HIV Testing: The State of the Art

Maurine M. Murtagh
6th Advanced Course on Diagnostics
Les Pensières, Veyrier-du-Lac, France
7 September 2015
Diagnostics for HIV/AIDS can generally be divided into three test categories: (i) tests to facilitate initial diagnosis; (ii) tests to stage the patient; and (iii) tests to monitor the patient, both before and after initiation onto ART. There are generally accepted algorithms and tests used at each stage.

This presentation reviews the current technologies and diagnostic platforms used for HIV screening and confirmation, CD4 and viral load testing for adults and children as well as early infant diagnosis (EID).

With the exception tests to diagnose HIV/AIDS, most testing is accessed through sophisticated laboratory-based platforms, even in resource-limited settings.
The Watchwords in State of the Art HIV Testing – Improving Access

The need for large, centralized and costly laboratory-based systems that require well-trained technicians and good sample transport networks to provide access to testing for those in some urban, and virtually all peri-urban and rural settings have combined to limit implementation of much HIV testing in resource-limited settings.

In order to improve access to testing for priority diseases, to simplify such testing and improve its efficiency, there has been a push to decentralize testing – to take testing nearer to the point-of-care (POC).

Therefore, much of the state of the art in testing for HIV/AIDS revolves around novel technologies designed for use at POC.
The state of the art in HIV rapid testing is the 4th generation “combo” test (like the Alere combo) that can detect both HIV 1/2 IgG and IgM antibodies, but can also detect p24 antigen, thus detecting infection earlier (by about 5 – 7 days) than 3rd generation tests.

There are more than a hundred HIV rapid tests commercially available today. They can be used effectively on patients >18 months and are typically used in an algorithm: screen, confirm, tie-break.

HIV rapid tests have been widely adopted, especially in resource-limited settings, where they can be used in decentralized settings like health posts and health centers to provide same-day results to patients.

Most HIV rapid tests are 3rd generation tests that can detect both HIV 1/2 IgG and IgM antibodies within about 3 weeks following infection. Third-generation assays are also more sensitive than first and second-generation assays, and since 2007 have replaced earlier assays.

The state of the art in HIV rapid testing is the 4th generation “combo” test (like the Alere combo) that can detect both HIV 1/2 IgG and IgM antibodies, but can also detect p24 antigen, thus detecting infection earlier (by about 5 – 7 days) than 3rd generation tests.
CD4 Testing – Why Needed

Once a patient is diagnosed as HIV-positive, CD4 testing has typically been used together with clinical staging to determine whether the patient is eligible for ART. The CD4 count considered to be the most important laboratory indicator of immune function in HIV-infected patients.

However, the WHO is expected to issue new HIV guidelines later this year that will recommend that all HIV positive patients be initiated onto ART regardless of their CD4 count or clinical stage. It is already the case under current WHO guidelines that certain key populations – e.g. all children aged under 5 years, pregnant and breastfeeding women, HIV-positive partners in serodiscordant couples and all individuals with active MTB disease – should be put on ART regardless of CD4 count or clinical stage.

As a result of the current and expected WHO guidelines on CD4 testing, many countries may cease to use CD4 testing altogether despite the desire of clinicians to get a baseline measure of CD4 in all HIV+ patients and despite its efficacy in managing OIs.
CD4 Testing – Current Options

Whether in low- or high-throughput settings, CD4 testing is primarily conducted on laboratory-based instruments, although there are several tests for use at POC.

In rural and peri-urban settings, and even in some urban settings, blood collection is done at clinics and blood samples are transported (via courier, or other services, including motorcycle services) to laboratories for testing; results are then returned, generally via the same mechanism, although mobile technologies (e.g. SMS) have been introduced at some sites for this purpose.

The most widely-used lab-based platforms are from BD and Coulter. In particular, the BD FACSCount pictured at right, is the most widely-used platform in resource-limited settings.

It is a medium-throughput benchtop analyzer used in relatively sophisticated labs.
There are three available CD4 platforms for use at POC: CyFlow® mini POC (Partec), the Pima™ Analyser (Alere) and the BD FACSPresto™. In addition, several more platforms are in the pipeline and should be launched in the next year or so, one of which is a semi-quantitative, disposable test from Omega Diagnostics.

The platforms are device-based, low-throughput instruments that can be used at primary healthcare facilities in resource-constrained settings.

For device-based POC platforms, one state of the art feature that is important for QA, supply chain and other reasons is connectivity.
POC CD4: available and pipeline*

- **Pima™ Analyser**
  - Alere
  - CE Mark
  - 2009

- **CyFlow® miniPOC**
  - Partec
  - CE Mark
  - 2009

- **BD FACSPresto™**
  - BD Biosciences
  - CE Mark
  - 2014

- **Visitect® CD4**
  - Omega/Burnet
  - 2015

- **Muse™ (RUO)**
  - EMD Millipore Merck
  - 2016

- **CD4 Counter**
  - Daktari™
  - 2016

- **ChipCare**
  - 2016

* Reported July 2015 - timeline and sequence may change
---

no specific market launch date
Viral Load Testing – Why Needed

Post-initiation onto ART, viral load testing ideally should be used to monitor patients, especially to detect early signs of virological failure, as it is the most important indicator of initial and sustained response to ART.

