HIV/AIDS Diagnostics Technology Landscape

5th edition

October 2015

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Contents

Glossary of terms and acronyms

AC	alternating current
ACD	acid citrate dextrose
AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
ARV	antiretroviral (drugs)
ASLM	African Society for Laboratory Medicine
BART	Bioluminescent Assay in Real-Time
bDNA	branched chain deoxyribonucleic acid
°C	degree Celsius
CE Mark	European Conformity (Conformité Européenne) (mark placed on products in the European Economic Area to indicate that a product conforms to the requirements of European Union directives)
CLIA	chemiluminescence immunoassay
CHAI	Clinton Health Access Initiative
cm	centimetre
cp	copies
CPA	Cross Priming Amplification
CPU	central processing unit
CRF	circulating recombinant forms
CT/NG	*Clamydia trachomatis/Neisseria gonorrhoeae*
CV	coefficient of variation
DBS	dried blood spot
DC	direct current
DFA	Diagnostics for All

dL	decilitre
DNA	deoxyribonucleic acid
DSP	digital signal processing
EDTA	ethylenediaminetetraacetic acid (a potassium salt that is contained in blood collection tubes and is a strong anticoagulant)
EID	early infant diagnosis
ELISA	enzyme-linked immunosorbent assay
EQA	external quality assurance
°F	degree Fahrenheit
fg	femtogram
g	gram
Global Fund	Global Fund to Fight AIDS, Tuberculosis and Malaria
GSM	global system for mobile communications
Hb	haemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HIS	hospital information system
HIV	human immunodeficiency virus
HIVDR	HIV drug resistance
HPV	human papillomavirus
HSV	herpes simplex virus
Hz	Hertz
ID	identification
in/”	inch
iNAAT	isothermal nucleic acid amplification technology

ISO	International Organization for Standardization
IT	information technology (computer network)
IU	international unit
IVD	in vitro diagnostics (tests that can detect diseases, conditions or infections)
kg	kilogram
L1 L2 L3 L4	Level 1 Level 2 Level 3 Level 4 (tiered laboratory system)
LAMP	loop-mediated amplification
lbs	pounds
LED	light emitting diode
LIMS	laboratory information management system (see LIS)
LIS	laboratory information system (see LIMS)
LOD	limit of detection (qualitative threshold)
LOQ	limit of quantitation (viral load)
LTR	long terminal repeat
mL	millilitre
mm	millimetre
MRSA	methicillin-resistant *Staphylococcus aureus*
MTB	*Mycobacterium tuberculosis*
MTCT	mother-to-child transmission (HIV)
N/A	not applicable
NASBA	nucleic acid sequence-based amplification
NAT	nucleic acid-based test
NWGHF	Northwestern Global Health Foundation
OPP	Open Polyvalent Platform
oz	ounce

PC	personal computer
PCR	polymerase chain reaction
PEPFAR	President's Emergency Plan for AIDS Relief
pg	picogram
PMTCT	prevention of mother-to-child transmission (HIV)
POC	point of care
QC	quality control
RDT	rapid diagnostic test
RIF	resistance to rifampicin
RNA	ribonucleic acid
RT	reverse transcriptase
SAMBA	simple amplification-based assay
SMS	short message service
SNP	single nucleotide polymorphism
STI	sexually transmitted infection
TBD	to be determined
TMA	transcription-mediated amplification
TNA	total nucleic acid (DNA and RNA)
µL	microlitre
UPS	uninterruptible power supply
US	United States
USAID	United States Agency for International Development
USB	universal serial bus
US CDC	United States Centers for Disease Control and Prevention
US FDA	United States Food and Drug Administration

V	volt
VL	viral load
w	watt
WBC	white blood cell
WHO	World Health Organization

Executive summary

There is growing demand within the global health community to find ways to improve access to diagnostics for HIV without diminishing the quality of patient care. HIV-infected individuals require testing for initial diagnosis, staging and ongoing monitoring throughout treatment. For low and middle-income country populations, and in particular for patients in peri-urban and rural settings, it can be a challenge to achieve timely diagnosis of infants under 18 months (early infant diagnosis or EID), viral load (VL) monitoring and CD4 staging. Access to VL, despite the clinical consensus on the importance of VL testing for detecting virological failure, is severely limited in many resource-limited settings *(1,2)*. Developers have responded to the need for increased access to robust, quality-assured diagnostics in resource-limited settings in order to facilitate early detection and treatment of HIV and a number of optimized solutions are emerging or in pipeline.

This report reviews diagnostic platforms for three key HIV testing modalities: CD4; EID; and VL. The majority of testing today is still performed in centralized laboratories using instrumentation that requires high-infrastructure support and maintenance with highly trained laboratory technicians. For peri-urban and rural settings, access to centralized testing requires additional transport networks for patients and/or specimens, connectivity for return of results in a timely manner and a subsequent follow-up consultation for patient counselling and treatment. In resource-limited settings, the transport requirements can often be quite expensive; each of these additional steps can significantly delay patient care and increase the risk of loss-to-follow-up. Given the limitations of centralized laboratory testing, there is general agreement that diagnostic tests need to be available closer to the primary site of patient care. This report examines new diagnostic technologies on the market and in the pipeline – most of which are designed for use at or near the point of patient care (POC). Information on technology development timelines, market release and validation/regulation are presented for each product, as disclosed by the developer. On the basis of their operational characteristics, each technology is also considered for appropriate fit within the tiered laboratory structure described for resource-limited settings.

With respect to CD4, there are a number of 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 for rural patients and to reduce patient loss-to-follow-up, there remains a need for quality-assured, cost-effective POC CD4 testing. Six compact benchtop CD4 options are already on the market, and other portable/disposable options are in development for anticipated release in the next few years.

With respect to EID, these tests are primarily performed in centralized reference laboratories on sophisticated laboratory equipment. The most widely used EID test is a DNA polymerase chain reaction (PCR) nucleic acid test; EID can also be performed on VL platforms with new qualitative

tests on the market for infant and adult "acute" diagnosis. These tests are subject to the challenges of centralized testing for resource-limited settings. However, the cost of EID has decreased, dried blood spot (DBS) sample transport networks have been developed, and EID training has been implemented, including through UNITAID-supported programs. As a result of these improvements, there has been considerable uptake of EID even in decentralized clinics. Access is far from universal, however, and access to EID at or near POC could improve access in hard-to-reach areas, decrease patient loss-to-follow-up and reduce the burden of presumptive diagnosis. In addition to the expanded use of VL platforms for qualitative EID, there are several POC assays being developed specifically for EID, three of which are now on the market.

With respect to VL, these plasma-based tests are almost exclusively performed in centralized laboratories, subject to the limitations described above, with the additional disadvantage of higher per-test cost. To simplify sample transport, new assays have been developed for VL using DBS – albeit with some tradeoff in detection sensitivity *(3,4)*. It is anticipated that point of care (POC) VL testing will significantly expand access for decentralized populations by eliminating the need for sample transport, infrastructure and extensive training. However, many POC settings lack the infrastructure for plasma separation, and a fingerprick or venipuncture whole blood sample may not accurately report VL below 5000 cp/mL with one notable exception *(1)*. There are a number of POC HIV VL platforms in development, several of which may come to market in 2015–2016.

Along with improvements in sample transport and connectivity networks, procurement strategies such as the Global Fund for VL and EID and the agreements reached by the Diagnostic Access Initiative, seek to significantly reduce the cost of VL testing.

Advances in POC testing for EID, CD4 and VL are likely to bring about significant changes in access to quality health care, particularly in resource-limited settings *(6–8)*. At the same time, advances in high-volume testing also are becoming available, allowing cost-effective consolidation of testing in high-volume centres. In addition, a number of modular or open molecular platforms are available. The level of CD4, VL and EID testing required over the coming years likely will necessitate scale-up in centralized testing facilities, including super-laboratories. The appropriate combination of POC versus centralized testing will certainly be country specific, and will depend on such factors as the urban/rural split of the population, the expected volume of each category of testing and the ability to effectively transport samples between collection sites and laboratories and ensure the return of laboratory results. Realistically, it also will depend on the comparative all-in cost of centralized versus decentralized options.

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

To improve the accessibility 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 treatment of people living with HIV 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 POC.

Access to diagnostics testing is crucial in facilitating early detection and treatment of HIV. Accurate detection, staging and monitoring 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 access to quality-assured diagnostics for HIV is still unfolding, however. Improvements in access likely will be achieved through a combination of 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 will likely be country specific, and new models for delivery also may emerge. It is essential that stakeholders (including ministries of health, UNITAID and other funders) understand current and pipeline diagnostic technologies in order to determine the most appropriate blend of investments to improve HIV care in resource-limited settings.

This report reviews the current technology landscape for HIV diagnostics, including: (i) the algorithms and tests required in HIV care and treatment, both before and after ART initiation; (ii) the diagnostic platforms used and price points for testing; and (iii) the intended uses and appropriate settings for testing. With this information as background, the report then reviews the current technologies and diagnostic platforms in three key testing areas: CD4 and viral load (VL) testing for adults and children as well as EID, including EID run on VL platforms – all of which have traditionally been available only through centralized, high-infrastructure laboratory platforms, even for resource-limited settings. The report also describes the POC and near-POC 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.

Methods

The material in this landscape was gathered from publicly available information, published and unpublished reports and prospectuses, and interviews with developers and manufacturers. The content on platforms and operational characteristics in this document was provided by the developers of the diagnostic technologies. Technologies are indicated where the supplier either did not provide updates or the systems are no longer available.

The *UNITAID HIV Diagnostics Technology Landscape* is published annually and is prepared as part of a broad and ongoing effort to understand the technology landscape for HIV. This report is an annual update on the POC technologies for CD4, VL and EID testing, as well as for the diagnostic pipeline. The complete *2015 HIV Diagnostics Technology Landscape*, together with previous editions and semi-annual updates, is available at: http://www.unitaid.org/resources/publications/technical-reports.

HIV diagnostics overview

Diagnostics for HIV 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. The clinical staging of HIV-related diseases are harmonized to a universal four-stage system that includes simplified standardized descriptors based on laboratory criteria combined with clinical or immunological criteria *(9)*. These stages are generally described as: (i) asymptomatic; (ii) mild symptoms; (iii) advanced symptoms; and (iv) severe symptoms. There are generally accepted algorithms and tests used at each stage as discussed below.[1]

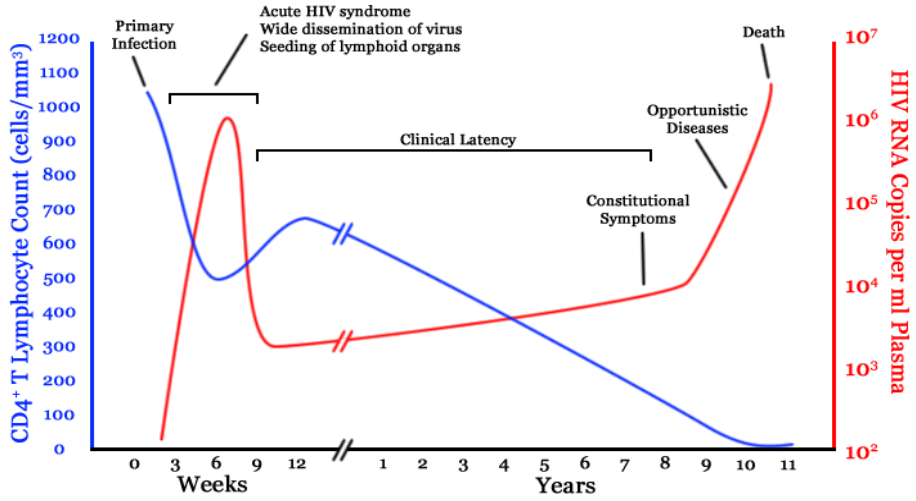
HIV disease involves a continuum of progressive damage to the immune system from the time of infection to the manifestation of significant immunologic damage by various opportunistic infections, wasting or CD4 lymphocyte count that marks the development of full-blown AIDS *(10)*. A typical, but approximate, clinical disease progression showing the relationship between the levels of HIV (VL) and CD4+ T-cell counts over the usual course of untreated HIV infection is presented below *(1)*. Untreated HIV infection is generally characterized by phases: (i) primary; (ii) acute; (iii) latency or chronic infection; and (iv) viral breakthrough/AIDS (Figure 1).[2] Within the first weeks following primary infection, the acute phase is described by a spike in HIV viraemia, when the virus replicates unchecked by any immune system response. The acute stage is characterized by extremely high VL (millions of cp/mL HIV RNA) and high concentrations of p24 antigens that are shed by replicating virus during the early weeks postinfection. The acute

[1] Portions of this overview are drawn from an unpublished report, entitled *ART 2.0 – Implications for diagnostics in resource-limited settings*, co-authored by Maurine Murtagh and Dr Trevor Peter of the Clinton Health Access Initiative.

[2] https://aids.gov/hiv-aids-basics/just-diagnosed-with-hiv-aids/hiv-in-your-body/stages-of-hiv/

phase can last 2–12 weeks, where the patient's extremely high VL can result in a "highly transmissible" state *(11)*.

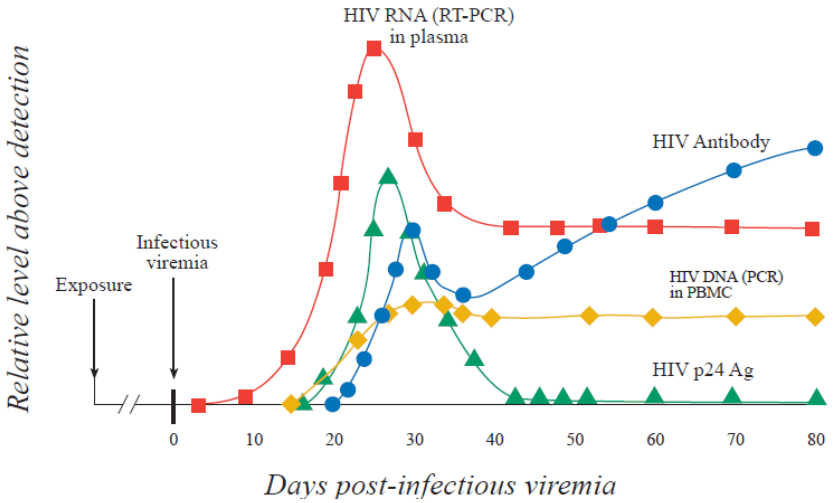
Figure 1. HIV viraemia and CD4 lymphocyte counts during infection



Source: Adapted from Pantaleo, Graziosi and Fauci *(12)*.

Subsequent to acute infection, antibodies against HIV infection appear (seroconversion) and are then present throughout the course of the disease; the antibody immune response partially suppresses the VL typically below 10 000 cp/mL and p24 levels become undetectable (Figure 2). Detection of HIV antibodies is the most common method to screen and diagnose HIV infection and can be performed in a simple lateral flow test (e.g. HIV; rapid diagnostic test/RDT) or laboratory test, however, these tests are not sensitive prior to seroconversion and will not identify HIV infection in the acute stage.

Figure 2. Sequence of appearance of laboratory markers for HIV-1 infection



Source: Adapted from www.hivguidelines.org.[3]

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) *(13)*.

The stage of HIV infection can be identified by clinical symptoms and more accurately by laboratory tests for the various laboratory markers. These tests can be used to identify persons at high risk of disease progression, detect in asymptomatic patients, and guide clinical decision-making such as when to initiate ART, and detect treatment failure. 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 key laboratory marker of disease progression until recently. Direct detection of the HIV viral RNA or pro-viral DNA has become as important a laboratory marker as CD4 count; it is now considered the best marker to use for ART monitoring and decision-making after initiation of therapy *(1,2)*. The measurement of the number of viral RNA cp/mL of patient plasma (commonly known as "viral load" or VL) provides a clinically useful range of values that can indicate the effectiveness of ART in HIV progression.

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 *(14,15)*. 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 or circulating recombinant forms (CRFs). In all, there are at least 43 HIV-1 CRFs with the more prevalent CRF01_AE and CRF02_AG strains included for detection in some tests.

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 and saliva), p24

[3] www.hivguidelines.org/clinical-guidelines/perinatal-transmission/acute-hiv-infection-in-pregnancy/#figure1 (origin: University of Washington, Department of Laboratory Medicine, Virology).

antigen tests and polymerase chain reaction (PCR) tests. Though not presented in this report, 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. HIV antibody rapid disposable tests are most commonly used for screening in decentralized settings as they can be performed with fresh blood or saliva and do not require laboratory infrastructure. If the individual is positive for HIV 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 second rapid disposable test. If the two screening tests are discordant, then a tie-breaker test is used, which can be a third HIV rapid disposable test, preferably from another manufacturer where available *(16)*.[4] In more centralized settings, the confirmatory test is an enzyme-linked immunosorbent assay (ELISA) and/or Western blot conducted in a central laboratory.

HIV rapid disposable tests generally come in the form of lateral flow strips or cassettes, which are convenient, self-contained tools for HIV serologic testing. They are relatively easy to use, can 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. HIV rapid disposable tests can typically be completed in 10–25 minutes. In resource-limited settings, the cost of HIV rapid disposable tests is about US$ 0.50–1.60 per test[5,6] for blood-based tests, but can be as much as US$ 5.00 per test for saliva-based tests. ELISA testing is laboratory based and generally costs US$ 1.50–2.00 per test, including consumables.

In addition, rapid tests are emerging that can be used for self-testing, proposed as an additional approach to help identifying people living with HIV not reached by current testing models. As per current guidance from WHO, all reactive self-test must be confirmed, receiving further testing from a trained provider (2).

HIV infection also can be detected by reverse transcriptase (RT) or p24 antigen. RT is an HIV-associated viral enzyme that is part of viral replication (transcription). RT activity can be quantified and levels can be correlated to the presence of HIV, however, this assay is not a POC test. Alternatively, one of the viral components in blood during the period of acute or early infection is the core structural protein of HIV-1, the p24 antigen, which is produced as a result of the initial, rapid viral replication and can typically be detected within two weeks of infection. It should be noted that levels of RT and p24 can decrease to undetectable levels with viral

[4] See the updated WHO 2015 Guidelines for testing recommendations *(16)*.

[5] In this report, costs for instruments and reagents are ex-works pricing, unless otherwise noted. The fully loaded cost of testing typically includes the cost of human resources, overhead, distributor markups, freight, insurance, taxes and other such ancillary charges. These ancillary costs can vary considerably from country to country.

[6] Distributors often play an important role in service and maintenance of the laboratory instruments, and in managing the supply chain (costs of importation, storage and handling). Distributor markups can range from 5% to 30% of the cost of the test or more. Disposable tests (HIV RDTs, etc.) require no instrument or service, and generally fall at the lower end of the range.

suppression, so both methods can be used for: (i) detecting early HIV infection; (ii) diagnosing infection in infants; and (iii) monitoring response to ART.

Initial diagnosis of HIV in infants (EID)

Compared with adults, a smaller proportion of HIV-infected children is less likely to be diagnosed or receive care *(17)*. Over 90% of paediatric HIV infection is during pregnancy, delivery or breastfeeding, known as mother-to-child transmission (MTCT). Prompt identification and treatment of infants who are infected via MTCT is crucial. In 2013, only 42% of children exposed to HIV was tested for the virus within the recommended first two months. Current EID testing requires complex laboratory technology that is often only available at central laboratories with weeks-to-months for return of results. Without knowing the HIV status of a child it is impossible to access life-saving treatment. Without treatment, half of all children born with HIV will die by the age of 2 years and the majority will die by the age of 5 years *(18)*.

Because of the persistence of maternal antibodies in infants under 18 months, the use of antibody tests such as HIV rapid disposable tests cannot be used to accurately screen infants for HIV. Instead, DNA or RNA testing (i.e. virological testing) should be used to determine the HIV status of infants in that age group. The World Health Organization (WHO) 2013 Consolidated Guidelines strongly recommended that all HIV-exposed infants have HIV virological testing at 4–6 weeks of age or at the earliest opportunity thereafter. Furthermore, it is strongly recommended 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 by virological testing, with confirmatory testing considered to be essential, especially as programmes for preventing MTCT improve and the prevalence of HIV infection among HIV-exposed infants continues to fall *(1,2)*.

The most widely used test for EID has been the DNA PCR molecular test, which is a qualitative nucleic acid test for the presence of pro-viral HIV. It also is possible to use qualitative RNA detection methods or p24 testing for EID (see further discussion below). The laboratory-based nucleic acid tests require relatively sophisticated instrumentation and a trained laboratory technician. Traditionally these nucleic acid tests (also referred to as molecular diagnostics) are restricted to centralized reference laboratories and limited for other settings; however, recent advances for dried blood spot (DBS) specimens and POC platforms are enabling molecular diagnostics for more decentralized settings *(3,4)*. The PCR DNA (and now RNA and TNA) tests are used primarily for EID for specimens obtained from prevention of mother-to-child transmission (PMTCT) centres, clinics and the like. The infant's blood is collected on DBS filter paper, which is transferred via couriers to the laboratory for testing, and test results are then returned to the clinic or other collection site for dissemination to caregivers. Because this process can sometimes be slow, especially the return of results from laboratories, some countries have introduced short message service (SMS) printers (or other mobile technologies) in order to achieve markedly improved turnaround time for return of results from laboratory to collection sites.

EID testing can be run on 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 about US$ 1.40–2.75 per test, depending on bundle configuration. It also does not include more general laboratory consumables (e.g. gloves or pipettes), which cost about US$ 0.35–4.00 per test, depending on the instrument platform.

The most universal EID method is virological testing that detects HIV DNA or TNA; these tests have the best accuracy in whole blood and DBS in almost all circumstances. For other testing options, there are caveats for ART exposure and timing. Infant exposure to neonatal prophylaxis and/or maternal ART may significantly reduce the amount of circulating virus and antigen, in which there is concern for the tests that detect RNA and p24, which rely on active viral replication. While almost all ART-naïve infants can be identified by DNA, RNA, TNA or p24 antigen testing at the recommended 4–6 week timepoint, breastfeeding infants with ongoing exposure may require repeat testing *(19,20)*.

Patient staging

After a primary HIV infection, the virus directly attacks CD4 T-lymphocyte cells (which coordinate the body's immune response) and begins to destroy them while at the same time using them as host cells for replication. After HIV infection, billions of CD4 T-lymphocytes can 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 *(23)*; for children under 5 years, the CD4% measure is considered more reliable, as healthy infants have considerably higher CD4 count than adults until about 6 years of age *(18,23)*.

CD4 testing is primarily conducted on larger laboratory-based instruments, although there are three POC CD4 test platforms currently available on the market. In rural and peri-urban settings, and even in some urban settings, blood collection is performed at clinics with blood samples and then transported (via courier, post or other services) to laboratories for testing. Results are then returned to the patient's primary clinic, 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 due to variability in the results and the failure to detect immature lymphocytes *(23,24)*.

The cost of laboratory-based CD4 testing varies based on testing volumes, reagents used and whether testing is conducted on high- or low-throughput instruments. Generally speaking, the cost of CD4 reagents varies from a low of about US$ 2–14 per test, excluding collection and

laboratory consumables. The cost of consumables will add between US$ 1–2 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.

As shown below, the cost of currently available POC CD4 testing ranges from about US$ 4 per test up to US$ 12 per test for the test reagents, with associated sample collection consumables adding approximately US$ 1 per test. The instrument cost ranges from US$ 500 to US$ 25 000 per device.[7] As additional POC CD4 products enter the market, including at least one disposable RDT, prices likely will fall. It is anticipated that a CD4 RDT could ultimately cost US$ 2–3 per test, but early pricing will be higher.

Initiation of treatment

As a result of latest scientific evidence showing the public and individual health impact of earlier treatment, WHO has revised in October 2015 the eligibility criteria for initiating treatment, recommending that ART should be initiated among all adults with HIV regardless of WHO clinical stage and at any CD4 cell count, replacing the use of CD4 testing for treatment eligibility *(1,21)*. CD4 count still has an important role in assessing baseline risk of disease progression, for starting and stopping prophylaxis and in prioritizing decisions regarding ART initiation in settings where universal treatment is not possible. CD4 cell count measurement may also be important for individuals for whom ART is failing. In some settings, CD4 cell count may still be necessary to guide initiation of ART outside of certain clinical situations *(2,22)*.

Patient monitoring

Chemistry and haematology testing

Though not presented in this report, the options for chemistry and haematology testing range from manual to fully automated laboratory instruments. Clinical chemistry and haematology tests are routinely used to monitor toxicities associated with ART, specifically haemoglobin and creatinine measurement (with additional tests recommended depending on patient symptoms) *(1)*. The cost of these platforms varies widely, from about US$ 9000 to US$ 32 000 for haematology instruments, and from about US$ 3000 to US$ 60 000 for chemistry instruments. There are POC chemistry and haematology benchtop platforms for about US$ 3000–10 000, depending on the features available. Simple handheld instruments exist for blood glucose, haemoglobin and fixed ranges of chemistry parameters for about US$ 1000–5000. The average cost of the basic full blood count is approximately US$ 1.15 per test, with consumables an additional US$ 2 per test. For chemistry testing, the costs range about US$ 0.10–0.45 per test, with consumables about US$ 1.50 per test.

[7] See also: www.msfaccess.org/sites/default/files/HIV_HCV_Report_Diagnostic_Guide_2015.pdf

Treatment monitoring

WHO recommends HIV VL measurement as the preferred approach over CD4 count to monitor patient response to ART *(1,2)*. CD4 cell counts typically do not decline for patients who are virally suppressed, so CD4 monitoring has less value post-treatment *(10,13)*. Along with a baseline CD4 count, VL testing is typically performed with basic blood tests for routine treatment monitoring. Where VL testing is not routinely available, CD4 count and clinical monitoring should be used to diagnose treatment failure, with targeted VL testing to confirm virological failure where possible.

VL testing

Left unchecked, HIV virus replication can produce billions of new HIV copies within one day. VL is traditionally defined as the number of HIV viral RNA copies per milliliter (cp/mL) in patient plasma, obtained by centrifugation of whole blood specimens. Generally tested by PCR or isothermal amplification methods, VL is the most sensitive method for detection of HIV.

ARV treatment failure is defined by a persistently detectable VL[8] exceeding after at least six months after ART initiation, as detected by two consecutive VL measurements within a three-month interval (with adherence support between measurements). The WHO 2013 Guidelines reduced the threshold for virological failure to 1000 cp/mL in plasma specimens.[9] Correspondingly, viral suppression is now defined as people living with HIV on ART for 12 months (+/-3 months) after ART initiation with VL <1000 cp/mL *(2)*.

Increasingly, VL platforms that were validated only for plasma are being validated for whole blood specimens as well. As described below, DBS specimens may result in a slight decrease in assay sensitivity compared to plasma, however, the benefit of DBS sample transport greatly simplifies the processing and transport of samples to central laboratories. Because of the possibility of reduced sensitivity of DBS for VL measurement at 1000 cp/mL, WHO suggests that programmes relying on DBS or whole blood specimens for VL testing may consider retaining a higher threshold (3000–5000 cp/mL) to define virological failure.

Due to the cost and complexity of VL testing, its implementation in resource-poor settings has been relatively limited. At the present time, virtually all VL testing is laboratory based, performed using sophisticated, high-throughput instruments.

One of the most important barriers to implementing VL testing in resource-limited settings is the current high cost of testing, with prices for reagents and non-commodity test consumables

[8] VL testing detects treatment failure well before CD4 count or clinical signs *(25)*.
[9] WHO emphasizes that patients must be on ART for at least six months before treatment failure can be determined.

averaging about US$ 28–29 per test, sometimes reaching heights of US$ 85 per test.[10] To put this in perspective, these per-test costs are 4–5 times greater than CD4, and do not include the large upfront investment required to purchase instruments and establish VL-ready laboratories. Instruments themselves generally cost about US$ 100 000–225 000, including installation and training. In addition, consumables for VL testing traditionally are not bundled and must be purchased separately by users. These items add approximately US$ 4.25 to the cost of VL testing.

Using laboratory testing, samples have to be collected and transported to central laboratories for plasma separation and VL testing. Although DBS has recently been introduced for several platforms, DBS uptake has been limited to date. Two near-POC platforms are now available for plasma-based VL testing. Currently, there are no validated POC testing options broadly available for VL (few Level 1 clinic settings[11] have centrifuges for plasma separation); POC VL platforms are under development, however, several developers have opted to first introduce similar but qualitative EID platforms.

Diagnostic access programs

In 2014, the Global Fund and the President's Emergency Plan for AIDS Relief (PEPFAR) developed a joint action plan to support the expansion of VL testing as recommended by WHO in 2013. Building on the 2014 HIV Diagnostic Access Program, the Global Fund Procurement Strategy for Viral Load and Early Infant Diagnosis focuses on optimizing existing equipment and investments and supporting scale-up. Expanding VL and EID testing can be complex and requires significant investment in the products, laboratory systems, sample transport networks and people involved across the entire testing process. The primary focus of the procurement process is to achieve simple, transparent and affordable pricing with optimal contracting models for analyzers and/or reagents for both existing and new investments, while at the same time maintaining a sustainable, competitive market.

In 2015, framework agreements were established between the Global Fund and diagnostic manufacturers[12] that aim to make the market for HIV VL testing more transparent and competitive, driving significant cost reductions.[13] The agreements, initially lasting three years, will provide clarity on prices, aiming for an all-inclusive price as low as US$ 15, including equipment and other costs such as consumables, maintenance and shipping. It establishes

[10] 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.

[11] Level 1 facilities are where most patients initially present for testing, care and treatment. These sites can include small hospitals, health centres and health posts that primarily serve outpatients.

[12] Initially, Abbott, Alere™, bioMérieux, Cepheid, Hologic, QIAGEN and Roche, following technical and commercial evaluation. Other public health funders and agencies will also be able to enter into agreements based on the benchmark prices negotiated.

[13] www.theglobalfund.org/en/mediacenter/newsreleases/2015-06-10_ New_Approach_on_HIV_Viral_Load_Testing/

procurement benchmarks for the Global Fund implementing partners, including government health departments, community health clinics and medical centres.

In addition, a multipartner initiative, led by UNAIDS was launched in 2014: the Diagnostic Access Initiative. This initiative (including UNAIDS, WHO, the Clinton Health Access Initiative (CHAI), US Centers for Disease Control and Prevention (CDC), the African Society for Laboratory Medicine (ASLM), UNICEF, PEPFAR and UNITAID) aims to leverage improved, accessible, affordable and optimally used diagnostic technologies and strategies contributing to reaching the global HIV Treatment targets.

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. Chemistry and haematology testing has a number of technologies available for use at POC, these do not represent a significant barrier to care and treatment. The tests that present the most persistent access challenges today are CD4, EID and VL. Increasing the availability of quality-assured POC technologies for these tests has the greatest potential to impact HIV care and treatment for patients worldwide.

This report focuses on CD4, EID and VL 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

Diagnostic platform selection and placement is effectively defined by the in-country laboratory system. It is useful to consider the available laboratory infrastructure before discussing diagnostic platforms in depth. The laboratory system in resource-limited settings is generally characterized as a tiered system as follows *(26)*:

Level 4 – National/central 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. With the highest laboratory infrastructure and capacity, national reference laboratories perform molecular and other sophisticated testing beyond the capabilities of most other level facilities (e.g. PCR and other nucleic acid assays; HIV drug resistance [HIVDR] studies; MTB drug susceptibility testing).

Level 3 – 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 some limitations in testing compared to Level 4 facilities, particularly with some high-throughput or unusual molecular diagnostics, however, Level 3 has more expansive test menus than those

found at Levels 1 and 2. In addition to performing all of the tests and services provided at Levels 1 and 2, regional and provincial facilities can usually provide additional testing capabilities such as blood cultures, full chemistry testing, acid-fast bacillus, solid culture and smear. For higher infrastructure or specialty sites, molecular testing also might be available.

Level 2 – 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; these sites 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 (Figure 3, left).

Level 1 – Primary: Level 1 facilities are where most patients initially present for testing, care and treatment. These sites can include small hospitals, health centres and health posts that primarily serve outpatients (Figure 3, centre and right).[14] Often, health posts have no laboratory capability but are able to perform some POC testing. Generally, no clinicians are onsite at a health post. Health centres 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.

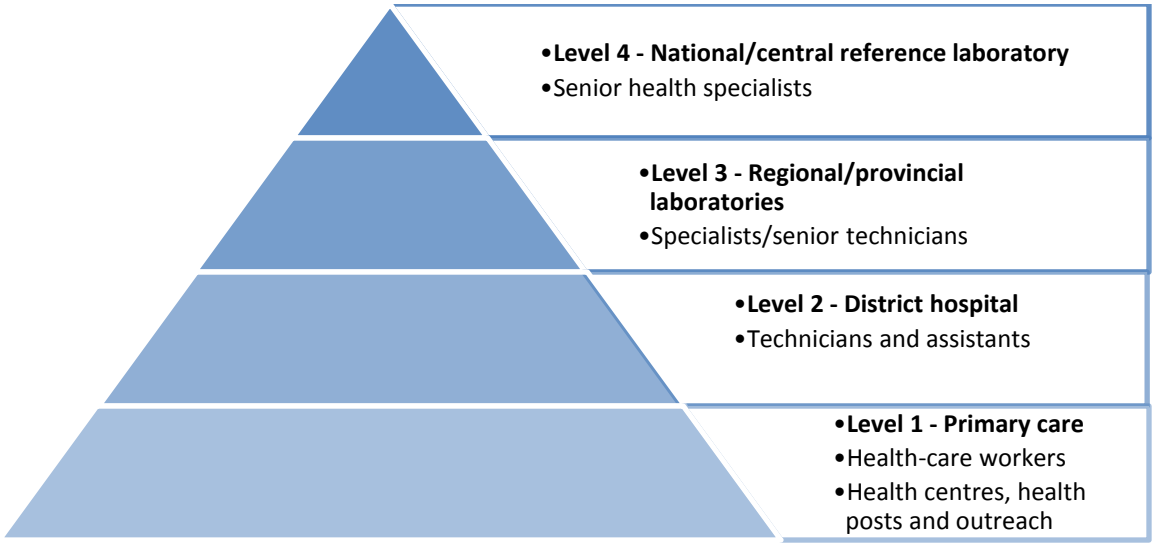
Figure 3. Primary care centres and health posts

Kandangan Hospital (Indonesia) | **Wamena Kota Health Centre (Indonesia)** | **Laalissa Health Post (Ethiopia)**

The laboratory system is often depicted as a pyramid, which illustrates that there are generally a large number of Level 1 facilities in-country and that they serve the most patients (Figure 4). As one migrates up the levels of the health 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).

