-minute 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 anticoagulated blood can be stored at room temperature (18–25 °C) for up to 48 hours; alternatively anticoagulated 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 °C) for up to 24 hours or alternatively refrigerated at 2–8 C° for at least 72 hours
Sample preparation and protocol complexity	Process for dry reagents only: (i) add 20 µL blood to Partec CD4 tube containing dry mAB reagent; (ii) incubate 15 minutes at room temperature in the dark; (iii) pour prefilled buffer to tube; (iv) run sample in CyFlow Counter For liquid reagents: (i) add 20 µL 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 800 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 can be stored at room temperature and have a maximum shelf life of 6 months Liquid reagents must be stored at 2–8 °C (36–46 °F) in the dark for up to 12 months maximum
Cost/test	€1.75 (~US$ 2.30 per test for absolute CD4 and €2.50 (~US$ 3.30) for CD4 absolute and percentage, high-volume discounts available
Cost/instrument	Approximately US$ 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 ~US$ 500
Electric power requirements	100–240 V AC mains 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: 3000 metres (9843 feet)
Data storage and data transfer	Dedicated Intel® CPU integrated into instrument; data storage of approximately 20 000 datasets; USB port
Monitor	8.4" TFT colour touchscreen 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)
EQA	Compatible with CD4 EQA programmes
Infrastructure requirements	Technology can be used at all levels of the health system, including central, regional, district and mobile laboratories 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 (sample volume per test is 50 µL); staining to take place within 48 hours of blood draw (24 hours for FACSCount CD4 reagents); analysis to take place within 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; (iv) sample is vortexed and run on the instrument
Reagent stability	Reagents are shipped to customers with an expiration date of 6 months or longer; reagents must be stored at 2–8 °C (36–46 °F)
Cost/test	Volume based; ranges from approximately US$ 3.50–10 per test
Cost/instrument	Approximately US$ 30 000
Regulatory status	FACSCount system is US-IVD cleared; FACSCount CD4 Reagents and FACSCount Reagent kits are US-IVD cleared and CE-IVD marked; single-tube CD4/CD3 reagents are neither FDA approved nor CE-IVD 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 lbs), fluid reservoirs empty
3rd party supplies	Refrigerator, vortex and pipettor; cost: approximately US$ 1500–2500
Electric power requirements	100–240 V AC mains 50–60 Hz 160 W (maximum rated power)
Environmental requirements	Temperature: 10–40 °C (50–104 °F) Humidity: Not available Maximum altitude: Not available
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, touchscreen 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
EQA	Compatible with CD4 EQA programmes
Infrastructure requirements	Technology can be used at central, regional, district laboratories and some well-developed primary sites with dedicated laboratory facilities and technicians

BD FACSClearCount™ System (preliminary specifications – FACSClearCount™ is under development – not for sale or use)	
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 (sample volume per test is 50 µL);staining to take place within 24 hours of blood draw; analysis to take place within 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; (iv) sample is vortexed and run on the instrument
Reagent stability	Reagents are shipped to customers with an expiration date of 6 months or longer; reagents must be stored at 4–35 °C (39–95 °F) in sealed pouches
Cost/test	Volume based; to be determined
Cost/instrument	To be determined
Regulatory status	FACSClearCount Systems and BD CD4 Assay Kit, will be FDA approved and CE marked
Physical dimensions (W x H x D)	Width: 59.44 cm Height: 57.24 cm Depth: 60.78 cm
Weight	43.1 kg (95 lbs), fluid reservoirs empty
3rd party supplies	For manual sample preparation only: vortex and pipettor; cost: approximately US$ 1000–1500
Electric power requirements	100–240 V AC mains 50–60 Hz 240 W (maximum rated power)
Environmental requirements	Temperature: 10–40 °C (50104 °F) Humidity: Not available Maximum altitude: Not available
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, touchscreen 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
EQA	Compatible with CD4 EQA programmes
Infrastructure requirements	Technology can be used at central, regional, district laboratories 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 50 µL 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	US$ 2.50 per test for absolute CD4 count; US$ 3.50 per test for %CD4
Cost/instrument	Approximately US$ 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 US$ 400
Electric power requirements	100–240 V AC mains (UPS with battery backup included) 50–60 Hz 550 W
Environmental requirements	Temperature: 5–35 °C (41–95 °F) Humidity: < 90% Maximum altitude: Not available
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
EQA	Compatible with CD4 EQA programmes (manual analysis only)
Infrastructure requirements	Technology can be used at central, regional, district laboratories with dedicated laboratory facilities and technicians

PointCare NOW™	
Type of technology	Desktop, flow cytometer
Output	Absolute and percentage CD4 counts, WBC, haemoglobin 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 bar code 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 US$ 10 per test, including Daily Check™ controls
Cost/instrument	Approximately US$ 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
Electric power requirements	UPS 110 V or 220 V, 60 W; portable battery power system available; solar charge system available
Environmental requirements	Temperature: 18–34 °C (64–93 °F) Humidity: <80% Maximum altitude: Not available
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, Portuguese; Indonesian under development
Monitor	LED colour touchscreen 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; thus should 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 2–42 °C (36–108 °F) for 6 months from date of manufacture
EQA	Yes; uses QC materials from Streck Laboratories
Infrastructure requirements	Technology can be used at central, regional, district and some well-developed primary sites with dedicated laboratory facilities and technicians

Alere Pima™ CD4 Test	
Type of technology	Portable bench-top, fixed volume cytometer
Output	Absolute CD4 counts only
Turnaround time	18–20 minutes
Capacity	Maximum of ~20 samples per day
Throughput per technician/ per day	~20 samples per technician per day; no batching capabilities; walk-away operation
Sample needed and stability	25 µL of capillary (fingerstick) blood wicked directly into the sample collector contained in the Pima cartridge or 25 µL of venous blood collected in EDTA anticoagulant 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 2–30 °C
Cost/test	US$ 6–12 per test
Cost/instrument	US$ 6500–12 000
Regulatory status	CE-IVD marked; WHO prequalified
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)
Electric power requirements	100–240 V (AC) at 47–63 Hz mains power Analyser contains onboard 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 2000 metres (~6500 feet); actual maximum operating altitude not evaluated
Data archive, export and connectivity	1000 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 USB powered GPRS modem for data export over mobile telephone networks Data point connectivity solution for instrument management, QC and cartridge consumption provided
Monitor	LED monocolour screen integrated into instrument
Printer	Separate printer (prints on thermal paper); powered by the instrument (with rechargeable batteries onboard) L 95 mm x W 93 mm x H 66 mm; weight: ~350 g, 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	Maintenance free instrument Care package for instrument is available Low cost and portability allows for direct swap-out replacement rather than onsite repair

Internal QC	Extensive internal controls: sample volume control; reagent control; automatic control of cartridge expiry date; internal process controls; automatic test identification
EQA	Known to be compatible with Pima: QASI and UK-NEQAS
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities
User interface	16 button keypad

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	14 minutes
Capacity	30–35 samples per day per instrument
Throughput per technician/ per day	One technician can operate three instruments without difficulty, or 90–100 samples per technician per day; no batching capabilities; walk-away operation
Sample needed and stability	16 µL of capillary (fingerstick) or venous blood transferred to Daktari™ cartridge
Sample preparation and protocol complexity	No manual sample preparation required Protocol: (i) lancet finger; (ii) transfer blood drop to cartridge; (iii) insert into CD4 counter; (iv) press "start"; (v) read result from LCD screen or printout Venipuncture blood also can be used via capillary tube transfer
Reagent stability	Dried reagents require no refrigeration Stable to 45 °C in preliminary studies Currently shipping with stability to 35 °C Final real-time stability studies are ongoing
Cost/test	US$ 9 per test (estimated), but might be lower with volume discounts
Cost/instrument	<US$ 8000 (estimated)
Regulatory status	ISO 13485 certification CE mark is expected in May 2014; WHO preqalification process has been started
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	Alcohol swabs, gauze, adhesive bandage (lancets and capillary transfer tubes are provided)
Electric power requirements	Regular AC mains long-life rechargeable battery self-contained in device that can operate for up to 3 days on a single battery charge Solar recharging option
Environmental requirements	Operating temperature: 4–40 °C Humidity: up to 90% relative humidity Maximum altitude: up to 3280 metres (10 000 feet)
Data station	Daktari™ CD4 system includes a data management system, with a keypad user interface, wireless data transmission and a back data package that can stand alone or be integrated with customer databases
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™ CD4 System includes an optional USB printer accessory for printed results
Bar code scanner	Daktari™ CD4 System includes an optional USB bar code scanner
Training	Minimal training required Lay person can be trained in less than 90 minutes Primary skill required is for correct lancet blood draw
Maintenance	No daily calibration required; CD4 assays can be run as soon as the instrument is powered on The device does not use lasers, but rather employs an electronic measurement system similar to a glucose meter and might be less prone to damage If damaged, the company plans to swap out the device rather than repair it onsite

