Technical Brief on HIV Viral Load Technologies

(June 2010)

Foreword

Viral load assays have been widely evaluated by research laboratories and the results of the evaluations published in scientific literature; however, what is needed is a single document summarizing the performance and operational characteristics of these technologies through a systematic review. This technical brief will help to facilitate the transfer of this information from the scientific community to policy-makers providing up to date information to help guide programmatic decisions. This will enable the limited resources available to health care systems to be put to the best use in order to accomplish the goals and objectives of the programme.

This brief is particularly relevant given the international effort to scale-up access to ART and the large unmet need of increasing laboratory capacity for performance of HIV monitoring technologies, Drug development generally takes 10-20 years but widespread use of drugs without monitoring for the development of resistance can result in the more rapid loss of the effectiveness of the drug and possibly to the entire class of drugs. Resistance to current first line therapies will leave countries with few therapeutic options and the requirement for more extensive use of expensive second and third line regimens for effective treatment of their HIV patients.
# Table of Contents

1. **Aims**
2. **Target audience**
3. **Objectives**
4. **Introduction**
   4.1 *Viral Load and the clinical course of HIV infection*
   4.2 *HIV Diversity*
   4.3 *HIV therapy*
   4.4 *HIV drug resistance*
   4.5 *VL testing and infant diagnosis*
   4.6 *VL testing and programme evaluation*
   4.7 *Dried Blood Spots and HIV diagnosis*
   4.8 *Current recommendations for VL testing*
5. **Background**
   5.1 *Laboratory testing in low income countries*
   5.2 *Difficulties associated with VL testing*
   5.3 *Advantages of Dried Blood Spots*
   5.4 *Disadvantages of Dried Blood Spots*
6. **Description of HIV Viral Load Technologies**
   6.1 *NAT technologies*
   6.2 *Non-NAT technologies*
7. **Considerations when choosing viral load technologies**
   7.1 *Performance characteristics*
   7.2 *Quality of testing*
8. **Viral Load testing using Dried Blood Spots**
9. **Summary of systematic review**
10. **Summary of DBS systematic review**
11. **Future Considerations for evaluations**
12. **Technologies on the horizon**
13. **Conclusions**
14. **References**
1.0 Aims:

To provide technical guidance on HIV viral load technologies and an assessment of their performance and operational characteristics, and their potential utility in HIV control programmes.

2.0 Target audience:

Ministries of health; national HIV/AIDS control programmes; laboratory managers and experts; manufacturers of viral load technologies; procurement agencies; and funders with particular focus on individuals living/working in resource-constrained countries.

3.0 Objectives:

To provide an evaluation of the performance of all commercial viral load assays currently available through a systematic review of published literature.

To outline the operating characteristics of all commercial available viral load assays and new technologies under development.

4.0 Introduction:

In an HIV-infected individual, the concentration of virus in the bloodstream or viral load (VL) can be a valuable tool for the clinical management of the infection. Broadly, there are three clinical uses for quantifying HIV in plasma: diagnosing acute HIV infection; determining prognosis and disease progression; and therapeutic monitoring. Unlike antibody detection, which is confounded by the trans-placental transfer of maternal IgG antibodies, VL can also be useful in diagnosing babies born to HIV-positive mothers. However monitoring VL is most relevant worldwide for its third use, as a biomarker to monitor the therapeutic efficacy of ART. Quantifying viral load in plasma enables a clinician to assess the success of treatment and detect treatment failure prior to the onset of clinical symptoms. This facilitates an evidence-based approach to patient management and can improve the specificity of treatment (WHO A guide for diagnostic evaluations).

The performance characteristics of VL technologies are available from manufacturers; however a systematic-review of evaluations published in the peer-reviewed literature could inform policy makers of the characteristics of diagnostic assays and facilitate evidence-based decision-making.

4.1 Viral Load and the clinical course of HIV infection

After entering the body, HIV infects a large number of CD4 T-lymphocytes and rapidly replicates within these cells. This produces a spike in the quantity of virus RNA in the bloodstream, as a result of progeny virus being spread throughout the body infecting cells and organs. HIV is able to integrate into the host cell’s genes and remain hidden from the host immune response initiated 2-4 weeks after the initial infection. The B-cells of an infected host produce antibodies in an attempt to contain the infection with the help of the CD8 T-lymphocytes (killer T-cells). This results in a drastic reduction of VL levels, allowing for recovery of CD4 cell numbers and partial reconstitution of the body’s immune system.
The level of viral replication that continues after the initial spike is brought under control by the immune system is called the virologic set-point. HIV will continue to replicate at this level, under the control of the immune system, throughout the asymptomatic period of infection (clinical latency).

Progression to AIDS occurs as a result of chronic depletion of CD4 cells, when the CD4 count falls below 200 cells/ul, at a functional level where opportunistic infections and malignancies cannot be controlled. The immune response becomes exhausted after years of maintaining control over VL levels, and as VL levels rise, CD4 cells are destroyed without being replaced at the same rate leading to reactivation or new opportunistic infection (Figure 1).

Figure 1. HIV Viral Load and CD4 T Lymphocyte count over the course of HIV infection.