[14] The Maputo Meeting Report does not specifically place outreach services in Level 1 of the tiered laboratory system. Although some experts place outreach activities at Level 1, others consider patient outreach to be below Level 1 and add a fifth tier to the system, referred to as sub-primary care, community-level, or Level 0.

Figure 4. Diagram of tiered laboratory system



Testing at the laboratory level

Centralized laboratory testing is performed at the Level 4 national reference laboratory level, enabling the greatest number of tests to be performed on a large number of patient samples. Level 4 facilities typically require a significant upfront investment in laboratory infrastructure, instrumentation and maintenance. In addition, these laboratories require expert technicians for daily calibration and management of high-throughput instrumentation: large, automated diagnostic instruments that can process hundreds of batched samples each day. The "batch mode" process enables maximized efficiency in instrument use, as samples can be collected until the instrument is at full capacity, reducing both per-test reagent and equipment costs. 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.

Centralized testing can become a challenge for health systems where patient specimens must travel long distances. Some of the sophisticated instrumentation can only process specimens in a specific format such as fresh whole blood or plasma, which requires a more expensive transport process to get the specimen to the testing site (see the Sample transport section below). Furthermore, to run the instruments at full capacity, it may be necessary to continuously collect specimens until a full "batch" can be run, which delays testing and diagnosis for some patients. And for patients in outlying areas, there may be challenges to return their test results for a follow-up consultation and treatment with their health-care provider (see the Return of results section below).

Sample transport

Most methods of laboratory testing require venous blood collection, cold chain and storage of specimens by trained personnel. Traditionally, tests such as VL that require instrument-based sample processing (centrifuging, etc.) will generally take place only in a national reference, or comparable, laboratory, which means that patient samples will have to be transported from urban, peri-urban and rural settings to the laboratory for processing. This can be 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 increase cost for delivery of samples to the central reference laboratory. In some instances where this transport chain breaks down, samples may be lost or spoiled before they reach the testing facility *(27,28)*. Poor roads sometimes make access to clinics and transport of specimens to the laboratory difficult, particularly during the rainy season.

The ability to use DBS samples is an important consideration in the implementation of blood-based diagnostics such as VL. Increasingly, testing platforms that were validated only for plasma or fresh blood are being validated for DBS specimens as well. As shown in the operational characteristic tables (Appendix 1), DBS specimens may result in a slight decrease in assay sensitivity compared to plasma, however, the benefit of DBS sample transport greatly simplifies the processing and transport of samples from POC. For DBS, drops of fingerprick or venipuncture blood are applied to a paper card, which are stable when dry, and transported by post or courier to the central laboratory. DBS testing eliminates the need for cold-chain or rapid transport, providing more reliable stability and ease of use for health-care workers. The use of DBS also is cost-effective. It should be noted that there are some valid concerns over the decrease in assay sensitivity for DBS (and for whole blood in general) compared to plasma (see the VL testing section below for a more detailed discussion).

Return of results

Combined with challenges of sample transport, centralized testing has additional complications in returning test results back to the primary care clinic and patient in a timely manner, which can often take weeks to months *(28)*. Delays or failure in information transfer increases the risk of negative outcomes and loss-to-follow-up, particularly for patients that must travel a long distance to the clinic. Some countries have introduced SMS printers or other mobile technologies (e.g. SMS; text; call clinic) in order to reduce turnaround time for return of results from laboratory to collection sites. Once the clinic receives the results, the patient must return to the clinic for a follow-up consultation. In some cases, the patient is automatically assigned a follow-up appointment within 2–4 weeks, in other cases the clinic will try to notify the patient directly once results are received (by cell phone if possible).

Decentralized molecular diagnostics

Decentralization of testing to Level 3 and Level 2 facilities can reduce some of the bottlenecks and transport challenges of centralized high-throughput testing. For these facilities, molecular diagnostic capability can be implemented using a modular "polyvalent" approach – combining small instrumentation to achieve the same functionality as an integrated platform, however, at a lower test throughput. This diagnostic platform approach can be flexible, rapid and easy to implement, with appropriate-sized machines for peripheral settings.

The Open Polyvalent Platform (OPP) model is currently being implemented by the OPP-ERA Project for decentralized VL testing in Burundi, Cameroon, Côte d'Ivoire and Guinea.[15] The initiative has validated off-the-shelf instruments and reagents from multiple suppliers to enable a more flexible approach for HIV VL and EID testing. The primary goal with the OPP-ERA initiative is to facilitate a modular laboratory-based approach to decentralized testing, to enable more cost-effective implementation for moderate to low-throughput testing. This modular instrument/reagent approach can readily be expanded for other molecular assays including tuberculosis, hepatitis B virus (HBV) and hepatitis C virus (HCV). The benefits of the modular "small batch" platforms include: (i) equipment size suitable for medium- and low-throughput laboratories; (ii) broad range of assay functionality (not limited to HIV-1 or subtype B); (iii) faster test results; and (iv) low environmental impact (reduced consumables and plastic waste).

This modular platform approach requires procurement from different suppliers, however, there can be benefits if redundant suppliers can be sourced. This approach also requires a higher level of education and training for laboratory technicians than some of the POC systems described below. Examples of the systems validated in the OPP-ERA initiative are listed in the VL technologies section.

Testing at POC

As described above, the majority of current laboratory diagnostics was created for markets where systems are operated in reference laboratories by highly trained technicians on costly, sophisticated instrumentation. 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 can improve access to testing in developing countries.

There is no universally accepted definition of POC testing *(29,30)*.[16] As a general description, POC devices process one test at a time and are intended for lower patient testing loads (4–20

[15] See http://www.unitaid.eu/en/opp-era-project.

[16] 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" *(29)*.

samples per day); some POC platforms can be installed with multiple processing modules for greater throughput. The time required for POC testing varies by test complexity, ranging from 10 minutes for protein tests to 120 minutes for nucleic acid (virological) tests. POC systems intended for resource-limited settings are generally designed for minimal laboratory infrastructure (Level 1 and Level 2 facilities) for health-care staff with minimal training. Typically, these POC devices can function as standalone systems without electricity, water or cold-chain storage/transport and do not require separate processing of patient samples. Many POC instrument platforms are designed with rechargeable batteries, self-contained reagents and disposable cartridges. Near-POC systems are generally described as requiring slightly higher laboratory infrastructure (Levels 2 and 3) and technician training, often requiring electricity and a clean room for sample processing, in some cases requiring cold-chain transport of reagents. These testing distinctions are somewhat blurred in resource-limited settings, where POC devices have been implemented for programmatic interventions across a range of laboratory settings.

It is often suggested that diagnostic tests for use at POC should meet the ASSURED criteria developed by WHO for the ideal rapid test *(31)*. The ASSURED criteria are:

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. suggested: “The technology as such does not define a POC test nor determine its use at POC. Rather it is the successful use at POC that defines a diagnostic process as POC testing” *(29)*. 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.

Connectivity

POC tests are designed for remote, decentralized settings. As such, they are typically performed without direct connection to a centralized health information network, often lacking basic laboratory information technology (IT) infrastructure such as power, Internet, computer education or IT support *(32)*. While POC diagnosis can have immediate impact on patient care and treatment, it is critical that health interventions can be tracked by the overall health system. Results from a POC test are often transcribed (traditionally handwritten into a logbook), then recorded to a patient file and ultimately reported to the central health database. Each step in the transcription process can introduce errors and miscommunication. As such, it can be a challenge for health ministries to know when POC tests are performed, and accumulate health data on the outcome and impact for overall programme management.

New approaches to POC testing can take advantage of instrumentation designed for field use that can also enable information connectivity *(7,33)*. By engineering built-in battery sources and wireless communication, POC systems can take advantage of the GSM or SMS networks to transmit data automatically, often in real time. Connectivity via a cellular network means that results can be automatically uploaded to a central, secure health systems database. Automatic tracking of results and instrument performance provides information not only on health data, but also on consumable use, system operation, QC and maintenance. The use of information can assist supply chain management and procurement, as well as oversight for training, service and maintenance for field instrumentation.

On a larger scale, electronic data tracking could greatly facilitate the monitoring of patients between facilities *(22)*. Electronic data systems could make it easier to trace patients from one health-care facility to the next, provided a system for unique patient identifiers could be implemented *(34,35)*. Testing platforms with connectivity could assist monitoring of patients and potential loss–to-follow-up across facilities. With these early steps forward, access and ownership of the data need to be further addressed *(36)*. To date, each developer has proprietary software and hardware for interfacing with health networks. While the data can typically be exported in a generic file format, there is currently no industry standard for POC connectivity or encryption.

Setting and appropriate technology

In this report, the appropriate target use setting for each of the technologies, both laboratory based and those intended for use at or near POC, is considered. There are a number of laboratory-based CD4, VL and EID technologies that are suited only for Level 3 and Level 4 facilities; on the other hand, there are a number of POC technologies that are targeted at Level 1 and Level 2 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 *(37)*. 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 analyzer itself (e.g. data station; printer; barcode 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, VL 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.[17]

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 *(38)*. Misclassification probabilities, which could be upward misclassification probability or downward misclassification probability, describe the likelihood that a test will incorrectly categorize a result as above or below a given cutoff value, respectively.

[17] 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.

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 VL 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

Overview of CD4 testing

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" *(23,37)*. 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). 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.[18] 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 *(39)*.

The consensus for the most important considerations for CD4 performance are:

- 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;

[18] Glover *(37)* 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.

- 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 *(40,41)* 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. (hereinafter Beckman Coulter),[19] EMD Millipore® and Partec GmbH (hereinafter Partec).

Flow cytometry is a member of a family of technologies known as automated, analytical or quantitative cytology. 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.

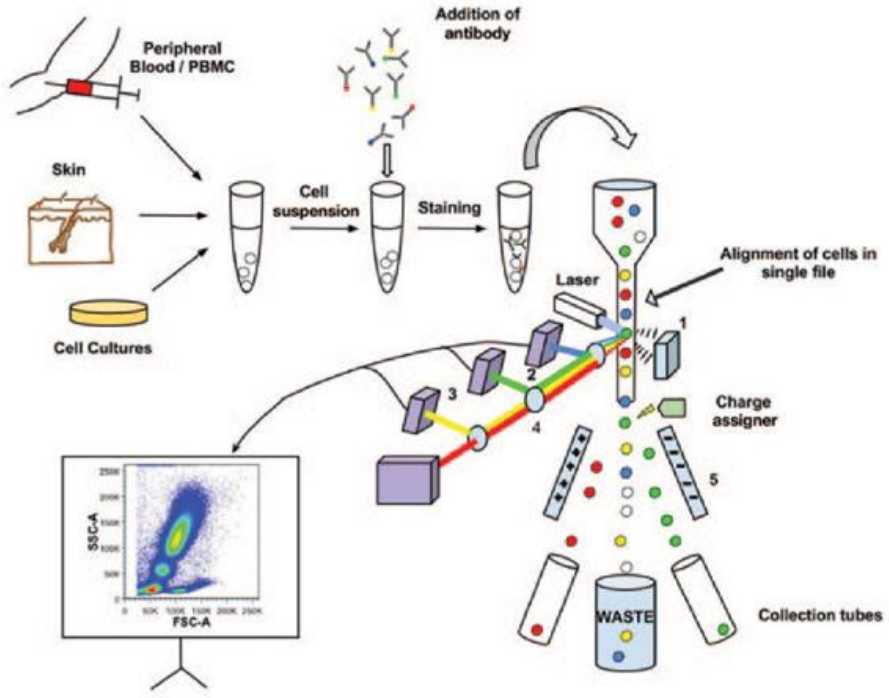
An important requirement of flow cytometry is the need to specifically label cell constituents with fluorescent molecules, which are then used to identify cells carrying this "label". Cell constituents can be made up of a number of cellular components, including DNA, which can be labelled by different 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 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) *(42,43)*. 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 *(44)*. Figure 5 presents a

[19] Beckman Coulter is a registered trademark of Beckman Coulter Inc.

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.

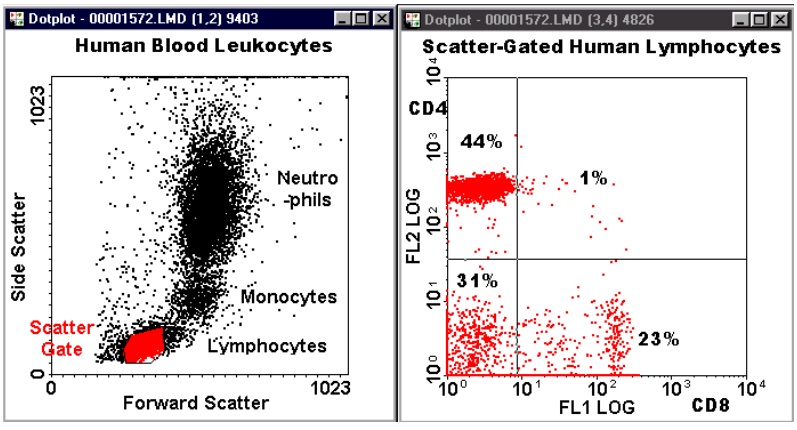
Figure 5. Schematic of flow cytometry



Source: Reprinted by permission from Macmillan Publishers Ltd. J Invest Dermatol. 2012;October;132(10):e1.

In a flow cytometer, as the fluorescing cells pass through the laser beam, the scattered light is detected, which is then converted by electronics converts into a digitized value for graphing on a two-dimensional plot. Cell data can be displayed in a number of formats, including dot plots, contour plots and density plots. Figure 6 shows two examples of dot-plot quadrant analysis for human blood lymphocytes *(30)*.

Figure 6. Examples of dot-plot quadrant analysis for human blood lymphocytes



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

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 Apogee Flow Systems (hereinafter Apogee), BD Biosciences, Beckman Coulter and Partec. In the developing world, BD Biosciences and Beckman Coulter have the largest CD4 testing market share.[20]

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 analyzer. With dual platform methodologies, either the total lymphocyte count (using the traditional method) or total white blood cell (WBC) count (using the PanLeucogating method) is obtained from the haematology analyzer. The CD4 T-lymphocyte percentage is obtained (in the traditional method) or the WBC 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 *(39)*. However, the PanLeucogating method is producing improved performance over the traditional approach *(45)*. In general, the dual platform method for CD4 enumeration is not particularly well suited to resource-limited settings because it is complex and requires significant training.

In addition to dual platform approaches to CD4 cell enumeration, there 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 Thermo Fischer Scientific 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 *(46–48)*. 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 analyzers (without the need for a microscope). For example, Dynabeads® can be used in conjunction with the pocH-100*i*™ haematology analyzer from Sysmex; and a team from Chiang Mai University has developed reagents, called CD4 Select, that can be used to enumerate CD4 cells on a haematology analyzer alone. Moderate training is required for this method of

[20] Unless otherwise noted, information on each of the CD4 technologies described 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.

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. 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,[21] 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 the 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™ platform. 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 health-care system in which the instruments can and should be used.

High-throughput laboratory platforms for CD4

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 (Multi-Carriage Loader) or Cytomics FC 500 MPL (Multi-Platform Loader). 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 4 facilities). Partec also manufactures a high-throughput CD4 platform and, due to its relative simplicity, can be used in small hospitals at the provincial and district levels (Level 2 facilities).

BD FACSCalibur™ system (BD Biosciences)

BD Biosciences manufactures the BD FACSCalibur™ system (Figure 7), which is a large, benchtop, 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 fluorescence detection and known concentrations of reference

[21] FC 500 is a registered trademark of Beckman Coulter Inc.

beads in each sample to obtain absolute T-cell concentrations *(49)*. 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 percentage 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 7. 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 *(50)*. 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.

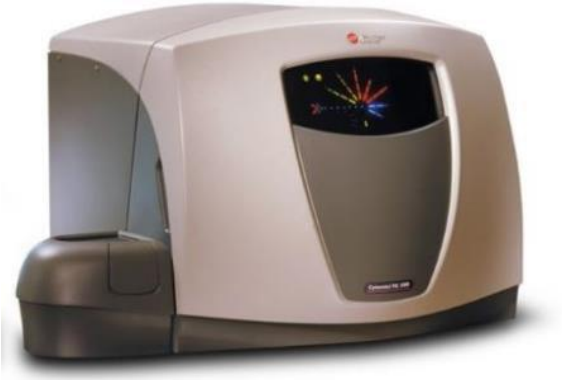
The cost of the FACSCalibur™ instrument is about US$ 75 000, but can be higher depending on the country/region, options chosen and whether there are any special negotiated prices available. For the basic three-colour reagent test (TruCount) used by most laboratories in

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 system (Beckman Coulter)

Like the BD FACSCalibur™, the Cytomics FC 500 MCL and Cytomics FC 500 MPL Systems, manufactured by Beckman Coulter, are large, benchtop flow cytometers. These systems are automated and can simultaneously analyse up to five colours of immunofluorescence from two lasers. The Cytomics FC 500 series platform (with either MCL or MPL sample loading capability) is a bead-based system that can perform absolute and percentage CD4 counts (using FlowCARE™ PLG reagents), but also can perform multiparametric DNA analysis, platelet studies, reticulocyte enumeration, cell biology/functional studies as well as a broad range of other research applications. The instrument is self-contained and biohazard safe.

Figure 8. Cytomics FC 500 system



The Cytomics FC 500 system (Figure 8) 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 or 24-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.

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; and from about US$ 5 to US$ 8 per test at volumes under 11 000 tests per instrument per annum.

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

The CyFlow® Counter (Sysmex Partec)

The CyFlow® Counter platform is intended for CD4 absolute count and CD4 percentage determination in HIV/AIDS patients (Figure 9).

The CyFlow® Counter is a compact and robust flow cytometer with a small footprint size. It is a device equipped with one green laser light source and three optical parameters for detection of side scatter channel and orange and red fluorescence signals. A sheath and waste bottle are placed outside of the equipment and, therefore, allow easy refill with carrier liquid Sheath Fluid and removal and disposal of the liquid waste. The optical components are placed on one bench and this align-free technique guarantees stable performance and easy installation of the device. The CyView™ software uses a script-based approach for Count Check Beads green, used as instrument quality check material, for CD4 absolute and CD4 percentage counting and for the cleaning procedure, which makes the handling of the overall procedure easy. A touchscreen monitor is used to keep the device compact and to avoid further accessories such as a keyboard and mouse, which can be installed using a USB connection. The CyFlow® Counter has a built-in thermal printer for printing results on request and it is supplied with accessories and a premium starter kit. The uninterruptible power supply (UPS) is necessary to protect the equipment from unexpected power fluctuations.

The CyFlow® Autoloading and Autopreparation Station (CyFlow® ALAPS II) is an automated sample preparation and loading system for the CyFlow® Counter. The CyFlow® ALAPS II operates independently once the sample tubes rack has been loaded. Sample preparation, incubation, mixing before loading and loading is fully automated. Each sample is measured and a cleaning mode starts automatically after the last sample.

Both for CD4 absolute count only and for CD4 absolute and CD4 percentage determination, the CyFlow® Counter is used in combination with the CD4 or CD4% easy count kits in dry or liquid format. The system is designed for a flexible sample throughput from single sample run up to 20 samples per hour. The CyFlow® Counter, the CyFlow® ALAPS II, the reagents mentioned above, the control materials and disposables are developed, produced and distributed by Sysmex

Partec GmbH as legal successor of Partec GmbH. Legal manufacturer of these products is Sysmex Partec GmbH with headquarters and production site in Görlitz, Germany.

The settings and working conditions in developing countries and emerging markets have been taken into consideration since the early stages of products development. Among those are environmental (temperature, humidity and dust) as well as infrastructure (instable power supplies) and labour (level of education and high turnover of laboratory personnel). Sysmex Partec GmbH experience gained in these settings with the forerunner models CyFlow® Counter I and CyFlow® SL_3 have contributed to the current level of development of the CyFlow® Counter.

Figure 9. CyFlow® Counter and CyFlow® ALAPS II

Published, peer-reviewed literature is available on the performance of the CyFlow® *(51–56)*.

Moderate-throughput platforms for CD4

BD FACSCount™ system (BD Biosciences)

The BD FACSCount™ system (Figure 10) 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 benchtop instrument, reagents and controls.

Figure 10. 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 under 5 years, as discussed earlier in this report) using a single-tube assay (Figure 11).

Figure 11. 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 *(57,58)*. 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 has recently decided not to pursue the development of the BD FACSClearCount™ system. However, next generation medium- to high-volume CD4 testing remains a priority for BD Biosciences.

Aquios CL™ (Beckman Coulter)

The Beckman Coulter Aquios CL™ Flow Cytometer with Tetra (4 colour CD4) was launched in 2014 (Figure 12). The PLG-CD4 application was launched in May 2015.[22] 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, then every 2 minutes thereafter.[23]

Figure 12. Aquios CL™ flow cytometry platform

In addition, the Aquios CL™ includes the SmartTrack reagent system. Reagents are preloaded with a range of barcoded reagents and consumables. The system automatically scans barcodes to track reagents, lot numbers and open and closed vial expiration dates, for example. There is continuous tracking of reagent usage per 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.

[22] Not available in the United States and certain other countries.

[23] Measured with Tetra-1 or Tetra-2+.

The Aquios CL™ system, which is a benchtop 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 increase automation of the most routine, repetitive tests, such as absolute CD4 and CD4%. Different specimen cassettes are available to accommodate a variety of blood collection tubes.

Apogee Auto40 Flow Cytometer (Apogee)

The Apogee Auto40 Flow Cytometer, manufactured by Apogee is a benchtop, 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 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 *(59,60)*.

POC platforms for CD4

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 POC.

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, including the Pima™ Analyser, Partec CyFlow® CD4 miniPOC and the BD FACSPresto™. The remaining technologies discussed, including those from Omega Diagnostics Ltd and others, are not yet available on the market. Due to shifting market forces, some CD4 products have been discontinued, as noted below.

Pima™ Analyser (Alere)

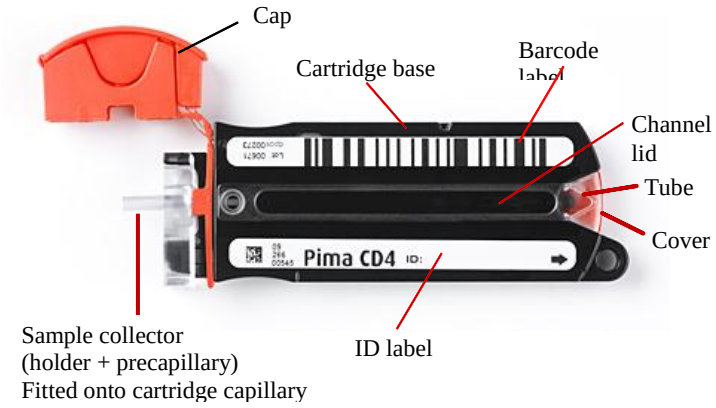
The Pima™ Analyser (Figure 14, with printer) is a small, portable benchtop, fixed-volume cytometer manufactured by Alere. The Pima™ Analyser employs the same immunological principles as existing CD4 enumeration systems combined with static image analysis and counting technology in a compact, portable and robust housing. A separate printer also is available.

The Pima™ CD4 system is made up of the Analyser and a disposable CD4 test cartridge (Figure 15) 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. 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 to 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.

Figure 14. Pima™ Analyser

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 15. Alere 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 via 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 16) and Alere™ SIM cards, providing the user with real-time

metrics for inventory planning and control, quality management and technical troubleshooting.[24]

Figure 16. Alere Datapoint™

A power extender (Figure 17), including an extended-life battery and adaptors for charging sources, including solar panels and mains, has been added to the product family.

Figure 17. Alere 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. Independent evaluations of the Pima™ system have been published since product launch *(54,61–80)*. 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]" *(65)*.

[24] For more information, see: http://alerehiv.com/connectivity.

CyFlow® CD4 miniPOC (Sysmex Partec)

The CyFlow® miniPOC is a very compact and portable CD4 counter (Figure 18). It is a flow cytometer using the same technology of the CyFlow® Counter such as fluidic and electronic. The CyFlow® miniPOC is designed for counting CD4 absolute and CD4% in people who are living in limited-resource areas. The instrument can run on main power supply, and it is also possible to connect it to a car battery or use a special battery pack with operation duration of 4–5 hours. The battery pack can be recharged using a foldable solar panel. The CD4 and CD4% testing kit contains all the consumables such as sheath fluid, Count Check Beads green for instrument checkup, cleaning solutions and disposables such as pipette tips and thermal printer paper.

Figure 18. CyFlow® CD4 miniPOC

CD4 absolute count and CD4 percentage determination is obtained by analysing one sample run. Prefilled reagent tubes for sample staining and preparation reduces pipetting steps. The sample is drawn in a syringe and a specific volume of the sample is transferred into the system via a step motor. At the end of each measurement, an automated analysing process starts providing the results of the sample run.

Peer-reviewed, independent performance evaluations of the CyFlow® miniPOC are 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 percentage count and haemoglobin (Hb) all on the same single-use disposable cartridge (Figure 19). Features of the automated device include touchscreen 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 19. BD FACSPresto Platform

Source: Photo courtesy of 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.

The CE-IVD marked BD FACSPresto™ was launched on 28 March 2014 and was WHO prequalified as of late September 2014. The price of the instrument is less than US$ 10 000 and the price per test of the assay is less than US$ 10 in resource-limited settings.

Millipore Muse® (Merck)

EMD Millipore (a division of Merck) is developing a new platform, the CD4/CD4% Muse® cell analyzer (Figure 20). The Muse® cell analyzer 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.

The Muse® cell analyzer is easy to use, requiring only pipetting and operating the software on the analyzer. 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 in 2–4 minutes.

Figure 20. Muse® cell analyzer

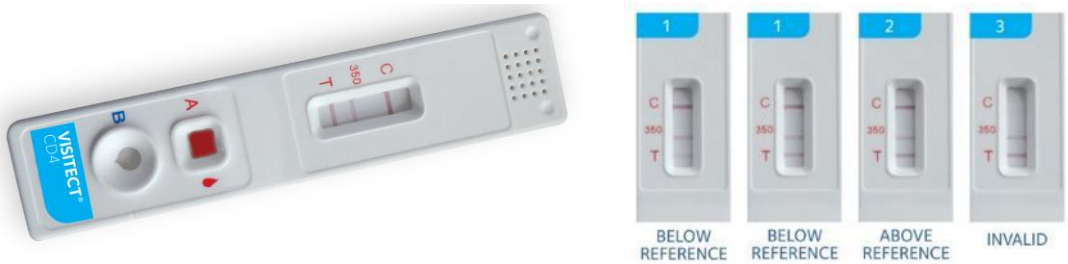
The Muse® Auto CD4/CD4% system will have two power sources. In the clinic laboratory, the Muse® system can be plugged into a UPS. However, for portability, the Muse® system offers an optional battery pack that will provide hours of operation. In addition, the system will have two new optional battery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

The Muse® Auto CD4/CD4% system is in final validation and is expected to obtain CE-IVD marking and be released in 2015–2016. 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).

Visitect® CD4 (Burnet Institute and Omega Diagnostics Ltd)

The Burnet Institute has licensed its semiquantitative CD4 technology to Omega Diagnostics Ltd (United Kingdom). The platform, which is now called the Visitect® CD4, is a rapid, disposable semiquantitative 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 RDT) with traditional rapid test format, including monocyte removal pad and immunogold conjugate (Figure 21).

Figure 21. 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 21, right), 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 22), 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 22. 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). Omega Diagnostics Ltd plans to conduct further trials of its Visitect® CD4 assay in southern Africa, the United Kingdom and India in the latter half of 2015. The company intends to seek CE-IVD marking for the assay once successful product evaluations have been completed. The per-test cost of the assay at release is expected to be about US$ 5.

Daktari™ CD4 Counter (Daktari Diagnostics Inc. [hereinafter Daktari])

Daktari has developed a portable and robust CD4 device, the Daktari™ CD4 Counter (Figure 23, with associated cartridge). The Daktari system will be capable of other assays, including HIV VL and HCV diagnosis; future tests may include full blood counts, CD4 percentages and bacterial diagnostics. Intended for use at POC, the Daktari system eliminates sample preparation through the use of a technology known as “microfluidic immunochromatography”, which isolates cells (or viruses) in a miniature sensing chamber. No pipetting, labels or reagents are required; the only user step is to apply one 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.

Figure 23. Daktari™ CD4 Counter

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$ 10, 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.

Independent validation studies on the Daktari™ system were completed in Kenya and Uganda in 2013 and the Daktari™ CD4 count test completed validation studies in Kenya in 2014, with additional studies expected in several countries in eastern and southern Africa in 2015. Daktari is in the process of completing the additional verification and validation studies for the CE Mark, and a completed WHO prequalification dossier is anticipated in 2015.

As a result of the global uncertainty in policy around CD4 testing and in the CD4 market, Daktari has recently decided to discontinue expansion of manufacturing capacity for the CD4 Counter cartridges, which would be necessary to drive costs and prices down to affordable levels. Daktari continues to pursue development of POC patient testing for HIV and HCV, and is a willing partner for commercialization of its POC CD4 system with other entities.

ChipCare-CD4 (ChipCare Corporation)

ChipCare Corporation is developing a mobile, easy-to-use, laboratory-quality blood testing platform for CD4 testing (Figure 24). The platform uses disposable cartridges that leverage recent advances in microfluidic and biomarker technologies to provide cell surface and blood

analyte tests. From a 20 µL sample of blood, health-care workers in remote health settings will be able to rapidly and accurately perform tests to diagnose or monitor a range of infectious diseases. The ChipCare Corporation initial test – absolute CD4 count – will stage HIV-positive patients for treatment. Research on blood analyte tests for neglected tropical diseases, sexually transmitted infections (STIs) and vaccination coverage is ongoing.

Figure 24. ChipCare hand-held platform

Designed for community-level health-care workers in remote or rural settings, the ChipCare-CD4 will weigh less than 2 kg and will be small and rugged enough to be carried in a small backpack. Time to result for the CD4 test will 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, refrigeration 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$ 5000 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.

MBio CD4 System (MBio Diagnostics Inc.)

Mbio Diagnostics Inc. has active development projects in Kenya and Mozambique, but has placed its launch schedule for CD4 on hold.

PointCare NOW™ (PointCare Technologies Inc.)

PointCare Technologies Inc. appears to be no longer in business.

CD4 Test (Zyomyx Inc.)

The Zyomyx Inc. CD4 test is no longer on the market, and Zyomyx Inc. has closed down as an operating company.

EID technologies

Overview of EID testing

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 DNA or RNA PCR testing) or ultrasensitive p24 antigen testing should be used to determine the HIV status of infants in that age group. 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 *(2).*[25]

EID by virological testing

Until recently, the most widely used test for EID was the DNA PCR molecular test. This qualitative HIV-1 DNA test detects the presence of HIV pro-viral 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 VL, but rather provides a qualitative "yes" or "no" answer with respect to whether the infant is infected with the HIV virus. The DNA PCR tests, similar to 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.

It is also possible to use VL (RNA) assays for initial diagnosis of HIV infection in infants, and the technologies discussed in the previous section on VL testing should also be considered viable options for EID. There are currently several validated HIV assays available for use in EID for high-throughput laboratory and POC platforms; these assays are presented below.

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[25] 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 *(2)*.

EID technologies/platforms

High-throughput platforms for EID

There are currently two laboratory platforms that have validated HIV-1 assays available in resource-limited settings that are used for EID (including DBS): the Roche Molecular Diagnostics (hereinafter Roche) COBAS® HIV-1 Qualitative Test and the Abbott RealTi*m*e Qualitative HIV-1 Test, both of which have CE-IVD marking (the Hologic Gen-Probe Inc [hereinafter Hologic] DBS test in development). 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*2000*rt* amplification system, using the *m*2000*sp*, *m*24*sp* or manual sample preparation. Technical specifications for these assays are set forth in Table 1.

Table 1. Technical specifications for commercial tests for EID

Assay name	**COBAS® TaqMan® HIV-1 Qualitative Test v2.0**	**Abbott RealTi*m*e Qualitative HIV-1 CE-IVD**
Type of assay	Real-time PCR, qualitative identification of HIV-1 DNA and RNA (total nucleic acid, TNA)	Real-time PCR, qualitative detection of HIV-1
HIV subtypes amplified	HIV-1 Group M, subtypes A through H; HIV-1 Group N, HIV-1 Group O	HIV-1 Group M subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, subtype H and Group N, and Group O
Intended use	HIV-1 infant diagnosis; adult aid in diagnosis	Aid in the diagnosis of HIV-1 infection in paediatric and adult subjects
Specimen type	1.0 mL plasma 70 µl DBS; 1 spot/test	200 µL plasma 0.1 mL for DBS (2 spots 50 µL each)
Limit of detection	Plasma: 16.5 cp/mL DBS: 222 cp/mL	110 cp/mL for plasma 2500 cp/mL for DBS
Sensitivity	N/A	100% for plasma 100% for DBS
Specificity	100%	100% for plasma 100% for DBS
Contamination control	Amperase	Not required due to system design
Controls	Run-in (negative, positive) internal control	Run-in (negative, positive) internal control
HIV genome amplified	Gag and LTR	Pol/INT
Time for result	5–6 hours	5.5–8 hours, depending on batch size
Number of samples/run	22–66 batch loading (176/8-hour day continuous loading)	1–94 patient samples (+2 external controls)
Cost/test[26]	US$ 10–16 per test in resource-limited settings; US$ 16–30 per test elsewhere	Refer to the Global Fund website for further information

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

Equipment required[27]	COBAS® AmpliPrep®, COBAS® TaqMan® 96, COBAS® TaqMan® 48	*m*2000*rt*; *m*2000*sp*, or manual sample preparation
Regulatory status	CE-IVD, WHO prequalified	WHO prequalified CE-IVD marked

COBAS® 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® HIV-1 Test v2.0.[28] The assay uses the AmpliPrep® instrument for automated viral nucleic acid extraction and the COBAS® TaqMan® analyzers (TaqMan® 48 or TaqMan® 96), both of which are discussed below, for automated amplification and detection of the viral nucleic acid target.