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 also will perform QC of capillary blood draw and inform user if fingerstick is inadequate prior to running assay
EQA	To be determined whether compatible with CD4 EQA programmes; cartridge cannot be retested to confirm results
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities

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-minute 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 anticoagulated blood can be stored at room temperature (18–25 °C) for up to 48 hours; alternatively anticoagulated 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 °C) 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 20 µL blood to Partec CD4 tube containing dry mAb reagents; (ii) incubate 15 minutes at room temperature in the dark; (iii) pour the two prefilled buffer tubes to specimen; (iv) after gently shaking the tube, refill volume from sample tube into syringe; (v) attach syringe to CyFlow® miniPOC
Reagent stability	Dry reagents can be stored at room temperature and have a maximum shelf life of 6 months
Cost/test	€3 (~US$ 3.96) per test for absolute CD4 and CD4 percentage combined, high-volume discounts available
Cost/instrument	~€8390 (~US$ 11 748) Partec offers a point-of-care package (including, instrument, reagents for 5000 tests, 36-month instrument warranty) with an effective instrument price of ~US$ 4000
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
Electric power requirements	100–240 V AC mains or 12 V DC power (car battery) 50–60 Hz
Environmental requirements	Temperature: 10–40 °C (50–104 °F) Humidity: <95% non-condensing Maximum altitude: 3000 metres (9843 feet)
Data storage and data transfer	Dedicated Intel® Atom™ CPU integrated into instrument; Windows™-based analysis software; data storage of approximately 20 000 datasets; USB port
Monitor	5.7″ colour touchscreen 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)
EQA	Compatible with CD4 EQA programmes
Infrastructure requirements	Technology can be used at all levels of the health-care system, including central, regional, district and mobiles laboratories and some well-developed primary sites with dedicated laboratory space and trained health workers

BD FACSPresto™	
Type of technology	Small, bench-top, fixed volume cytometer
Output	Absolute CD4, %CD4 and Hb
Turnaround time	3–4 minutes reading; plus incubation of cartridge (18 minutes)
Capacity	Maximum of ~60–80 samples per day
Throughput per technician/ per day	~50–60 samples per technician per day; flexible throughput; walk-away operation
Sample needed and stability	~20 µL of capillary (fingerstick) blood wicked directly into BD cartridge or ~20 µL of venous blood collected in EDTA anticoagulant tube Cartridge must be inserted and tested within 2 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) enter patient ID; (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	To be determined
Cost/instrument	To be determined
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 (~11 lbs) (instrument only)
3rd party supplies	For venous samples: transfer pipette For capillary samples: sterile lancets, alcohol swabs, cotton gauze, Band-Aid
Electric power requirements	100 to 240 V (AC) at 45–65 Hz mains power Analyser contains onboard rechargeable battery; can be charged with cigarette lighter
Environmental requirements	Operating temperature: 10–40 °C (50–104 °F) (ongoing validation) Humidity: 5–95% (ongoing validation) Maximum altitude: 2500 metres (8200 feet) (ongoing validation)
Data station	Dedicate CPU integrated into instrument; approximately 1000 test results can be stored on the instrument archive; results can be downloaded via USB USB port also can be used to support an external Bluetooth 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 multicolour screen integrated into instrument
Printer	Onboard 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 onsite repair
Internal QC	Yes; instrument will check itself each day and each cartridge will have onboard QC
EQA	Will be compatible with CD4 EQA programmes (ongoing validation)
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities
User interface	Touchscreen keyboard on the device

MyT4™ CD4 Test	
Type of technology	Disposable cartridge that is used with a portable, mixing/spinning Accessory and self-metering blood collection tube Measurement is by selectively binding heavy particles with CD4+ T-cells, then isolating and stacking the conjugated cells in a microcapillary to deliver a fully quantitative result
Output	Absolute CD4 count, quantitative
Turnaround time	~10 minutes
Capacity	~40 samples per day
Throughput per technician/ per day	~40 samples per technician per day
Sample needed and stability	100 µL of venous or capillary whole blood
Sample preparation and protocol complexity	Protocol: (i) use self-metering blood collection tube (BCT) to collect venous or capillary sample and inject sample into MyT4 CD4 Test; (ii) put Test into MyT4 Accessory station-A and press button to initiate self-timed 4-minute mix; (iii) put test into station-B and rotate the twist-handle; (iv) press button to initiate self-timed 4-minute spin; (v) use reader-lens to read quantitative results
Reagent stability	2–30 °C for 12 months, 2–40 °C for up to 2 weeks, 2–50 °C for up to 48 hours
Cost/test	US$8
Cost/instrument	US$500
Regulatory status	CE-IVD marked
Physical dimensions of Test (W x H x D)	Width 0.5″ Height 2.5″ Depth 0.5″
Weight	MyT4 CD4 Test is 11.3 grams, MyT4 CD4 Accessory is 6.5kg (2014 units are a few kgs heavier)
3rd party supplies	For venous blood transfer: small plastic tube and transfer pipette For capillary blood collection: kit available with sterile lancets, alcohol swabs, gauze pads, adhesive bandages
Electric power requirements	110–220 V AC mains current or DC power with rechargeable battery for MyT4 CD4 Accessory Battery holds 50 tests per charge
Environmental requirements	Temperature: store MyT4 CD4 Test and blood collection tubes at 2–30 °C; use Test at room temperature Store MyT4 CD4 Accessory at 2–50 °C; operate Accessory at 10–40 °C Humidity: 10–95% non-condensing
Data station	Not available
Monitor	Not available
Printer	Not available
Bar code scanner	Not available
Training	Minimal training required for any level of health-care personnel Novice user can be trained in less than 2 hours Primary skill required is correct capillary blood collection No maintenance training required
Maintenance	MyT4 CD4 Accessory is service-free and does not require any maintenance or calibration Accessories will be replaced if broken, with no swap of broken units required MyT4 CD4 Test is disposable
Internal QC	Internal QCs are built into both the MyT4 Test and the MyT4 Accessory Three IQC indicators in the MyT4 Test show whether the test has run to completion or whether an error has occurred Internal QC indicators in the MyT4 Accessory check the calibration of the key parameters, time and speed for every test run Indicator lights inform the operator of any errors
EQA	Evaluating compatibility with existing EQA programme
Infrastructure requirements	Can be used at all levels of health facility, including hospitals, health centres, mobile facilities, and in the field

MBio CD4 system	
Type of technology	First release product will deliver absolute CD4 count Future releases will provide a panel of immunoassay results (e.g. HIV; syphilis)
Output	Absolute CD4 count (quantitative) Future releases will provide haemoglobin and %CD4
Turnaround time	~23 minutes, including a room temperature incubation period of 20 minutes that is controlled by the CD4 Rack, followed by a 3-minute analysis by the CD4 Reader Read window is 100 minutes after the incubation period
Capacity	~10–15 tests per hour
Throughput per technician/ per day	~80–100 tests per technician per 8-hour day
Sample needed and stability	15 µL of capillary (by fingerstick) or venous whole blood
Sample preparation and protocol complexity	Complexity comparable to that of CLIA-waived diagnostic instruments in the United States No buffers or liquid reagents are necessary Only one incubation period is required
Reagent stability	The lyophilized reagents on the cartridge do not require cold-chain transportation or storage CD4 cartridges can be stored in package at 2–40 °C for 12 months at 70% relative humidity
Cost/test	US$ 6 per test (estimated); volume discounts
Cost/instrument	Less than US$ 5000 per system; reagent rental or leasing plans are available
Regulatory status	Refer to company website for latest information
Physical dimensions (cytometer only) (L x W x H)	Length: 25 cm (~10″) Width: 15 cm (~6″) Height: 17 cm (~7″)
Weight	2.5 kg (5.5 lbs)
3rd party supplies	Sterile lancets (for capillary blood samples), alcohol swabs, dry swabs, gauze, Band-Aid
Electric power requirements	Rechargeable battery operation (8 hours) or plug-in to electrical supply (100–220 V AC mains)
Environmental requirements	Operating Temperature: 15–35 °C Humidity: 5–95%, non-condensing Maximum altitude: 4000 metres (~6900 feet)
Data station and reports	Onboard 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 bar code readers
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 bar code reader captures cartridge information Capable of supporting an external bar code reader
Training	Formal training can be achieved in one day Basic competency can be acquired in 10 minutes
Maintenance	No routine maintenance or service; system replacement via depot/distributor swap-out
Internal QC	Internal QC on every cartridge for multiple parameters, including sample volume, reagent quality, lot expiration, etc.
EQA	Compatible with pre-identified, third-party external QC materials
Infrastructure requirements	None provided that environmental requirements are met