### 4.2 HIV Diversity

There are two types of HIV, HIV-1 and HIV-2, which differ in their genetic composition but are both transmitted in bodily fluids and able to infect humans.

HIV-1 is responsible for the majority of infections around the world and is divided into four groups (M, N, O, P), and 9 subtypes and two sub-subtypes within the M group (A1, A2, B, C, D, F1, F2, G, H, J, K). There are also at least 43 circulating recombinant forms (CRF) or inter-subtype recombinant HIV-1 (CRF01_AE to CRF43_02G). Figure 2 shows the global distribution of HIV-1 subtypes and the relative contribution of each of the subtypes and recombinants to the overall burden of HIV-1 infection (Hemelaar et al, 2006).
HIV-2 is much less virulent and has lower rates of transmission than HIV-1 and is the cause of a minority of HIV cases in Western Africa as well as sporadic cases in other countries (e.g. India and the United States).

The prevalence of HIV-1 and HIV-2 groups and subtypes differ across geographical regions and the choice of diagnostic assays should reflect the geographic distribution of the groups and subtypes if the assay cannot measure all groups and subtypes. Versatility is also an important factor given the movement of subtypes and emergence of unidentified recombinant forms. For example, subtype B is the main cause of the HIV epidemic in Western Europe and North America but non-B strains and CRFs have been identified in HIV-1 positive patients in these regions. VL assays should therefore detect and quantify all known HIV-1 subtypes, inter-subtype recombinants and emerging variants to provide the greatest benefit.

4.3 HIV Therapy

Antiretroviral therapy (ART) interrupts viral replication. This leads to a decrease in the level of virus in the bloodstream thereby slowing the progression to AIDS and improving the prognosis. Initiation of therapy is determined by the patient’s CD4 T-cell count, the presence of HIV-related symptoms, and only rarely by high VL. Once therapy has commenced, reduction in VL is used as a primary indicator of therapeutic efficacy. (CD4 counts and clinical status may also be used but are less sensitive indicators of therapeutic failure or success). The current aim of ART should be to maintain the VL below the level of detection of available assays. Upon initiation of treatment, VL reduction indicates treatment efficacy normally correlating with an improved clinical outcome. VL is increased
in individuals on ART if there is a low drug concentration in the body because of good adherence and treatment failure, non-adherence, or poor pharmacokinetics, these factors often resulting in the emergence of drug resistance. Other factors that can increase VL include acute infection (e.g. Tuberculosis, pneumococcal pneumonia) and immunizations; however, these increases are transient, usually declining after treatment of the infection or within several months after vaccination.

4.4 HIV drug resistance

HIV is a rapidly replicating virus that has error-prone reverse transcription giving rise to mutations throughout its genome. Several billion new viruses are made every day; the enzyme, reverse transcriptase (RT), that transcribes viral RNA into DNA, introduces random mistakes during the process of replication leading to the development of new circulating strains and variants in a single individual.

Sub-optimal concentrations of antiretroviral drugs in blood or tissues favour the selection of viruses harbouring mutations conferring resistance to the circulating drugs. The drug resistant strain will dominate and continue to replicate irrespective of the presence of the therapeutic agent. Sub-optimal concentrations of the drug can be the result of poor adherence, drug quality, bioavailability or drug interactions. The result is virologic failure and rising VL which eventually causes declines in CD4 counts and clinical progression.

4.5 VL testing and programme evaluation

Through population level VL testing, HIV treatment programs can be evaluated. In this way, VL testing can act as a form of quality assessment for specific centres or for regions and identify areas with poor or exceptional performance facilitating the conveyance of additional support or the sharing of information and problem solving strategies. Drug resistance can be monitored through VL testing and those specimens with increases in VL indicative of virologic failure can be selected for further testing and resistance typing.

4.6 Dried Blood Spots in HIV diagnosis

DBS samples are widely applied for the diagnosis of HIV-1 with antibody and/or antigen detection [1-24] and have been used in large scale HIV surveillance studies for over a decade. The performance of DBS has been validated against plasma/serum samples with various commercially available assays and showed excellent correlation. The performance of PCR for infant diagnosis[25-39] using DBS and detecting HIV DNA has shown excellent results compared to plasma samples.

4.7 VL testing and infant diagnosis

Each year an estimated 800,000 infants are infected with HIV through vertical transmission from their mother. Antibody testing, which is widely used in HIV diagnostics, is not conclusive in the first year of life because maternal antibodies transferred to the infant during pregnancy or breastfeeding persist for up to 18 months. Detection of HIV RNA (or DNA) can provide confirmation of an infection within neonates and infants. As VL testing requires advanced technical expertise and a laboratory infrastructure, it is often not available outside of tertiary laboratory facilities. The use of Dried Blood Spots (DBS) has enabled the collection and transport of specimens for VL testing from primary
care settings to referral centres, benefiting populations with otherwise limited access to laboratory services.

4.8 Current Recommendations for VL testing

The WHO estimates that in 2008, 4 million people had access to ART in low- and middle-income countries. As access to therapy increases and more and more of the 9.7 million eligible individuals receive ART therapy, the need for the development of robust laboratory infrastructure to allow laboratory monitoring also increases. Although costly, laboratory monitoring can greatly benefit clinical decision monitoring, infant diagnosis and program evaluation (Calmy 2007).