COBAS® TaqMan® HIV-1 Qualitative Test v2.0

The COBAS® AmpliPrep®/COBAS® TaqMan® HIV-1 Qualitative Test v2.0 is an IVD, TNA amplification test for the qualitative detection of HIV-1 DNA and RNA (or total nucleic acid, TNA) in human EDTA plasma or DBS in combination with the COBAS® AmpliPrep®/COBAS® TaqMan® Specimen Pre-Extraction Reagent (SPEX). The test is a diagnostic test, indicated for individuals who are suspected to be actively infected with HIV-1. Detection of HIV-1 TNA is indicative of active HIV infection. Infants born to mothers infected with HIV-1 may have maternal antibodies to HIV-1, and the presence of HIV-1 nucleic acid in the infant indicates active HIV-1 infection. In adults, the test may be used as an aid in the diagnosis of HIV-1 infection.

The COBAS® AmpliPrep®/COBAS® TaqMan® HIV-1 Qualitative Test v2.0 is based on three major processes: (i) specimen preparation to isolate HIV-1 nucleic acids; (ii) reverse transcription of target RNA to generate complementary DNA (cDNA); and (iii) PCR amplification of target DNA and cDNA, and simultaneous detection of cleaved dual-labelled oligonucleotide detection probes specific to the target and internal control. The workflow is automated using the COBAS® AmpliPrep® Instrument with the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 96 Analyzer. For DBS specimens, a manual pre-extraction step is required. The test is performed using the COBAS® AmpliPrep® Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer for automated amplification and detection, as described above. The Roche HIV-1 Qualitative Test simultaneously amplifies and detects two separate regions of the HIV-1 genome, which are not subject to selective drug pressure. This unique, dual target design allows for more reliable results to confidently and effectively diagnose HIV-1 infection. HIV subtype detection includes Group M subtypes A-H, CRF01-AE, Group N, and Group O. The lower limits of detection for the COBAS® AmpliPrep®/COBAS®

[27] Each of the assays requires pipettes, a vortex mixer and a refrigerator.

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

TaqMan® HIV-1 Qualitative Test is 16.5 cp/mL for plasma samples, and 222 cp/mL for whole blood samples using the DBS procedure.

The COBAS® AmpliPrep® Instrument is an automated sample preparation technology (Figure 25) for use in conjunction with the Roche COBAS® TaqMan® analyzers discussed below. The company considers the AmpliPrep® to provide "walk-away" sample preparation/extraction capability, which can significantly reduce hands-on time of laboratory technicians.

Figure 25. 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 2 hours to process. However, because the instrument allows for parallel processing, subsequent batches of 24 can be completed every hour as one rack of specimens will begin processing before the previous rack processing has been completed. The system is closed and requires the use of test-specific, barcoded, 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).

Roche manufactures two versions of its TaqMan® Analyzer: the COBAS® TaqMan® 96 Analyser and the COBAS® TaqMan® 48 Analyzer. Each of the analyzers is a fully automated, closed-tube system. The COBAS® TaqMan® 96 (Figure 26) is a large instrument, weighing about 450 lbs.[29] It has higher capacity and can run up to 96 samples at a time in a run time of approximately 180

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

minutes with automated transfer from the COBAS® AmpliPrep® via a docking station. The TaqMan® 48 (Figure 27) is relatively compact and can run 6–48 samples at a time. The instrument is equipped with two thermal cyclers that operate independently and provide run times of 90–120 minutes.

Figure 26. COBAS® TaqMan® 96

The cost of the COBAS® TaqMan® 96 Analyzer is approximately US$ 100 000–110 000, which includes the cost of a docking station.

Figure 27. COBAS® TaqMan® 48

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

In September 2014, Roche announced a global access programme for certain organizations in eligible low- and low middle-income countries and/or those with a high disease burden. The cost per test for EID reagents and consumables is US$ 9.40, but may vary depending on local

conditions.[30] In addition, pricing will vary depending on factors such as outright instrument purchase, reagent rental and volume-based, tiered pricing arrangements.

*m*2000 System (Abbott Laboratories [hereinafter Abbott])

Abbott is the manufacturer of the quantitative RealTi*m*e HIV-1 VL and qualitative RealTi*m*e HIV-1 assays. Both assays are based on the RT-PCR technology and can be fully automated on either *m*2000 or *m*24 systems. The RealTi*m*e HIV-1 qualitative assay allows for qualitative detection of HIV-1 in plasma and DBS, which can be 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 a highly conserved integrase region of the polymerase (or pol) gene. The combination of the unique probe design (a partially double-stranded probe) and cycling conditions ensure a high degree of mismatch tolerance that allows the assay to reliably detect HIV-1 groups and subtypes. The Abbott RealTi*m*e HIV-1 assay uses an external calibration strategy shown to demonstrate high precision across the linear range and especially at the clinical decision points. A more precise assay can help identify virologic failure earlier and determine when therapy is failing or drug resistance may be developing. In a recent study, at VL of 50 cp/mL the relative odds of the Abbott RealTi*m*e HIV-1 assay identifying confirmed virologic failure in patients was three times higher (6%; 23/389) than a comparator assay (2%; 8/389) p=0.007 *(81)*.

RealTime HIV-1 Qualitative test

The Abbott RealTi*m*e HIV-1 Qualitative test is an in vitro amplification assay for the qualitative detection of human immunodeficiency virus type 1 (HIV-1) nucleic acids from human plasma and DBS. The RealTi*m*e HIV-1 Qualitative test is intended to be used as an aid in the diagnosis of HIV-1 infection in paediatric and adult subjects (not intended to be used as a donor screening test for HIV-1). HIV subtype detection includes Group M subtypes A, B, C, D, CRF01-AE, F, CRF02-AG, G, subtype H and Group N, and Group O. The lower limits of detection for the RealTi*m*e HIV-1 Qualitative test is 110 cp/mL for plasma samples and 2500 cp/mL for whole blood samples using the DBS procedure. The test is performed using the *m*2000 system using manual sample preparation or the *m*2000*sp*, as described below.

The Abbott RealTi*m*e assays are designed to be used with the *m*2000*rt* 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).

[30] For details, see: http://molecular.roche.com/globalaccessprogram/Pages/default.aspx.

The Abbott *m*2000*sp* (Figure 28) is a 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 barcoded laboratory tube through patient result. *m*Plus extended reagent use is offered for more efficient reagent use with low volumes.

Figure 28. *m*2000*sp* instrument

The *m*24*sp* (Figure 29) is a benchtop 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 29. *m*24*sp* instrument

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 30) can run 96 samples at a time in about 3 hours of cycling time for HIV-1 assays (not including time for sample preparation).

Figure 30. *m*2000*rt* instrument

The system will run both quantitative and qualitative analyses and offers key validity parameters such as maxRatio. Like other laboratory-based VL systems, the operator must have a thorough knowledge of the applications run on the instrument (and on the sample preparation instrument) and must follow good laboratory practices when operating them.

The RealTi*m*e HIV-1 is included in a Global Fund framework agreement as part of an expanded assay menu – together with HIV EID, MTB, HBV, HBC, human papillomavirus (HPV) and *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CT/NG) – at the same low access price. Abbott offers scale-up planning as well as assistance with scale-up, including training and performance monitoring based on country needs. Terms vary dependent upon volumes and specific needs.

POC platforms for EID

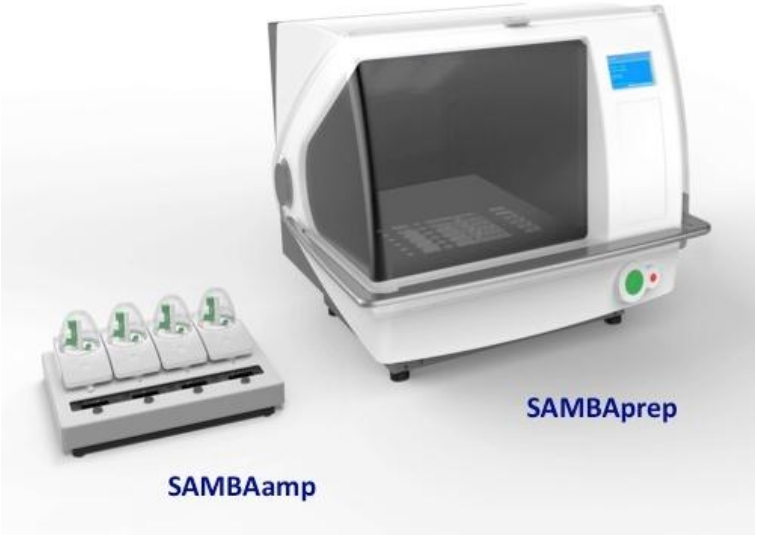
SAMBA platforms (Diagnostics for the Real World Ltd)

SAMBA has been developed by a team led by Dr Helen Lee, Director of the Diagnostics Development Unit at the University of Cambridge. Its spinoff company, Diagnostics for the Real World Ltd, is located in California and is the manufacturer of the SAMBA system. Four nucleic acid-based test (NAT)-based HIV assays have been developed: (i) a semiquantitative test with a cutoff of 1000 cp/mL for monitoring ART using plasma; (ii) a semiquantitative 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.

SAMBA I system

SAMBA I (Figure 31) is for semi-automated batch testing with a throughput of 16–48 samples per day (see the SAMBA I product profile). It automates extraction (SAMBAprep) and integrates amplification and detection (SAMBAamp) into a benchtop analyzer with amplification and detection taking place in a hermetically sealed cartridge.

Figure 31. SAMBA I system



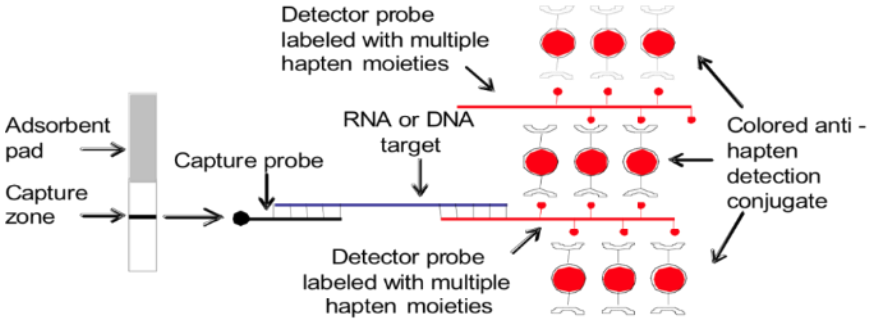
SAMBA II system

SAMBA II (Figure 32) is a fully automated "sample-in, result-out" system. It is suitable for both low- and medium-volume testing sites with the throughput of 4–32 samples per day. A display unit controls the assay module and each display unit can control up to eight assay units. It is a modular, random access system that allows the throughput to be adjusted as per the requirements of the site. A phone module can also be used as the controller to run up to two assay modules.

Figure 32. SAMBA II system

The SAMBA HIV tests use 500 µL of plasma or 100 µL of whole blood 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 hermetically sealed cartridge to prevent amplicon contamination. Amplification targets the long-terminal repeat (LTR) region of the HIV genome, which is detected on a lateral flow strip using the Diagnostics for the Real World Ltd patented SAS technology (Figure 33).

Figure 33. 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 ensure visual detection of the RNA or DNA target, which can be visually read off of a test strip within 25 minutes. The visual detection 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 clinical evaluations for EID have taken place:

- 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 Roche COBAS® AmpliPrep®/COBAS® TaqMan® carried out by laboratories in Uganda and Zambia; field evaluation using infant blood completed in MU-JHU, Uganda; KEMRI/CDC, Kisumu, Kenya; and NMRL, Harare Hospital, Zimbabwe;
- evaluations on EID are currently ongoing in Malawi and Nigeria (EID only).

Currently, the total assay time is 2 hours for the SAMBA EID assay. The SAMBA II system is best suited for use at Level 2 facilities or in large clinics (Level 1 facilities) in sub-Saharan Africa where laboratory technicians and electricity are available.

The SAMBA EID assay has recently received product approval in Kenya, Uganda and Zimbabwe. The assays are currently being evaluated in a number of additional countries in sub-Saharan Africa, including Nigeria. CE Mark is anticipated in 2015. Pricing information is available from the company and is volume dependent.

Alere™ q HIV-1/2 Detect (Alere™)

The Alere™ q system (Figure 34) is a generic platform for the implementation of nucleic acid testing. The first test on this platform was recently commercialized and is an integrated test for the qualitative detection of HIV-1 and HIV-2 simultaneously from 25 µl of whole blood. The Alere™ q device 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 34. Alere™ q instrument (left) and HIV-1/2 Detect cartridge (right)

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 company expects sensitivity and specificity will be comparable to current virological testing reference technologies (e.g. Roche 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. 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 analyzer, thus greatly reducing any possibility for cross-contamination. The actual hands-on time for the device is expected to be less than 3 minutes (i.e. sample collection and loading of the cartridge onto the analyzer).

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 analyzer; and (iv) entering the operator and sample IDs on the analyzer. 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 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.

The Alere™ q analyzer has been CE-IVD marked since July 2014; the HIV-1/2 Detect assay received CE marking in March 2015 for qualitative detection of HIV-1 and HIV-2. So far, data from one study using the prototype assay have been published *(82)*. The HIV Detect cartridge and analyzer are also currently undergoing the WHO prequalification process. Additional tests in development include the quantitative detection of HIV-1 and HIV-2 simultaneously from 25 µl of whole blood, a Filovirus (Ebola and Marburg viruses) assay and others.

GeneXpert® System (Cepheid)

The Cepheid GeneXpert® System is a fully automated and integrated system for PCR-based nucleic acid testing (Figure 35, left). The GeneXpert® System integrates and automates sample preparation, amplification and detection in a single-use, self-contained cartridge (Figure 35, right). Most liquids and dry reagents, along with enzymes, are prefilled so that pre-analytical steps are minimized, greatly reducing opportunities for sample mix-ups and operational errors. GeneXpert® cartridges can handle a variety of sample volumes (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. The GeneXpert® system is available in a 1, 2, 4 or 16-module configuration.

Figure 35. GeneXpert® 4-4 module instrument (left) and Xpert® HIV-1 Qual cartridge (right)

The GeneXpert® System is sufficiently simple so that training can usually be completed within half a day. Furthermore, although the system was designed to use AC 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 a laboratory information system (LIS).

Cepheid also sells the GeneXpert® Infinity-48 and GeneXpert® Infinity-80 systems, which can incorporate up to 48 and 80 modules, respectively. The GeneXpert® Infinity systems include an automated cartridge-handling feature that utilizes a robotic arm than is not available on the other systems. The GeneXpert® Infinity-80 can accommodate more than 183 samples onboard at one time and process more than 2000 samples per day for high-throughput laboratory applications.

Cepheid has recently developed a portable version of the GeneXpert® instrument called the GeneXpert® Omni (Figure 36). Leveraging existing cartridge technology, the GeneXpert® Omni expands accurate clinical molecular diagnostic testing into disseminated locations around the world that demand portability, connectivity and ease of use. Approximately 23 centimetres tall (about 9”) and 1 kg (2.2 lbs), the GeneXpert® Omni provides the freedom to operate, deliver and store real-time PCR test results in and outside the laboratory environment without direct power supply or Internet connectivity. At the heart of the GeneXpert® Omni, solid state digital electronic architecture enhances durability, portability and connectivity, while lowering power consumption. The GeneXpert® Omni has a simple, intuitive user interface driven by a dedicated mobile device (included) with wireless and web-enabled connectivity to transmit instrument and test information in real time.

Figure 36. GeneXpert® Omni

The GeneXpert® Omni can be powered with an AC-DC power supply or via battery that has the capacity to operate it for up to 4 hours without an external power source. A supplemental external rechargeable battery (included) can extend operation for up to an additional 12 hours,

providing up to 2 days of total run time without electricity. Due to its closed design, the GeneXpert® Omni is operable in resource-limited settings and is designed for heat, humidity and dusty environments. The GeneXpert® Omni standalone, single module system will run the same PCR-based cartridge tests in the Cepheid assay menu, including assays for Xpert® MTB/RIF Ultra, Xpert® HIV-1 Qualitative, Xpert® HIV-1 Viral Load, Xpert® HCV Viral Load, and Xpert® Ebola in 2016, Xpert® CT/NG and Xpert® HPV in 2017, and the full Xpert® menu by 2018. The GeneXpert® Omni system is expected to be available outside the United States for emerging market customers in the first half of 2016 at a price point of US$ 2895.

The GeneXpert® System currently has 16 FDA-cleared and 22 CE-IVD-approved assays.[31] CE-IVD tests include Xpert® MRSA, Xpert® SA Nasal Complete, Xpert® MRSA/SA SSTI, Xpert® MRSA/SA BC, Xpert® C. difficile, Xpert® vanA/vanB, Xpert® Norovirus, Xpert® Carba-R, Xpert® Flu/RSV XC, Xpert® Flu, Xpert® EV, Xpert® MTB/RIF, Xpert® TV, Xpert® HPV, Xpert® CT/NG, Xpert® CT, Xpert® GBS, Xpert® BCR-ABL Monitor, Xpert® FII and FV, Xpert® HIV-1 Viral Load, Xpert® HIV-1 Qual and Xpert® HCV Viral Load. In addition to the tests listed, Cepheid has 14 tests in active development including Bladder CA Monitor/Sympt, BCR-ABL Ultra, SA Nasal Complete-Next Gen, Group A/C Strep, Meningitis/Encephalitis, Pertussis, GI Panel, HSV 1/2 Typing, Vaginitis, GBS Ultra, HBV Quant, Breast CA Stratifier, Breast CA Signature. Any of these tests can be run on virtually all of the more than 9000 GeneXpert® systems placed worldwide.

Xpert® HIV-1 Qual Test

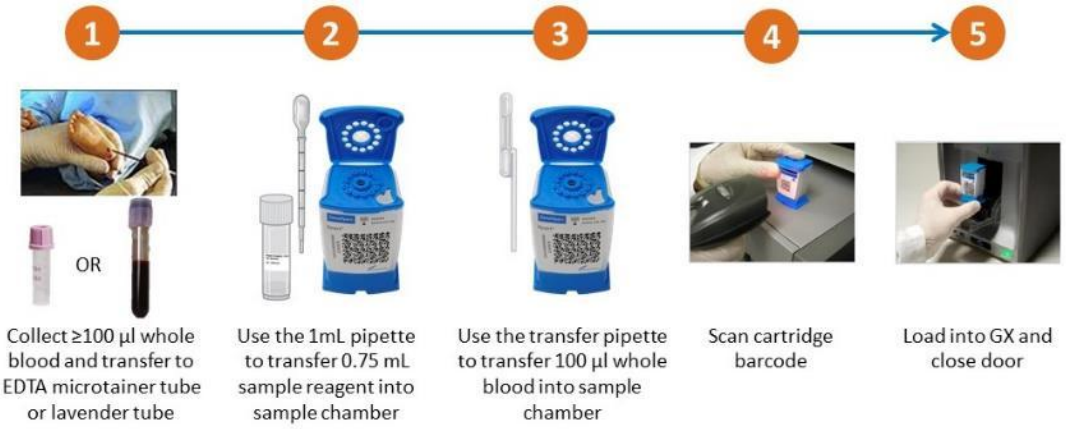
The Xpert® HIV-1 Qual assay (using whole blood and DBS specimens) targets one genomic region of HIV-1 that is proven both in silico and in vitro to detect the vast majority of all HIV-1 strains independent of group and subtype. The forward and reverse primer and the TaqMan® probe are located in the most 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 the Cepheid 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, A/E, CRF AG/AH and Group N and Group O. The Xpert® HIV-1 Qual assay has a limit of detection (LOD) of 203 cp/mL (VQA reference material) in 100 µL of whole blood and an LOD of 531 cp/mL (VQA reference material) with one DBS specimen. Each test includes a Sample Volume Adequacy (SVA), a Sample Processing Control (SPC) and Probe Check Control (PCC). There are two workflows for the Xpert® HIV-1 Qual assay: one for whole blood and one for DBS.

For whole blood specimens (Figure 37): (i) collect whole blood in an EDTA microtainer or EDTA tube; (ii) transfer 750 µl of sample reagent with the pipette provided directly to the Xpert® HIV-1 Qual cartridge; (iii) transfer 100 µl of the whole blood with the micropipette provided directly

[31] For more information, see http://cepheid.com/en/cepheid-solutions-uk/clinical-ivd-tests/healthcare-associated-infections

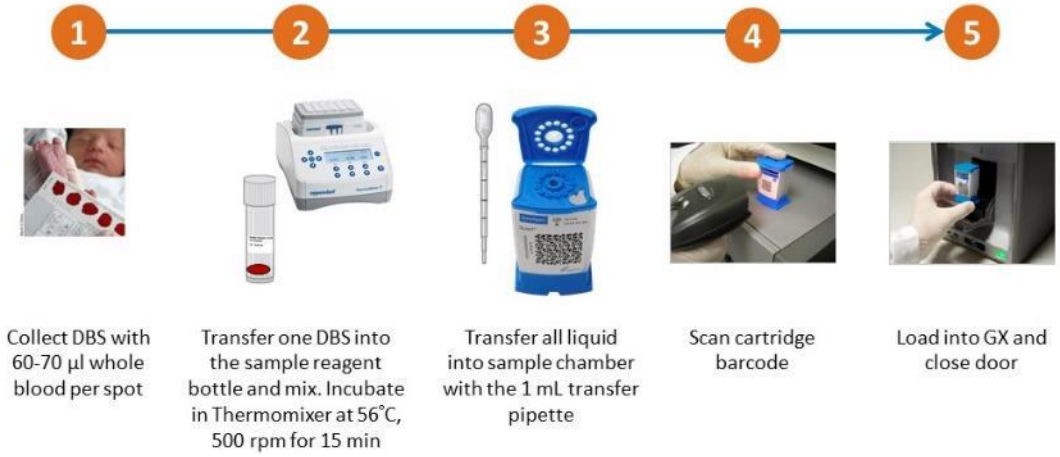
into the cartridge; (iv) scan the cartridge barcode; and (v) load the cartridge into the GeneXpert® module and close the door with a 90-minute time to result.

Figure 37. Xpert® HIV-1 Qual Whole Blood Workflow



For DBS (Figure 38): (i) collect DBS with whole blood; (ii) add DBS to the provided sample reagent vial and mix, incubate in Thermomixer for 15 minutes at 56 °C and rotate at 500 revolutions per minute (rpm); (iii) transfer all of the mixture with the pipette provided directly into the Xpert® HIV-1 Qual cartridge; (iv) scan the cartridge barcode; and (v) load the cartridge into the GeneXpert® module and close the door with a 90-minute time to result.

Figure 38. Xpert® HIV-1 Qual Dried Blood Spot Workflow



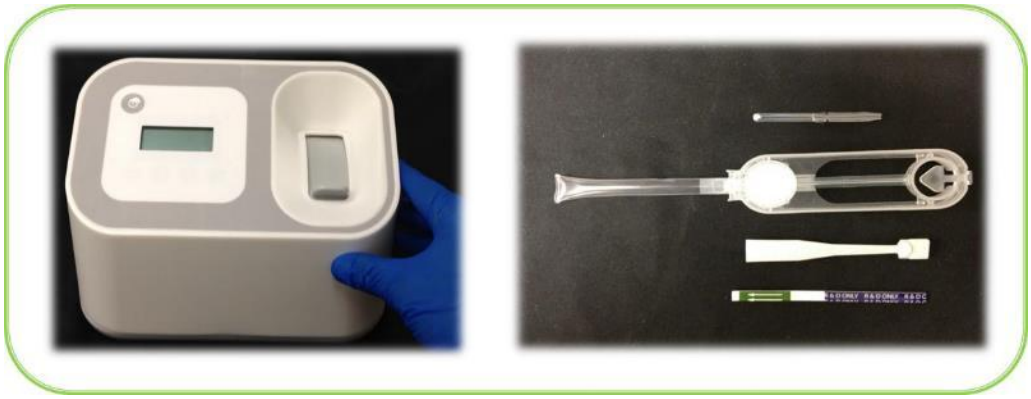
The Cepheid Xpert® HIV-1 Qual test received CE-IVD clearance in April 2015.

LYNX HIV p24 Antigen Assay (Northwestern Global Health Foundation [NWGHF])

NWGHF is developing an ultrasensitive p24 antigen rapid lateral flow assay for use at POC. The LYNX technology (Figure 39) 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 39. LYNX HIV p24 Antigen Assay and processor



The assay procedure involves: (i) collecting about 80 µL of heelstick blood from the infant using a blood collection tube; (ii) separating plasma from the sample; (iii) adding buffer to the sample and "heat shocking" it in a small, battery-powered processor device; (iv) inserting the rapid test strip into the device; and (v) waiting approximately 30–40 minutes to read the result. The total assay duration is about 45–50 minutes (Figure 40).

Figure 40. 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 2015. In 2015, NWGHF will: (i) evaluate the laboratory and field performance of the LYNX p24 system with the addition of an automated reader in 2015; (ii) obtain ISO 13485 certification; and (iii) prepare for high-volume manufacturing of the LYNX p24 system.

Q-POC™ (QuantuMDx)

QuantuMDx is developing the handheld Q-POC™ device to provide gold standard molecular diagnostic testing to populations who urgently need a rapid, cheap and accurate way of diagnosing disease and drug resistance. QuantuMDx strives to address the humanitarian health challenges such as malaria, tuberculosis, HIV and cancer. The Q-POC™ is a small battery-operated handheld unit that can fit into a laboratory coat pocket (Figure 41). Q-POC™ has an intuitive graphical touchscreen interface (GUI) and is used with disposable cartridges that contain all the reagents and sensors required to process samples, including urine, blood, plasma, sputum and swabs. Q-POC™ can provide a molecular diagnosis in less than 20 minutes, depending on the assay. Operation is simple: input the sample into the cartridge; insert the cartridge into the device; and press go. The result will be presented onscreen.

Q-POC™ incorporates a novel and rapid mechanical lysis technology, allowing DNA to be rapidly released from a range of biological samples. Lysis occurs in less than 1 minute; sample homogenization in less than 2 minutes prior to automated nucleic acid extraction. The Q-FILTER™ captures cellular debris in a lysed sample, allowing the DNA to continue flowing through the fluidic channel in solution. This simple methodology rapidly purifies PCR-quality DNA for amplification in less than 3 minutes. The fluidics of Q-POC™ are simple, robust and reproducible. The QuantuMDx proprietary continuous flow fluidic thermal cycler achieves rapid cycling (28 PCR cycles in approximately 10 minutes).

Figure 41. Q-POC™

The PCR reaction mix is flowed through a fluidic channel with cycles through three temperature zones at 65 °C, 72 °C and 95 °C. Unlike traditional PCR methods, these blocks are kept at a constant temperature, eliminating the substantial time and energy requirements for thermal ramping.

The QuantuMDx proprietary label-free nanowire biosensor microarray can detect from tens to thousands of features, printed with DNA probes associated with disease or drug resistance. When the amplified DNA flows over the biosensor, target sequences hybridize to the probes in less than 5 minutes. As DNA is naturally negatively charged, this causes a change in charge density at the surface of the nanowire, which in turn causes a change in resistance in the nanowires. The nanowires are individually calibrated and monitored in real time with the signal processed by an onboard application-specific integrated circuit (ASIC) and analysed by the proprietary analytical algorithms to give a diagnostic result. Single nucleotide polymorphism (SNP) mutations are easily differentiated, allowing for parallel detection of hundreds of SNP mutations – effectively sequencing specimens for genotyping and drug resistance.

Diagnostics for All (DFA)

DFA is a non-profit enterprise fusing biotechnology and development, dedicated to creating low-cost, easy-to-use, POC diagnostics designed specifically for the 60% of the developing world that lives beyond the reach of urban hospitals and medical infrastructure. DFA develops simple and low-cost paper-based tests that will benefit those living in resource-poor regions. These diagnostic tests are designed to be portable, easy to use, inexpensive, disposable and scalable, with results available in minutes. Additionally, these tests require no external power, water or trained technicians.

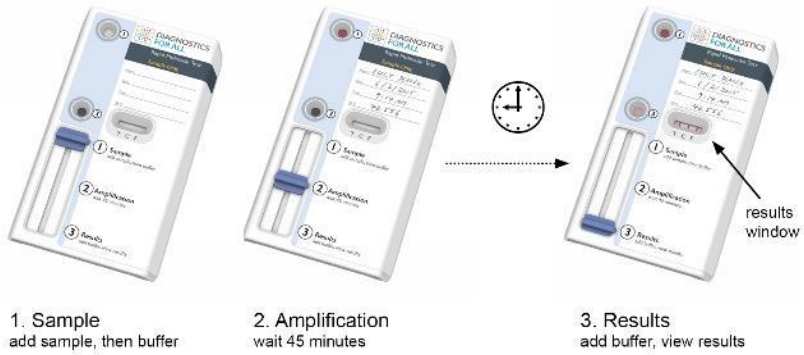
With support from Saving Lives at Birth partners: the United States Agency for International Development (USAID), the Government of Norway, the Bill & Melinda Gates Foundation, Grand Challenges Canada, and the Government of the United Kingdom, DFA is developing an equipment-free, qualitative, nucleic acid test for EID HIV (Figure 42).

Figure 42. DFA working prototype patient-side test

With only one drop of whole blood, this test will deliver results at the patient's side in less than 60 minutes. The disposable test kit is designed for use by people with minimal training, making it

ideal for use in remote clinics and community settings in sub-Saharan Africa (Figure 43). This EID HIV test has a low-cost, disposable, onboard electric heater and battery to enable reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), providing a test capable of detecting both viral RNA and pro-viral DNA. As a TNA test, the DFA test holds promise to serve not as a screening test, but as a definitive confirmatory diagnostic not requiring any other testing before proceeding to treatment.

Figure 43. Operation of the DFA rapid nucleic acid test



DFA anticipates the cost of the final kit to be competitive with current ex-works consumable costs for central laboratory testing *(83,84)*. DFA will endeavour to find partnerships that enable the kit to be sold at cost. The DFA kit also eliminates expenses for specimen transport, laboratory, training and ongoing machine maintenance. DFA will be pursuing FDA and CE Mark approval and, if deemed necessary, approval from the WHO prequalification of the In Vitro Diagnostics Programme. DFA is aiming to conduct its first field trial of this product in 2017, with market launch expected in 2020. Though currently being developed for EID HIV, the same product could be adapted to identify other infectious diseases such as HCV and Ebola.

As a non-profit organization, the DFA mission is supported by public contributions, project-specific funding, and philanthropic support. DFA also supports its mission through public–private partnerships with manufacturers and distributors to sell its devices at low margins to high-need developing markets. DFA intends to generate revenue through licensing technology to corporate partners for use in the developed world, with proceeds from these licenses used to further support technology development and its mission for the developing world.

PanNAT® Platform (Micronics Inc.)

Micronics Inc., a subsidiary of Sony Corporation of America, has developed the PanNAT® System (Figure 44), which is a small, portable microfluidic platform 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 test cartridges, each of which is designed to perform a single and/or multiplexed nucleic acid assay. The cartridge includes all necessary reagents and controls onboard. The system is lightweight, mains powered, can store up to 1000 test results before prompting the user to download or delete results, and can provide results

within about 60 minutes, depending upon assay parameters. The system includes battery backup as well as full HIS/LIS connectivity, including Wi-Fi and USB data storage.

Figure 44. 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 Inc. has a number of tests in its development pipeline, including an assay for Shiga toxin-producing *Escherichia coli*, as well as other infectious disease diagnostics. Commercial launch for a first test and system is targeted for 2016. Micronics Inc. has been funded to develop qualitative assays for HIV, HBV and HCV; and also has quantitative assays scheduled for HIV and hepatitis on the platform.

VL testing technologies

Overview of VL testing

As discussed earlier in this report, VL testing is now the recommended method 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 *(15)*. 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 VL levels can be used as an indicator of the efficiency of therapy, along with clinical symptoms and CD4 counts. VL testing is used to determine whether the virus is "undetectable" in the patient's blood (below the LOD 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% *(85–87)*.

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. VL testing also enables clinicians to switch failing patients earlier to new drug regimens, before the accumulation of drug resistance mutations, thereby reducing the spread of highly resistant virus. In other words, VL 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. Furthermore, it should be noted that unlike antibody detection of HIV, which is limited by the transfer of maternal antibodies across the placenta to the fetus, VL 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 VL testing, several factors have traditionally limited access to VL testing in low-resource settings; however, efforts have been achieved in workarounds and new developments to surmount these barriers. A key barrier has been the high cost of VL diagnostics – which in part is being addressed by current programmes, including those supported by the Global Fund, PEPFAR, UNITAID and partners, including the Diagnostics Access Initiative. A second barrier to VL implementation is the complexity of conventional assays that require sophisticated laboratory instrumentation and expertise that is generally only found in centralized reference laboratories – which in part is being addressed by DBS sample transport and return-of-results connectivity. In addition, new POC technologies are enabling testing at decentralized clinics, mobile sites and health centres. With greater access via these VL testing modalities, the 2013 WHO recommendation for VL monitoring treatment will accelerate more countries to implement VL testing over the next few years.