EMD Millipore® Muse™ Auto CD4/%CD4 System	
Type of technology	Small, bench-top flow cytometer
Output	Absolute and percentage CD4 counts
Turnaround time	2–4 minutes, after 2 15-minute incubations
Capacity	Approximately 16 samples per day
Throughput per technician/ per day	16 samples per technician per 8-hour day
Sample needed and stability	10 µL whole blood collected in EDTA anticoagulant
Sample preparation and protocol complexity	Process: (i) add reagents to tube; (ii) add 10 µL of blood from patient; (iii) incubate 15 minutes; (iv) add lyse solution; (v) incubate sample 15 minutes in darkness; (iv) sample is run on the instrument
Reagent stability	Reagents must be stored at 2–8 °C (36–46 °F); reagents are shipped with 12 months of shelf life
Cost/test	~€2 (~US$2.72) per test for CD4/%CD4, regardless of volume
Cost/instrument	Approximately €10 000 (~US$13,605)
Regulatory status	CE-IVD marking being sought
Physical dimensions (cytometer only) (W x H x D)	Width: 20.62 cm (8.12 in) Height: 22.07 cm (8.69 in) Depth: 28.22 cm (11.11 in)
Weight	5.94 kg (13.1 lbs)
3rd party supplies	Refrigerator, vortex and pipettor
Electric power requirements	100–240 V AC mains 50–60 Hz 80 W Or optional battery pack
Environmental requirements	Temperature: 15–35 °C (59–95 °F) Humidity: 10%–90% Maximum altitude: Not available
Data station	Dedicated CPU integrated into instrument; results can be downloaded via USB
Monitor	Colour touchscreen integrated into instrument
Printer	Not included
Bar code scanner	No
Training	Less than one day of training is required
Maintenance	Routine preventative maintenance required If damaged, device will be sent to a regional repair laboratory Repair and return of device should be effected within one week
Internal QC	Yes; extensive internal controls, including reagent control, automatic control of cartridge expiry date; internal process controls
EQA	To be determined
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities

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	~1 test per hour per technician without batching
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	US$ 5 per test (estimated)
Cost/smartphone and reader	Cost of Android smartphone; application is available as a free download US$ 3000 for reader (eventual price estimated to be US$ 2000) Reader will be provided free of charge dependent on committed volumes Note that tests also can be read by eye
Regulatory status	To be determined
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
Electric power requirements	None for cartridge; reader 12V DC via adapter (110–240 V), optional battery pack
Environmental requirements	Operating temperature: To be determined Humidity: To be determined Maximum altitude: To be determined
Data station and connectivity	None (reader stores most recent 1000 tests; downloadable via USB/Ethernet; smartphone storage limited to available memory on device) Smartphone application will include data handling and interface LIMS or cloud database
Monitor	None (reader 2.4″ colour touchscreen)
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 skills required are for correct lancet blood draw and for visual test reading (automated with reader) Reader provides onboard 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)
EQA	To be determined
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities

II. 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® COBAS® TaqMan Analyser
Turnaround time	3 racks of 24 specimens in approximately 5 hours; with 216 seconds processing time per specimen
Capacity (per run)	72 samples per run (maximum) that 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	1000 µL of plasma or 70 µL DBS for Taqman® analysers Plasma can 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	Not available
Cost/instrument	Approximately US$ 80 000–100 000
Regulatory status of assays	FDA approved; WHO prequalified; CE-IVD marked (DBS is RUO)
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
Electric power requirements	100–125 V AC mains and 200–240 V AC mains (+10, -15%) 50–60 Hz
Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: <80% (for temperatures up to 32 °C) Maximum altitude: 2000 meters (6500 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 COBAS® AmpliPrep: onboard bar code scanner for reagent racks, reagent cassettes and specimen clips AMPLILINK data station: handheld bar code scanner for original specimen/specimen clip
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	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 and 5 minutes
Capacity (per run)	2 independent segments of 24 samples each up to 2 different tests onboard 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 setup 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 can be stored in the output tubes at 2–8 °C for up to 1 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 2–8 °C (36–46 °F); all reagents are stable until expiration date
Cost/test	TaqMan® HIV-1 Test v2.0: US$ 11–25 in resource-limited settings; range is dependent on instrument purchase, reagent rental and volume-based tiered pricing
Cost/instrument	US$ 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)	Width 50cm (19.7″) Depth 79cm (31.1″) Height 58cm (22.8″)
Weight	55 kg (121 lbs)
3rd party supplies	Microtiter plate centrifuge (not supplied by Roche) and other general supplies
Electric power requirements	120 or 240 V AC mains 50–60 Hz
Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: <80% (for temperatures up to 32 °C) Maximum altitude: 2000 metres (6500 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	AMPLILINK handheld bar code scanner for original specimen/specimen clip
Training	Fully trained laboratory technician 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
EQA	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 and 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 setup 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 can be stored in the output tubes at 2–8 °C for up to 1 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: US$ 20–30 per test (least developed countries); US$ 35–90 per test elsewhere
Cost/instrument	US$ 100 000–110 000, including docking 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)
Electric power requirements	Analyser: 100–125 and 200–240 V AC mains (+10%; -15%); 50 or 60 Hz (± 2 Hz) Data station: 100–125 and 200–240 V AC mains (+10%; -15%); 47–63 Hz (± 2 Hz)
Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: <80% (for temperatures up to 32 °C) Maximum altitude: 2000 metres (6500 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 laboratory technician 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
EQA	Amenable to EQA
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Abbott *m24*sp Automated extraction instrument	
Type of technology	Automated extraction and sample preparation (magnetic particle technology)
Output	HIV-1 RNA VL: quantification HIV-1 RNA levels
Turnaround time (full run)	HIV-1 RNA VL = 400 minutes/6 hours and 40 minutes (total turnaround time including *m*2000rt); extraction time (including loading of instrument) = 210 minutes/3 hours and 30 minutes
Capacity (per run)	1 minimum–24 maximum
Throughput per technician/ per day	HIV-1 VL: within 8-hour shift: 2 full runs = 48 samples
Sample needed and stability	HIV-1 VL: freshly drawn whole blood can 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 can 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, can be stored at -70 °C
Sample preparation and protocol complexity	Moderately complex Steps include vortexing (internal control, calibrators, controls and specimens) pipetting, centrifuge, etc.
Reagent stability	HIV-1 VL: reagents (liquid), as well as controls and calibrators, must be stored at 0 °C or colder when not in use and must be shipped on dry ice All reagents can be reused Extraction reagents are ready to use and can be stored at 15–30 °C All reagents are stable until expiration date
Cost/test	Not available
Cost/instrument	US$ 80 000
Regulatory Status	Abbott RealTi*m*e HIV-1 viral load with *m24sp* is WHO prequalified, 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; freezer
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 2000 metres (6600 feet)
Peripherals/supporting instrumentation	Data station, monitor and printer are supplied with the instrument
Bar code scanner	Handheld bar code scanner is supplied with the instrument
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are available and required for each preparation run Internal control: a defined, consistent quantity of internal control is introduced into each specimen and control at the beginning of sample preparation and detected on the Abbott *m*2000*rt* instrument to demonstrate proper specimen processing and assay validity Internal control is comprised of an RNA sequence unrelated to the HIV-1 target sequence
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 (magnetic particle technology)
Output	Quantification HIV-1 RNA levels (HIV-1 viral load); HIV-1 qualitative: TNA extraction
Turnaround time	HIV-1 VL: extraction inclusive PCR plate preparation: depends on number of samples from 2 hours and 30 minutes for 24 samples to 4 hours and 45 minutes for 96 samples Amplification and detection: 3 hours per run (up to 96 samples) HIV-1 Qualitative: Extractions: depends on number of samples from 2 hours and 39 minutes for 24 samples to 4 hours and 54 minutes for 96 samples Amplification and detection: 3 hours per run (up to 96 samples)
Capacity (per run)	HIV-1 VL: 96 samples (1–93 patient samples + 3 controls) HIV-1 Qualitative: 96 samples (1–94 patient samples + 2 controls)
Throughput per technician/ per day	HIV-1 VL and HIV-1 Qualitative 192 samples (2 batches of 96 samples)
Sample needed and stability	HIV-1 VL: freshly drawn whole blood can 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 can 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, can be stored at -70 °C HIV-1 Qualitative: plasma: same conditions as for HIV-1 VL DBS can be prepared on a Whatman 903 card (or equivalent) using blood obtained from a heelstick or fingerstick or collected in a blood collection tube Freshly drawn specimens (whole blood) can be held at 15–30 °C for up to 6 hours or at 2–8 °C for up to 24 hours
Sample preparation and protocol complexity	Moderately complex Steps include vortexing (internal control, calibrators if applicable, controls and specimens), pipetting, centrifuge, etc. Once all required consumables, reagents and samples are placed in the *m*2000*sp*, each process is walk away (extraction and mastermix addition)
Reagent stability	HIV-1 VL: reagents (liquid), as well as controls and calibrators, must be stored at 0 °C or colder when not in use and must be shipped on dry ice *m*Plus (Amplification Reagent Extended Use) allows reuse of amplification reagent HIV-1 Qualitative: amplification reagents, controls must be stored at -10 °C or colder when not in use Reagents are shipped on dry ice Extraction reagents are ready to use and can be stored at 15–30 °C All reagents are stable until expiration date
Cost/test	Not available
Cost/instrument	US$ 162 000
Regulatory status	Abbott RealTi*m*e HIV-1 viral load (*m*2000system) is WHO prequalified, CE-IVD marked and FDA approved Abbott RealTi*m*e HIV-1 qualitative is WHO prequalified, CE-IVD marked
Physical dimensions (W x D x H)	Width: 145 cm (57.1 in.) Depth: 78 cm (30.7 in.) Height: 174.5 cm (68.7 in)
Weight	211 kg (465 lbs)
3rd party supplies	Pipettes, vortex mixer and refrigerator, freezer
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 2000 metres (6600 feet)
Peripherals/supporting instrumentation	Data station, monitor and printer are supplied with the instrument
Bar code scanner	Supplied with instrument (integrated on work desk)

Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are available and required for each preparation run, 2 (HIV-1 Qualitative) or 3 (HIV-1 VL) controls per run up to 96 batch size Internal control: a defined, consistent quantity of internal control is introduced into each specimen and control at the beginning of sample preparation and detected on the Abbott *m*2000*rt* instrument to demonstrate proper specimen processing and assay validity; the internal control is comprised of an RNA sequence unrelated to the HIV-1 target sequence
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	Quantification HIV-1 RNA levels (HIV-1 viral load); HIV-1 Qualitative use TNA extraction
Turnaround time	Amplification and detection cycle takes 3 hours
Capacity (per run)	HIV-1 VL: up to 96 (1–93 patient samples, + 3 controls); HIV -1 Qualitative: up to 96 (1–94 patient samples, + 2 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 (nucleic acid from *m*2000*sp*, *m*24*sp* or manual sample preparation/extraction protocol including mastermix addition)
Sample preparation and protocol complexity	Manual sample preparation: Moderately complex Steps include vortexing (internal control, calibrators, controls and specimens), pipetting, centrifuging, etc. *m*2000*rt*: easy to use. User friendly software, multiple languages available, very limited hands on time needed. Work list can be imported via network, CD-ROM or created manually Once 96-well plate is loaded in the *m*2000*rt*, process is walk away
Reagent stability	No onboard reagents are required on the instrument All reagent addition is performed during the sample preparation process
Cost/test	HIV-1 VL: ~US$ 25–40 per test, dependent on volumes and subject to negotiations with Abbott Molecular
Cost/instrument	US$ 45 000
Regulatory status	Abbott RealTi*m*e HIV-1 viral load (*m*2000system) is WHO prequalified, CE-IVD marked and FDA approved Abbott RealTi*m*e HIV-1 Qualitative (*m*2000system) is WHO prequalified and CE-IVD marked
Physical dimensions (W x D x H)	Width 34 cm (13.4 in) Depth 48 cm (17.8 in) Height 49 cm (19.3 in)
Weight	75.2 lbs (34.1 kg)
Electric power requirements	100–240 V
Environmental requirements	Temperature: 15–30 °C (59–86 °F) Humidity: 30–80% relative humidity, non-condensing Maximum altitude: not exceeding 3000 metres (9800 feet) 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 laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are available and required for each run, 2 (HIV-1 Qualitative) or 3 (HIV-1 VL) controls per run up to 96 batch size Internal control: a defined, consistent quantity of internal control is introduced into each specimen and control at the beginning of sample preparation and detected on the Abbott *m*2000*rt* instrument to demonstrate proper specimen processing and assay validity The internal control is comprised of an RNA sequence unrelated to the HIV-1 target sequence
EQA	Amenable to EQA
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

Real-time PCR: VERSANT® kPCR Molecular System automated sample preparation and amplification/ detection modules	
Type of technology	Automated real-time extraction, amplification and detection (kinetic PCR [(kPCR technique])
Output	HIV-1 RNA quantification
Turnaround time	Sample preparation system setup <10 minutes; sample extraction <3 hours; amplification detection <3 hours
Capacity (per run)	96 tests per run (89 clinical samples and 4 calibrators and 3 controls) run in less than 6 hours; flexible run sizes of 1–96 tests per batch
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 (50–100 µL); whole blood collected in EDTA tubes can be stored for 6 hours at room temperature or for up to 24 hours at 2–8 °C before centrifugation; plasma can be stored for up to 24 hours at room temperature or for up to 5 days at 2–8 °C
Sample preparation and protocol complexity	Steps: (i) load the dedicated sample preparation reagents into a trough; (ii) place the reagents on the module; (iii) load plasma samples onto the sample carrier; (iv) place the sample carriers on the auto load tray of the VERSANT Sample Prep module – from that point on, sample prep module is fully automated
Reagent stability	Reagents are stored frozen (from -30 °C to -10 °C); calibrators and controls are stored frozen (from -90 °C to -60 °C)
Cost/test	Not available
Cost/instrument	Not available
Regulatory status	VERSANT® HIV-1 RNA 1.0 Assay (kPCR) is WHO prequalified and CE-IVD marked
Physical dimensions: sample preparation module; application/detection module (W x D x H)	Width 112.4 cm (44 in)/ 36.8 cm (14.5 in) Depth 100.6 cm (39.5 in)/53.4 cm (21 in) Height 90.5 cm (35.5 in)/45.7 cm (18 in)
Weight: sample preparation module; application/detection module	320 lbs (145 kg)/55 lbs (25 kg)
Electric power requirements	100–240 V; 50 or 60 Hz
Environmental requirements	Temperature: 18–30 °C Humidity: 30–80% non-condensing Maximum altitude: 0–2000 metres (6560 feet)
Peripherals/supporting instrumentation	Computer supplied 17 in screen and separate keyboard Printer optional
Physical dimensions (W x D x H)	38.1 cm × 14.0 cm × 33.0 cm (15 in × 5.5 in × 13 in)
Weight	12 kgs (26 lbs)
Bar code scanner	Supplied with the instrument
Training	Fully trained laboratory technician required; dedicated training on instrument; electronic training for VERSANT kPCR is widely available using Siemens Personalized Education Program (PEP)
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
EQA	Amenable to EQA
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

QIAsymphony® SP/AS: sample preparation and assay setup instruments	
Type of technology	Automated sample preparation and assay setup
Output	Samples ready for amplification and detection on Rotor-Gene Q
Turnaround time	Approximately 3 hours for extraction and assay setup
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 3 specimens per 8-hour shift, based on testing combinations and laboratory workflow
Sample needed and stability	Up to 1000 µL of plasma Plasma can 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	Not available
Cost/instrument	Not available
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) Integrated: 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
Electric power requirements	100–240 V AC mains, 50–60 Hz
Environmental requirements	Temperature: 15–32 ° C (59–89 °F) Humidity: 15-% (for temperatures up to 31 °C, decreasing linearly to 50% humidity at 32 °C) non-condensing Maximum altitude: 2000 metres (6500 feet)
Data station	Not available
Monitor	Not available
Printer	Not available
Bar code scanner	Supplied with instrument
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Not available
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

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 setup 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 2–8 °C (36–46 °F)
Cost/test	Not available
Cost/instrument	Not available
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
Electric power requirements	100–240 V AC mains and 200–240 V AC mains, 50–60 Hz; 560 V AC (peak)
Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: 15–75% (for temperatures up to 31 °C, decreasing linearly to 50% humidity at 32 °C) non-condensing Maximum altitude: 2000 metres (6500 feet)
Peripherals/supporting instrumentation	
Bar code scanner	
Training	Fully trained laboratory technician 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
EQA	
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

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 onboard: 60 minutes 24 samples, lysis offboard: 40 minutes
Capacity (per run)	1–24 patient samples per run
Throughput per technician/ per day	Up to 168 extractions per shift – lysis onboard workflow Up to 240 extractions – lysis in tube workflow
Sample needed and stability	100–1000 µL plasma for NucliSENS® EasyQ® HIV assay (LOD is better with larger sample) DBS protocol available (CE-marked protocol 100 µL EDTA whole blood and on 100 µL 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	The reagents can be stored at 2–30 °C, except the wash buffer 3 and the silica must be stored at 2–8 °C All reagents are stable until expiration date
Cost/test	Not available
Cost/instrument	Approximately €72 000 (US$ 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	Dedicated pipettes and filter tips, vortex mixer and refrigerator
Electric power requirements	100–240 V AC mains 50–60 Hz
Environmental requirements	Operating temperature: 15–30 °C Humidity: maximum relative humidity: 80%, non-condensing at 30 °C Maximum altitude: 2500 metres (8202 feet)
Data station	Yes; can be linked with LIS using NucliSENtral™ software
Monitor	Onboard monitor
Printer	None supplied
Bar code scanner	Supplied with the system
Training	Fully trained laboratory technician required; dedicated training on instrument that 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 regional/central or national reference (or comparable) laboratories Access to decentralized settings via DBS