WHO now recommends routine viral load testing as the preferred monitoring approach to diagnose and confirm ART failure and recommends that viral load testing be done at 6 months after initiating patients onto ART and every 12 months thereafter.

Currently, viral load testing is almost exclusively laboratory-based, with most testing done on sophisticated, high-throughput instruments by highly-trained technicians in national reference laboratories.
In general, the current viral load testing market is dominated by four companies: Abbott, bioMérieux, Roche and Siemens. Although these are laboratory-based systems, the use of DBS is now an option on the bioMérieux platform, which greatly simplifies the transport of samples and helps to improve testing access. Roche and Abbott will also introduce DBS options soon.

A laboratory-based assay (Aptima®) for viral load has been introduced by Hologic for its Panther platform. Roche has introduced a new series of molecular instruments (6800/9800).

The state of the art buzz words are: absolute automation, ultra high throughput, flexibility, work-away time, integration, random access/mixed batching, connectivity and multiplexing.
A number of new Viral Load POC diagnostics are in the market or in development. They are primarily molecular-based tests on platforms many of which will be able to multi-plex.

These will have lower instrument and per-test costs, but will also have lower throughput than lab-based systems.

The Alere™ q (near right) and GeneXpert® (Cepheid) have been launched. The SAMBA semi-quantitative VL platform from DFW is in limited release in Kenya, Malaria, and Uganda.

Additional platforms will follow over the next few years, including a new, smaller platform Cepheid – the Omni – pictured below right.
Viral Load: available and pipeline*

* Reported July 2015 - timeline and sequence may change
+ Dried blood spot assay CE Marked

---

- EasyQ® HIV-1 v2.0
  bioMerieux
  DBS+ CE Mark - 2009
- SAMBA I Semi-Q VL
  DDU/Cambridge
  Kenya, Malawi, Uganda
- Xpert® HIV-1 VL
  Cepheid
  CE Mark
- TaqMan® HIV-1 v2.0 DBS/FVE
  Roche (DBS)
- RealTime HIV-1 DBS
  Abbott
- Alere™ q VL
  Alere
- Savanna VL
  NWGHF
- Truelab Uno
  Molbio Dx
  India
- ZIVA RT
  Cavidi
- Aptima® HIV-1 Quant DBS
  Hologic

---

Under development
- Roche/iquum
- Ustar
- Daktari
- Wave 80
- Micronics
- Lumora
- Nanobiosim

---

* no specific market launch date
Because of the persistence of maternal antibodies in infants aged under 18 months, the use of antibody tests, such as commercially available HIV RDTs, cannot be used to accurately screen infants for HIV/AIDS.

Instead, DNA PCR or RNA PCR testing (i.e. virological testing) should be used to determine the HIV status of infants in that age group. The most commonly used tests are DNA PCR molecular tests.

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. Birth testing is also under consideration.

Infant testing is almost exclusively laboratory-based, but access to testing has been improved with the use of DBS technology.
Infant Testing – Testing Options

There are currently two HIV-1 DNA assays available in resource-limited settings and that are used for EID: the Roche cobas® AmpliPrep/cobas® TaqMan® HIV-1 Qualitative Test and the Abbott RealTime Qualitative HIV-1 Test, both of which have CE-IVD marking.

The Roche cobas® test is designed to be run with the Roche cobas® AmpliPrep and cobas® TaqMan® amplification instruments, while the Abbott RealTime assay is designed to be run on the Abbott RealTime m2000rt amplification system, using the m2000sp, m24sp or manual sample preparation.
Infant Testing – New Options for Testing at POC

A number of new EID diagnostic platforms for use at or near the point of patient care are either in the market or in development.

For example, the SAMBA I platform from Diagnostics for the Real World offers an EID assay. The assay is already available in Kenya, Malawi and Uganda.

Alere™ q and GeneXpert® also offer qualitative DNA PCR assays for infant testing.

NWGHF LYNX HIV p24 Antigen Test is the only platform currently in the pipeline dedicated entirely to EID.
EID: available and pipeline*

* Reported July 2015 - timeline and sequence may change
+ Dried blood spot assay CE Marked

---

2014

RealTime Qual HIV-1
Abbott
DBS+ CE Mark - 2011

SAMBA II EID
DRW
Kenya, Uganda

2015

TaqMan® HIV-1 Qual v2.0
Roche
DBS+ CE Mark

Alere™ q HIV-1/2 Detect
Alere
CE Mark

Xpert® HIV-1 Qual
Cepheid
CE Mark

2016

LYNX HIV p24
NWGHF

---

no specific market launch date

ZIVA™ RT
Cavidi

Under development

Iquum/Roche
Ustar
Lumora
Micronics
QuantumDx
DFA
Resources on HIV Diagnostics

UNITAID HIV/AIDS Diagnostics Landscapes:


Information on HIV rapid tests: http://www.who.int/diagnostics_laboratory/evaluations/150729_prequalified_products_list.pdf?ua=1.

Thank you