VL test complexities

The first molecular assay for quantifying HIV viral RNA was approved by the FDA in 1999. Since then, a number of assays have been developed and are considered here in some detail. First, it is worth considering some of the complicating factors that characterize VL assays and platforms, which should inform the choice of platforms for a given setting. These include HIV diversity (including HIV-1 subtypes, circulating recombinants, and HIV-2) and certain practical challenges, including laboratory infrastructure and transport of samples.

The high level of genetic heterogeneity of HIV-1 and the emergence of recombinant strains of the virus complicate VL assay development *(88,89)*. In an ideal world, VL assays would detect and quantify all known HIV 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 VL assay.

Laboratory infrastructure

Currently available VL platforms are laboratory based and require significant infrastructure, including continuous power, clean running water and climate control/airconditioning. For example, the typical, non-POC VL platform based on nucleic acid technology (discussed below) will require two to three dedicated rooms in a laboratory.[32] 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.

DBS versus plasma VL

As discussed above, VL assays were developed for viral extraction from plasma after centrifugation of >1 mL venous blood specimen (venipuncture), typically with cold-chain storage and transport of specimens to the central laboratory. For resource-limited settings, expanding capacity of the central laboratory to include decentralized collection sites is of critical importance for greater access to VL testing and monitoring. The use of DBS is an important resource in the implementation of testing because it greatly simplifies the transport of samples from outlying areas to the reference laboratory.

Several studies have been performed to correlate VL obtained from plasma and DBS. Most assays demonstrated good correlation between the two sample types for sensitivity ranges above 3 log HIV-RNA cp/mL *(5,90–92)*; however, for some assays the correlation falls away at low cp/mL because of interference from contributions from cell-associated viral RNA and pro-viral DNA *(93–95)*. A specific comparison of the commercial assay performance has been published; Figure 45 shows the DBS VL components according to assay technique *(96)*. The contribution of DNA and RNA to VL depends on the selectivity of the extraction method. Extraction methods that are selective for RNA (closer correlation with plasma) include RNA-specific nucleic acid extraction, DNAse pretreatment to remove DNA, and virus particle elution.[33]

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[32] 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.

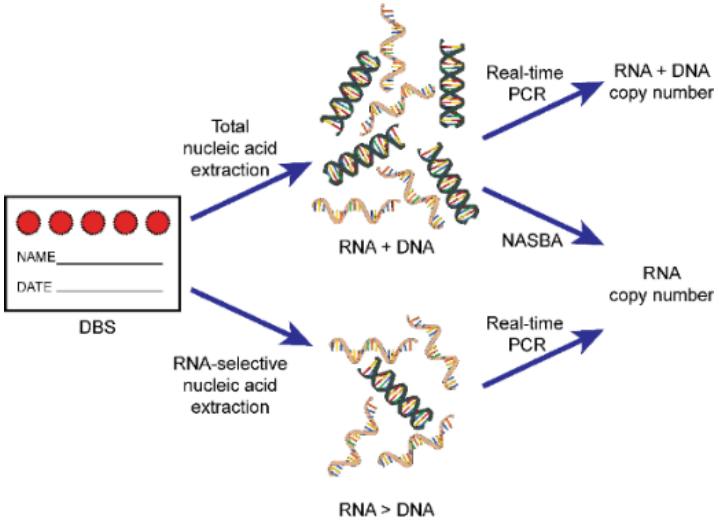
[33] bioMérieux uses TNA extraction with NASBA; Abbott uses RNA-selective extraction with RT-PCR (both methods close to plasma RNA). Roche uses TNA extraction followed by RT-PCR (RNA + DNA copy number); Roche is now validating a “free virus elution” (FVE) method with RT-PCR for results closer to plasma RNA.

It should be noted that VL tests using fresh whole blood will have the same issues with cell-associate RNA and pro-viral DNA as DBS, unless a plasma separation step is included.

For non-selective nucleic acid assays, the non-plasma contributions of cell-associated RNA and pro-viral DNA can add a "baseline" to the plasma-only RNA VL detected below 3000–5000 cp/mL (plasma RNA dominates the assay above 5000 cp/mL). In addition, these relative contributions can vary for treated versus untreated patients and healthy versus immune compromised patients. Because of these issues with low-end sensitivity for DBS VL, the WHO 2013 Guidelines suggest that programmes relying on DBS technology for VL testing may consider retaining a higher threshold (3000–5000 cp/mL) until DBS sensitivity at lower thresholds is established *(1)*.

In a review of VL monitoring technologies, Médecins sans Frontières noted 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" *(97)*, as many have recently done.

Figure 45. DBS copy number contribution (RNA, DNA) for different assay methods



Note: Top: non-selective nucleic acid extraction; bottom: selective for RNA (closer to plasma copy number).
Source: Used with permission from Parkin 2014 *(96)*.

POC infrastructure

For POC testing, most platforms have been designed for whole blood obtained (fingerprick or venipuncture) or saliva to be applied immediately to the test cartridge and processed onsite. It is presumed that in many POC settings, a centrifuge may not be available for plasma separation from whole blood, which complicates the measurement of VL. Similar to the concern with the accuracy of DBS at the lower LOD, studies have found cases of poor correlation between whole blood and plasma VL below 3000–5000 cp/mL because of interference from non-plasma-associated virus as described above. Some developers are investigating the possibility of

integrating plasma separation into the cartridge design – see the individual developer sections for further information.

Connectivity

Some of the newer POC instruments have been designed for field-enabled information connectivity by engineering built-in battery sources and wireless communication (see specific product operational characteristics in Appendix 1). POC systems take advantage of the GSM or SMS networks to transmit data automatically, often in real time. Connectivity via a cellular network means that results can be automatically or periodically uploaded to a health systems database. Automatic tracking of results and instrument performance provides information not only on health data, but also on consumable use, system operation, QC and maintenance. This information can assist supply chain management and procurement as well as oversight for training, service and maintenance for field service.

VL test methods

HIV VL technologies can be categorized broadly as NAT and non-NAT-based technologies (Table 2). 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.

Table 2. Commercial NAT- and non-NAT VL technologies for laboratory use

NAT-based technologies	
Type	**Assay name**
RT-PCR	COBAS® Taqman® v2.0 (Roche)
	Abbott RealTi*m*e HIV-1 (Abbott)
	VERSANT® HIV RNA 1.0 (kPCR) (Siemens)
	VERIS^{MDx} (Beckman Coulter)
	artus™ HIV-1 QS-RGQ (QIAGEN)
NASBA	NucliSens® EasyQ® HIV-1 v2.0 (bioMérieux)
RT-TMA	Panther® system (Hologic)
Non-NAT-based technologies	
Type	**Assay name**
RT	ExaVir™ Load version 3.0 (Cavidi AB)
p24 antigen	HIV-1 p24 Ultra ELISA (PerkinElmer) (research use only)

NAT-based technologies

NAT-based assays have become the core VL monitoring technology used in high-income settings as well as resource-limited settings.[34] 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) *(15)*. Currently, the bulk of commercially available VL 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) pre-amplification sample preparation and/or viral nucleic acid extraction; (ii) amplification of either the nucleic acid target or detection signal; and (iii) post-amplification detection and/or quantification of the amplified viral nucleic acids.

Pre-amplification. Pre-amplification methods (sample preparation and/or viral nucleic acid extraction) are critical to the VL 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 *(15)*. Although HIV nucleic acids are relatively stable, molecular detection methods require prompt processing of samples (generally within 6 hours of collection), a rapid extraction method and appropriate storage of plasma or cells prior to assessing.

Amplification. 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 RT-PCR method used in the Abbott, QIAGEN N.V. (hereinafter QIAGEN) and Roche assays, the nucleic acid sequence-based amplification (NASBA) method used in the bioMérieux assay and the transcription-based nucleic acid amplification method used in the Hologic 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 Healthcare Diagnostics Inc. (hereinafter Siemens).

Post-amplification. 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). Detection can be achieved

[34] The NAT-based systems manufactured by Abbott, bioMérieux, Roche and Siemens currently dominate the market.

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.[35]

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". 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 VL 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.

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

[35] 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.

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 (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 *(15)*. 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.

VL technologies/platforms

High-throughput laboratory platforms for VL

COBAS® System (Roche)

The Roche COBAS® system and instrument platforms are described in detail in the preceding EID section.

Roche currently manufactures a single real-time PCR assay, the COBAS® AmpliPrep®/COBAS® TaqMan® HIV-1 Test v2.0.[36] The assay uses the AmpliPrep® instrument for automated viral nucleic acid extraction and the COBAS® TaqMan® analyzers (TaqMan® 48 or TaqMan® 96), both of which are discussed below, for automated amplification and detection of the viral nucleic acid target.

COBAS® AmpliPrep®/COBAS® TaqMan® HIV-1 Test v2.0 test

The COBAS® AmpliPrep®/COBAS® TaqMan® HIV-1 Test v2.0 test is an in vitro nucleic acid amplification test for the quantitation of HIV-1 RNA in human plasma. It is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV patients. In order to specifically address HIV-1 mutations, a dual-target approach is used. The dual-target technology provides additional confidence in results in the event of mutation. The assay is able to co-amplify two target regions of HIV-1 (known as the gag and 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. It is able to quantify HIV-1 group M (subtypes A through H) and HIV-1 group O, and has an LOD 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. Performance of the test has proven to have good correlation with the AMPLICOR HIV-1 MONITOR™ v1.5 assay[37] (hereinafter MONITOR assay), which had been considered to be the gold standard *(98)*.

The COBAS® AmpliPrep®/COBAS® TaqMan® HIV-1 Test v2.0 is CE marked, FDA approved and prequalified by WHO for use plasma specimens on both TaqMan® 48 and TaqMan® 96 systems.

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

[37] Roche has globally discontinued the COBAS® AMPLICOR HIV-1 MONITOR™ v1.5 (the MONITOR assay).

Use of the TaqMan® HIV-1 Test v2.0 assay with the Free Virus Elution protocol on DBS has been described *(99)* and the July 2014 WHO Technical Update on Implementing Viral Load Testing (refer to EID section for the HIV-1 Qualitative Test with DBS).

In addition to the COBAS® AmpliPrep® and Taqman® systems described in the EID section, in 2014 Roche released the cobas® 6800/8800 systems, two integrated and fully automated molecular testing systems for blood and plasma donor screening in markets that accept the CE Mark (Figure 46). The cobas® 6800/8800 systems are commercially available in markets that recognize CE Mark and are not currently available in the United States. The 6800/8800 systems offer the fastest time to results with the highest throughput available. They are fully automated solutions designed for donor screening, VL monitoring, women's health and microbiology testing and are available in medium- and high-throughput models. Each system provides results for the first 96 tests in less than 3.5 hours, with the 6800 system delivering up to 384 results in an 8-hour shift, and the 8800 system generating up to 960 results in the same amount of time. Both systems also allow for simultaneous processing of multiple assays and are designed to enable up to 8 hours (cobas® 6800) and 4 hours (cobas® 8800) of "walk-away" time* with minimal user interaction.

Figure 46. cobas® 6800/8800 systems

cobas® 6800 system *cobas® 8800 system*

Assays launched with the 6800/8800 platforms include the quantitative cobas® HIV-1, HBV, and HCV assays. cobas® HIV-1 is an in vitro nucleic acid amplification test for the quantitation of HIV-1 RNA in human plasma. The test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV patients. cobas® HIV-1 is not available in all markets. The CE Mark version can also be used for confirmation of HIV-1 infection in antibody reactive individuals. Similar to the COBAS® AmpliPrep®/COBAS® TaqMan® HIV-1 Test v2.0 assay, cobas® HIV-1 uses dual-target technology to co-amplify two target regions of HIV-1 (known as the gag and 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. It is able to quantify HIV-1 group M (subtypes A through H), HIV-1 group N, and HIV-1 group O. The test has two sample process volumes, 500 µL and 200 µL, with an LOD of 13.2 cp/mL for 500 µL and 35.5 cp/mL for 200 µL. Both sample volumes have a linear range of up to 10 million cp/mL.

In September 2014, Roche announced a global access programme for certain organizations in eligible low- and low-middle income countries and/or those with a high disease burden. The cost per test for VL reagents and consumables is US$ 9.40, but may vary depending on local conditions. In addition, pricing will vary depending on factors such as outright instrument purchase, reagent rental and volume-based, tiered pricing arrangements.[38]

*m*2000 System (Abbott)

The Abbott *m*2000 system and instrument platforms are described in detail in the preceding EID section.

Abbott RealTime HIV-1 assay

The Abbott RealTi*m*e HIV-1 assay can be fully automated on the *m*2000*sp* for sample preparation and the *m*2000*rt* for amplification and detection. The assay uses an internal control that is a non-competitive RNA sequence unrelated to the HIV-1 target. This unrelated RNA sequence is added to each sample, control and calibrator and it is monitored so that the sample has been correctly processed through the extraction and amplification/detection. 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 from 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. A publication is available for detection of group P *(100)*. The sensitivity of the assay is dependent on specimen volume. The LOD 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 *(101)*. 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 is CE marked and FDA approved, and has been prequalified by WHO. The RealTi*m*e HIV is included in a Global Fund framework agreement as part of an expanded assay menu (together with HIV EID, MTB, HBV, HCV, HPV and CT/NG) at the same low access price. Abbott offers scale-up planning as well as assistance with scale-up, including training and performance monitoring based on country needs. Terms vary dependent upon volumes and specific needs (refer to the Global Fund website for further information on global access programmes and pricing).

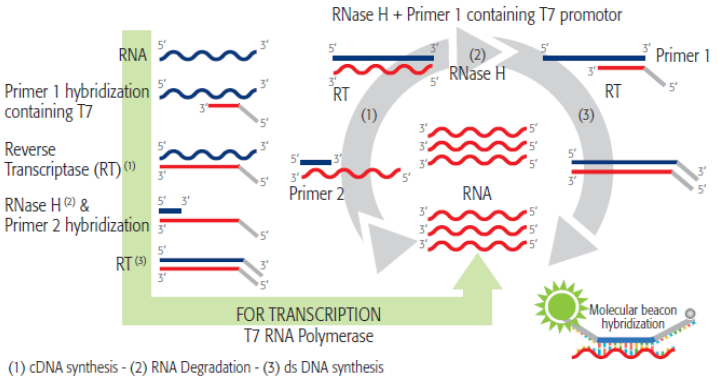
[38] For details, see: http://molecular.roche.com/globalaccessprogram/Pages/default.aspx.

NucliSENS® HIV Solution (bioMérieux)

The NucliSENS® HIV solution is manufactured by bioMérieux. The NucliSENS® EasyQ® HIV-1 v2.0 assay targets a well-conserved region of the gag gene and is based on NASBA® technology. Following sample extraction with proprietary magnetic BOOM® technology, the highly efficient real-time NASBA amplification reaction ensures very sensitive test results in only 60 minutes.

NASBA is an isothermal transcription-based amplification method that 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 *(102)*. 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 (Figure 47).

Figure 47. NASBA schematic



The NucliSENS® EasyQ® HIV-1 v2.0 (Automated) is a nucleic acid amplification assay for the quantitative determination of HIV-1 RNA in human EDTA plasma and EDTA whole blood spotted on cards (DBS). It is intended to be used for NASBA-based amplification and real time detection of isolated HIV-1 RNA. The test can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of ART by measuring changes in plasma/DBS (from EDTA whole blood) HIV-1 RNA levels during the course of antiretroviral treatment.

The linear range of the EasyQ® HIV assay v2.0 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 *(103,104)*. The assay is CE marked and FDA approved, and prequalified by WHO for use on either the semi-automated or automated systems described below. The average price of the EasyQ® HIV assay v2.0, including extraction and amplification/detection, is about €18 (~US$ 23.75) per test.

The NucliSENS® easyMAG® and miniMAG® 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. Both systems employ an extraction process

using magnetic silica-based beads with proprietary BOOM® technology. For high-throughput needs, the easyMAG® is an automated benchtop nucleic acid extraction device that is able to perform 24 extractions in as little as 40 minutes (and offers the possibility to extract different samples types, to be used in several applications, in the same run). The easyMAG® system (Figure 48) 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 48. NucliSENS® easyMAG® extraction system

The average price of the easyMAG® instrument is approximately €72 000 (~US$ 95 000).

The miniMAG® system (Figure 49) is a small, semi-automatic extraction device for both DNA and RNA in various specimens. 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 a standardized extraction protocol for multiple downstream applications and is considered to have an easy workflow for operators.

Figure 49. NucliSENS® miniMAG® extraction system

The price of the miniMAG® extraction device is about €6800 (~US$ 9000).

The NucliSENS® EasyQ® is a closed system made up of a real-time NASBA amplification step with automated data analysis (Figure 50). 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 VL of each sample is calculated automatically and displayed on a computer.

Figure 50. NucliSENS® EasyQ® Amplification and Detection system

The EasyQ® analyzer 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 analyzer is approximately €37 100 (~US$ 49 000).

bioMérieux also provides NucliSENtral™, which is an integrated software system that can be used to link NucliSENS® easyMAG® and NucliSENS® EasyQ® with LIS.

VERSANT® kPCR Molecular System (Siemens)

The VERSANT® kPCR Molecular System and the VERSANT® HIV RNA 1.0 Assay (kPCR) are manufactured by Siemens. The Siemens HIV assay is an automated amplification method based on reverse transcription and real-time PCR technology. The system (Figure 51) consists of two modules: the Sample Preparation Module used to extract both RNA and DNA from plasma as well as a wide variety of other samples; and the Amplification Detection Module, along with VERSANT® MiPLX software. The system is flexible and allows for either a “one-room” technology with no need for clean room operations due to closed-tube processing and other physical and chemical contamination controls or two separate rooms, depending on the individual laboratory’s setting.

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 nanobeads coated with silica that provide for efficient and high-quality extraction of

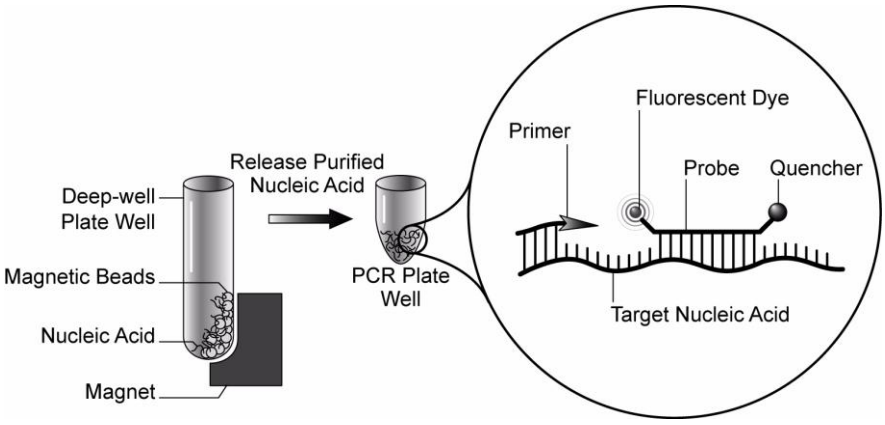
nucleic acids. Extraction consists of a lysis step that utilizes proteinase K and a chaotropic buffer, and several washes to remove non-nucleic acid components of the sample and elution.

Figure 51. VERSANT® kPCR Molecular System

Source: Photo courtesy of Siemens Healthcare Diagnostics Inc. ©

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 kinetic PCR technique. The RT-PCR step uses primers and probes that target a highly conserved region of the pol integrase gene. Figure 52 shows a schematic representation of the assay principle.

Figure 52. Schematic of VERSANT® HIV RNA 1.0 Assay principle



Source: Schematic courtesy of Siemens Healthcare Diagnostics Inc. ©

The VERSANT® kPCR Molecular System provides the flexibility to process samples in run 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 6 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 *(105–107)*.

The VERSANT® kPCR Molecular System and the VERSANT® HIV RNA 1.0 Assay (kPCR) are CE-IVD marked and available globally with the exception of the United States. The VERSANT® HIV-1 RNA 1.0 assay has been prequalified by WHO. An updated version of the VERSANT® HIV RNA 1.0 Assay (kPCR) is currently under development by the manufacturer.

VERSANT® 440 Molecular System (Siemens)

Siemens, as announced in April 2014, discontinued manufacturing (as of 31 December 2014) and support (as of 31 March 2015) of its VERSANT® 440 Molecular system and HIV-1 RNA 3.0 bDNA Assay.

RT-TMA technology Panther® system (Hologic)

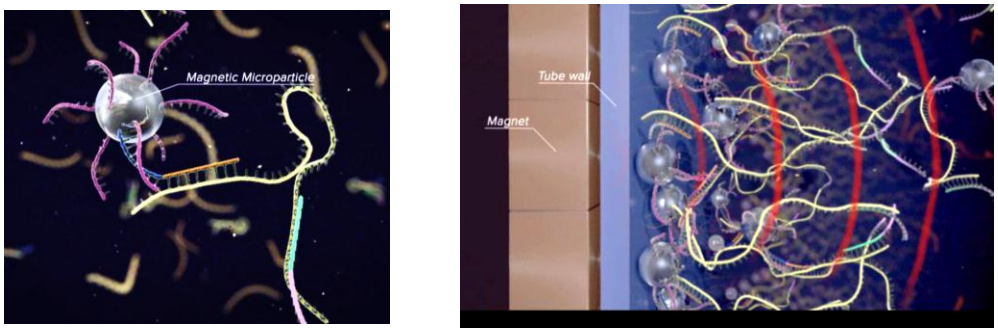
Hologic has introduced the Panther® system, a molecular diagnostic platform with true random access testing capability on a fully integrated and automated NAT system (Figure 53). The platform brings the flexibility of clinical chemistry instrumentation to molecular diagnostics.

Figure 53. Panther® system

Hologic has developed a fully quantitative VL assay, the Aptima® HIV-1 Quant Dx Assay (Aptima® assay), for the Panther® system. The Aptima® assay involves three main steps (Figure 54), all of which take place in a single tube on the Panther® system: (i) target capture; (ii) target amplification by transcription-mediated amplification (TMA); and (iii) detection of the amplification products (amplicon) by the fluorescent-labelled probes (torches).

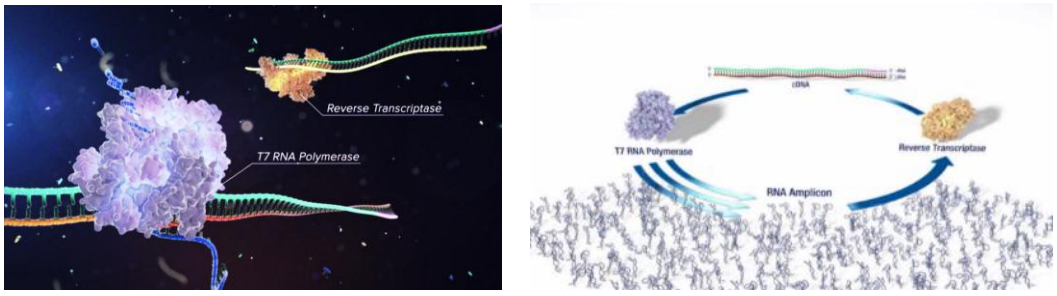
Target capture. During target capture, viral RNA is isolated from samples (Figure 54, left). The sample is treated with a detergent to release viral genomic RNA. Oligonucleotides capture and hybridize to highly conserved regions of HIV-1 RNA, if present, in the sample. The hybridized target is then captured onto magnetic microparticles that are separated from the sample in a magnetic field. Finally, wash steps remove extraneous components from the reaction tube.

Figure 54. 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 55). 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 (polymerase 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.

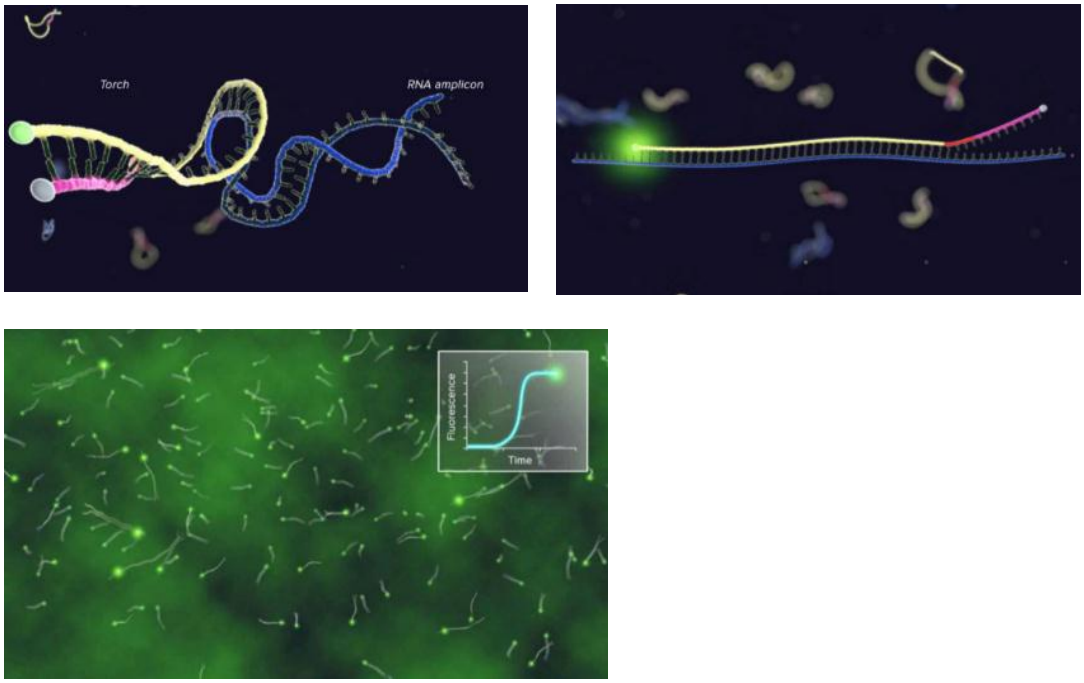
Figure 55. TMA transcription-based nucleic acid amplification method



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 56). 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.

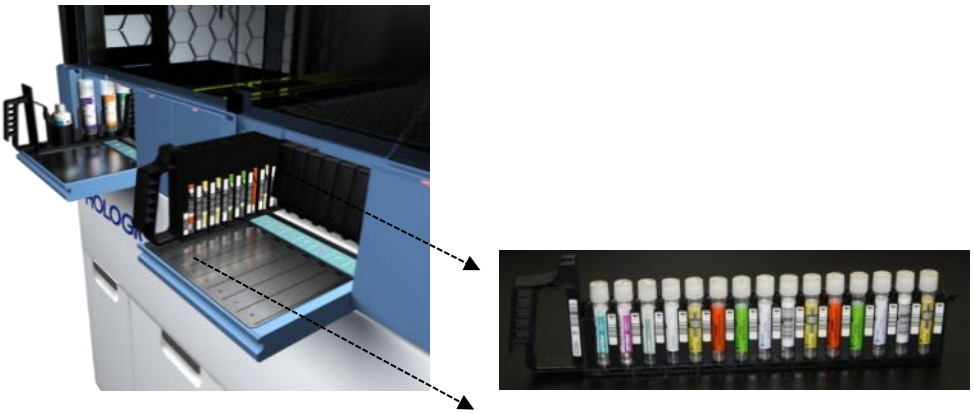
Figure 56. Real-time detection of amplicon



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 3 hours after loading samples, and five results every 5 minutes thereafter. Samples can be continuously loaded with up to 120 samples at a time (Figure 57). Reagent controls and calibration are valid for 24 hours. At least 320 samples can be run within an 8-hour shift or 560 in a 12-hour period (an additional 240 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.[39]

[39] The HCV Quant Dx, HBV Quant and HSV1/2 assays are in development.

Figure 57. Panther® continuous loading system



The Panther® intuitive task-driven software with touchscreen (Figure 58) interface simplifies setup, adding or removing reagents or samples, and onboard inventory management of reagents and consumables; bi-directional 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 of refrigeration for assay reagents. Up to 2000 tests of common system fluids are managed by radio frequency identification tags and Panther® software.

Figure 58. Panther® touchscreen

The Aptima® HIV-1 Quant Dx Assay on the Panther® system is now CE-IVD marked and available for sale in the European Union and in other countries that observe CE-IVD marking. Hologic has also submitted a dossier for WHO prequalification and is pursuing FDA approval for the assay. Hologic has developed a DBS protocol and is investigating regulatory certification for DBS. Pricing will be variable and will depend on variables such as instrument purchase, reagent rental and volume-based pricing.

VERIS MDx (Beckman Coulter)

Beckman Coulter has introduced a new fully automated random access molecular diagnostics system, the VERIS MDx (Figure 59). The DxN VERIS integrates sample introduction, nucleic acid extraction, reaction setup, real-time PCR amplification and detection, and results interpretation into one system saving space, time and cost – no specialized expertise is required. There is no lengthy set-up – all consumables/reagents are refrigerated onboard. The system's QC trending and auto-verification results features reduce the risk of errors from manually entered results. The DxN VERIS molecular diagnostics system delivers greater productivity and flexibility to the laboratory, ensuring that results are available to physicians as quickly as possible, for faster clinical decision-making and positively impacting the patient pathway.

Figure 59. VERIX MDx

The VERIS MDx accepts several sample containers for plasma, serum and culture tubes; 48 samples can be lined up on 12 racks of four samples each. The time to result for DNA tests is approximately 70 minutes and for RNA tests is approximately 110 minutes. For multiplex analysis, five different detection colours are available with a bandwidth of 505–720 mm. The onboard capacity consists of 96 extraction and purification cartridges and reagents are covered for 20 assays with 48 tests per assay. Reagents are stable in the machine for up to 14 days.

Depending on the assay, the VERIS MDx can process up to 450 samples in 24 hours. The system features walk-away time of at least 2 hours. The system also includes intuitive graphical touchscreen user interface and has LIS interface capabilities.

CE-IVD marked assays for the VERIS MDx system include HIV-1, HCV, HBV and cytomegalovirus (CMV). Other assays in development include VL and qualitative CT/NG, methicillin-resistant *Staphylococcus* (MRSA), *Clostridium difficile* and HPV.

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

QIAGEN has 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 kit) extraction and sample preparation system (QIAamp® DSP Virus Kit) or an automated (artus™ HIV-1 QS-RGQ RT-PCR kit) 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 60.

Figure 60. 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 an LOD of approximately 35 cp/mL. The time to result is about 5–6 hours for 24 samples. Performance of the artus™ assay has been evaluated and is comparable to that of the Abbott RealTi*m*e system *(108)*.

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 artus™ HIV assay can be run on the real-time PCR Rotor-Gene Thermocycler RGQ.

In addition to HIV, the artus™ panels for QIAsymphony™ Rotor-Gene Q (RGQ) include assays for HBV and HCV, plus a transplantation/immunosuppressed panel, with assays for detection and quantification of CMV, Epstein-Barr virus, herpes simplex virus (HSV) 1 and 2, varicella-zoster virus and BK virus.

ExaVir™ Load (Cavidi AB)

Cavidi AB manufactures the ExaVir™ Load (Version 3.0), 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 VL. The principle is based on the synthesis of a product that can be detected by an alkaline phosphatase conjugated antibody. An advantage of the assay is that because the ExaVir™ Load determines VL 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 HIV-1 group O and N, and HIV-2. 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 Roche AMPLICOR assay *(109,110)*.

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 ARV. 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 61).

Figure 61. ExaVir™ Separation equipment

The ExaVir™ Load assay is more manual than most of the other VL 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 5-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. 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 Analyzer software is required (supplied with the first order) as well as a computer with Microsoft® Excel® and Adobe® Reader®.

The Cavidi ExaVir™ is CE marked. The cost of the ExaVir™ equipment is approximately US$ 4 500. The cost per test, which varies according to volume, is about US$ 12–25. Although the assay is manual, it is reasonably priced and relatively easy to implement, especially in Level 2 settings.

ZIVA™

Cavidi AB is developing an easy-to-use, benchtop platform, the ZIVA™, for near-patient HIV monitoring, a prototype is pictured in Figure 62. The ZIVA™ is targeted at district hospitals (Level 2 facilities) and large clinics to provide high-quality VL test results using existing facilities and staff in decentralized settings.

Figure 62. ZIVA™ platform

ZIVA™ uses a proven technology as it builds upon the existing Cavidi platform, the 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 VL. The assay principle is based on three steps: (i) isolation of HIV from plasma and preparation of RT-lysate; (ii) RT reaction; and (iii) detection and quantification of RT-reaction product by chemiluminescence immunoassay (CLIA).

The ZIVA™ VL assay is a 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 AB), starts the assay run and leaves the machine. The operator only needs to return after the run for the results and to empty waste. Sample volume is 500 µl of plasma and the system will offer two kits, one for 20 and one for 48 patient samples. The system will be easy to integrate with a laboratory information management system (LIMS) and will offer UPS and battery backup options to

ensure reliable testing results even when power is lost during a run. This will provide reliable and robust VL monitoring for all HIV types and subtypes.

The company's planned launch date for the VL assay on the ZIVA™ system is set for mid-2016. 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.

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 the 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.[40]

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 VL assays. Both the HIV-1 p24 ELISA kit and the ELAST® Amplification System kit are currently available for research use only purposes and not for patient results.

POC platforms for VL

SAMBA platforms (Diagnostics for the Real World Ltd)

The SAMBA I and II platforms are described in detail in the preceding EID section.

The SAMBA HIV tests use 200 µL of plasma or 120 µL of whole blood for the semiquantitative VL assay. The amplification and detection process is integrated into a hermetically sealed cartridge to prevent amplicon contamination. Amplification targets the LTR region of the HIV genome, which is detected on a lateral flow strip using the Diagnostics for the Real World Ltd patented SAS technology (see Figure 33 in the preceding EID technologies/platforms section).

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 clinical evaluations for VL have taken place:

[40] 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)*.

- SAMBA semiquantitative VL test: evaluated in clinical samples from St. Thomas Hospital, Royal London Hospital, and two Médecins sans Frontières sites (Chiradzulu, Malawi, and Arua, Uganda);
- SAMBA semiquantitative VL test for whole blood: evaluated in clinical samples from KEMRI/CDC, Kisumu, Kenya;
- evaluations on VL are currently ongoing in Nigeria.