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 miniMAG®s at the same time)
Sample needed and stability	100–1000 µL plasma for NucliSENS® EasyQ® HIV assay (sensitivity is higher with larger sample volume) 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 2–8 °C (36–46 °F); all reagents are stable until expiration date
Cost/test	Not available
Cost/instrument	Approximately €6800 (US$ 9000)
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	Dedicated pipettes and filter tips, vortex mixer and refrigerator; bench-top centrifuge (1.5 mL tubes), thermoshaker, centrifuge (2 mL lysis tubes)
Electric power requirements	100–240 V AC mains 50–60 Hz
Environmental requirements	Operating temperature: 4–45 °C Humidity: maximum of 90% relative humidity Maximum altitude: 2000 metres (6500 feet)
Data station	None
Monitor	None
Printer	None
Bar code scanner	Not available
Training	Fully trained laboratory technician 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 levels or national reference (or comparable) laboratories Access to decentralized settings via DBS

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	Samples are extracted with miniMAG® or easyMAG® The obtained eluates can be stored at 2–8 °C or at -20 °C
Sample preparation and protocol complexity	Moderate complexity Dehydrated reagents are quickly reconstituted
Reagent stability	NucliSENS EasyQ® HIV-1 v 2.0 assay storage at 2–8 °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 (US$ 23.75)
Cost/instrument	Approximately €37 100 (US$ 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 in) 42 cm (16.5 in) 22 cm (8.7 in)
Weight	20.5 kg (45 lbs)
Electric power requirements	100–240 V
Environmental requirements	Operating temperature: 15–30 °C Humidity: no greater than 80% relative humidity Maximum altitude: 2000 metres (6500 feet)
Peripherals/supporting instrumentation	Data station and monitor are supplied with the instrument Printer not supplied with instrument Can be linked with LIS using NucliSENtral™ software
Bar code scanner	Not supplied with instrument
Training	Fully trained laboratory technician 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
EQA	
Infrastructure requirements	Technology can be used at central/national reference (or comparable) laboratories Access to decentralized settings via DBS

bDNA: VERSANT® 440 Molecular System Automated amplification/detection instrument	
Type of technology	Automated signal amplification and detection based on bDNA 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 two 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	200–1000 µL plasma
Sample preparation and protocol complexity	Technology/assay does not require viral RNA purification/extraction
Reagent stability	Box 1 stored at 2–8 °C; Box 2 stored from -80 °C to -60 °C
Cost/test	Not available
Cost/instrument	Not available
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
Electric power requirements	100–120 V AC mains ±10%; 200–240 V AC mains ±10%; 50/60 Hz; 500 V AC maximum
Environmental requirements	Temperature: 18–30 °C Humidity: 24–80%, non-condensing Maximum altitude: 0–2000 metres above sea level
Peripherals/supporting instrumentation	Onboard 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 laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Yes
EQA	Amenable to EQA
Infrastructure requirements	Technology can be used at central/national reference (or comparable) laboratories

Aptima® HIV-1 Quant Dx Assay on the Panther® System	
Type of technology	Real-time TMA (transcription mediated amplification)
Output	HIV-1 RNA quantitation in plasma and HIV-1 RNA detection in plasma or serum
Turnaround time	Time to first result: less than 3 hours (for first 5 results), with 5 additional results every 5 minutes
Capacity	Panther® can hold up to 120 patient specimens with continuous loading after first rack of 15 specimens is pipetted
Throughput per technician/ per day	275 samples per 8-hour day and 500 samples per 12-hour day (additional 225 samples processed without operator attendance) Random, continuous loading of samples eliminates the need for batching
Sample needed and stability	Minimum volume for primary collection tubes is 1200 µL and for secondary specimen aliquot tube (SAT) the minimum volume is 700 µL Dilution feature allows quantitation with 240 µL of plasma Whole blood, plasma or serum in primary collection tubes can be stored at 2–30 °C for up to 24 hours after specimen collection Plasma can be stored in the primary collection tube at 2–8 °C for up to 3 days; or in the SAT at 2 °C for up to 5 days; or in the SAT at -20 °C or -70 °C for up to 90 days Serum can be stored in the primary collection tube at 2–8 °C for up to 3 days; or in the SAT at 2–8 °C for up to 5 days; or in the SAT at -20 °C for up to 7 days
Sample preparation and protocol complexity	Plasma in primary blood tubes or secondary tubes can be placed on Panther® after centrifugation to separate red blood cells Test protocol is fully automated following reagent reconstitution and instrument setup Reagents are shipped lyophilized with paired reconstitution solution and collar Once reconstituted, reagents are placed on Panther® and ready for use
Reagent stability	Reagents stable until expiration (typically >6 months shelf life) Once reconstituted, reagents are stable for up to 48 hours on the Panther® and for up to 30 days refrigerated
Cost/test	To be determined
Cost/instrument	To be determined
Regulatory status	Pursuing CE-IVD marking, FDA approval, WHO prequalification for Aptima® HIV-1 Quant Dx Assay
Physical dimensions (W x H x D)	Width: 122 cm (48 in) Height: 175 cm (69 in) Depth: 81.5 cm (32 in)
Weight	363 kg (~800 lbs)
3rd party supplies	None
Electric power requirements	100–230 V
Environmental requirements	Operating temperature: 15–30 °C (59–86 °F) Humidity: 20–85% Maximum altitude: 2000 metres (6000 feet) above sea level
Data station	Built-in Dell computer running Windows Vista
Monitor	Touchscreen monitor attached to Panther® for easy access
Printer	Supplied: HP Office Jet Pro 8000
Bar code scanner	Built-in scanners automatically read reagents and samples on loading Handheld scanner attached to Panther® for master lot input Both read Code 39, Code 93, Code 128 (isbt 128), Interleaved 2 of 5, and Codabar
Training	5-day operator training is provided at certified training facility or customer site
Maintenance	Panther® provides ability to schedule many routine maintenance procedures Routine preventative maintenance by authorized service representative every 6 months Hologic Secure PRO360° allows remote issue evaluation to either resolve or send out appropriate applications or engineering support

Internal QC	An internal calibrator/control is added to each sample at the beginning of the processing to control for nucleic acid capture, amplification and detection and is used to normalize target signals for quantitation Panther® also has multiple in process and validity checks to ensure proper performance of the system
EQA	Compatible with EQA programmes Verified with College of American Pathologists, National Institute for Biological Standards and Control, Quality Control for Molecular Diagnostics, United States Nuclear Regulatory Commission, Accrometrix
Infrastructure requirements	Suitable for a centralized and some decentralized settings
User interface	Touchscreen user interface in English

RT: 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 prepared from EDTA or CPD anticoagulated 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 from -14 °C to -25 °C; reagent kits are stable 24 months at -20 °C If stored at 4–8 °C, must be used within one week
Cost/test	Approximately US$ 12–25
Cost/instrument	Approximately US$ 4500
Regulatory status	CE-IVD marked
Physical dimensions	Not available
Weight	Not available
3rd party supplies	ELISA plate reader with A405 filter, incubator set at 33 °C, freezer set from -4 °C to -25 °C, end-over-end mixing table, vortex, computer
Electric power requirements	100–240 V; 50–60 Hz
Environmental requirements	Temperature: 16–33 °C Humidity: Not available Maximum altitude: Not available
Peripherals/supporting instrumentation	ExaVir™ Viral Load Analyser software for processing results; computer required, but not supplied; no printer supplied
Bar code scanner	None
Training	4 days of training required
Maintenance	Routine preventative maintenance required
Internal QC	Yes
EQA	Available in some regions
Infrastructure requirements	Technology can be used at central, regional, district and some well-developed Level I sites with dedicated laboratory facilities and technicians

SAMBA I – Analyser	
Type of technology	SAMBAprep: sample extraction SAMBAamp: Isothermal target/signal amplification and visual detection
Output	Qualitative for EID or acute infection (limit of detection ~400 cp/mL RNA with 100 µL of whole blood, also detects DNA) Qualitative for acute infection (limit of detection ~100 cp/mL with 500 µL of plasma) Semi-quantitative viral load test for monitoring of patients on ART (1000 cp/mL cutoff with 200 µL of plasma)
Turnaround time	90–120 minutes, depending on the assay
Capacity	SAMBAprep: 6 samples per run (batch) SAMBAamp: 4–8 samples run individually (random access)
Throughput per technician/ per day	30 samples per day for EID or acute infection tests; 42 viral load tests per day
Sample needed and stability	200 µL (plasma) for viral load test; 500 µL (plasma) for acute infection test; 100 µL (whole blood) for EID or acute infection test Sample is stable at room temperature for 6–8 hours
Sample preparation and protocol complexity	Simple, preloaded, disposable cartridges containing all required liquid or dry reagents
Reagent stability	Transport stability up to 50 °C for 1 month Reagents do not require cold-chain storage and are stable up to 2–37 ° for 15 months
Cost/test	Volume dependent
Cost/instrument	Volume dependent
Regulatory status	Malawi and Uganda: product approval obtained Kenya: technical dossier approved, site audit completed, pending issuance of certificate Cameroon and Nigeria: pending in-country validation CE marking and WHO prequalification planned
Physical dimensions (W x H x D)	SAMBAprep: 600 mm x 516 mm x 600 mm SAMBAamp: 582 mm x 114 mm x 305 mm
Weight	SAMBAprep: 50 kg SAMBAamp: 4.5 kg
3rd party supplies	Plasma viral load and qualitative assay requires a centrifuge to separate plasma
Electric power requirements	SAMBAprep: 100–250 V, 50 Hz SAMBAamp: 100–250 V, 50 Hz
Environmental requirements	Operating temperature: 10–35 °C Humidity: up to 95% Maximum altitude: Not available
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
EQA	Monthly EQA panel available for blinded testing
Infrastructure requirements	Can be used at various levels of health facility, including health centres or in mobile facilities Electricity is required
User interface	Touchscreen