Currently, the total assay time is 90 minutes for the semiquantitative VL assay. The SAMBA II system is best suited for use at Level 2 facilities or in large clinics (Level 1 facilities) in sub-Saharan Africa where laboratory technicians and electricity are available.

Pricing information is available from the company and is volume dependent. The SAMBA VL assays have recently received product approval in Kenya and Uganda, and are currently being evaluated in a number of additional countries in sub-Saharan Africa, including Nigeria.

Xpert® HIV-1 Viral Load Test (Cepheid)

The GeneXpert® platforms are described in detail in the preceding EID section.

The Xpert® HIV-1 Viral Load (VL) assay (using plasma specimens) 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 (Figure 63). 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 Roche TaqMan® probe was designed. The HIV genome target forward primer and the two probes included in the assay incorporate the Cepheid 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 Xpert® HIV-1 VL assay has an LOD of 18.3 cp/mL (WHO reference method) and a limit of quantitation (LOQ) of 40 cp/mL with a 1 mL plasma sample input volume. The assay includes two internal quantification standards: Internal Quantitative Standard High and Low (IQS-H and IQS-L). The IQS-H and IQS-L are standards calibrated against the WHO third international standard. They are used for quantification by using lot-specific parameters for the calculation of HIV-1 RNA concentration in the sample. Additionally, IQS-H and IQS-L detect specimen-associated inhibition of the RT-PCR reaction. The IQS-H and IQS-L pass if they meet the validated acceptance criteria. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability.

Figure 63. GeneXpert® 4-4 module instrument (left) and Xpert® HIV-1 Viral Load cartridge (right)

The workflow for Xpert® HIV-1 VL assay (Figure 64) consists of: (i) collecting whole blood in acid citrate dextrose (ACD) or EDTA tube; (ii) centrifuging the tube; (iii) transferring 1 mL of plasma using the pipette provided directly into the Xpert® HIV-1 VL cartridge; (iv) scanning the cartridge barcode; and (v) loading the cartridge into the GeneXpert® module and closing the door with a 90-minute time to result. No special instrumentation or handling is required for the Xpert® HIV-1 Viral Load test.

Figure 64. Xpert® HIV-1 Viral Load workflow

The Cepheid Xpert® HIV-1 Viral Load test received CE-IVD clearance in December 2014.

Alere™ q system (Alere™)

The Alere™ q platform is described in detail in the preceding EID section.

The Alere™ q tests are disposable cartridges that contain all reagents required for the assay in a stabilized form. The HIV Detect and HIV VL (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 company expects sensitivity and specificity will be comparable to current

virological testing reference technologies (e.g. Roche COBAS® AmpliPrep®/COBAS® TaqMan®). The system detects HIV-1 Groups M, N and O and HIV-2.

Figure 65. Alere™ q Analyser

The Alere™ q Analyser has been CE-IVD marked since July 2014. Additional tests in development include the VL test for quantitative detection of HIV-1 and HIV-2 simultaneously from 25 µL of whole blood (Figure 65).

cobas® Liat™ System (Roche)

The cobas® Liat™ System, now manufactured by IQuum Inc. for Roche, 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. The cobas® Liat™ System is small and portable weighing 3.76 kg (Figure 66). The system currently has assays clinically validated, CE-IVD marked and FDA cleared for the detection of influenza A/B and Strep A. The cobas® Strep A test has received CLIA Waiver from the FDA. A CLIA waiver determines that there is little risk of error due to the simple use of the test. Assays for HIV are under development and additional assays are being planned.

As illustrated in Figure 67, the test procedure is straightforward, with no sample manipulation or reagent loading steps other than inputting the sample directly into the cobas® Liat™ tube. The cobas® 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. The cobas® Liat™ System executes all required assay steps and reports a test result in 20 minutes (influenza A/B) and 15 minutes (Strep A).

Figure 66. cobas® Liat™ System

To aid the operator and provide reliable results, the cobas® Liat™ System incorporates a variety of intelligent and advanced features: barcode 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 cobas® 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.

Figure 67. cobas® Liat™ test procedure

As illustrated above, the test procedure is straightforward, with no sample manipulation or reagent loading steps other than inputting the sample directly into the cobas® Liat™ tube. The cobas® 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. The cobas® Liat™ System has an internal optical system that provides 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. It can be powered by AC mains or by battery, allowing mobile use.

No pricing information is available on either the instrument or the individual assays for resource-limited settings. Roche launched the system in the United States at the end of 2014 and is planning to expand globally. Stability profile for future assays is also not available at this time.

Savanna Viral Load Platform (NWGHF and Quidel Corporation)

NWGHF and Quidel Corporation are developing a POC rapid RT-PCR testing platform, Savanna, which will be both easy to use and low cost. The product (Figure 68) can accommodate 14 tests in an 8-hour day. The proposed VL assay will achieve an LOD of 1000 cp/mL of plasma, using approximately 170 µL of whole blood that is converted into plasma with simple sample preparation materials provided by the Quidel Corporation.

Figure 68. Savanna Platform for HIV Viral Load

Savanna is designed and manufactured for use in the developing world by laboratory technicians with limited training, nurses and community health-care workers treating HIV patients. The system will provide low-cost HIV VL and tuberculosis testing options to high-burden regions, while building assay and feature sets that appeal to the rest of the world. The system is rugged enough to endure high heat/humidity, unstable electricity and a lack of cold storage that are common in developing world environments. Savanna will use the Quidel Corporation proprietary extraction technology, Phase Gate, to allow rapid and integrated molecular

extraction. The system is expected to cost US$ 10 000, with a cartridge cost of under US$ 10 per test.

Laboratory evaluations of the Savanna HIV Viral Load test and platform are expected to begin in Africa in 2016. NWGHF/Quidel Corporation expect to launch the Savanna HIV Viral Load test and platform in 2016 or 2017.

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

Based on its unique assay threading technology, Wave 80 Biosciences is developing the cartridge-format EOSCAPE-HIV™ test, running on its advanced EOSCAPE molecular platform. The EOSCAPE platform is described by the company as a sample-to-answer multiplexed assay system incorporating automated sample metering; a no-spin, cartridge-integrated nucleic acid extraction process; and multiplexed photonic signaling/detection system. The system processes each specimen within a single-use, enclosed cartridge. The cartridges, which do not require cold-chain transport, ships as two snap-together components containing all reagents necessary to run the test. The proprietary assay threading technology speeds time-to-result by running multiple assay processes simultaneously.

The EOSCAPE instrument product line includes two systems, both designed for use in diverse clinical laboratory settings. The single-cartridge EOSCAPE-1 system (Figure 69) is targeted for smaller laboratories; the larger EOSCAPE-16 (16-cartridges, random access) supports laboratories with higher throughput requirements. The single-cartridge system configuration is shown in Figure 69, along with a partially disassembled cartridge showing the built-in liquid micropistons used to drive assay processes. The single-cartridge configuration analyzer footprint is 6” x 6.5” (15 cm x 16 cm), with a touchscreen display; it can run on a rechargeable 8-hour battery or mains power. With leading-edge ease-of-use, the system requires between 60 minutes and one day of training for operators, depending on prior training and experience.

The testing process is straightforward: the operator inserts a single-use cartridge into the receptacle in the analyzer; 100 µL of whole blood (typically obtained using a fingerstick lancet) is applied directly into the cartridge; no external sample preparation is required. The analyzer hatch is swung shut, patient data entered through the touchscreen display and the run initiated.

Processing takes approximately 65 minutes, after which the results are read out and/or transferred electronically, and the cartridge is discarded. Equipped with an easy-to-use touchscreen interface, the analzyer has full LIS capabilities, including transmitting test results through wired and wireless connectivity. Internal assay and amplification controls, along with volume partitioning, enable thresholding at 400 cp/mL and quantitation over the range from 1000 to 50 000 cp/mL, without the need for external calibration.

Figure 69. EOSCAPE-1 analyzer and EOSCAPE-HIV™ cartridge

Beyond the EOSCAPE-HIV™, assays in development for the EOSCAPE system include an assay for diagnosing active MTB infection and a 2-plex assay for chlamydia and gonorrhoea, an MTB drug susceptibility test and a 20-plex acute respiratory infection test as well as other infectious and non-infectious diseases. Early versions of the EOSCAPE-HIV™ have been shipped to beta customers. Full product launch is currently scheduled for 2016–2017, depending on the availability of funding to support clinical trials.

Truelab™ Real Time micro PCR System (Molbio Diagnostics Pvt Ltd)

Molbio Diagnostics Pvt Ltd has developed the Truelab™ Real Time micro PCR System, a comprehensive, rapid, near-patient RT-PCR platform, and is currently developing a PCR test for HIV VL. 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 60 minutes. 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 VL, 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 Analyzer (Figure 70). Thermal cycling takes place automatically within the analyzer.

During amplification, the Truenat™ micro PCR chip exponentially releases fluorophores. 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.

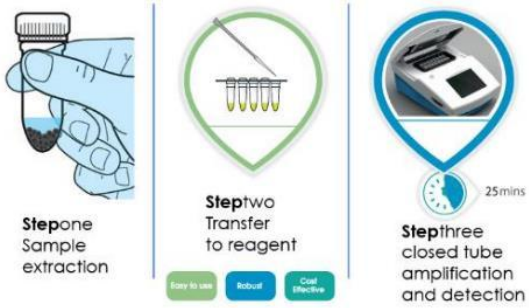
Figure 70. Truelab™ Uno Real Time micro PCR System

Test results are automatically stored in the analyzer 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 VL assay is expected to launch in the third quarter of 2015. The assay is currently undergoing laboratory-based trials in India.

BART platform (Lumora Ltd)

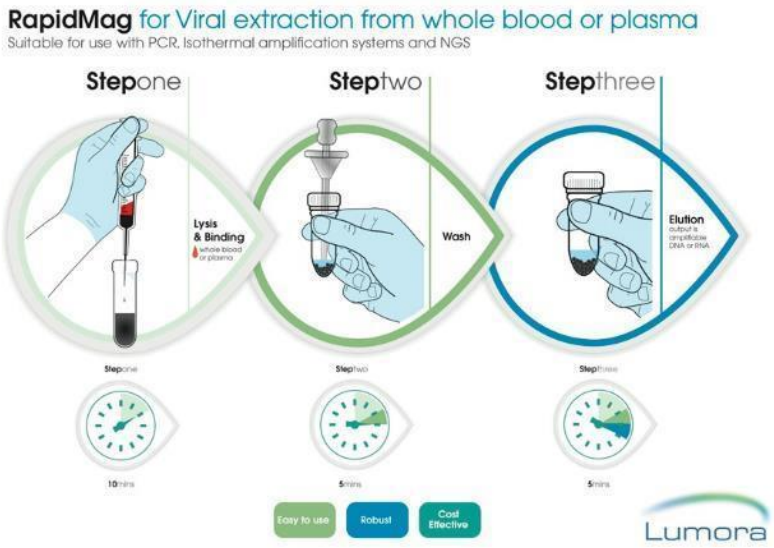
Lumora Ltd is developing the BART (Bioluminescent Assay in Real-Time) platform for performing molecular diagnostics that allows real-time closed-tube quantitative detection. BART is designed for use with isothermal nucleic acid amplification technologies (iNAAT), using a bioluminescent reporter system for disease monitoring in resource-constrained settings. 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 (Figure 71). Lumora Ltd has developed two novel and proprietary approaches, including heat elution for whole blood and DBS, and bead-based viral extraction from whole blood or plasma.

Figure 71. BART integrated assay



Through the utilization of the Lumora Ltd proprietary heat elution sample preparation technology it is possible to extract a sample from whole blood or DBS in 10 minutes using only a heating block and Lumora Ltd sample preparation kits. The processed sample can then be added directly to freeze-dried amplification reagents. The Lumora Ltd novel manual three-step viral extraction technology (Figure 72) 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 72. BART manual three-step viral extraction technology



BART employs exponential generation of pyrophosphate 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 been 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.

Assays in development include a quantitative HIV-1 VL test and a qualitative HIV-1 assay for EID, malaria, chagas, leptospirosis, tuberculosis, leishmaniasis and trypansomiasis.

RT CPA HIV-1 Viral Load test (Ustar Biotechnologies [hereinafter Ustar])

Ustar has developed Cross Priming Amplification (CPA), a novel isothermal amplification technology using multiple crossing primers and probes to rapidly amplify target DNA sequences at a uniform temperature (typically 63 °C) in an easy-to-use protocol with high sensitivity and specificity. 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. Ustar also possesses a proprietary glassification process that stabilizes enzymes for ambient temperature transport and storage. RT CPA can effectively amplify an RNA template with similar performance to existing DNA-based assays, 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.

The Ustar goal is to develop a quantitative RT CPA HIV VL assay and test cartridge in conjunction with a robust and user-friendly portable instrument that will provide VL measurements from fingerstick whole blood or plasma. For this purpose, Ustar is developing a fully automated sample-in, answer-out system (Figure 73).

Figure 73. Ustar RT CPA HIV-1 viral load system

The latest 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 one 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 VL measure will be available in as little as 20 minutes (depending on the LOD required), and the sample can be run for 45 minutes to ensure a VL 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 – “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 developing CPA assays for tuberculosis, HIV, HCV and chlamydia. Completion of the Ustar VL assay and launch is expected in 2016–2017.

Gene-RADAR® Platform (Nanobiosym® Diagnostics)

Nanobiosym® Diagnostics has developed a portable nanotechnology platform called the Gene-RADAR® (Figure 74). This fully integrated chip-based system, which is about the size of an iPad, can recognize the genetic "fingerprint" (DNA or RNA signature) of any disease, using approximately one drop of specimen; in the case of HIV, with less than 50 µL of fingerprick blood. The Gene-RADAR® system is easy to use; the sample is collected via fingerprick with a sterile lancet and deposited directly onto a disposable testing chip. The chip is then inserted into the platform where the Gene-RADAR® proprietary nanobiophysics-based technology analyses DNA/RNA present in the sample and determines whether it matches the DNA/RNA of a particular pathogen. The Gene-RADAR® platform does not require sophisticated laboratory infrastructure, trained laboratory technicians, running water or continuous power.

Figure 74. Gene-RADAR® Platform

In 2013, Nanobiosym® Diagnostics received the first health care XPRIZE for its Gene-RADAR® technology. Recently the company was awarded the top prize in the first Galactic Grant Competition to detect the presence or absence of a target pathogen cultivated in zero gravity to support the development of predictive models for antibiotic resistant pathogen mutations. In addition, the company has been awarded grants from USAID and Grand Challenges Canada under the programme Saving Lives at Birth: A Grand Challenge for Development for implementation of EID and HIV VL validation trials in Rwanda using the Gene-RADAR® platform. The company expects its first product approval for the Gene-RADAR® v1.0 platform to be for an Ebola application, closely followed by a fully quantitative HIV POC VL test in less than 60 minutes.

Conclusions and future directions for HIV testing

Expanding HIV care and treatment requires expanding access to optimal tools to enable adequate clinical management of HIV-infected individuals. The testing required in resource-limited settings likely will necessitate both a scale-up in centralized testing facilities as well as a drive towards POC testing. Centralized high-throughput testing typically has the lowest cost per test, provided that sample transport does not significantly increase the associated cost; smaller benchtop CD4 platforms can provide a solution for Level 2 facilities (district hospitals) high- to

moderate- throughput needs. POC platforms may be the best viable alternative for hard-to-reach populations, particularly in outlying rural areas. The appropriate combination of high-volume laboratories and POC testing will likely be country specific, and will depend on strategic factors such as the urban/rural division in population, the volume of testing needed per site, effective sample transport samples between collection sites and laboratories, and efficient return of results to the site of care. Each of the testing modalities has specific strengths and challenges, as described below.

Future of CD4

CD4 cell count is the primary indicator to assess the strength of the immune system and overall health, and will remain a key parameter in HIV care for establishing baselines for ART initiation and risk stratification, along with monitoring immune recovery through the early stages of ARV clinical management. CD4 testing will also retain an important role in management of opportunistic infections, drug toxicity and virological failure.

However, the role of CD4 testing will inevitably shift with adoption of the new WHO guidelines: (i) ART should be initiated based on diagnosis alone[41] among all adults with HIV regardless of WHO clinical stage and at any CD4 cell count; (ii) similarly ART initiation is recommended for HIV-infected children under 5 years, pregnant women, serodiscordant couples and coinfected tuberculosis and chronic hepatitis patients *(1,2)*; and (iii) for the general population, where VL testing is available, the frequency of CD4 testing could be reduced after the early stages of ARV clinical management. Since CD4 levels typically remain high for patients with stable virological suppression, long-term CD4 testing could be replaced by routine or targeted VL monitoring *(111)* with the exception of specific failure cases *(112)*. It has been suggested that programmatic resources could be conserved by reducing the frequency of CD4 testing in favour of VL monitoring *(113)*.

Laboratory resources for CD4

Until VL testing becomes widely available, health guidelines may continue to require CD4 testing for ART monitoring as well as initiation. In addition, POC alternatives for CD4 testing need to be expanded as the majority of HIV clinics are dependent on the central laboratories for analysis. In rural and peri-urban settings, blood collection is done at the clinic and blood samples are transported the same day via courier to laboratories for testing. It is not currently recommended to use DBS sample collection for CD4 testing due to variability in the results and the failure to detect immature lymphocytes *(23,24)*. The process can be hindered because of insufficient, delayed, lost or spoiled samples. CD4 test results are returned to the collection site, generally via the same courier, although mobile technologies (such as cellphone and SMS) have been introduced for faster return of results. In many cases, patients are asked to return to their health post for their CD4 test result four weeks after the initial visit to ensure sufficient

[41] The “test and treat” model, regardless of CD4 count.

turnaround time. Often ART-eligible patients have to visit health-care facilities several times before they can be initiated onto ART.

Laboratory-based CD4 platforms account for virtually the entire market for CD4 testing in resource-limited settings (though the POC platforms have made inroads). These laboratory systems from Apogee, BD Biosciences, Beckman Coulter and Partec can process 250–500 samples per day for the high-throughput models at the central laboratories and 80–100 samples per day for the moderate-throughput models that can be placed in some of the Level 2 district hospitals. Laboratory-based CD4 testing has the lowest cost per test of US$ 3–7, however, with the caveat of the added burden of sample or patient transportation and return of results. Given the well-established and competitive market, the cost of conventional laboratory-based CD4 is unlikely to fall significantly from current levels, except in settings where transport and testing can be made more efficient.

POC alternatives for CD4

POC testing is generally intended for lower-throughput Level 1 facilities, processing one sample at a time within a 2-hour turnaround time (20–30 tests per system per day). POC CD4 systems are now on the market from Alere™, BD Biosciences and Partec. Decentralized CD4 testing using the Alere Pima™ has been shown to both increase and accelerate treatment coverage for HIV-infected patients. POC testing decreases the return of results from weeks to days, with same-day results significantly reducing loss-to-follow-up for treatment initiation *(67,114,115)*. The Alere Pima™ system also has extensive field evaluations; the results suggest that the Pima™ is a useful platform for ART initiation at true POC *(72,77,116)*. Recent laboratory evaluations have been published for the Partec CyFlow® miniPOC, *(55,56)*; however, performance data for other POC CD4 platforms have been limited. While some developers are discontinuing CD4 product development due to shifting market focus to VL, other POC CD4 alternatives are in development (including a disposable CD4 RDT, see Appendix 2) that are likely to become available in 2015–2016.

Future of EID

PMTCT is an essential programme for decreasing the number of new infections, reducing mortality for the most vulnerable and averting a lifetime of HIV-associated health issues. As a result of scaled-up HIV prevention services from 2001 to 2013, there was about a 60% decline in the number of new HIV infections among children. Despite this significant progress, the number of children becoming newly infected with HIV remains unacceptably high. In 2013, about 250 000 children became infected with HIV and less than half were tested within the recommended first two months of age *(18)*. Timing is critical to reduce HIV-related infant mortality, which peaks at 2–3 months of age.

Laboratory resources for EID

EID is a key parameter of PMTCT, enabling infants to received proper care (and avoid unnecessary treatment) as early as possible. EID platforms based on virological testing (RNA, DNA, TNA) or p24 can be used for early diagnosis at the recommended 4–6 week infant checkup. Until recently, the only EID testing available was virological testing at central laboratories. DBS sample transport has greatly facilitated the transport of EID samples to the central laboratory (and most high-throughput platforms are now validated for DBS analysis), however, the return of results can still take weeks to months, especially for the outlying areas. HIV testing and treatment need to be available closer to those most affected to ensure rapid intervention. It is hoped that new POC platforms will increase the rate of infant diagnosis by enabling testing at the primary care clinic, as well as strengthen PMTCT for women during pregnancy by initiating care at their first visit.[42]

POC alternatives for EID

New platforms for POC EID testing are on now the market from Alere™ (Alere™ q HIV-1/2 Detect, March 2015) and Cepheid (Xpert® HIV-1 Qual, April 2015), with the Diagnostics for the Real World Ltd SAMBA I and II available in limited markets. These virological tests qualitatively detect HIV and can be used for infant diagnosis and "acute" detection of HIV. POC EID tests should significantly reduce the return of results from weeks to days, preferably enabling on-the-spot diagnosis, while the mother and child are in the clinic; ongoing laboratory and field validation will provide further insight into the platforms for ease of use and test accuracy *(82,117)*. Given the similarity between tests for qualitative EID and quantitative VL testing, the ability to perform both EID and VL on the same POC platform may expedite programme implementation and uptake. Alternative virus detection methods such as p24 (NWGHF/Quidel Corporation LYNX HIV p24 Antigen Assay) and RT (Cavidi ExaVir™ Load) testing will provide a complementary approach to virological EID and are targeted for market release in 2016. Other POC technologies in the pipeline, including the MolbioDx TrueLab™ Uno, the Roche cobas® Liat™ and the Ustar Biotechnologies CPA, among others (see Appendix 3), should become available after 2016. As with all testing platforms, the issues of POC test accuracy, ease of use, and instrument/per-test costs will be key towards implementation into existing HIV programmes for care and treatment.

[42] There is some suggestion of virological testing at birth in addition to the 4–6 week testing algorithm to accelerate HIV intervention. However, most virological tests detect HIV only after about 2 weeks postinfection, once the virus has replicated to detectable levels in the blood. It is estimated that only 10–15% of infants becomes infected in utero and could likely test positive at birth; the majority of transmissions occur during labour or postpartum breastfeeding, which would not be detected at delivery. There may be programmatic barriers to birth testing in resource-limited settings, however, WHO encourages countries to consider pilot assessments and consideration of whether testing infants at birth could be implemented in the future *(2)*.

Future of VL

In resource-limited settings, VL testing is seldom performed within the public health sector with the notable exception of Botswana, Brazil, South Africa and Thailand, which were early adopters for expanded access to VL monitoring. Given the WHO recommendation towards routine VL monitoring, it is expected that more countries will begin to scale up VL testing using both centralized and decentralized methods as available. Until very recently, VL platforms were only available in centralized reference laboratories, and validated only for plasma specimens (which require centrifugation and cold-chain transport). For laboratory-based tests, there are innovations in sample transport, cost and resource allocation that can contribute to increasing access for people in resource-limited settings.

Laboratory resources for VL

The recent validation of DBS with laboratory platforms from Abbott, bioMérieux, Roche and Siemens will significantly reduce the burden and cost of sample transport to the centralized laboratories. As noted previously, the use of DBS or whole blood for quantitative VL testing generally imposes a slightly higher threshold for the lower limit of quantitative detection, due to the presence of cell-associated RNA and DNA that would otherwise be extracted during centrifugation. In some cases, the DBS lower limit of quantitative detection may be above the WHO 2013 Guidelines recommendation of 1000 cp/mL threshold for monitoring virological failure (see Appendix 1 for specific operational characteristics). Given the imperative for increased access to VL testing, coupled with the existing investments in VL and DBS infrastructure, it may make sense to rationalize a more flexible threshold for virological monitoring. In anticipation, the WHO 2013 Guidelines suggest that programmes relying on DBS or whole blood specimens for VL testing may consider retaining a higher threshold (3000–5000 cp/mL) until lower test thresholds can be achieved.

VL procurement strategies

The paramount challenge of laboratory-based VL testing is the cost of instrumentation, reagents and ancillary supplies. For low and middle-income countries, the purchase of VL platforms can cost more than US$ 150 000, with test costs varying from US$ 10 to more than US$ 85 per test for reagents alone *(111,118)*. Several approaches have sought to reduce the cost of VL testing by leveraging existing laboratory capacity, employing DBS transport alternatives, combining EID/VL platforms, encouraging competition between suppliers, and negotiated volume-based pricing.

Towards the latter option, the Global Fund procurement strategy for VL and EID is likely to drive significant cost reductions in laboratory testing and foster a more transparent and competitive market. The programme seeks to establish benchmarks for test cost and support through optimized procurement strategies for infrastructure, sample transport, supply networks and scale-up. Framework agreements with developers will provide clarity on prices, aiming for an all-inclusive price as low as US$ 15, including equipment and other costs such as consumables,

maintenance and shipping. Initial participants in the Global Fund programme include Abbott, Alere™, bioMérieux, Cepheid, Hologic, QIAGEN and Roche. Other public health funders and agencies will also be able to enter into agreements based on the benchmark prices negotiated. The procurement framework seeks to establish baseline prices for all-inclusive VL testing, driving up to one third reduction in current costs. These cost savings should enable the Global Fund and partners to re-deploy those savings into expanded VL testing as mandated by the WHO 2013 Guidelines and in alignment with the UNAIDS 90-90-90 treatment targets *(119)*.

It has been suggested that programmatic resources could be conserved by reducing the frequency of CD4 testing in favour of VL monitoring *(120)*. Much of the discussion depends on the cost of testing, and it is too early to predict whether POC devices will ultimately drive down the cost of VL testing – preferably below US$ 15 per test. Over time, competition among POC and non-POC platforms could eventually lead to pricing similar to CD4 pricing levels.

POC alternatives for VL

The availability of POC technologies is expected to overcome some of the technological limitations to improving access to VL, particularly in remote rural areas. Some of the early systems are designed for near-POC implementation and slightly higher processing volumes and are validated for plasma processing alone (requiring a laboratory centrifuge). Other POC systems will include validation for DBS and whole blood samples that are more typically obtained at POC facilities. As mentioned above, tradeoffs in test sensitivity may be necessary for the convenience (or necessity) of DBS/whole blood samples, at least until POC plasma separation becomes feasible. At the time of this report, a limited number of platforms are on the market for POC/near-POC VL: Cepheid (Xpert® HIV-1 Viral Load, December 2014) and Diagnostics for the Real World Ltd (SAMBA I/II, 2013 limited release). The Alere™ VL platform is anticipated for release in 2015, with the NWGHF Savanna p24 and the Cavidi RT testing alternatives anticipated in 2016 (see Appendix 4).

HIV genotyping for drug resistance

As more people are initiated onto a lifetime of ART, the emergence of HIVDR becomes a concern. HIVDR undermines the effectiveness and cost of ART and negatively impacts transmission, morbidity and mortality. In tandem with expanded access to treatment, there is evidence of increasing prevalence of transmitted HIVDR *(121)*. Since 2004, levels of resistance have been slowly increasing – not yet endangering the effectiveness of ART programmes – however, efforts to maintain the first-line ART and prevent resistance are becoming a priority.

HIVDR can be detected through routine monitoring of the VL of patients on ART. Antiretroviral treatment failure is defined by a persistently detectable VL[43] at least six months after ART initiation, as measured by two consecutive VL measurements within a 3-month interval (with

[43] Preferably benchmarked above a threshold of 1000 cp/mL, otherwise 3000–5000 cp/mL for DBS and whole blood.

adherence support between measurements). Patients with acquired HIVDR are characterized by VL that is initially suppressed by ART, but fails at a later date, typically because an interruption in drug regimen (poor adherence or ARV stockout) that enables replication of drug resistant mutations. Patients who were initially infected with a drug-resistant strain generally never achieve ART suppression. HIVDR can also be detected through routine monitoring at the population level. WHO recommends that HIVDR surveillance be integrated into national HIV programmes to assess prevalence of HIVDR in key populations: (i) newly initiating ART (pretreatment HIVDR) to inform the national choice for first-line ART; (ii) already on ART (acquired HIVDR) to inform selection of second-line regimens; (iii) recently infected with HIV (transmitted HIVDR) to document the transmission of drug-resistant virus; and (iv) infants under 18 months to inform selection of the first-line regimen.

In tandem with the VL tests discussed above, laboratory genotyping tests are designed to confirm HIVDR through the detection of specific drug resistance mutations.[44] Standard commercial genotyping assays (ViroSeq® from Abbot; TruGene® from Siemens) and "home brew" laboratory tests are found primarily in central reference laboratories, as well as the more costly sequencing approach *(122)*. As VL programmes mature, HIV genotyping can play an important tool for surveillance and targeted patient treatment, provided the process can be made cost-effective for resource-limited settings *(25,123)*. However, less expensive and more accessible options for HIV genotyping are necessary, with alternatives for DBS *(124,125)*, in-house testing *(126,127)* and POC[45] in development.

Coinfections and platform polyvalency

Coinfections are common among immune-compromised populations, and people living with HIV are at higher risk in areas of high disease prevalence. Coinfections such as tuberculosis, hepatitis, meningitis, malaria and STIs can have complications for HIV treatment in the timing and choice of ART. In endemic regions, reflex testing for suspected coinfections may be included in a clinical algorithm. Cases such as malaria may be diagnosed with a rapid test, however, many other coinfections require molecular diagnostics.

The ability to perform multiple tests on a single platform (polyvalency) becomes important for instrument-based platforms in resource-limited settings. To minimize capital equipment investment, it is more cost-effective for a laboratory to run multiple assays on one platform rather than invest in a different platform for each test. In addition, a single platform minimizes issues with training, maintenance and supply chain. Given the differences in chemistry and processing requirements for proteins and nucleic acids, most instrument platforms support either polyvalent protein testing or polyvalent nucleic acid testing.[46] Both qualitative and

[44] Particularly for the prevalence of transmitted drug resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) such as nevirapine or efavirenz.

[45] PANDAA assay: www.aldatubio.com.

[46] A protein/NAT test in development: www.rheonix.com.

quantitative tests are useful for centralized, POC and near-POC patient management. In some cases, a qualitative test (EID) may have different clinical utility than a quantitative test (VL). In other applications, a single test can probe with multiple markers for simultaneous detection of disease as well as specific mutations for drug resistance.

Most commercial laboratory platforms (Abbott, bioMérieux, Hologic, QIAGEN, Roche, Siemens) offer a menu of reagent-based tests for their instruments, including HIV, HBV, HCV, HPV, chlamydia and gonorrhoea *(2)*. These large laboratory platforms are capable of performing multiple tests in parallel, while most POC instruments perform only one test at a time. Most new POC platforms such as Alere™ begin with a single test cartridge as the first product, and then develop additional tests for high-priority or coinfective indications. Some of the near-POC platforms such as Cepheid Xpert® combine attributes of both, with a menu of cartridge-based tests that can be performed using multiple modules for parallel processing. Somewhere in the middle are the open PCR platform assemblies, with the capability of varying reagents as necessary for different tests.

As new platforms become available, more tests are needed for coinfection indications such as tuberculosis, HCV, HBV, meningitis and STIs (including cervical cancer). Particularly for POC and near-POC settings, it is important to enable multiple choices for instrument platforms and healthy competition in the marketplace – at a cost that would enable diagnostics to become part of routine comprehensive HIV care in the future.

The HIV diagnostic landscape

This report describes the current HIV diagnostic landscape from initial diagnosis through staging and monitoring of the disease for the people living with HIV. With emphasis towards broader access to HIV treatment and care for patients worldwide, the diagnostic landscape must adapt in order to achieve cost-effective and accessible services necessary to implement high-quality comprehensive HIV care. Improved access to quality diagnostics will play a critical role in: (i) early detection and treatment of HIV to reduce morbidity and mortality, and maximize the preventive impact of treatment; (ii) detection of drug resistance to enable the appropriate ARTs, and reduce the spread of drug-resistant strains; (iii) preservation of drug regimens to increase programme effectiveness and successful treatment for each patient; and (iv) detection and care of co-morbidities for a more comprehensive approach to patient health. As platforms and test modalities become available, diagnostic services should have strategic implementation to increase the number of patients served regardless of location. The most effective delivery will include a combination of high-volume centralized testing and low-volume POC access, with the appropriate combination depending on country-specific factors such as geography, demographics, transportation and efficiencies of scale.

Significant advances have recently been made towards increasing access to diagnostics testing, and testing options should continue to expand. A combination of new methodologies (DBS) and new technologies (POC) for ART staging and monitoring are now available, with additional

platforms in the pipeline that should ensure a multiplicity of options and a robust market in the future. Improved transport efficiencies for centralized test centres are enabling cost-effective consolidation of high-volume automated processing. The pace at which countries can implement a strategic blend of these diagnostic services will determine the impact on HIV treatment and care over the next decade.

There are a number of key areas for future work to improve diagnostics for HIV, including: (i) quality improvements at all levels of diagnostic testing; (ii) continued improvement for sample transport and return of results for central laboratories; (iii) access to tools for comprehensive HIV care including coinfection; (iv) mapping of barriers and acceleration potential for new technology introduction, especially for POC; and (v) strategic analysis of diagnostic options relative to country characteristics.

Strategic funding on the part of UNITAID and other funders could make a difference in a number of these areas, including advances in laboratory instrumentation and new POC technologies. UNITAID has committed to funding programmes to expand access to diagnostics worldwide, including projects to facilitate optimization, evaluation, regulatory approval, procurement and adoption of improved tools. By helping to fast-track access and reduce costs of more effective technologies and treatment, UNITAID can assist the worldwide effort to reduce the global health burden in underserved markets and resource-limited settings.

"The author notes no conflicts of interest."

Appendix 1: Operational characteristics

CD4 technologies

BD FACSCalibur™ System	
ASSAY	
Type of technology	Large, benchtop, 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)
Intended use	Determining percentages or counts of helper/inducer T-lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
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)
Turnaround time	60 minutes for 40 tests run on a rack, including incubation time
Capacity (per run)	~200–250 samples per day
Throughput per technician/per day	40 per hour, after ~30-minute incubation time
INSTRUMENT	
Physical dimensions, weight	Width: 91.4 cm; height: 61.5 cm; depth: 67.3 cm; 109.1 kg (~240 lbs)
Power requirements, battery if applicable	100–240 V AC mains; 50–60 Hz
Environmental requirements	Temperature: 16–29 °C (60–85 °F); humidity: 10–90% relative non-condensing
Robustness	Large laboratory instrument
Display	In workstation
Peripherals/supporting instrumentation	Separate FACSCalibur™ workstation (BD FACStation™); computer and colour printer separate from instrument
Barcode scanner	Optional
Connectivity	LIMS
SAMPLE PREPARATION	
Sample type and volume	At least 100 µL whole blood collected in either 2 mL or 4 mL K2 EDTA anticoagulant tubes
Sample preparation	Process: (i) blood is collected and added to tube to which reagent has been added; (ii) sample is vortexed and incubated; (iii) fixative is added to the tube, then vortexed and incubated; and (iv) sample is vortexed and run on the instrument
Sample stability	Staining to take place within 72 hours of blood draw; analysis to take place within 6 hours of staining
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central and L3 regional laboratories
3rd party supplies	Refrigerator, vortex and pipettor; cost: ~US$ 1500–2500
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
PRODUCT STATUS	
Development status	On market
Regulatory status	FDA-IVD, CE-IVD
Cost/test	Volume- and assay-based; ranges from ~US$ 3–7 per test
Cost/instrument	~US$ 75 000–100 000
Other tests available (polyvalency)	Cell analysis, cell sorting
More information (website link)	http://www.bdbiosciences.com/us/instruments/clinical/cell-analyzers/bd-facscalibur/m/744696

BD FACSPresto™	
ASSAY	
Type of technology	Small, benchtop, fixed volume cytometer
Output	Absolute CD4, CD4% and Hb
Intended use	Enumeration of absolute and percentage CD4 count and haemoglobin in venous and capillary whole blood
Limit of detection/quantitation/linear range	CD4: 50–4000 cell/µL; CD4%: 5–60% Hb: 2–20 g/dL
Sensitivity	N/A
Specificity	N/A
Reagent stability	Dried reagents require no refrigeration; stable for 12 months at 10–31°C
Turnaround time	3–4 minutes reading; plus incubation of cartridge (18 minutes)
Capacity (per run)	Maximum of ~60–80 samples per day
Throughput per technician/per day	~50–60 samples per technician per day; flexible throughput capabilities; walk-away operation
INSTRUMENT	
Physical dimensions, weight	Length: ~26 cm (10.2”); height: ~28.5 cm (11.2”); depth: ~25 cm (9.8”); ~7 kg (~15 lbs) (instrument only)
Power requirements, battery (if applicable)	100–240 V AC at 45–65 Hz mains power; analyzer contains onboard rechargeable battery that 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)
Robustness	Robust, portable POC instrument
Display	LED multicolour screen integrated into instrument; touchscreen keyboard on the device
Peripherals/supporting instrumentation	Dedicated CPU integrated into instrument; onboard printer (prints on thermal paper)
Barcode scanner	Integrated into instrument for test cartridges only
Connectivity	Results can be downloaded via USB; the USB port also can be used to support an external Bluetooth or GPRS/GSM module to communicate with SMS printer or the port could 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
SAMPLE PREPARATION	
Sample type and volume	~20 µL of capillary (fingerstick) blood wicked directly into BD cartridge or ~20 µL of venous blood collected in EDTA anti-coagulant tube

Sample preparation	For capillary blood: (i) lancet finger; (ii) apply blood drops to cartridge; (iii) close cartridge; (iv) incubate cartridge; (v) insert cartridge into analyzer; (vi) enter patient ID; (vii) read result from LED screen; and (viii) print result
Sample stability	Blood stable up to 24 hours if in EDTA tube at 20–25 °C; cartridge must be inserted and tested within 2 hours of sample application
IMPLEMENTATION	
Infrastructure requirements	Can be used at all levels L4-L1 health facilities, including health centres or in mobile facilities
3rd party supplies	For venous samples: transfer pipette; for capillary samples: sterile lancets, alcohol swabs, cotton gauze, Band-Aid
Training	Minimal training required; lay person can be trained in less than half a day; primary skill required is for correct lancet blood draw
Maintenance	Analyzer contains an integrated camera and 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)
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD; FDA 510(k) in process; WHO prequalified
Cost/test	<US$ 10 in resource-limited settings
Cost/instrument	<US$ 10 000 in resource-limited settings
Other tests available (polyvalency)	Cell analysis, cell sorting
More information (website link)	http://www.bdbiosciences.com/sg/instruments/facspresto/index.jsp

BD FACSCount™ System	
ASSAY	
Type of technology	Benchtop, 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
Intended use	N/A
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
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)
Turnaround time	60–90 minutes incubation, 2–3 minutes per test
Capacity (per run)	~30–80 samples per day
Throughput per technician/per day	20 per hour, after initial 60–90 minutes incubation
INSTRUMENT	
Physical dimensions, weight	Width: 43.2 cm; height: 38.1 cm; depth: 55.9 cm; 25.9 kg (57.1 lbs); fluid reservoirs empty
Power requirements, battery (if applicable)	100–240 V AC mains; 50–60 Hz; 160 W (maximum rated power)
Environmental requirements	Temperature: 10–40 °C (50–104 °F); humidity: N/A

Robustness	Benchtop laboratory instrument
Display	Display screen integrated into instrument
Peripherals/supporting instrumentation	Dedicated CPU, printer (thermal paper) integrated into instrument
Barcode scanner	Optional
Connectivity	N/A
SAMPLE PREPARATION	
Sample type and volume	0.5–5 mL whole blood collected in EDTA anticoagulant (sample volume per test is 50 µL)
Sample preparation	Process: (i) blood is collected and added to tube; (ii) sample is vortexed and incubated; (iii) fixative is added to the tube, then vortexed and incubated; and (iv) sample is vortexed and run on the instrument
Sample stability	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
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites
3rd party supplies	Refrigerator, vortex and pipettor; cost: ~US$ 1500–2500
Training	Moderate training required for laboratory technicians; skills required for phlebotomy, touchscreen data entry; prompts on the instrument display guide the 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
PRODUCT STATUS	
Development status	On market
Regulatory status	FACSCount™ system is FDA-IVD; FACSCount™ CD4 Reagents and FACSCount™ Reagent kits are FDA-IVD and CE-IVD; WHO prequalified
Cost/test	Volume based; ranges from ~US$ 3.50–10 per test
Cost/instrument	~US$ 30 000
Other tests available (polyvalency)	Cell analysis, cell sorting
More information (website link)	http://www.bdbiosciences.com/us/instruments/clinical/cell-analyzers/bd-facscount/m/744703

Beckman Coulter CYTOMICS FC 500 MCL and MPL Systems	
ASSAY	
Type of technology	Large, benchtop, bead-based flow cytometer; two different loaders (MCL and MPL)
Output	Absolute and percentage CD4 counts
Intended Use	Specify assay; the Cytomics FC 500 is a system intended for use as an in vitro diagnostic device for the qualitative and quantitative measurement of biological and physical properties of cells and other particles; these properties are measured when the cells pass through one or two laser beams in single file
Sensitivity	Scatter: detects 0.5–40 µm diameter particles Fluorescence: 600 MESF (FITC), 300 MESF (PE) and 600 MESF (APC)
Specificity	N/A

Reagent stability	Reagents must be stored at 2–8 °C (36–46 °F); PLG reagents are shipped with an expiration date of 1 year
Turnaround time	About 30 minutes, after 20–30 minutes incubation
Capacity (per run)	47 samples per hour with the MCL; >60 samples per hour with the MPL
Throughput per technician/per day	The Cytomics FC 500 system is a high-volume (on average, 45 samples per hour with the MCL, and one plate of 96 samples in less than 90 minutes with the MPL and the Beckman Coulter CellMek automated preparation system), high-performance system that is geared for use in busy reference laboratories
INSTRUMENT	
Physical dimensions, weight	Width: 90 cm (35.5”); with MPL 97.8 cm (38.5”); height: 61 cm (24”); with MPL 61 cm (24”); depth: 88.9 cm (35"); with MPL 88.9 cm (35”); 84.8 kg (~187 lbs) (cytometer with MPL; computer/monitor and power supply are separate)
Power requirements, battery (if applicable)	115–220 V AC mains; two 50–60 Hz lines required; power supply weighs 54.4 kg (120 lbs)
Environmental requirements	Operating temperature: 16–32 °C (60–90 °F); humidity: 30–80%
Robustness	Large benchtop laboratory instrument
Display	External monitor with 17” flat screen display with printer
Peripherals/supporting instrumentation	Operating system: Microsoft® Windows™ 7
Barcode scanner	Included
Connectivity	LIMS/Data Innovations Instrument Manager
SAMPLE PREPARATION	
Sample type and volume	>1 mL (100 µL used) whole blood collected in EDTA anticoagulant
Sample preparation	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 20–30 minutes; (iv) sample is lysed; and (v) the test is run on the instrument Alternatively, CellMek automation can be used for PLG reagents
Sample stability	PLG application: 5 days post draw
IMPLEMENTATION	
Infrastructure requirements	Significant training required for laboratory technicians
3rd party supplies	Refrigerator, vortex and pipettor; cost: ~US$ 1500–2500
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 IMMUNO-TROL) are provided by Beckman Coulter; WBC count should be performed to determine whether cell counts are outside the normal range, which could influence CD4 count results
EQA	Compatible with CD4 EQA programmes
PRODUCT STATUS	
Development status	On market
Regulatory status	FDA-IVD, CE-IVD for FC 500 MPL
Cost/test	Volume based; ranges from ~US$ 2.50–8.00 per test
Cost/instrument	~US$ 90 000; ~US$ 200 000 with CellMek
Other tests available (polyvalency)	PLG Application: CD45/CD4 can be measured as well as multiparametric DNA analysis, platelet studies, reticulocyte enumeration, cell biology/functional studies and a broad range of other research applications
More information (website link)	http://www.beckmancoulter.com/wsrportal/WSR/diagnostics/clinical-products/flow-cytometry/flow-cytometers/index.htm

Beckman Coulter Aquios CL™	
ASSAY	
Type of technology	Benchtop, bead-based flow cytometer
Output	AQUIOS Tetra-1 Panel Monoclonal Antibody Reagent: Total CD3+, CD3+CD4+,CD3+CD8+, CD3+CD4+/CD3+CD8+ (ratio only) lymphocyte percentages and absolute counts; CD45+ absolute count; CD45+ Low SS (lymphocytes) percentage and absolute count
Intended use	Use in the immunologic assessment of patients having, or suspected of having, immune deficiency
Limit of detection/quantitation/linear range	Total CD3+ 55-4700; CD3+/CD4+ 35-3000; CD3+/CD8+ 45-1600; CD45 350-26500 cells/µL
Sensitivity	N/A
Specificity	N/A
Reagent stability	Antibody onboard 72 hours cumulative, 8 hours continuous
Turnaround time	~20 minutes for first result, 25 results/hour thereafter up to one full 96-well plate (measured with Tetra-1 or Tetra-2+)
Capacity (per run)	Autoloader holds up to 8 cassettes at a time with up to 5 sample tubes each and allows for continuous loading and unloading
Throughput per technician/per day	N/A
INSTRUMENT	
Physical dimensions, weight	System: width: 32” (82 cm); depth: 22” (56 cm); height: 22” (56 cm) Workstation: width: 23” (59 cm); depth: 5” (13 cm); height: 18” (46 cm) Supply cart: width: 21” (54 cm); depth: 18” (46 cm); height: 16” (41 cm)
Power requirements, battery (if applicable)	See system IFU, part number B21896, Appendix B https://www.beckmancoulter.com/wsrportal/page/techdocSearch
Environmental requirements	Operate at room temperature between 18 °C and 26 °C (64.4 °F and 78.8 °F); humidity 35–85%, without condensation, operational altitude 700–1060 mbar (from -1253 to +9878 feet above sea level) For complete information, see system IFU, part number B21896, Chapter 1
Robustness	Benchtop laboratory instrument
Display	All-in-one touchscreen
Peripherals/supporting instrumentation	AQUIOS System with reagent cart and computer loaded with AQUIOS System software, database, and Tetra tests (Tetra-1, Tetra-2, and Tetra Combo)
Barcode scanner	Included
Connectivity	LIMS
SAMPLE PREPARATION	
Sample type and volume	EDTA anticoagulated whole blood for a 13 x 75 mm tube; Aquios CL™ requires 750 µL of whole blood
Sample preparation	Fully automated, onboard
Sample stability	Maximum of 24 hours from draw, stored at 20–25 °C (68–77 °F)
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites (test results should be reviewed by a qualified flow cytometrist)
3rd party supplies	None required
Training	Computer-cased training course + 1 applications specialist visit; Aquios CL™ can be operated safely by minimally trained laboratory staff
Maintenance	No routine maintenance calibration required annually; in case of breakdown, vendor-trained technician required to repair

Internal QC	AQUIOS IMMUNO-TROL Cells and AQUIOS IMMUNO-TROL Low Cells (positive cell controls)
EQA	Beckman Coulter IQAP, NEQAS, CAP and AFREQAS
PRODUCT STATUS	
Development status	On market
Regulatory status	FDA-IVD, CD-IVD
Cost/test	US$ 4–12, dependent on test volume, pay terms available
Cost/instrument	US$ 60–100 000, dependent on test volume; reagents rentals, leases and other programmes available
Other tests available (polyvalency)	AQUIOS Tetra-2+ Panel Monoclonal Antibody Reagent: Total CD3+, CD3-CD19+, CD3-CD56+ and/or CD16+ lymphocyte percentages and absolute counts; CD45+ absolute count; CD45+ Low SS (lymphocytes) percentage and absolute count
More information (website link)	www.beckmancoulter.com http://www.aquioscl.com

Beckman Coulter Aquios CL™: FlowCare PLG Application	
ASSAY	
Type of technology	Benchtop, bead-based flow cytometer
Output	Identification and enumeration of CD4+ absolute cell count and CD4+ lymphocyte percentage when used in combination with AQUIOS Flow-Count fluorospheres as a single platform measurement
Intended use	Use in the immunologic assessment of patients having, or suspected of having, immune deficiency
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
Reagent stability	Antibody onboard 72 hours cumulative, 8 hours continuous
Turnaround time	Time from loading a blood specimen to result for the first sample is ~35 minutes, including sample preparation and analysis; subsequent sample results (including sample preparation) are reported at a rate of 24 results per hour for up to 1 full microplate with no interruptions
Capacity (per run)	Autoloader holds up to 8 cassettes at a time with up to 5 sample tubes each and allows for continuous loading and unloading
Throughput per technician/per day	N/A
INSTRUMENT	
Physical dimensions, weight	System: width: 32” (82 cm); depth: 22” (56 cm); height: 22” (56 cm) Workstation: width: 23” (59 cm); depth: 5” (13 cm); height: 18” (46 cm) Supply cart: width: 21” (54 cm); depth: 18” (46 cm); height: 16” (41 cm)
Power requirements, battery (if applicable)	See system IFU, part number B21896, Appendix B https://www.beckmancoulter.com/wsrportal/page/techdocSearch
Environmental requirements	Operate at room temperature between 18 °C and 26 °C (64.4 °F and 78.8 °F); humidity 35–85%, without condensation; operational altitude 700–1060 mbar (from -1253 to +9878 feet above sea level) For complete information, see system IFU, part number B21896, Chapter 1 https://www.beckmancoulter.com/wsrportal/page/techdocSearch
Robustness	Benchtop laboratory instrument
Display	All-in-one touchscreen
Peripherals/supporting instrumentation	AQUIOS System with reagent cart and computer loaded with AQUIOS System software, database and tetra Tests (Tetra-1, Tetra-2, and Tetra Combo); printer not supplied

Barcode scanner	Included
Connectivity	LIMS
SAMPLE PREPARATION	
Sample type and volume	EDTA anticoagulated whole blood for a 13 x 75 tube mm; Aquios CL™ requires 750 µL of whole blood
Sample preparation	Fully automated, onboard
Sample stability	May be processed up to 72 hours (3 days) of sample collection
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites (test results should be reviewed by a qualified flow cytometrist)
3rd party supplies	None required
Training	Computer-based training course + 1 applications specialist visit Aquios CL™ can be operated safely by minimally trained laboratory staff
Maintenance	No routine maintenance; calibration required annually; in case of breakdown, vendor-trained technician required to repair
Internal QC	AQUIOS IMMUNO-TROL Cells (PLG/Tetra) and AQUIOS IMMUNO-TROL Low Cells (PLG/Tetra), which are positive cell controls
EQA	Beckman Coulter IQAP, NEQAS, CAP and AFREQAS
PRODUCT STATUS	
Development status	On market
Regulatory status	CD-IVD
Cost/test	Dependent on test volume, pay terms available
Cost/instrument	US$ 60–100 000, dependent on test volume; reagents rentals, leases and other programmes available
Other tests available (polyvalency)	See Aquios Tetra table
More information (website link)	www.beckmancoulter.com http://www.aquioscl.com

Sysmex Partec CyFlow® Counter	
ASSAY	
Type of technology	Desktop, volumetric flow cytometer
Output	CD4 absolute and CD4% (CD3 and CD8 on request)
Intended use	In vitro diagnostic use
Limit of detection/quantitation/linear range	5–5000 cells/µL
Sensitivity	N/A
Specificity	N/A
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 in the dark for up to 12 months maximum
Turnaround time	After 15 minutes incubation time (outside the instrument), 3 minutes per test for counting, analysing and saving
Capacity (per run)	20 test per hour for sample preparation in batches
Throughput per technician/per day	20 tests per hour = 160 tests per day
INSTRUMENT	
Physical dimensions, weight	Width: 325 mm; height: 330 mm; depth: 265 mm; 11.5 kg (~25.3 lbs) (cytometer only)
Power requirements, battery (if applicable)	100–240 V AC mains or 12 V DC/5A power (on car battery or solar panels); 50–60 Hz

Environmental requirements	Operating temperature: 15–30 °C; humidity: 20–85% non-condensing
Robustness	Small benchtop laboratory instrument
Display	8.4" TFT colour touchscreen integrated into instrument
Peripherals/supporting instrumentation	Dedicated Intel® CPU integrated into instrument; data storage of ~20 000 datasets; built-in thermal printer integrated into instrument
Barcode scanner	N/A
Connectivity	USB port
SAMPLE PREPARATION	
Sample type and volume	20 µL venous EDTA whole blood
Sample preparation	Dry reagents: (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; and (iv) run sample in CyFlow® Counter 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; and (v) run sample on the Partec device In either case, the process for CD4% requires the addition of a second buffer
Sample stability	48 hours at 2–8 °C
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites
3rd party supplies	Refrigerator (only required when using liquid mAB reagents); cost ~US$ 500
Training	Training is required
Maintenance	Routine preventative maintenance required; in case of breakdown, vendor-trained technician required to repair (generally available locally)
Internal QC	Instrument supports QC (Count Check Beads green as non-biological controls and Control Blood – dry as biological controls)→ONLY for CD4 absolute
EQA	Compatible with CD4 EQA programmes
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD
Cost/test	€1.75 (~US$ 1.94) per test for absolute CD4 and €2.50 (~US$ 2.78) for CD4 absolute and CD4 percentage combined; high-volume discounts available on request
Cost/instrument	€18 800 (~US$ 20 868) for CyFlow® Counter + Starterkit, including installation and user training of €1950 (~US$ 2164); CyFlow® ALAPS II (CyFlow® Autoloading and Auto Preparation Station) optionally available
Other tests available (polyvalency)	Total lymphocytes and WBC, CD3 and CD8 optional
More information (website link)	http://www.sysmex-partec.com/instrumentation/flow-cytometry.html

Sysmex Partec CyFlow® miniPOC	
ASSAY	
Type of technology	Portable and compact flow cytometer
Output	CD4 absolute and CD4%
Intended use	In vitro diagnostic use
Limit of detection/quantitation/linear range	5–5000 cells/µL
Sensitivity	N/A
Specificity	N/A

Reagent stability	Dry reagents can be stored at room temperature and have a maximum shelf life of 6 months
Turnaround time	After 15 minutes incubation time (outside the instrument), 3 minutes per test for counting, analysing and saving
Capacity (per run)	20 test per hour for sample preparation in batches
Throughput per technician/per day	20 tests per hour = 160 tests per day
INSTRUMENT	
Physical dimensions, weight	Width: 270 mm; height: 240 mm; depth: 188 mm; 6.2 kg
Power requirements, battery (if applicable)	100–240 V AC mains or 12 V DC power (battery pack available); 50–60 Hz
Environmental requirements	Temperature: 15–30 °C (operative); humidity: 20–85% relative non-condensing
Robustness	Robust, portable
Display	5.7” colour touchscreen integrated into instrument
Peripherals/supporting instrumentation	Dedicated Intel® Atom™ CPU integrated into instrument; Windows™-based analysis software; data storage of ~20 000 datasets; built-in thermal printer integrated into instrument
Barcode scanner	None
Connectivity	USB port
SAMPLE PREPARATION	
Sample type and volume	20 µL whole blood collected in EDTA anticoagulant
Sample preparation	Dry reagents: (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; and (v) attach syringe to CyFlow® miniPOC
Sample stability	48 hours at 2–8 °C
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites
3rd party supplies	None
Training	Training is required
Maintenance	Routine preventative maintenance required; in case of breakdown, vendor-trained technician required to repair (generally available locally)
Internal QC	Supports internal QC (Count Check Beads green – dry as non-biological controls)
EQA	Compatible with CD4 EQA programmes
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD
Cost/test	€3.00 (~US$ 3.33) per test for absolute CD4 and CD4 percentage combined, including all needed consumables and disposables, high-volume discounts available on request
Cost/instrument	€10 255 (~US$ 11 383) for CyFlow® miniPOC + Starterkit, including user training of €1365 (~US$ 1515)
Other tests available (polyvalency)	None
More information (website link)	http://www.sysmex-partec.com/instrumentation/flow-cytometry.html

Alere Pima™ CD4 Test	
ASSAY	
Type of technology	Portable benchtop, fixed volume cytometer
Output	Absolute CD4 counts only
Intended use	Intended for the rapid in vitro quantitative measurement of CD3+/CD4+ T cells (T -helper cells) in capillary or venous whole blood
Limit of detection/quantitation/linear range	3–2168 cells/µL
Sensitivity	N/A
Specificity	N/A
Reagent stability	Freeze-dried reagents require no refrigeration; stable for 12 months at 2–30 °C
Turnaround time	18–20 minutes
Capacity (per run)	Maximum of ~20 samples per day
Throughput per technician/per day	~20 samples per technician per day; no batching capabilities; walk-away operation
INSTRUMENT	
Physical dimensions, weight	Length: 22 cm (8.7”); height: 16 cm (6.3”); depth: 13 cm (5.1”); 2.54 kg (~5.6 lbs) (instrument only)
Power requirements, battery (if applicable)	100–240 V AC at 47–63 Hz mains power; analyser contains onboard rechargeable battery with sufficient capacity to run ~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
Robustness	Robust, portable POC instrument
Display	LED monochrome screen integrated into instrument with 16 button keypad
Peripherals/supporting instrumentation	1000 test results can be stored on the instrument archive; separate printer (prints on thermal paper); powered by the instrument (with rechargeable batteries onboard); length: 95 mm; width: 93 mm; height: 66 mm; ~350 g, including paper roll
Barcode scanner	Integrated into instrument for test cartridges only
Connectivity	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
SAMPLE PREPARATION	
Sample type and volume	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
Sample preparation	Blood is wicked directly into the sample collector contained in the Pima™ cartridge or 25 µL of venous blood collected in EDTA anticoagulant tube
Sample stability	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
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	For venous samples: volumetric or transfer pipette; for capillary samples: sterile lancets, alcohol swabs, dry swabs (also available from Alere™)
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
PRODUCT STATUS	
Development status	On market
Regulatory status	CD-IVD, WHO prequalified
Cost/test	US$ 6–12 per test
Cost/instrument	US$ 6500–12 000
Other tests available (polyvalency)	None
More information (website link)	http://alerehiv.com/hiv-monitoring/alere-pima-cd4/

Omega Diagnostics Group PLC/Burnet Institute VISITECT® CD4	
ASSAY	
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
Intended use	N/A
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
Reagent stability	N/A
Turnaround time	~40 minutes, including incubation
Capacity (per run)	~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)
INSTRUMENT	
Physical dimensions, weight	Width: 12 cm (4.7”); height: 8.5 cm (3.3”); depth: 7.7 cm (3.0”); 390 g (~14 oz)
Power requirements, battery (if applicable)	None for cartridge; reader 12V DC via adapter (110–240 V), optional battery pack
Environmental requirements	TBD
Robustness	Robust, portable POC instrument
Display	None for cartridge; 2.4” colour touchscreen for reader
Peripherals/supporting instrumentation	None included; reader can support peripheral printer
Barcode scanner	Optional with reader
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
SAMPLE PREPARATION	
Sample type and volume	30 µL of capillary (fingerstick) blood, or peripheral blood into EDTA anticoagulant
Sample preparation	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; and (vii) read results
Sample stability	N/A

IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	None required; sterile lancets (for capillary blood samples) and alcohol swabs are provided in the test kit
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 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	TBD
PRODUCT STATUS	
Development status	In development
Regulatory status	TBD
Cost/test	US$ 5 per test (estimated)
Cost/instrument	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 by eye
Other tests available (polyvalency)	None
More information (website link)	http://www.omegadiagnostics.com/cd4/

ChipCare Corporation ChipCare-CD4	
ASSAY	
Type of technology	CD4 quantitative cell counting
Output	Quantitative
Intended use	Remote health settings, community-level health centres, district hospitals
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
Reagent stability	>12 months
Turnaround time	<15 minutes
Capacity (per run)	single use
Throughput per technician/per day	20+ tests
INSTRUMENT	
Physical dimensions, weight	<2 kg
Power requirements, battery operation	Battery charged via outlet, vehicle or solar panel
Environmental requirements	Operating temperature: 10–40 °C (50–104 °F); humidity range: 10–95% non-condensing; maximum altitude: 2000 metres (6500 feet)
Robustness	Designed and built to military specifications (MIL STD 810G)
Display	2.7” LCD
Peripherals/supporting instrumentation	Thermal printer, computer via USB
Barcode scanner	Yes
Connectivity	3G, Bluetooth
SAMPLE PREPARATION	
Sample type and volume	Whole blood – 20 µL

Sample preparation	On cartridge
Sample stability	Use cartridge immediately after sample application
IMPLEMENTATION	
Infrastructure requirements	L2 district hospital, L1 clinic
3rd party supplies	Lancet and pipette to be shipped with cartridges
Training	Minimal training required, half-day
Maintenance	None required
Internal QC	Yes
EQA	Compatible
PRODUCT STATUS	
Development status	In development
Regulatory status	N/A
Cost/test	US$ 6–8 (estimated)
Cost/instrument	US$ 3500–5000 (estimated)
Other tests available (polyvalency)	In pipeline – STIs, NTDs
More information (website link)	www.chipcare.ca

EID/VL technologies

Roche COBAS® AmpliPrep® System Automated extraction instrument	
ASSAY	
Type of technology	Automated extraction and sample preparation
Output	Samples ready for amplification and detection on COBAS® TaqMan® Analyzer
Intended use	N/A
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
Reagent stability	Varies by reagent, but most must be stored at 2–8 °C (36–46 °F); all reagents are stable until expiration date
Turnaround time	3 racks of 24 specimens in ~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
INSTRUMENT	
Physical dimensions, weight	Width: 165 cm (65”); depth: 75 cm (29.5”); height: 95 cm (37.4”); 373 kg (822 lbs); trolley table: 167 cm (65.7”) x 76 cm (29.9”) x 55 cm (21.7”)
Power requirements, battery (if applicable)	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 metres (6500 feet)
Robustness	Large laboratory instrument
Display	Monitor VGA 14"
Peripherals/supporting instrumentation	Custom-built PC (included) with Microsoft® Windows™ XP and AMPLILINK®; software to control COBAS® AmpliPrep® System; printer HP 1320
Barcode scanner	Supplied with instrument: COBAS® AmpliPrep®: onboard barcode scanner for reagent racks, reagent cassettes and specimen clips; AMPLILINK data station: hand-held barcode scanner for original specimen/specimen clip
Connectivity	Printer interface: LPT interface via parallel port

SAMPLE PREPARATION	
Sample type and volume	1000 µL of plasma or 70 µL DBS for Taqman® analyzers
Sample preparation	Plasma transferred to a properly identified, sterile screw-cap, polypropylene tube after centrifugation; requires test-specific, barcoded, 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
Sample stability	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
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Pipettors, vortex mixer, refrigerator, gloves and other lab consumables
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
EQA	Fully compatible with existing EQA programmes
PRODUCT STATUS	
Development status	On market
Regulatory status	FDA-IVD, CE-IVD; WHO prequalified
Cost/test	N/A
Cost/instrument	~US$ 80 000–100 000
Other tests available (polyvalency)	Broad range of Taqman® assays
More information (website link)	http://molecular.roche.com/instruments/Pages/COBASAmpliPrepInstrument.aspx

Roche COBAS® TaqMan® 48 Automated amplification/detection instrument	
ASSAY	
Type of technology	RT-PCR; fully automated real-time amplification and detection
Output	HIV-1 RNA quantification; DNA qualitative measure
Intended use	TaqMan® HIV-1 Test v2.0 is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma, for the clinical management of HIV-1 group M and HIV-1 group O infected patients
Limit of detection/quantitation/linear range	The test can quantitate HIV-1 RNA over the range from 20 to 10 million cp/mL
Sensitivity	98.3% (465/473; 95% CI = 96.7% to 99.3%)
Specificity	99.4% (516/519; 95% CI = 98.3% to 99.9%)
Reagent stability	No onboard reagents are required on the analyzer; all reagent addition is performed during the sample preparation process
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 (per an 8-hour shift)
INSTRUMENT	
Physical dimensions, weight	Width: 50 cm (19.7”); depth: 79 cm (31.1”); height: 58 cm (22.8”); 55 kg (121 lbs)

Power requirements, battery (if applicable)	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)
Robustness	Large benchtop laboratory instrument
Display	PC screen
Peripherals/supporting instrumentation	Custom-built PC supplied with the analyzer; data station runs Microsoft® Windows™ XP Professional operating system and AMPLILINK Software
Barcode scanner	AMPLILINK hand-held barcode scanner for original specimen/specimen clip
Connectivity	AMPLILINK software is a Windows™-based, LIS-compatible user interface that manages up to 3 COBAS® TaqMan® 48 analyzers
SAMPLE PREPARATION	
Sample type and volume	PCR-ready setup samples from COBAS® AmpliPrep®
Sample preparation	AmpliPrep®
Sample stability	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)
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Microtiter plate centrifuge (not supplied by Roche) and other general supplies
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
PRODUCT STATUS	
Development status	On market
Regulatory status	TaqMan® HIV-1 Test v2.0: FDA-IVD, CE-IVD; Canada-IVD; Japan-IVD; WHO prequalified
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
Other tests available (polyvalency)	Broad range of Taqman® assays, including HCV and HBV
More information (website link)	http://molecular.roche.com/instruments/Pages/COBASTaqMan48Analyzer.aspx http://molecular.roche.com/assays/Pages/COBASAmpliPrepCOBASTaqManHIV-1Testv20.aspx

Roche COBAS® TaqMan® 96 Automated amplification/detection instrument	
ASSAY	
Type of technology	RT-PCR; fully automated real-time amplification and detection
Output	HIV-1 RNA quantification; DNA qualitative measure
Intended use	TaqMan® HIV-1 Test v2.0 is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma, for the clinical management of HIV-1 group M and HIV-1 group O infected patients
Limit of detection/quantitation/linear range	The test can quantitate HIV-1 RNA over the range from 20 to 10 million cp/mL

Sensitivity	98.3% (465/473; 95% CI = 96.7% to 99.3%)
Specificity	99.4% (516/519; 95% CI = 98.3% to 99.9%)
Reagent stability	No onboard reagents are required on the analyzer; all reagent addition is performed during the sample preparation process
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 (per 8-hour shift)
INSTRUMENT	
Physical dimensions, weight	Analyzer: 45" x 30" x 37" (114.3 x 76.2 x 94 cm); table: 45" x 30" x 20" (114.3 x 76.2 x 50.8 cm); 448 lbs (203 kg) with PC
Power requirements, battery (if applicable)	Analyzer: 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)
Robustness	Large laboratory instrument
Display	PC screen
Peripherals/supporting instrumentation	Custom-built PC supplied with the analyzer; data station runs Microsoft® Windows™ XP Professional operating system
Barcode scanner	Hand-held barcode scanner for original specimen/specimen clip
Connectivity	LIS
SAMPLE PREPARATION	
Sample type and volume	PCR-ready setup samples from COBAS® AmpliPrep®
Sample preparation	AmpliPrep®
Sample stability	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)
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Microtiter plate centrifuge (not supplied by Roche) and other general supplies
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
PRODUCT STATUS	
Development status	On market
Regulatory status	TaqMan® HIV-1 Test v2.0: FDA-IVD, CE-IVD; Canada-IVD; Japan-IVD; WHO prequalified
Cost/test	TaqMan® HIV-1 Test v2.0: US$ 9.40 in resource-limited settings; range is dependent on instrument purchase, reagent rental and volume-based tiered pricing http://molecular.roche.com/globalaccessprogram/Pages/default.aspx
Cost/instrument	US$ 100 000–110 000, including docking station
Other tests available (polyvalency)	Broad range of Taqman® assays, including HCV and HBV