SAMBA II – Analyser	
Type of technology	Sample extraction, isothermal target/signal amplification and detection
Output	Qualitative for EID or acute infection (limit of detection ~400 cp/mL RNA with 100 µL of whole blood, also detects DNA) Qualitative for acute infection (limit of detection ~100 cp/mL with 500 µL of plasma) Semi-quantitative viral load test for monitoring of patients on ART (1000 cp/mL cutoff using 200 µL of plasma) Semi-quantitative viral load test for monitoring of patients on ART (1000 cp/mL cutoff using 120 µL of whole blood)
Turnaround time	90–120 minutes, depending on the assay
Capacity	Flexible, random access, modular
Throughput per technician/ per day	4 tests/assay module/day Each display module controls up to 16 assay modules giving a potential throughput of up to 64 tests/day
Sample needed and stability	200 µL (plasma) for viral load test; 500 µL (plasma) for acute infection test; 100 µL (whole blood) for EID test; 120 µL (whole blood) for viral load test Sample is stable at room temperature for 6–8 hours
Sample preparation and protocol complexity	Simple, unit-dose, disposable cartridges containing all required liquid or dry reagents
Reagent stability	Transport stability up to 50 °C for one month Reagents do not require cold-chain storage and are stable up to 2–37 °C for 15 months
Cost/test	Volume dependent
Cost/instrument	Volume dependent
Regulatory status	Same as SAMBA I, pending equivalency testing (same chemistry as SAMBA I)
Physical dimensions (W x H x D)	Assay module: 190 mm x 330 mm x 330 mm Display module: 215 mm x 170 mm x 180 mm
Weight	Assay module: 8.5 kg Display module: 1.8 kg
3rd party supplies	Plasma viral load and qualitative assays requires a centrifuge to separate plasma
Electric power requirements	Assay module: 100–250 V, 50 Hz Display module: 100–250 V, 50 Hz
Environmental requirements	Operating temperature: 10–35 °C Humidity: up to 95% Maximum altitude: Not available
Data station	USB memory stick
Monitor	LCD screen integrated into instrument
Printer	Yes; integrated
Bar code scanner	Yes; integrated
Training	Minimal training required – up to 4 hours
Maintenance	No maintenance required; swap-out of instrument if needed
Internal QC	Synthetic, non-target nucleic acid internal controls
EQA	Monthly EQA panel available for blinded testing
Infrastructure requirements	Can be used at various levels of health facility, including health centres or in mobile facilities Electricity is required
User interface	Touchscreen

Alere q HIV-1/2 Detect	
Type of technology	Alere q is a portable automated bench-top real-time RT RNA PCR system used for the processing and analysis of Alere q HIV-1/2 test cartridges Alere q HIV-1/2 Detect cartridges provide for sample collection, cell lysis, target capture, reverse transcription, real-time PCR amplification and real-time fluorescence detection based on competitive reporter probe hybridization on an integrated microchip array
Output	Alere q HIV-1/2 Detect test provides qualitative HIV-1, Groups M, N and O, and HIV-2 results
Turnaround time	<60 minutes
Capacity	Maximum of ~8 samples per day
Throughput per technician/ per day	~8 samples per technician per day; no batching capabilities; walk-away operation
Sample needed and stability	Whole blood assay: 25 µL of capillary (fingerprick or heelprick) blood or EDTA venous blood Plasma assay: 25 µL EDTA plasma
Sample preparation and protocol complexity	Whole blood: no manual sample preparation or pretreatment The required 25 µL of blood can be collected directly into the test cartridge's sample collection capillary (for fingerprick or heelprick) or via transfer capillary or volumetric pipette (venous blood) Plasma: centrifugation required Steps: (i) apply sample to cartridge; (ii) close cartridge; (iii) insert cartridge into analyser; (iv) analysis starts automatically; (v) enter operator and sample ID; (vi) after assay is finished remove cartridge from analyser; (vii) read result from screen Hands-on time <3 minutes
Reagent stability	Freeze-dried reagents require no refrigeration Stable for 12 months at 4–30 °C
Cost/test	To be determined
Cost/instrument	To be determined
Regulatory status	Alere will seek regulatory approval for CE-IVD marking and FDA approval
Physical dimensions (analyser only) (L x H x D)	Length: 20 cm (7.87″) Height: 22 cm (8.66″) Depth: 31 cm (12.2″)
Weight	<7.8 kg
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs (also available from Alere)
Electric power requirements	100–240 V (A/C) at 50–60 Hz mains power Analyser contains onboard rechargeable battery; additional external battery available
Environmental requirements	Operating temperature: 15–40 °C (59–104 °F) Humidity: <90% relative humidity Maximum altitude: Not available (permissible atmospheric pressure: 800–1060 hPa)
Data station	1000 test results can be stored on the instrument archive; results can be downloaded via USB Results can be printed immediately, but results also are stored in an onboard 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 Data point connectivity solution for instrument management, QC and cartridge consumption provided
Monitor	Colour touchscreen integrated into instrument
Printer	Separate printer (prints on thermal paper); USB/battery powered Length 95 mm x width 93 mm x height 66 mm; weight: ~350 g, including paper roll
Bar code scanner	Integrated into instrument for test cartridges and compatible to external bar code readers
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	Maintenance free instrument Care package for instrument is available If damaged portability of device allows for direct swap-out replacement rather than onsite repair
Internal QC	Yes
EQA	Will be fully compatible with existing EQA programmes
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities
User interface	Touchscreen colour 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 ~80 cp/mL from plasma sample)
Turnaround time	30 minutes for plasma sample and 35 minutes for whole blood sample
Capacity	~15 samples per 8-hour day
Throughput per technician/ per day	~15 samples per technician per 8-hour day per analyser
Sample needed and stability	150 µL of plasma or 75 µL of fingerstick 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 ~30–35 minutes automatically
Reagent stability	Liat™ assay cartridge expected to be shipped with an expiration date of at least 6 months if stored at 4 °C (39.2 °F) or 15 months if stored at -20 °C (-4 °F); but 37 °C (98.6 °F) storage allowed for 3 weeks
Cost/test	To be determined
Cost/instrument	~US$ 25 000, but can be priced lower for resource-limited settings
Regulatory status	FDA 510 (k) clearance for Liat™ Influenza A/B Assay, To be determined for Liat HIV Quant Assay
Physical dimensions (W x H x D)	Width: 11.4 cm (4.5 in) Height: 19 cm (7.5 in) Depth: 24.1 cm (9.5 in)
Weight	3.75 kg (~8.3 lbs)
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs
Electric power requirements	AC mains or battery powered
Environmental requirements	Operating temperature: 15–40 °C (59–104 °F) Humidity: 15–80% (non-condensation) Maximum altitude: 2000 metres (6500 feet) above sea level (Expanding operation conditions is possible, but requires further validation)
Data station	Dedicated central processing unit 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 colour screen integrated into instrument
Printer	No printer provided; can be connected via USB or Ethernet (optional)
Bar code scanner	Integrated into instrument for operator bar code, patient bar code and Liat™ tube bar code
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 alert the operator in the event of malfunction or error Remote system monitoring/diagnosis performed via the Liat Analyser'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
EQA	To be determined whether compatible with EQA programmes
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities within the limits of reagent storage requirements.
User interface	LCD touchscreen with 4 hard keys and 4 arrow buttons

WAVE 80 EOSCAPE-HIV™ System	
Type of technology	Nucleic acid test
Output	HIV-1 RNA level (quantitative/qualitative)
Turnaround time	70 minutes
Capacity	1 cartridge per processing unit; multiple processing units can be run in parallel with single analyser unit
Throughput per technician/ per day	>40 per 8-hour day (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 40° C
Cost/test	<US$ 17 per test (viral load 1000–50 000 cp/mL) <US$ 20 per test (EID)
Cost/instrument	<US$ 10 000 for one analyser unit with two processing units (typical)
Regulatory status	In process for WHO prequalification and CE-IVD marking; FDA To be determined
Physical dimensions (W x H x D)	Width: 250 mm (9.8″) Height: 90 mm (3.5″) Depth: 150 mm (5.9″)
Weight	1.4 kg (~3.1 lbs)
3rd party supplies	Lancet, alcohol swab (supplied in kit)
Electric power requirements	Mains power with 8-hour rechargeable battery backup Solar charging capable
Environmental requirements	Operating temperature: <40 °C Humidity: Not available Maximum altitude: Not available
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 United States 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
EQA	Separate cartridges required to run external positive/negative controls; separate standards can be required for laboratory quality assurance programmes
Infrastructure requirements	Biohazard disposal; cartridge storage
User interface	Touchscreen interface; power on/off switches on processing unit and analyser unit

Truelab™ Real Time micro PCR system	
Type of technology	Nucleic acid amplification test (real-time PCR)
Output	HIV-1 RNA level (quantitative)
Turnaround time	60 minutes (sample to result)
Capacity	1 chip per processing unit (company plans a 4 chip version)
Throughput per technician/ per day	About 12 per 8-hour day (about 50 with 4 chip version)
Sample needed and stability	100 µL plasma for viral load or 100 µL of blood for screening Sample must be processed immediately upon collection or stored at -20 °C Alternatively, for transport, 100 µL of specimen can be transferred to a tube to which 500 µL of lysis reagent has been pre-added
Sample preparation and protocol complexity	Extraction process currently involves multiple pipetting steps that require operator interventions, including adding reagents, aspirating liquid, adding buffer, etc. (an automatic sample prep is expected to be introduced soon) Once extracted, the nucleic acid is dispensed into a chip that is inserted into the PCR analyser and the thermal cycling and analysis takes place automatically within the analyser
Reagent stability	Reagents are ready to use, shelf stable for 1 year when stored at 2–30 °C and for 3 months at temperatures up to 40 °C
Cost/test	US$ 15
Cost/instrument	US$ 8000 (includes sample preparation, PCR analyser, printer, pipettes)
Regulatory status	Manufacturing facility is ISO 13485 and ISO 9001 certified Indian test manufacturing license obtained and registration process under way
Physical dimensions (analyser) (W x H x D)	Length: 21 cm (8.27 in) Width: 14 cm (5.5 in) Height: 10.9 cm (4.29 in)
Weight (analyser)	0 .9 kg (~2 lbs)
3rd party supplies	Powder-free disposal gloves, waste disposal container with lid, sterile lancets, alcohol swabs, dry swabs
Electric power requirements	Continuous power supply not required Rechargeable lithium ion battery pack: 7.5 V; 2200 mAh provides for over 8 hours of backup on a full charge
Environmental requirements	Operating temperature: 15–~35 °C Humidity: 10–80% Maximum altitude: Not available
Data station	Dedicated CPU integrated into instrument; approximately 5000 test results can be stored on the instrument archive Support wireless connectivity (Wi-Fi, Bluetooth, GPRS).
Monitor	Integrated touchscreen colour monitor (3.2″); touchscreen interface; power on/off switches on analyser unit
Printer	External 2″ Bluetooth Thermal Printer
Bar code scanner	No
Training	2–3 hours; high school diploma or equivalent
Maintenance	Yearly contract, warranty 1 year
Internal QC	Full process internal control that validates the sample preparation and PCR
EQA	Universal control kit containing positive and negative controls must be ordered separately Positive and negative controls should be run from time to time; it is advised to run controls under the following circumstances: (i) whenever a new shipment of test kits is received; (ii) when opening a new test kit lot; (iii) by each new user prior to performing testing on a clinical specimen
Infrastructure requirements	None

Viral Load Assay for GeneXpert®	
Type of technology	PCR-based NAAT test
Output	Quantitative HIV-1 (viral load): limit of detection ~20 cp/mL Qualitative HIV-1: limit of detection whole blood ~200 cp/mL Both assays detect Group M Subtypes A-H, Group O, Group N and recombinants AB, AE and AG
Turnaround time	<95 minutes
Capacity	Dependent on GeneXpert® system and number of modules ranging 1–80 per system, comparable to GeneXpert® MTB
Throughput per technician/ per day	Dependent on GeneXpert® system and number of modules; e.g. 397 results per 8-hour shift with an Infinity-80* (80 modules)
Sample needed and stability	Quantitative HIV-1: 1 mL plasma sample input volume Qualitative HIV-1: 100 µl whole blood and 1 DBS
Sample preparation and protocol complexity	Automated within cartridge
Reagent stability	No refrigeration required; in development
Cost/test	To be determined
Cost/instrument	Comparable to GeneXpert® MTB
Regulatory status	Regulatory submissions for CE-IVD marking in 2014 and FDA approval expected in 2015
Physical dimensions (W x H x D)	See www.cepheid.com for brochure on systems available Specifications for GX-IV Processing Unit: Length: 11.00″ Height: 12.00″ Depth: 13.25″
Weight	GX-IV Processing Unit: 25 lbs
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
EQA	Will be fully compatible with existing EQA programmes
Infrastructure requirements	Can be used at all levels of health facilities that have electricity, including health centres or in mobile facilities

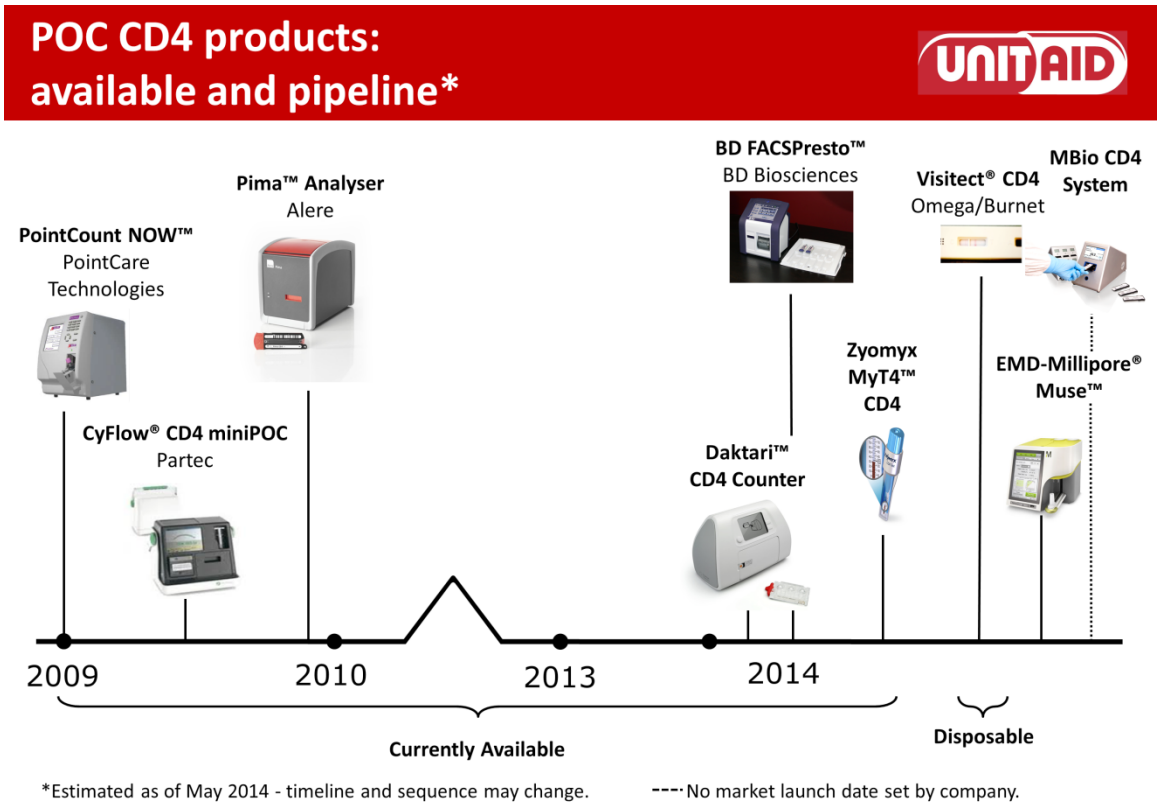
NWGHF Savanna HIV Viral Load Test and Platform	
Type of technology	A bench-top automated cartridge-based system that extracts, amplifies and detects nucleic acid targets for IVD applications
Output	Quantitative HIV-1
Turnaround time	~60–90 minutes
Capacity	Processor will accommodate 13 tests per 8-hour workday
Throughput per technician/ per day	13 tests per 8-hour workday
Sample needed and stability	To achieve 1000 cp/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	Steps: (i) add sample to mini-cartridge; (ii) close sample port and cap to seal mini-cartridge; (iii) place the mini-cartridge into sample preparation device for 2–3 minutes; (iv) remove mini-cartridge from sample preparation device and attach to cartridge; (v) place cartridge onto the loading/unloading position on the system; (vi) read the results on the screen
Reagent stability	Shelf life of the assay kit is expected to be 12–18 months at 30–40 °C, 70–90% humidity
Cost/test	<US$ 10 per test
Cost/instrument	<US$ 12 000
Regulatory status	To be determined
Physical dimensions (W x H x D)	To be determined
Weight	To be determined
3rd party supplies	Blood collection supplies
Electric power requirements	Processor is powered by an external power transformer that connects to either an AC mains or DC power cable that connects to an AC mains or DC power socket in the clinic or laboratory 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
EQA	Will be fully compatible with existing EQA programmes
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities
User interface	Onboard display