More information (website link)	http://molecular.roche.com/instruments/Pages/COBASTaqManAnalyzer.aspx http://molecular.roche.com/assays/Pages/COBASAmpliPrepCOBASTaqManHIV-1Testv20.aspx

Roche cobas® Liat™	
ASSAY	
Type of technology	RT-PCR; portable benchtop, sample preparation, amplification, detection
Output	Qualitative HIV-1 and HIV-2 or quantitative HIV-1 (viral load)
Intended use	TBD
Limit of detection/quantitation/linear range	TBD
Sensitivity	TBD
Specificity	TBD
Reagent stability	TBD
Turnaround time	TBD
Capacity (per run)	TBD
Throughput per technician/per day	TBD
INSTRUMENT	
Physical dimensions, weight	Width: 11.4 cm (4.5”); height: 19 cm (7.5”); depth: 24.1 cm (9.5”); 3.76 kg (~8.3 lbs)
Power requirements, battery (if applicable)	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
Robustness	Small benchtop laboratory instrument
Display	LCD colour touchscreen with 4 hard keys and 4 arrow buttons integrated into instrument
Peripherals/supporting instrumentation	Dedicated central processing unit integrated into instrument; ~20 000 test results can be stored on the instrument archive
Barcode scanner	Integrated into instrument for operator barcode, patient barcode and cobas® Liat™ tube barcode
Connectivity	TBD
SAMPLE PREPARATION	
Sample type and volume	TBD
Sample preparation	Operation: (i) apply sample to Liat™ tube; (ii) scan the tube’s barcode on the device; and (iii) insert tube into cobas® Liat™ analyzer; the analyzer will start assay and the result will be reported automatically in 20 minutes, depending on assay
Sample stability	TBD
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	Sterile lancets (for capillary blood samples), alcohol swabs, dry swabs, gauze, Band-Aid
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 the assay run alert the operator in the event of malfunction or error; remote system monitoring/diagnosis performed via the cobas®Liat™ analyzer’s built-in network connectivity interface

Internal QC	Extensive internal controls: sample volume control, internal process controls, and more
EQA	TBD whether compatible with EQA programmes
PRODUCT STATUS	
Development status	HIV tests in development
Regulatory status	TBD
Cost/test	TBD
Cost/instrument	TBD
Other tests available (polyvalency)	Strep A (CLIA WAIVED, FDA-IVD and CE-IVD), influenza A/B, (FDA-IVD and CE-IVD) A/B and RSV (CE-IVD)
More information (website link)	http://molecular.roche.com/instruments/Pages/cobasLIATsystem.aspx

Abbott *m*2000*sp* Automated extraction instrument	
PLATFORM	
Type of technology	Automated extraction and sample preparation (magnetic particle technology)
Output	HIV-1 RNA for Abbott *m*2000*rt*
Intended use	N/A
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
Reagent stability	HIV-1 VL: reagents (liquid), as well as controls and calibrators, must be stored at ‑10 °C or colder when not in use and must be shipped on dry ice; extraction reagents are ready to use and can be stored at 15–30 °C; all reagents are stable until expiration date
Turnaround time	Extraction inclusive PCR plate preparation: depends on number of samples from 2 hours and 30 minutes for 24 samples to 4 hours and 40 minutes for 96 samples
Capacity (per run)	96 samples (1–93 patient samples + 3 controls)
Throughput per technician/per day	192 samples (2 batches of 96 samples)
INSTRUMENT	
Physical dimensions, weight	Width: 179 cm (70.5”); height 187 cm (72.3”); depth 124.4 cm (49.1”)
Power requirements, battery (if applicable)	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)
Robustness	Large laboratory instrument, calls per year (CPY) ~1.7 CPY for the *m*2000*sp*
Display	PC monitor
Peripherals/supporting instrumentation	Data station, monitor and printer are supplied with the instrument
Barcode scanner	Hand-held barcode scanner is supplied with the instrument
Connectivity	LIS connection available
SAMPLE PREPARATION	
Sample type and volume	RealTi*m*e HIV-1: human plasma (ACD-A and EDTA) specimens may be used with the assay; DBS is in preparation (LBE available Q2 2016);the assay offers 4 different plasma input protocols: 0.2 mL; 0.5 mL; 0.6 mL; 1.0 mL
Sample preparation	Steps include vortexing (internal control, calibrators, controls and specimens) pipetting, centrifuge, etc.
Sample stability	HIV-1 VL: freshly drawn whole blood can be held at 15–30 °C for up to 6 hours or at 28 °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 or lower

IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Pipettes, vortex mixer and refrigerator; freezer
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
EQA	Amenable to EQA
PRODUCT STATUS	
Development status	On market
Regulatory status	FDA-IVD, CE-IVD, WHO prequalified
Cost/test	N/A
Cost/instrument	Refer to the Global Fund website for further information.
Other tests available (polyvalency)	Broad RealTi*me* assay menu, including HCV, HBV, HIV-1 Qualitative, MTB, MTB RIF/INH Resistance, HPV, CT/NG (outside the United States)
More information (website link)	https://www.abbottmolecular.com/products/instrumentation-automation/realtime-pcr/m2000-sp-rt.html

Abbott *m*24*sp* Automated extraction instrument (not available in the United States)	
PLATFORM	
Type of technology	Automated extraction and sample preparation (magnetic particle technology)
Output	HIV-1 RNA for Abbott *m*2000*rt*
Intended use	N/A
Limit of detection/quantitation/linear range	N/A
Sensitivity	N/A
Specificity	N/A
Reagent stability	HIV-1 VL: reagents (liquid), as well as controls and calibrators, must be stored at -10 °C when not in use and must be 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
Turnaround time	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	2 full runs = 48 samples per 8-hour shift
INSTRUMENT	
Physical dimensions, weight	Width: 88.1 cm (34.7”); height: 75.9 cm (29.9”); depth: 69.6 cm (27.4”); 185 lbs (84 kg)
Power requirements, battery (if applicable)	100–240 V
Environmental requirements	Temperature: 15–30 °C (59–86 °F); humidity: 30–86% relative non-condensing at 30 °C (86 °F) or below; maximum altitude: up to 3000 metres (9800 feet)
Robustness	Mid-size laboratory instrument, calls per year (CPY) *m*24*sp*: 0.8 CPY
Display	PC monitor
Peripherals/supporting instrumentation	Data station, monitor and printer are supplied with the instrument
Barcode scanner	Hand-held barcode scanner is supplied with the instrument

Connectivity	Connectivity to AbbottLink available
SAMPLE PREPARATION	
Sample type and volume	RealTi*m*e HIV-1: human plasma (ACD-A and EDTA) specimens may be used with the DBS is in preparation (LBE available in the second quarter of 2016); the assay offers 4 different plasma input protocols: 0.2 mL; 0.5 mL; 0.6 mL; 1.0 mL
Sample preparation	Steps include vortexing (internal control, calibrators, controls and specimens) pipetting, centrifuge, etc.
Sample 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 or lower
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Pipettes, vortex mixer and refrigerator; freezer
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
EQA	Amenable to EQA
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD, WHO prequalified
Cost/test	N/A
Cost/instrument	Refer to the Global Fund website for further information
Other tests available (polyvalency)	Broad RealTi*m*e assay menu, including HCV, HBV, HIV-1 Qualitative, MTB, MTB RIF/INH Resistance, HPV, CT/NG (outside United States)
More information (website link)	https://www.abbottmolecular.com/products/instrumentation-automation/realtime-pcr/m24sp.html

Abbott *m*2000*rt* Automated amplification/detection instrument	
ASSAY	
Type of technology	RT-PCR; fully automated real-time amplification and detection
Output	RealTi*m*e HIV-1 assay: quantification HIV-1 RNA levels (HIV-1 viral load) in plasma; assay results can be reported in cp/mL, log (cp/mL), IU/mL or log (IU/mL)
Intended use	Intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to ART as measured by changes in plasma HIV-1 RNA levels
Limit of detection/quantitation/linear range	Range from 40 to 10 million cp/mL
Sensitivity	LOD of the Abbott RealTi*m*e HIV-1 assay is 40 cp/mL with the 0.6 mL sample volume procedure (plasma)
Specificity	100% (95% CI 99.28 to 100%)
Reagent stability	No onboard reagents are required on the instrument; all reagent addition is performed during the sample preparation process
Turnaround time	Amplification and detection cycle takes 3 hours

Capacity (per run)	HIV-1 VL: up to 96 (1–93 patient samples, + 3 controls)
Throughput per technician/per day	288 samples per day; sample preparation and extraction can be the limiting factor
INSTRUMENT	
Physical dimensions, weight	Width: 34 cm (13.4”); height: 49 cm (19.3”); depth: 45 cm (17.8”)
Power requirements, battery (if applicable)	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
Robustness	Small laboratory instrument, calls per year (CPY) ~0.5 CPY for the *m*2000*rt*
Display	PC monitor
Peripherals/supporting instrumentation	Data station, monitor and printer are supplied with the instrument
Barcode scanner	Hand-held barcode scanner is supplied with the instrument
Connectivity	LIS connectivity available
SAMPLE PREPARATION	
Sample type and volume	RealTi*m*e HIV-1: human plasma (ACD-A and EDTA) specimens may be used with the assay; DBS is in preparation (LBE available in the second quarter of 2016); assay offers 4 different plasma input protocols: 0.2 mL; 0.5 mL; 0.6 mL; 1.0 mL
Sample preparation	Abbott *m*24*sp* or *m*2000*sp*
Sample 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 or lower
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Pipettes, vortex mixer and refrigerator; freezer
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
EQA	Amenable to EQA
PRODUCT STATUS	
Development status	On market
Regulatory status	RealTi*m*e HIV-1 assay: FDA-IVD, CE-IVD, WHO prequalified
Cost/test	Refer to the Global Fund website for further information
Cost/instrument	Refer to the Global Fund website for further information
Other tests available (polyvalency)	Broad RealTi*me* assay menu, including HCV, HBV, HIV-1 Qualitative, MTB, MTB RIF/INH Resistance, HPV, CT/NG (outside the United States)
More information (website link)	https://www.abbottmolecular.com/products/instrumentation-automation/realtime-pcr/m2000-sp-rt.html

bioMérieux NucliSENS® easyMAG® Automated extraction instrument	
PLATFORM	
Type of technology	Automated extraction instrument

Output	Purified nucleic acids (RNA and DNA)
Intended use	N/A
Limit of detection/quantitation/linear range	N/A
Sensitivity	Refer to sensitivity of the EasyQ® HIV
Specificity	Refer to specificity of the EasyQ® HIV
Reagent stability	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
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
INSTRUMENT	
Physical dimensions, weight	Width: 100 cm (39.4”); depth: 65 cm (25.6”); height: 53 cm (20.9”); 106 kg (233.7 lbs); PC monitor and keyboard: 8 kg (17.6 lbs)
Power requirements, battery (if applicable)	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)
Robustness	Large benchtop laboratory instrument
Display	Onboard monitor
Peripherals/supporting instrumentation	None
Barcode scanner	Supplied with the system
Connectivity	Can be linked with LIS using NucliSENtral® software
SAMPLE PREPARATION	
Sample type and volume	100–1000 µL plasma for NucliSENS® EasyQ® HIV assay; DBS protocol for 100 µL EDTA whole blood and on 100 µL capillary whole blood
Sample preparation	Entire extraction process takes place in a single sample compartment, which minimizes potential sample loss and cross-contamination
Sample stability	EDTA plasma specimens can be stored in NucliSENS® Lysis Buffer for a maximum of 14 days at 2–8 °C; 24 hours at ambient temperature (2–30 °C) Venous DBS can be stored with desiccant sachets in an air-impermeable bag without a significant loss of HIV-1 RNA at room temperature (15–30 °C) for a maximum period of 9 months Packed venous DBS can alternatively be stored without a significant loss of HIV-1 RNA for a maximum of: 3 weeks at 2–8 °C; 9 weeks at 37±3 °C (in case of high humidity: maximum 3 weeks); 3 months at -20 °C Capillary DBS can be stored with desiccant sachets in an air impermeable bag at room temperature (15–30 °C) for a period of 7 weeks
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Dedicated pipettes and filter tips, vortex mixer and refrigerator
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
EQA	Amenable to EQA
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD, WHO prequalified

Cost/test	N/A
Cost/instrument	~€72 000 (US$ 95 000)
Other tests available (polyvalency)	Broad application for DNA and RNA extraction
More information (website link)	http://www.biomerieux-diagnostics.com/nuclisensr-easymag

bioMérieux NucliSENS® miniMAG® Semi-automated extraction instrument	
PLATFORM	
Type of technology	Semi-automated extraction instrument
Output	Purified nucleic acids (RNA and DNA)
Intended use	N/A
Limit of detection/quantitation/linear range	N/A
Sensitivity	Refer to sensitivity of the EasyQ®HIV
Specificity	Refer to specificity of the EasyQ® HIV
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
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 with 2 miniMAG®s at the same time)
INSTRUMENT	
Physical dimensions, weight	Width: 43.8 cm (17.2”); depth: 11.4 cm (4.5”); height: 15.3 cm (6”); .6 kg (8 lbs)
Power requirements, battery (if applicable)	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)
Robustness	Small benchtop laboratory instrument
Display	Integrated readout
Peripherals/supporting instrumentation	None
Barcode scanner	None
Connectivity	None
SAMPLE PREPARATION	
Sample type and volume	100–1000 µL plasma for NucliSENS® EasyQ® HIV assay; DBS protocol for 100 µL EDTA whole blood and on 100 µL capillary whole blood
Sample preparation	Entire extraction process takes place in a single sample compartment, which minimizes potential sample loss and cross-contamination
Sample stability	EDTA plasma specimens can be stored in NucliSENS® Lysis Buffer for a maximum of 14 days at 2–8 °C; 24 hours at ambient temperature (2–30 °C) Venous DBS can be stored with desiccant sachets in an air-impermeable bag at room temperature (15–30 °C) without a significant loss of HIV-1 RNA for a maximum period of 9 months Packed venous DBS can alternatively be stored without a significant loss of HIV-1 RNA for a maximum of 3 weeks at 2–8 °C; 9 weeks at 37±3 °C (in case of high humidity: maximum 3 weeks); 3 months at -20 °C Capillary DBS can be stored with desiccant sachets in an air impermeable bag at room temperature (15–30 °C) for a period of 7 weeks
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Dedicated pipettes and filter tips, vortex mixer and refrigerator; benchtop centrifuge (1.5 mL tubes), thermoshaker, centrifuge (2 mL lysis tubes)

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
EQA	Amenable to EQA
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD, WHO prequalified
Cost/test	N/A
Cost/instrument	~€6800 (US$ 9000)
Other tests available (polyvalency)	Broad application for DNA and RNA extraction
More information (website link)	http://www.biomerieux-diagnostics.com/nuclisens-minimag

bioMérieux NucliSENS® EasyQ® Automated amplification/detection instrument	
ASSAY	
Type of technology	NASBA; automated, real-time amplification and detection
Output	NucliSENS® EasyQ® HIV-1 assay v 2.0: HIV-1 viral load for subtypes A-H and CRFO2_AG
Intended use	To assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of ART be measuring changes in plasma/DBS (from EDTA whole blood) HIV-1 RNA levels during the course of ART
Limit of detection/quantitation/linear range	1 mL plasma: 25 to 7.9 x 10^6 cp/mL; 0.5 mL plasma: 50 to 1.5 x 10^7 cp/mL; 0.1 mL plasma: 292 to 7.1 x 10^7 cp/mL DBS: 500 to 21 x 10^6 cp/mL
Sensitivity	25 VQA cp/mL LOD (95% detection level), 1 mL input volume
Specificity	Plasma (1 mL input): 100% (95% CI 97.2–100) DBS: 100% (95% CI 95.4–100)
Reagent stability	NucliSENS® EasyQ® HIV-1 v 2.0 assay: storage at 2–8 °C; all reagents are stable until expiration date
Turnaround time	~1.5 hours for 48 samples
Capacity (per run)	Up to 48 patient samples (minimum is 8 patient samples)
Throughput per technician/per day	192 samples (4 runs of 48)
INSTRUMENT	
Physical dimensions, weight	42 cm (16.5“); 42 cm (16.5“); 22 cm (8.7“); 20.5 kg (45 lbs)
Power requirements, battery (if applicable)	100–240 V
Environmental requirements	Operating temperature: 15–30 °C; humidity: no greater than 80% relative humidity; maximum altitude: 2000 metres (6500 feet)
Robustness	Several small benchtop laboratory instruments
Display	PC monitor
Peripherals/supporting instrumentation	Data station and monitor are supplied with the instrument; printer not supplied with instrument
Barcode scanner	Not supplied with instrument
Connectivity	Can be linked with LIS using NucliSENtral® software
SAMPLE PREPARATION	

Sample type and volume	Plasma: 1 mL, 0.5 mL, 0.1 mL; DBS: 2 spots 50 µL each
Sample preparation	Samples are extracted with miniMAG® or easyMAG®
Sample stability	The obtained eluates can be stored at 2–8 °C or at -20 °C
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Dedicated pipettes and filter tips
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	Amenable to EQA
PRODUCT STATUS	
Development status	On market
Regulatory status	NucliSENS® EasyQ® HIV-1 v 2.0: CE-IVD, WHO prequalified
Cost/test	The average price per test of EasyQ® HIV assay v2.0, including extraction and detection/amplification is about €18 (US$ 23.75)
Cost/instrument	~€37 100 (US$ 49 000)
Other tests available (polyvalency)	Broad range of NucliSENS® assays
More information (website link)	http://www.biomerieux-usa.com/clinical/nuclisens-easyq

Siemens VERSANT® kPCR Molecular System automated sample preparation and amplification/detection modules	
ASSAY	
Type of technology	RT-PCR; automated real-time extraction, amplification and detection (kinetic PCR/(kPCR technique)
Output	HIV-1 RNA quantification (viral load) of Group M subtypes A-G and CRF01-AE, Group O
Intended use	For the quantitation of human immunodeficiency virus type 1 (HIV-1) RNA in plasma from HIV- 1 infected individuals
Limit of detection/quantitation/linear range	From 37 to 11 million cp/mL
Sensitivity	100%
Specificity	99.5%
Reagent stability	Reagents are stored frozen (from -30 °C to -10 °C); calibrators and controls are stored frozen (from -90 °C to -60 °C)
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, 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
INSTRUMENT	
Physical dimensions, weight	Sample preparation module/Detection module: width: 112.4 cm (44”)/ 36.8 cm (14.5”); depth: 100.6 cm (39.5”)/53.4 cm (21”); height: 90.5 cm (35.5”)/45.7 cm (18”); 320 lbs (145 kg)/55 lbs (25 kg)
Power requirements, battery (if applicable)	100–240 V; 50–60 Hz
Environmental requirements	Temperature: 18–30 °C; humidity: 30–80% non-condensing; maximum altitude: 0–2000 meters (6560 feet)

Robustness	Large benchtop laboratory instrument
Display	PC monitor
Peripherals/supporting instrumentation	Computer supplied, 17 in screen and separate keyboard; printer optional
Barcode scanner	Supplied with the instrument
Connectivity	LIMS
SAMPLE PREPARATION	
Sample type and volume	Up to 500 µL plasma or 1 DBS (50–100 µL); whole blood collected in EDTA tubes
Sample preparation	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; and (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
Sample stability	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
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories
3rd party supplies	Dedicated pipettes and filter tips
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
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD, WHO prequalified
Cost/test	Not disclosed
Cost/instrument	Not disclosed
Other tests available (polyvalency)	HCV, HBV, CT/GC, CMV, EBV, HSV 1 and 2, VZV, HHV-6, BKV, JCV, adenovirus, and parvovirus B19, open channel for laboratory developed assays and 3rd party assays
More information (website link)	http://www.healthcare.siemens.com/molecular-diagnostics/molecular-diagnostics-systems/versant-kpcr-molecular-system

Hologic Aptima® HIV-1 Quant Dx Assay on the Panther® System	
ASSAY	
Type of technology	Real-time TMA
Output	HIV-1 RNA quantitation in plasma and HIV-1 RNA detection in plasma or serum for HIV-1 group M (subtypes A, C, D, F, G, CRF01_AE, CRF02_AG) and groups N and O
Intended use	Aptima® HIV-1 Quant Dx Assay: in vitro nucleic acid amplification test for the detection and quantitation of human immunodeficiency virus type 1 (HIV-1) RNA on the fully automated Panther® system; it is intended for use as an aid in the diagnosis of HIV-1 infection, including acute or primary infection, as a confirmation of HIV-1 infection and as an aid in clinical management of patients infected with HIV-1

Limit of detection/quantitation/linear range	LOD: 13 cmL; linear range: 30 to 1 x 106 cp/mL
Sensitivity	LOD: 13 cp/mL (95% detected in 500 µL plasma)
Specificity	100% (95% CI: 99.4–100%)
Reagent stability	Reagents stable until expiration (typically >6 months shelf life); once reconstituted, reagents are stable for up to 72 hours on the Panther® and for up to 30 days refrigerated
Turnaround time	Time to first result: 2 hours and 40 minutes (for first 5 results), with 5 additional results every 5 minutes
Capacity (per run)	Panther® can hold up to 120 patient specimens with continuous loading after first rack of 15 specimens is pipetted
Throughput per technician/per day	320 samples per 8-hour day and 560 samples per 12-hour day (additional 240 samples processed without operator attendance); random, continuous loading of samples eliminates the need for batching
INSTRUMENT	
Physical dimensions, weight	Width: 122 cm (48”); height: 175 cm (69”); depth: 81.5 cm (32”); 363 kg (~800 lbs)
Power requirements, battery (if applicable)	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
Robustness	• direct tube sampling capability of primary tube and no processing of sample prior to loading onto the instrument • random access capability using onboard sample barcode readers with samples and assay requests performed in a random manner, allowing samples to be loaded and tested as they are received throughout the day • a reagent identification system (barcode or other) to automatically link reagent lot and expiration date information to the sample report • positive sample identification with ability to load samples and let the system run by itself automatically • reagent dispense verification and liquid level sensing capability to verify proper dispense of sample and reagents into reaction tube • ability to automatically decontaminate and remove amplification reaction tubes from the assay processing area without operator intervention in a closed system • ability to perform maintenance steps automatically at times scheduled by operator
Display	Touchscreen monitor attached to Panther® for easy access
Peripherals/supporting instrumentation	Built-in Dell computer running Windows™ Vista; HP Office Jet Pro 8000 printer
Barcode scanner	Built-in scanners automatically read reagents and samples on loading; hand-held scanner attached to Panther® for master lot input; both read Code 39, Code 93, Code 128 (isbt 128), Interleaved 2 of 5, and Codabar
Connectivity	LIMS, Hologic Secure PRO360° allows remote issue evaluation to either resolve or send out appropriate applications or engineering support, mobile device connectivity available through a 3rd party software tool
SAMPLE PREPARATION	
Sample type and volume	1200 µL plasma; dilution feature allows quantitation with 240 µL of plasma; secondary specimen minimum volume is 700 µL
Sample preparation	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

Sample stability	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
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional laboratories; can be run in general purpose laboratory
3rd party supplies	Centrifuge, refrigerator, freezer, and optional tube rocker
Training	3-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 the College of American Pathologists, National Institute for Biological Standards and Control, Quality Control for Molecular Diagnostics, United States Nuclear Regulatory Commission, Accrometrix
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD certified, WHO prequalification and US FDA in process
Cost/test	US$ 10–25 in resource-limited settings; range depends on instrument purchase, reagent rental and volume-based tiered pricing
Cost/instrument	US$ 150 000–175 000
Other tests available (polyvalency)	FDA-IVD: Aptima® Combo 2 assay for CT/NG, Trichomonas vaginalis, HPV, HPV 16 18/45 genotyping assay CE-IVD: Aptima® CT assay and Aptima® GC assay
More information (website link)	http://www.aptimavirology.com http://www.hologic.com/products/clinical-diagnostics-and-blood-screening/assays-and-tests/aptima-hiv-1-quant-dx-assay http://www.hologic.com/products/clinical-diagnostics-and-blood-screening/instrument-systems/panther-system

Diagnostics for the Real World Ltd SAMBA I system	
PLATFORM	
Type of technology	SAMBAprep: sample extraction; SAMBAamp: Isothermal amplification and visual detection for HIV-1
Output	Semi-quantitative detection of HIV-1 RNA from plasma; qualitative detection HIV-1 total nucleic acid (RNA + DNA) from whole blood
Intended use	VL monitoring for patients on ART from plasma and detection of HIV-1 total nucleic acid (RNA + DNA) from whole blood for EID
Limit of detection/quantitation/linear range	EID LOD: ~400 cp/mL RNA (whole blood) VL LOD: 1000 cp/mL cutoff (plasma)

Sensitivity	EID: clinical sensitivity: 95.7–100% (from three independent clinical evaluations in Kenya, Uganda and Zimbabwe) VL concordance: overall concordance: 98%, 96.4%, 95.9%, 94.8% in independent clinical evaluations performed in Malawi, Zimbabwe, Kenya, Uganda, respectively
Specificity	EID: clinical specificity: 99.2–100% (from three independent clinical evaluations in Kenya, Uganda and Zimbabwe)
Reagent stability	Transport stability up to 55 °C for 1 month; reagents do not require cold-chain storage and are stable at 2–37 °C for 12 months
Turnaround time	90–120 minutes, depending on the assay
Capacity (per run)	SAMBAprep: 6 samples per run (batch); SAMBAamp: 4–8 samples run individually (random access)
Throughput per technician/per day	1 SAMBAprep + 1 SAMBAamp = 16–20 tests/day; 1 SAMBAprep + 2 SAMBAamp = 28–32 tests/day; 1 SAMBAprep + 3 SAMBAamp = 42–48 tests/day
INSTRUMENT	
Physical dimensions, weight	SAMBAprep: 68 X 65 X 51 cm; SAMBAamp: 41 X 32 X 11 cm SAMBAprep: 53 kg; SAMBAamp: 3.8 kg
Power requirements, battery (if applicable)	100–250 V, 50 Hz
Environmental requirements	Operating temperature: 10–35 °C; humidity: up to 95%
Robustness	Suitable for resource-limited settings
Display	SAMBAprep: 4.3”back-lit LCD touch panel showing operational status, step by step instructions and any system errors SAMBAamp: two-line alpha-numeric back-lit display screen that reports status, operator instructions and any errors such as temperature
Peripherals/supporting instrumentation	None
Barcode scanner	N/A
Connectivity	N/A
SAMPLE PREPARATION	
Sample type and volume	EID: 100 µL of whole blood; VL: 200 µL plasma
Sample preparation	Simple, preloaded, disposable cartridges containing all required liquid or dry reagents
Sample stability	At room temperature: whole blood up to 8 hours; plasma up to 24 hours
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites with electricity
3rd party supplies	Plasma VL requires a centrifuge to separate plasma
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
PRODUCT STATUS	
Development status	Available in limited markets
Regulatory status	CE-IVD (VL and EID) in process, WHO prequalification in process (VL and EID), ERPD Category 3 approval (VL and EID) In-country approval obtained in Kenya, Malawi, Uganda and Zimbabwe (VL); and Kenya, Uganda and Zimbabwe (EID); pending in Cameroon and Nigeria
Cost/test	Volume dependent
Cost/instrument	Volume dependent
Other tests available (polyvalency)	N/A
More information (website link)	http://www.drw-ltd.com/#!samba-i/c11zc

Diagnostics for the Real World Ltd SAMBA II system	
PLATFORM	
Type of technology	Integrated sample prep and isothermal amplification and visual detection for HIV-1
Output	Semi-quantitative detection of HIV-1 RNA from plasma and/or whole blood; qualitative detection HIV-1 total nucleic acid (RNA + DNA) from whole blood
Intended use	VL monitoring for patients on ART from plasma or whole blood and EID for HIV-1 from whole blood
Limit of detection/quantitation/linear range	EID LOD: ~400 cp/mL RNA (whole blood) VL LOD: 1000 cp/mL cutoff (plasma or whole blood)
Sensitivity	Equivalent to SAMBA I in-house testing (EID) and field evaluations in Uganda (VL)
Specificity	Equivalent to SAMBA I in-house testing (EID)
Reagent stability	Transport stability up to 55 °C for 1 month; reagents do not require cold-chain storage and are stable at 2–37 °C for 12 months
Turnaround time	90–120 minutes, depending on the assay
Capacity (per run)	Flexible, random access, modular
Throughput per technician/per day	4 tests/assay module/day; each display module controls up to 8 assay modules giving a potential throughput of up to 32 tests/day
INSTRUMENT	
Physical dimensions, weight	Display module: 22 X 22 X 19 mm; assay module: 22 X 40 X 36 cm; Display module: 2.1 kg; assay module: 9.9 kg
Power requirements, battery (if applicable)	100–250 V, 50 Hz
Environmental requirements	Operating temperature: 10–40 °C; humidity: up to 95%
Suitable for resource-limited settings	Suitable for resource-limited settings
Display	• display module has a 7” back-lit touch panel with alphanumeric display • results can be sorted by patient name, patient ID, date of test, assay type, etc. • display module reports system errors • assay module has a LED strip that indicates instrument status (white = machine available; green = in use; red = system error) • in-built printer in display module • in-built camera in assay module for automated results
Peripherals/supporting instrumentation	Integrated printer
Barcode scanner	Integrated
Connectivity	USB port, Ethernet and SMS using the phone module
SAMPLE PREPARATION	
Sample type and volume	EID: 100 µL of whole blood; VL: 200 µL plasma or 120 µL whole blood
Sample preparation	Simple, unit-dose, disposable cartridges containing all required liquid or dry reagents
Sample stability	At room temperature: whole blood up to 8 hours; plasma up to 24 hours
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities with electricity
3rd party supplies	Plasma VL and qualitative assay requires a centrifuge to separate plasma
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
PRODUCT STATUS	
Development status	Available in limited markets
Regulatory status	Same as SAMBA I (except ERPD), pending equivalency testing in Zimbabwe and Malawi for in-country approval
Cost/test	Volume dependent
Cost/instrument	Volume dependent
Other tests available (polyvalency)	In development: HIV-1 Acute infection assay, chlamydia and gonorrhoea duplex assay, influenza A and B duplex assay
More information (website link)	http://www.drw-ltd.com/#!samba-ii/c20x

Alere™ q HIV-1/2 Detect	
ASSAY	
Type of technology	RT-PCR; portable automated benchtop real-time RT RNA PCR system used for the processing and analysis of Alere™ q HIV-1/2 test cartridges: sample collection, cell lysis, target capture, reverse transcription, real-time PCR amplification and real-time fluorescence detection
Output	Alere™ q HIV-1/2 Detect test provides qualitative detection of HIV-1, Groups M (subtypes A-H), N and O, and HIV-2 group A, A/B
Intended use	Qualitative detection of HIV-1 M/N and O and HIV-2 simultaneously from whole blood
Limit of detection/quantitation/linear range	HIV-1 M/N: 2491 cp/mL; HIV-1 O: 943 cp/mL; HIV-2: 952 cp/mL
Sensitivity	N/A
Specificity	N/A
Reagent stability	Freeze-dried reagents require no refrigeration; stable for 12 months at 4–30 °C
Turnaround time	<60 minutes
Capacity (per run)	Maximum of ~8 samples per day
Throughput per technician/per day	~8 samples per technician per day; no batching capabilities; walk-away operation
INSTRUMENT	
Physical dimensions, weight	Length: 20 cm (7.87”); height: 22 cm (8.66”); depth: 31 cm (12.2”); <7.8 kg
Power requirements, battery (if applicable)	100–240 V AC at 50–60 Hz mains power; analyzer contains onboard rechargeable battery; additional external battery available
Environmental requirements	Operating temperature: 15–40 °C (59–104 °F); humidity: <95% relative humidity;
Robustness	Robust, portable POC instrument
Display	Integrated touchscreen colour display to enter patient information, view results, adjust settings, download results and navigate system software
Peripherals/supporting instrumentation	Separate printer (prints on thermal paper); USB/battery powered; length 95 mm x width 93 mm x height 66 mm; ~350 g, including paper roll
Barcode scanner	Integrated into instrument for test cartridges and compatible to external barcode readers
Connectivity	Results can be 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
SAMPLE PREPARATION	

Sample type and volume	Whole blood assay: 25 µL of capillary (fingerprick or heelprick) blood or EDTA venous blood; plasma assay: 25 µL EDTA plasma
Sample preparation	Whole blood: no manual sample preparation or pretreatment Plasma: centrifugation required; steps: (i) apply sample to cartridge; (ii) close cartridge; (iii) insert cartridge into analyzer; (iv) analysis starts automatically; (v) enter operator and sample ID; (vi) after assay is finished remove cartridge from analyzer; and (vii) read result from screen; hands-on time <3 minutes
Sample stability	Venous EDTA whole blood: 24hrs at 18–28 °C
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs (also available from Alere™); centrifuge for plasma
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	Fully compatible with existing EQA programmes
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD; WHO prequalification in process
Cost/test	N/A
Cost/instrument	N/A
Other tests available (polyvalency)	In development: quantitative HIV VL
More information (website link)	http://alerehiv.com/hiv-screening-detect/alereq-detect-cartridge/