CPA: Ustar RT CPA HIV-1 Viral Load Test	
Type of technology	Nucleic acid amplification – isothermal CPA
Output	Viral cp/mL of blood (dynamic range of 1000–20 000 cp/mL)
Turnaround time	<1 hour
Capacity (per run)	8 tests plus 4 controls
Throughput per technician/ per day	>36 tests
Sample needed and stability	50–100 µL of whole blood fingerstick; or 1 mL of venous blood
Sample preparation and protocol complexity	No more than 3–5 steps from sample to result
Reagent stability	Stable for 24 months at 0–40 ˚C, 90% humidity, including transport stress (48 hours with fluctuations up to 50 ˚C and down to 0 ˚C
Cost/test	US$ 3–5 per test (ex-works)
Cost/instrument	<US$ 5000
Regulatory status	Under development
Physical dimensions	Width: To be determined Height: To be determined Depth: To be determined
Weight	<2 kg (<4.4 lbs)
3rd party supplies	None
Electric power requirements	110–220 V AC mains current or DC power with rechargeable battery lasting 8 hours
Environmental requirements	Temperature: 15–40 ˚C Humidity: no requirement Maximum altitude: >2000 metres (6500 feet)
Peripherals/supporting instrumentation	None
Bar code scanner	To be determined
Training	Approximately half a day
Maintenance	System is swapped for a new one upon malfunction
Internal QC	Internal amplification control; fluorescent control to ensure probes are working
EQA	Three quantitative standards and one negative control
Infrastructure requirements	Intermittent power; bench to store instrument; incineration for medical waste

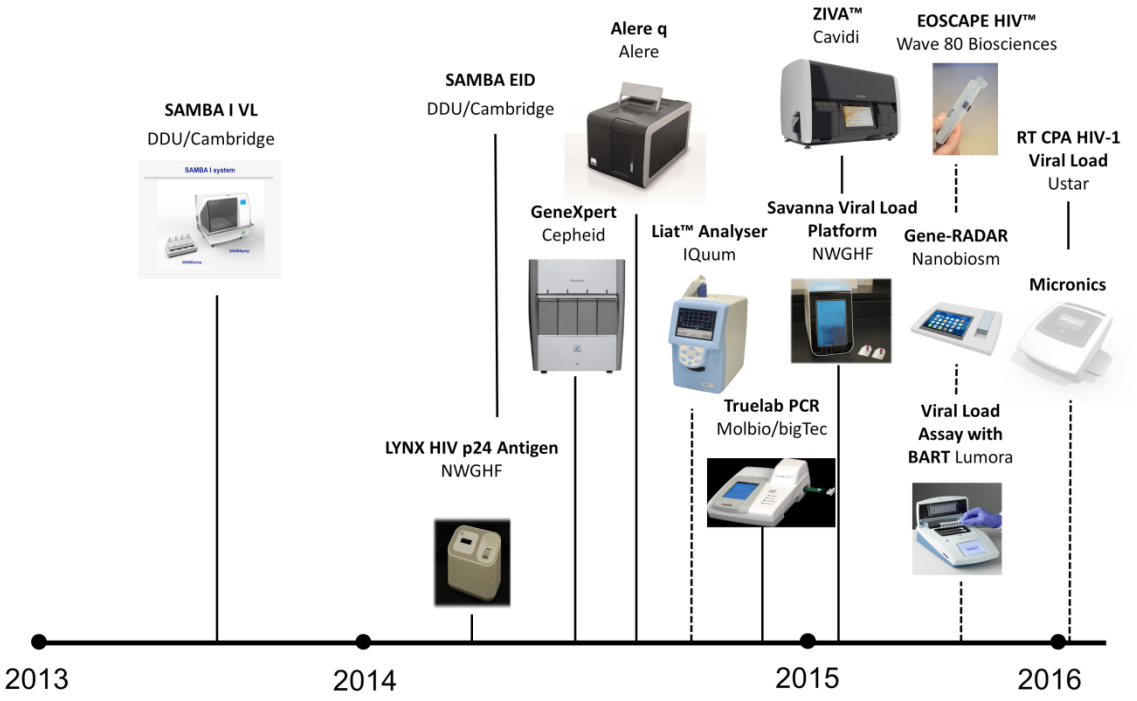
ZIVA™ Automated RT Viral Load	
Type of technology	Fully automated measurement of RT activity
Output	Quantification of HIV RT enzyme activity, type- and subtype independent
Turnaround time	5 hours
Capacity (per run)	20 or 48 patient samples + controls
Throughput per technician/ per day	48 samples in 6 hours, 96 samples with overnight mode (start second run of 48 samples before end of day and collect results first thing the morning after)
Sample needed and stability	500 µL plasma; plasma prepared from EDTA or CPD anticoagulated whole blood
Sample preparation and protocol complexity	Load patient samples, reagents and consumables; start assay and leave machine to run automated test Return to collect results and empty waste
Reagent stability	Reagent kits must be stored at 2–8 °C; reagent kits are stable 12 months at 2–8 °C Consumables kits can be stored at room temperature
Cost/test	To be determined
Cost/instrument	To be determined
Regulatory status	In process Plan for CE-IVD marking and WHO prequalification
Physical dimensions	Maximum dimensions (width x depth x height): 100 x 60 x 70 cm
Weight	To be determined
3rd party supplies	None to run assay Centrifuge needed for preparation of plasma
Electric power requirements	100–240 V; 50–60 Hz
Environmental requirements	Temperature: 16–33 °C Humidity: Not available Maximum altitude: Not available
Peripherals/supporting instrumentation	Stand-alone machine Onboard computer with touchscreen
Bar code scanner	Supplied with instrument
Training	Half-day needed Onscreen tutorial and run support
Maintenance	Routine preventative maintenance required
Internal QC	Yes
EQA	Available in some regions
Infrastructure requirements	Technology can be used at central, regional, district and some well-developed Level I sites with laboratory facilities and technicians

NWGHF LYNX HIV p24 Antigen Test (EID)	
Type of technology	p24 Antigen Assay for EID
Output	Qualitative detection of HIV infection
Turnaround time	45–50 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	Steps: (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	To be determined
Cost/test	Estimated to be: US$ 7–15 per test, depending on volume
Cost/instrument	~US$ 700–2000 for sample processor, depending on volume
Regulatory status	To be determined
Physical dimensions (W x H x D)	Width: 202 mm (~8 in) Height: 156 mm (6.1 in) Depth: 134 mm (5.3 in)
Weight	1.7 kg (~3.7 lbs)
3rd party supplies	None
Electric power requirements	Sample processor is battery powered with 12 V DC (e.g. solar or car battery) or 100–240 V AC mains recharging
Environmental requirements	To be determined
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
EQA	To be determined whether compatible with EQA programmes
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities
User interface	Display with timer and battery indicator

Appendix 2: Pipeline for POC diagnostics



POC viral load & EID products: available and pipeline*



*Estimated as of May 2014 - timeline and sequence may change. ----No market launch date set by company.

Appendix 3: Technical specifications for HIV qualitative assays

Assay name	COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 v2.0 Qualitative CE-IVD	Abbott RealTi*me* Qualitative HIV-1 CE-IVD
Type of assay	Real-time PCR, qualitative	Real-time PCR, qualitative
Dynamic range (cp/mL)	N/A	N/A
Contamination control	Amperase	Not required due to system design
Controls	Run-in (negative, positive) internal control	Run-in (negative, positive) internal control
Specimen type	Whole blood/DBS	DBS, EDTA and ACD-A plasma
Specimen volume	1.0 mL plasma70 µl DBS	200 µL plasma or DBS
Area of HIV genome amplified	Gag and LTR	Pol/INT
HIV-1 subtypes amplified	Group M, subtypes A through H	Group M, subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, and H, Group O and Group N
Time for result	5–6 hours	5 hours and 39 minutes up to 7 hours and 54 minutes depending on batch size
Cost/test[1]	US$ 12–16 per test in resource-limited settings; US$ 16–30 per test elsewhere	US$ 18–20 per test depending on volumes and subject to negotiations with Abbott
Number of samples/run	22–66 batch loading (176/8-hour day continuous loading)	1–94 patient samples (+2 external controls)
Equipment required[2]	COBAS® AmpliPrep with COBAS® TaqMan® 96 COBAS® TaqMan® 48	*m*2000rt; *m*2000sp, *m*24sp or manual sample preparation
Equipment Cost ($US)	COBAS TaqMan 48: US$ 45 000–100 000 COBAS TaqMan 96: US$ 80 000–150 000 COBAS AmpliPrep: US$ 80 000–150 000	*m*2000sp: US$ 162 000; *m*2000rt: US$ 45 000
Regulatory status		WHO prequalified CE-IVD marked

N/A = Not applicable.

1 Prices will vary considerably depending on quantities, infrastructure and support required plus special negotiations.
2 Each of the assays requires pipettes, a vortex mixer and a refrigerator.

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