Cepheid Xpert® HIV-1 Qual	
ASSAY	
Type of technology	RT-PCR amplification test
Output	Qualitative detection of HIV-1 from whole blood and DBS; HIV-1 Group M subtypes A, B, C, D, F, G, H, A/E and AB, CRF AG/GH, J, K, Group N and Group O
Intended use	The Xpert® HIV-1 Qual Assay, performed on the GeneXpert® Instrument Systems, is a qualitative IVD test designed to detect human immunodeficiency virus type 1 (HIV-1) total nucleic acids on the automated GeneXpert® Systems using human whole blood and DBS specimens from individuals suspected of HIV-1 infection and is validated for specimens across Group M subtypes A, B, C, D, F, G, H, A/E and A/B, CRF AG/GH, J, K, Group N and Group O; it is intended to aid in the diagnosis of HIV-1 infection in conjunction with clinical presentation and other laboratory markers
Limit of detection/quantitation/linear range	HIV-1 Qual LOD (whole blood): 203 cp/mL (VQA reference method) HIV-1 Qual LOD (DBS): 531 cp/mL (VQA reference method)
Sensitivity	Positive percent agreement: 98.2% (95% CI: 90.3–100) Negative percent agreement: 98.0% (95% CI: 89.6–100) (Comparator assay: RT-PCR HIV-1 Assay with DBS)
Specificity	100% (95% CI: 99.6–100)
Reagent stability	2–28 °C
Turnaround time	92 minutes with less than 1 minute hands-on time

Capacity (per run)	Dependent on GeneXpert® system and number of modules ranging 1–80 per system, comparable to GeneXpert® MTB
Throughput per technician/per day	GeneXpert® 4-4 Module: 21 GeneXpert® 16-16 Module: 83 GeneXpert® Infinity-80 Module: 394
INSTRUMENT	
Physical dimensions, weight	Specifications for GX-IV Processing Unit: length: 11.00”; height: 12.00”; depth: 13.25”; GX-IV Processing Unit: 25 lbs
Power requirements, battery (if applicable)	Mains power required: 100–240 V
Environmental requirements	Operating temperature: 15–30 °C; relative humidity: 10–95%, non-condensing
Robustness	Compact benchtop instrument
Display	PC monitor
Peripherals/supporting instrumentation	PC computer
Barcode scanner	Included with system
Connectivity	LIS and cloud system connectivity
SAMPLE PREPARATION	
Sample type and volume	100 µL whole blood or 1 DBS
Sample preparation	Automated within cartridge
Sample stability	EDTA-anticoagulated whole blood may be stored at 31–35 °C for up to 8 hours, 15–30 °C for up to 24 hours or at 2–8 °C for up to 72 hours, prior to preparing and testing the specimen DBS cards may be stored at 2–25 °C or -15 °C or colder for up to 12 weeks Cards may also be stored at 31–35 °C for up to 8 weeks
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	Phlebotomy supplies
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	Compatible with existing EQA programmes
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD
Cost/test	Global Access Pricing at US$ 19.90
Cost/instrument	Comparable to GeneXpert® MTB
Other tests available (polyvalency)	Broad range of Xpert® assays available, including Xpert® HIV-1 Qual, Xpert® MTB/RIF, Xpert® HCV Viral Load
More information (website link)	http://www.cepheid.com/en/cepheid-solutions-uk/clinical-ivd-tests/virology/xpert-hiv-1-qual http://www.cepheid.com/en/cepheid-solutions-uk/systems/genexpert-systems/genexpert-iv

Cepheid Xpert® HIV-1 Viral Load	
ASSAY	
Type of technology	RT-PCR amplification test

Output	Quantitative detection of HIV-1 (viral load) from plasma; HIV-1 Group M subtypes A, B, C, D, AE, F, G, H, AB, AG, J, K and Group N and Group O
Intended use	The HIV-1 VL Assay, performed on the GeneXpert® Instrument Systems, is an IVD test designed for the rapid quantitation of human immunodeficiency virus type 1 (HIV-1) in human plasma from HIV-1 infected individuals over the range of 40 to 10 million cp/mL, and is validated for specimens across Group M subtypes A, B, C, D, AE, F, G, H, AB, AG, J, K and Group N and Group O; it is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to ART as measured by changes in plasma HIV-1 RNA levels
Limit of detection/quantitation/linear range	HIV-1 VL LOD: 18.3 cp/mL (WHO reference method); HIV-1 VL LOQ: 40 cp/mL; HIV-1 VL linear range: 40 to 10 million cp/mL
Sensitivity	See LOD/LOQ
Specificity	100% (95% CI: 96.7–100) (determined from HIV seronegative blood donor specimens)
Reagent stability	2–28 °C
Turnaround time	90 minutes with less than 1 minute hands-on time
Capacity (per run)	Dependent on GeneXpert® system and number of modules ranging 1–80 per system, comparable to GeneXpert® MTB
Throughput per technician/per day	GeneXpert® 4-4 Module: 20; GeneXpert® 16-16 Module: 84; GeneXpert® Infinity-80 Module: 403
INSTRUMENT	
Physical dimensions, weight	Specifications for GX-IV Processing Unit: length: 11.00”; height: 12.00”; depth: 13.25”; GX-IV Processing Unit: 25 lbs
Power requirements, battery (if applicable)	Mains power required: 100–240 V
Environmental requirements	Operating temperature: 15–30 °C; relative humidity: 10–95%, non-condensing
Robustness	Compact benchtop instrument
Display	PC monitor
Peripherals/supporting instrumentation	PC computer
Barcode scanner	Included with system
Connectivity	LIS and cloud system connectivity
SAMPLE PREPARATION	
Sample type and volume	1 mL plasma sample input volume
Sample preparation	Automated within cartridge
Sample stability	Whole blood may be held at 15–30 °C for up to 8 hours or at 2–8 °C for up to 72 hours, prior to preparing and testing the specimen After centrifugation, plasma may be held at 15–30 °C for up to 24 hours or at 2–8 °C for up to 6 days, prior to testing Plasma specimens are stable frozen (≤-18 °C and ≤-70 °C) for 6 weeks Plasma specimens are stable up to three freeze/thaw cycles
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	Phlebotomy supplies
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	Compatible with existing EQA programmes

PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD, FDA-IVD in process
Cost/test	Global Access Pricing at US$ 19.10
Cost/instrument	Comparable to GeneXpert® MTB
Other tests available (polyvalency)	Broad range of Xpert® assays available, including Xpert® HIV-1 Qual, Xpert® MTB/RIF, Xpert® HCV Viral Load
More information (website link)	http://www.cepheid.com/en/cepheid-solutions-uk/clinical-ivd-tests/virology/xpert-hiv-1-viral-load http://www.cepheid.com/en/cepheid-solutions-uk/systems/genexpert-systems/genexpert-iv

Cavidi ExaVir™ Load separation and RT assay	
ASSAY	
Type of technology	RT ELISA-based manual measurement of RT activity
Output	Quantification of HIV-1 RT enzyme activity
Intended use	Intended for determination of the activity of the enzyme RT as a marker of retroviral replication; ExaVir™ Load kit is not intended to be used as a screening test for HIV-1 nor is it to be used as a diagnostic test to confirm the presence of HIV-1 infection
Limit of detection/quantitation/linear range	1 to 3000 fg/mL equivalent to 200–600 000 cp/mL
Sensitivity	1 fg/mL equivalent to 200 cp/mL
Specificity	>99%
Reagent stability	Reagent kits must be stored from -14 °C to -25 °C; reagent kits are stable for 24 months at -20 °C; if stored at 4–8 °C, must be used within one week
Turnaround time	48 hours for up to 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
INSTRUMENT	
Physical dimensions, weight	Footprint on bench: < 0.6 square metres
Power requirements, battery (if applicable)	100–240 V; 50–60 Hz
Environmental requirements	Temperature: 16–33 °C
Robustness	Multi-component benchtop laboratory equipment
Display	None
Peripherals/supporting instrumentation	ExaVir™ Load Analyzer software for processing results; computer required, but not supplied; no printer supplied
Barcode scanner	None
Connectivity	None, offline software for data processing
SAMPLE PREPARATION	
Sample type and volume	1 mL plasma prepared from EDTA or CPD anticoagulated whole blood
Sample preparation	Complex: sample preparation requires about 20 steps over 2 days
Sample stability	Plasma should be separated within 6 hours of the blood collection; plasma samples must be frozen once before being analysed and should be frozen at or below -20 °C
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites

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, pipettes, tips, absorbing paper, 25 mL tubes
Training	4 days of training required
Maintenance	Routine preventative maintenance required
Internal QC	Yes
EQA	Available in some regions
PRODUCT STATUS	
Development status	On market
Regulatory status	CE-IVD
Cost/test	~US$ 12–25
Cost/instrument	~US$ 4500
Other tests available (polyvalency)	Drug resistance testing in pipeline
More information (website link)	http://www.cavidi.se/exavir-load/

Cavidi ZIVA™ Automated RT Viral Load	
PLATFORM	
Type of technology	RT; fully automated measurement of RT activity
Output	Quantification of HIV-1 RT enzyme activity
Intended use	Ziva™ HIV viral load assay is intended for the quantification of human immunodeficiency virus (HIV)
Limit of detection/quantitation/linear range	TBD
Sensitivity	TBD
Specificity	TBD
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
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)
INSTRUMENT	
Physical dimensions, weight	Maximum dimensions (width x depth x height): 100 x 60 x 70 cm; weight: TBD
Power requirements, battery (if applicable)	100–240 V; 50–60 Hz
Environmental requirements	Temperature: 16–33 °C
Robustness	Large benchtop laboratory equipment
Display	Onboard computer with touchscreen
Peripherals/supporting instrumentation	Onboard computer with touchscreen
Barcode scanner	Supplied with instrument
Connectivity	LIMS, remote monitoring
SAMPLE PREPARATION	
Sample type and volume	500 µL plasma; plasma prepared from EDTA or CPD anticoagulated whole blood
Sample preparation	Load patient samples, reagents and consumables; start assay and walk away; return to collect results and empty waste
Sample stability	Plasma should be separated within 6 hours of the blood collection
IMPLEMENTATION	

Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites
3rd party supplies	Centrifuge needed for preparation of plasma
Training	Half-day of training required
Maintenance	Routine preventative maintenance required
Internal QC	Yes
EQA	Available in some regions
PRODUCT STATUS	
Development status	In development
Regulatory status	CE-IVD in process, will submit for WHO prequalification
Cost/test	TBD
Cost/instrument	TBD
Other tests available (polyvalency)	Drug resistance testing in pipeline
More information (website link)	http://www.cavidi.se/ziva/

NWGHF LYNX HIV p24 Antigen Test	
ASSAY	
Type of technology	p24 Antigen Assay for EID
Output	Qualitative detection of HIV infection
Intended use	Birth to 18 months but no evaluations have taken place in infants less than 4 weeks (studies planned)
Limit of detection/quantitation/linear range	N/A
Sensitivity	TBD pending external/independent evaluations
Specificity	TBD pending external/independent evaluations
Reagent stability	TBD
Turnaround time	51 minutes
Capacity (per run)	1 sample tested sequentially
Throughput per technician/per day	~12 samples per day
INSTRUMENT	
Physical dimensions, weight	Width: 202 mm (8.0”); height: 156 mm (6.1”); depth: 134 mm (5.3”); 1.7 kg (~3.7 lbs)
Power requirements, battery (if applicable)	Sample processor is battery powered with 12 V DC (e.g. solar or car battery) or 100–240 V AC mains recharging
Environmental requirements	15–35 °C
Robustness	Robust, portable POC instrument
Display	Display with timer and battery indicator
Peripherals/supporting instrumentation	None
Barcode scanner	None
Connectivity	Optional reader with connectivity
SAMPLE PREPARATION	
Sample type and volume	~80 µL of blood from the infant’s heel
Sample preparation	(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; and (v) read test
Sample stability	TBD
IMPLEMENTATION	

Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	Blood collection supplies
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	In process of determining whether compatible with EQA programmes
PRODUCT STATUS	
Development status	In development
Regulatory status	TBD
Cost/test	US$ 7–15 per test, depending on volume (estimate)
Cost/instrument	US$ 700–2000 for sample processor, depending on volume (estimate)
Other tests available (polyvalency)	HCV test under development
More information (website link)	http://www.nwghf.org/

NWGHF/Quidel Savanna HIV Test and Platform	
ASSAY	
Type of technology	A benchtop automated cartridge-based system that extracts, amplifies and detects nucleic acid targets for IVD applications
Output	Quantitative HIV-1 viral load results
Intended use	Aid in assessing viral response to ART as measured by changes in HIV-1 RNA levels to (i) identify virological failure; (ii) enable clinicians to provide adherence counseling; or (iii) switch failing patients to new drug regimens
Limit of detection/quantitation/linear range	LOD: 1000 cp/mL cutoff
Sensitivity	N/A
Specificity	N/A
Reagent stability	Shelf life of the assay kit is expected to be 12–18 months at 15–40 °C; humidity: 70–90%
Turnaround time	~60 minutes
Capacity (per run)	Each Savanna can run up to 2 concurrent runs with the ability to expand to more integrated systems if needed
Throughput per technician/per day	14 tests per 8-hour work day
INSTRUMENT	
Physical dimensions, weight	Width: 24 cm; height: 59 cm; depth: 62 cm; weight: TBD
Power requirements, battery (if applicable)	Powered by AC or DC mains with external battery backup; standard: external battery completes the cartridges in the instrument; optional: expanded external battery with 8-hour capacity
Environmental requirements	No cold chain or humidity control is required for shipping and transport
Robustness	Benchtop laboratory instrument completely enclosed for operation in dusty environments; comply with EN 60529
Display	Monitor and PC integrated into the instrument
Peripherals/supporting instrumentation	Internal EDGE/3G modem provided upon request, optional printer
Barcode scanner	Integrated into the instrument
Connectivity	Internal Wi-Fi modem, cellular or wired data connection. Data can be sent via cellular, data cable, Wi-Fi or USB
SAMPLE PREPARATION	

Sample type and volume	~170 µL of whole blood (converted to plasma)
Sample preparation	Whole blood will be converted into plasma with simple sample preparation materials provided with the assay kit
Sample stability	TBD
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories and some well-developed L1 primary sites
3rd party supplies	None
Training	Minimal training required; primary skill required is for correct lancet blood draw
Maintenance	TBD
Internal QC	Yes
EQA	Will be fully-compatible with existing EQA programmes
PRODUCT STATUS	
Development status	In development
Regulatory status	TBD
Cost/test	<US$ 10 per test (estimate)
Cost/instrument	<US$ 10 000 (estimate)
Other tests available (polyvalency)	TB and other assays in development
More information (website link)	http://www.nwghf.org/ www.quidel.com

Micronics PanNAT® Platform	
PLATFORM	
Type of technology	PCR
Output	Qualitative or quantitative
Intended use	Near patient diagnosis of 3+ infectious targets from sample
Limit of detection/quantitation/linear range	TBD
Sensitivity	TBD
Specificity	TBD
Reagent stability	Integrated on test cartridge; store at room temperature
Turnaround time	Assay dependent; ~60 minutes includes quality testing and sample processing
Capacity (per run)	Low to medium, with anticipated multiplex capacity for up to 5 detectors
Throughput per technician/per day	~8 assays per instrument
INSTRUMENT	
Physical dimensions, weight	Height: 30”; depth: 46”; width: 30.5”; ~24 lbs
Power requirements, battery operation	100–240 V AC mains at frequencies of 50–60 Hz with built in battery backup for ~1 hour
Environmental requirements	12–32 °C; humidity: (non-condensing) of 10–95% relative humidity; altitudes 0–2000 metres above sea level
Robustness	Built-in handle for portability
Display	Touchscreen on instrument
Peripherals/supporting instrumentation	Optional printer accessory
Barcode scanner	Optional accessory; system read
Connectivity	Full; LIS/HIS; local network; Ethernet and Wi-Fi
SAMPLE PREPARATION	
Sample type and volume	Dependent on assay (faeces, blood, urine, respiratory, cerebral spinal fluid, urethral and vaginal swabs); less than 200 µL sample size

Sample preparation	Integrated
Sample stability	Dependent on assay and sample type)
IMPLEMENTATION	
Infrastructure requirements	Target setting: L3-L4 reference lab, L2 district hospital, L1 clinic
3rd party supplies	None
Training	Quick reference guide, video, other materials available through Customer Portal of website; WebEx training
Maintenance	Minimal to none
Internal QC	Included
EQA	TBD
PRODUCT STATUS	
Development status	In development
Regulatory status	None yet; CE market and US FDA planned as well as in-country approvals
Cost/test	Varies depending on assay
Cost/instrument	~US$ 30 000 (estimate); reagent rental
Other tests available (polyvalency)	None yet
More information (website link)	www.micronics.net

WAVE 80 EOSCAPE-HIV™ System	
ASSAY	
Type of technology	PCR, integrated sample extraction and detection
Output	HIV-1 RNA level (quantitative/qualitative)
Intended use	Clinical management of adult HIV infection
Limit of detection/quantitation/linear range	VL: 400–50 000 cp/mL
Reagent stability	Cartridges are shelf stable for 1 year at 40 °C
Turnaround time	65 minutes
Capacity (per run)	1 sample per cartridge; 1 cartridge or 16 cartridges (random access) can run at a time, depending on analyzer model purchased
Throughput per technician/per day	>40
INSTRUMENT	
Physical dimensions, weight	Width: 150 mm (6.5”); depth: 150 mm (5.9”); 1.4 kg (~3.1 lbs)
Power requirements, battery (if applicable)	Mains power with 8-hour rechargeable battery backup; solar charging capable
Environmental requirements	Operating temperature: <40 °C
Robustness	Robust, portable POC instrument
Display	Integrated 7” touchscreen
Peripherals/supporting instrumentation	External printer
Barcode scanner	Integrated
Connectivity	LIMS; USB; integrated wireless connectivity
SAMPLE PREPARATION	
Sample type and volume	100 µL fingerstick whole blood
Sample preparation	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 100 µL sample from fingerstick requires specialized technique (e.g. massaging of finger; positioning of hand below heart)
Sample stability	Collection method dependent
IMPLEMENTATION	

Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	Lancet, alcohol swab (supplied in kit)
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; some laboratories may choose to include testing with reference materials as part of their quality assurance programmes
PRODUCT STATUS	
Development status	In development
Regulatory status	CE-IVD in process, WHO prequalification in process
Cost/test	VL: <US$ 12 per test (estimate)
Cost/instrument	<US$ 10 000 for EOSCAPE-1; EOSCAPE-16 pricing TBD
Other tests available (polyvalency)	In development: CT/NG, MTB, acute respiratory infection
More information (website link)	http://www.wave80.com/technology.php

MOLBIO Dx Truelab™ Real Time micro PCR system	
PLATFORM	
Type of technology	RT-PCR nucleic acid amplification
Output	HIV-1 RNA level (quantitative)
Intended use	EID/VL estimation/HIV treatment monitoring
Limit of detection/quantitation/linear range	TBD
Sensitivity	TBD
Specificity	>99%
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
Turnaround time	60 minutes (sample to result)
Capacity (per run)	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)
INSTRUMENT	
Physical dimensions, weight	Length: 21 cm (8.27”); width: 14 cm (5.5”); height: 10.9 cm (4.29”); 0.9 kg (~2 lbs)
Power requirements, battery (if applicable)	Continuous power supply not required; rechargeable lithium ion battery pack: 7.5 V; 2200 mAh provides over 8 hours of backup on a full charge
Environmental requirements	Operating temperature: 15–~35 °C; humidity: 10–80%
Robustness	N/A
Display	Integrated touchscreen colour monitor (3.2”); touchscreen interface; power on/off switches on analyzer unit
Peripherals/supporting instrumentation	Dedicated CPU integrated into instrument; ~5000 test results can be stored on the instrument archive; external 2” Bluetooth thermal printer
Barcode scanner	None
Connectivity	Support wireless connectivity (Wi-Fi, Bluetooth, GPRS)
SAMPLE PREPARATION	
Sample type and volume	100 µL plasma for VL or 100 µL of blood for screening

Sample preparation	Extraction process currently involves multiple pipetting steps that require operator interventions, including adding reagents, aspirating liquid, adding buffer, etc. (an automatic sample preparation is expected to be introduced soon); once extracted, the nucleic acid is dispensed into a chip that is inserted into the PCR analyzer and the thermal cycling and analysis takes place automatically within the analyzer
Sample stability	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
IMPLEMENTATION	
Infrastructure requirements	Appropriate for L4 central, L3 regional, L2 district laboratories, including L1 primary sites and mobile facilities
3rd party supplies	Micropipette tips, gloves, waste disposal container
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 and run from time to time; preferably under the following circumstances: (i) whenever a new shipment of test kits is received; (ii) when opening a new test kit lot; and (iii) by each new user prior to performing testing on a clinical specimen
PRODUCT STATUS	
Development status	On market
Regulatory status	Manufacturing facility is ISO 13485 and ISO 9001 certified; Indian test manufacturing license obtained and registration process under way
Cost/test	US$ 15
Cost/instrument	US$ 8000 (includes sample preparation, PCR analyser, printer, pipettes)
Other tests available (polyvalency)	Broad range of TrueNAT tests, including HBV, MTB, MTB/RIF, malaria pf, H1N1, Dengue, Chikungunya
More information (website link)	http://www.molbiodiagnostics.com

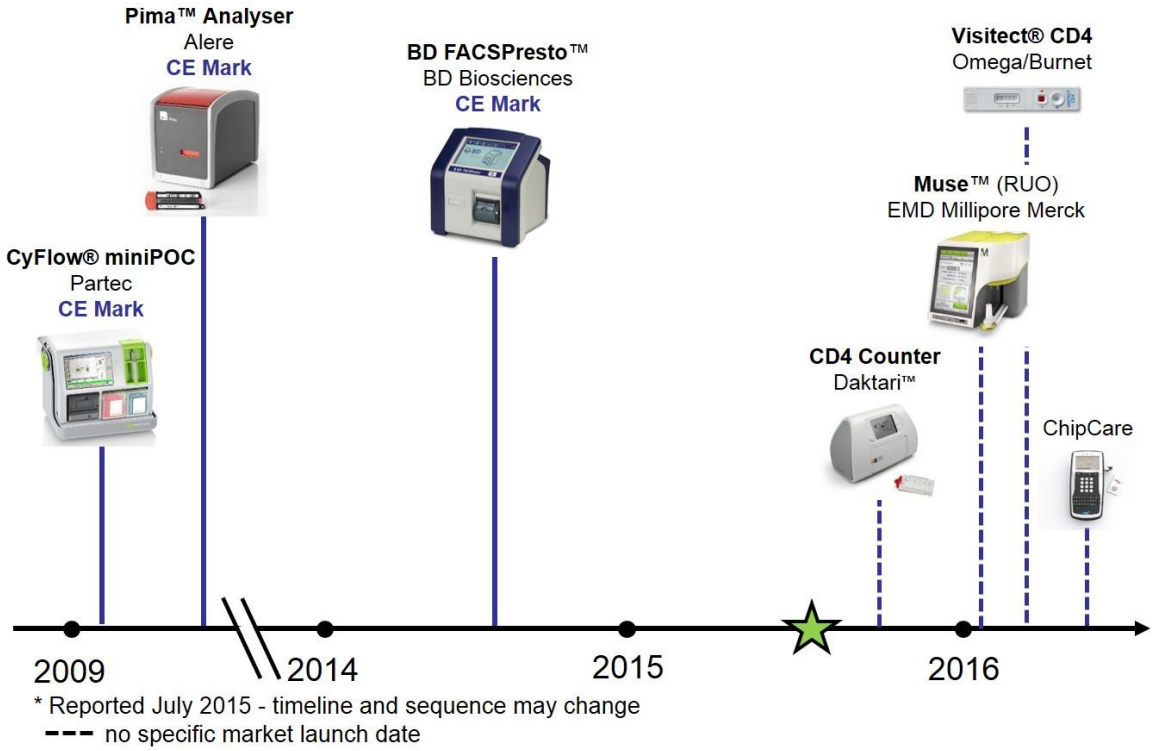
Ustar RT CPA HIV-1 Viral Load Test	
ASSAY	
Type of technology	Isothermal CPA
Output	Quantitative HIV-1 RNA (viral load)
Intended use	ART monitoring, HIV diagnosis
Limit of detection/quantitation/linear range	1000–20 000 cp/mL
Sensitivity	>95% at 1000 cp/mL cut-off
Specificity	>95%
Reagent stability	Stable for 24 months at 2–30 °C; 90% humidity, including transport stress (48 hours with fluctuations up to 50 °C and down to 0 °C)
Turnaround time	<1 hour
Capacity (per run)	4 tests with internal controls
Throughput per technician/per day	>24 tests
INSTRUMENT	
Physical dimensions, weight	TBD
Power requirements, battery (if applicable)	110–220 V AC mains current or DC power with rechargeable battery lasting 8 hours
Environmental requirements	Temperature: 4–40 °C

Robustness	Small benchtop laboratory instrument
Display	Touchscreen
Peripherals/supporting instrumentation	None
Barcode scanner	Yes
Connectivity	None
SAMPLE PREPARATION	
Sample type and volume	50–100 µL of whole blood fingerstick; or 1 mL of venous blood
Sample preparation	No more than 3–5 steps from sample to result
Sample stability	TBD
IMPLEMENTATION	
Infrastructure requirements	TBD
3rd party supplies	None
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	TBD
PRODUCT STATUS	
Development status	In development
Regulatory status	TBD
Cost/test	US$ 5.5 per test (ex-works, estimate)
Cost/instrument	<US$ 5000 (estimate)
Other tests available (polyvalency)	Broad range of assays in development, including TB
More information (website link)	http://www.bioustar.com/en/

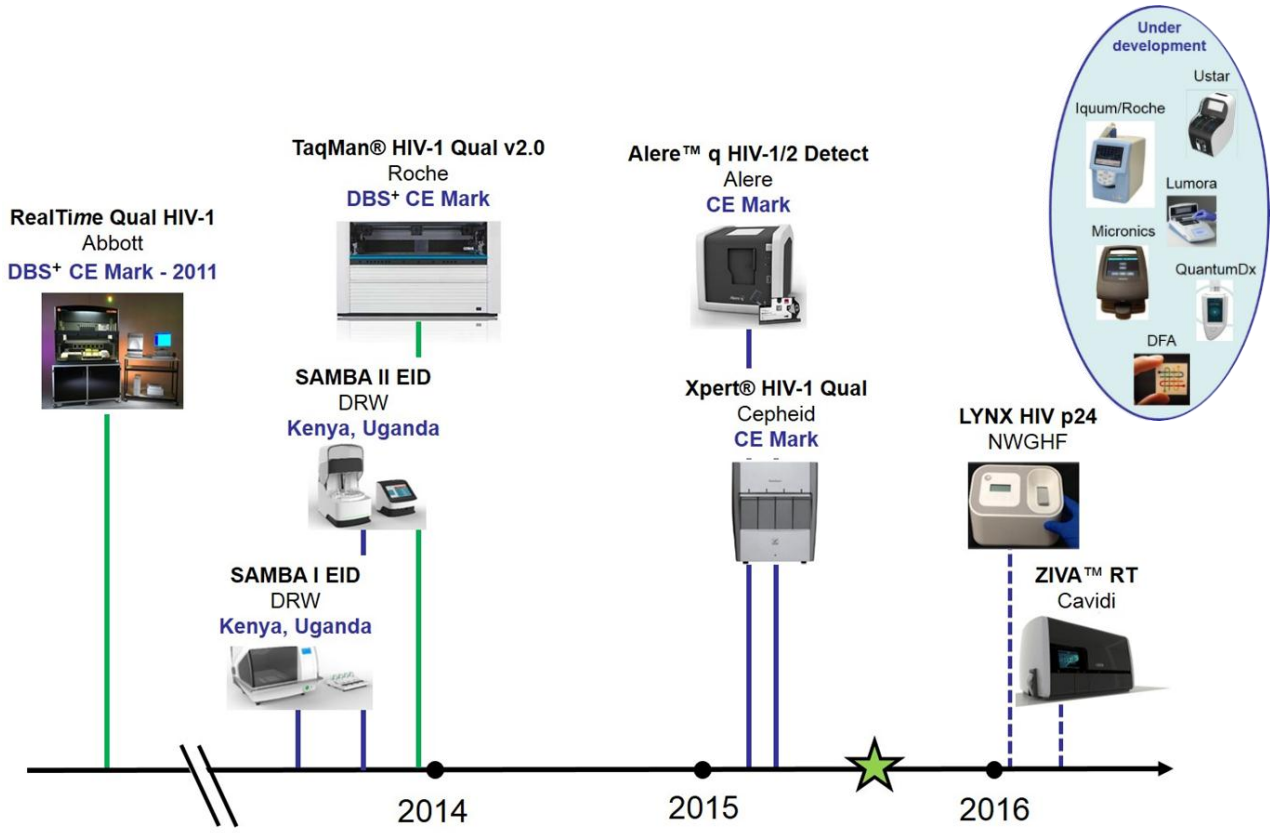
Nanobiosym Gene-RADAR® platform	
PLATFORM	
Type of technology	Nanobiophysics
Output	Quantitative and qualitative
Intended use	HIV VL and EID
Sensitivity	Target: 99%
Specificity	Target: 99%
Reagent stability	Target: up to 12 months at room temperature
Turnaround time	<1 hour
Capacity (per run)	Moderate throughput
INSTRUMENT	
Physical dimensions, weight	Height: less than 7”; length: less than 14.5”; width: less than 12.5”; <10 lbs
Power requirements, battery operation	AC mains or battery, battery life >12 hours
Display	LCD colour screen integrated into instrument
Peripherals/supporting instrumentation	None required
Connectivity	Wireless (internet and/or cellular networks)
SAMPLE PREPARATION	
Sample type and volume	Finger prick/capillary blood; <50 µL
Sample preparation	Integrated
IMPLEMENTATION	
Infrastructure requirements	L1 or higher
3rd party supplies	Sterile lancet, alcohol swab

Training	Minimal, <4 hours for lay person, <1 hour for trained medical/laboratory professional
PRODUCT STATUS	
Development status	Validation
Regulatory status	Anticipated: US FDA, CE Mark, WHO prequalification, and in-country approvals
Cost/test	Target: ~US$ 15
Cost/instrument	Target: US$ 10 000
Other tests available (polyvalency)	Currently in development: EID, Ebola and H7N9 Pipeline: HIV drug resistance, TB, HCV
More information (website link)	www.nanobiosym.com

Appendix 2: Point-of-care (POC) CD4 technologies in the pipeline

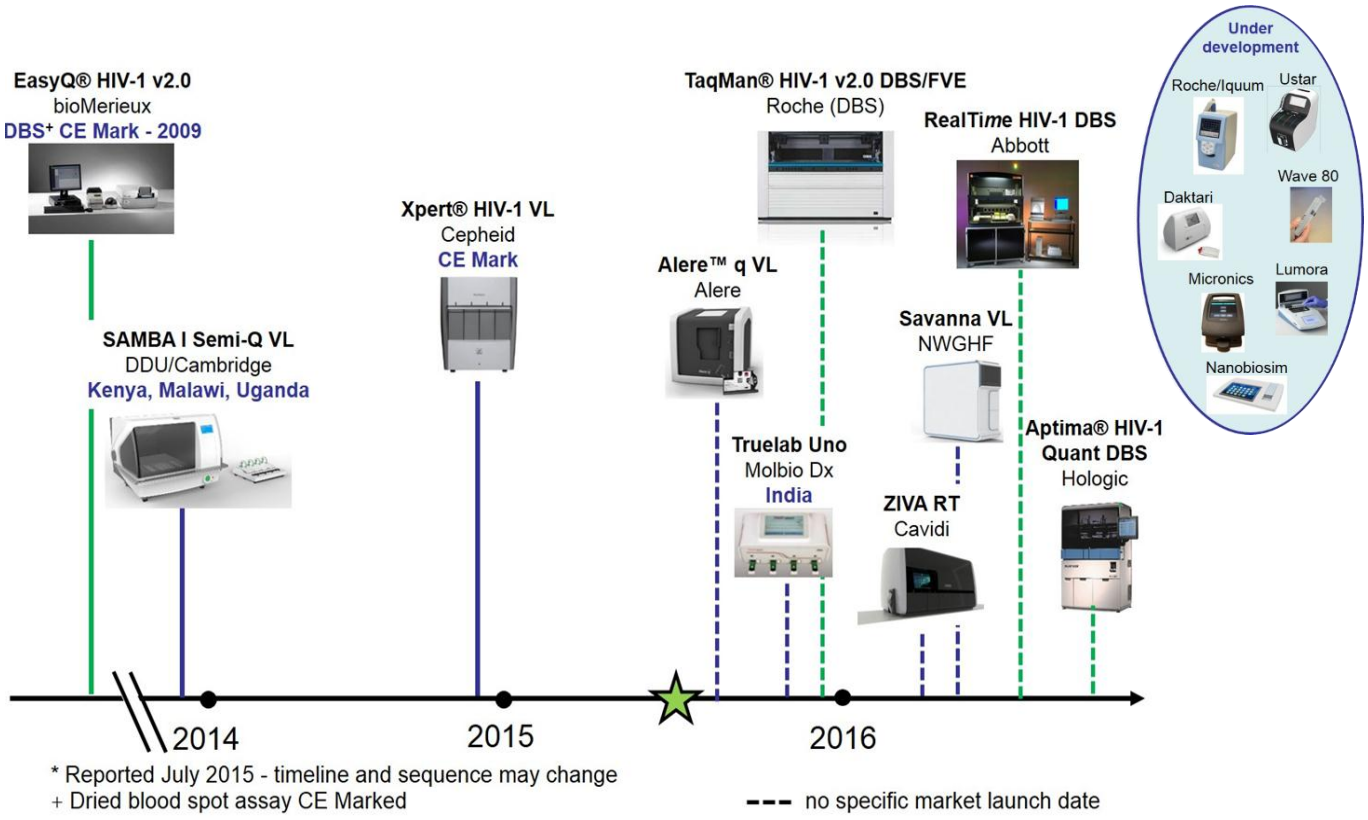


Appendix 3: Early infant diagnosis (EID) technologies in the pipeline



* Reported July 2015 - timeline and sequence may change
\+ Dried blood spot assay CE Marked
--- no specific market launch date
** In addition, open polyvalent platforms are currently available for HIV VL and EID testing

Appendix 4: Point-of-care (POC) viral load (VL) technologies in the pipeline



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