In the 2006 revision of ‘Antiretroviral Therapy for HIV Infection and Adults’, the WHO acknowledged the importance of laboratory monitoring in managing HIV infection and advocated wider access to virologic testing. Although viral load testing can assist in assessing the efficacy, managing side-effects, and identifying treatment failure in patients receiving ART, regular VL monitoring was not recommended given the high cost and complexity of the available technology at the time. It was feared that the lack of laboratory services and infrastructure in developing countries would limit access to therapy particularly in regions of the world most affected by HIV.

Viral load testing is one aspect of managing ART that should complement clinical observations and immunologic assessments. Four decision-making time points have been highlighted in the management of patients with respect to ART: when to start, when to substitute for toxicity, when to switch for failure, and when to stop therapy. Although VL monitoring has not been recommended by the WHO because of the aforementioned limitations of cost and complexity, its importance as part of monitoring HIV infection in patients receiving ART is becoming widely accepted. VL monitoring can be of great benefit in determining when to switch for failure or, if confirmed by resistance testing, when to stop administration of therapy because of the emergence of drug resistance. Decreasing CD4 T-lymphocyte counts are a surrogate marker of virus replication and have been shown to be a poor predictor of clinical endpoints such as opportunistic infections, malignancies and death. Testing VL provides a more valuable prognostic tool and a better guide for assisting clinical decision making (Saag, 1996).

Increases in plasma viral load occur before immunologic decline and the appearance of clinical signs and symptoms. Before ordering virological testing, physicians need to carefully consider the potential use of the results they will receive. For example, in countries where third-line regimens are not available, and one switch has already been made, early identification or confirmation of virologic failure is of limited use.

The International AIDS Society- USA Panel recommends performing baseline HIV-1 RNA measurements and repeat testing within a month of initiating treatment. Effective therapy should result in at least a 10-fold (1.0 log_{10}) decrease in HIV-1 RNA copies/mL in the first month and suppression to less than 50 copies/mL by 24 weeks, depending on pretreatment viral load (Hammer et al, 2008). Viral load should then be assessed at regular intervals, every 3 to 4 months. Episodes of low-level viremia, “blips”, do not constitute virologic failure; however, consistent elevations greater than 50 copies/mL satisfy the definition of virologic failure. Given the challenges of viral load testing in resource poor settings, guidelines will need to be developed at a national level to reflect the laboratory capabilities, affordability of testing and therapeutic options available.
5.0 Background:

5.1 Laboratory testing in low-income countries

Laboratory services are one of the most neglected areas of health care provision in low income countries and are disproportionately affected by staff shortages, poor communications, inadequate equipment, lack of funding, low morale, and a lack of training. The absence of a developed laboratory infrastructure in many countries has led to the current practice of monitoring therapy based most often on clinical progression and, where the resources are available, changes in to immunological status. CD4 counts vary among individuals and reports of discordance between virologic and immunologic responses to antiretroviral treatment have been reported. Viral Load measurements can provide an early indication of treatment failure but are of optimal use in guiding treatment only when the following two criteria can be fulfilled: (1) effective plasma drug levels can be assured and (2) alternative drugs are available. While VL testing can identify virologic failure, there is a need to establish a threshold to guide clinicians in deciding when to switch therapy. This will depend on the cost and availability of second line drugs (Petti 2006).

Acknowledging that the major initiatives to expand HIV care and prevention services require a concurrent strengthening and expansion of laboratory services and infrastructure, the CDC has published a document outlining the development of laboratory capacity in low-income countries (Building Laboratory Capacity in Support of HIV/AIDS Care Programs in Resource-limited Countries). The scale up of laboratory services must be accessible, providing accurate and reliable results in a timely fashion in order for programs to succeed. The recommendations made by the CDC were the first step towards strengthening the capacity of laboratories in many resource limited settings.

Countries are advised to initiate laboratory capacity building first with an assessment of present capabilities and infrastructure, and then to formulate a strategic plan to specifically address the national situation. The plan is to provide ongoing coordination to the effort of all partners involved and provide a framework for advocacy and partnerships at a country level. Key elements of the plan include: addressing the personnel needs, developing guidelines and consensus protocols, monitoring and evaluation, supply management, data management, and quality assurance (Table 1).

Table 1. CDC recommendations for components of a strategic plan for laboratory capacity building.

<table>
<thead>
<tr>
<th>Component</th>
<th>Details to address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personnel</td>
<td>Pre-service and in-service training</td>
</tr>
<tr>
<td></td>
<td>Recruitment and retention plans</td>
</tr>
<tr>
<td></td>
<td>Certification</td>
</tr>
<tr>
<td></td>
<td>Training requirements</td>
</tr>
<tr>
<td></td>
<td>Qualifications</td>
</tr>
<tr>
<td></td>
<td>Support and development of leadership</td>
</tr>
<tr>
<td>Guidelines and Consensus protocols</td>
<td>Standard operating procedures</td>
</tr>
<tr>
<td></td>
<td>Guidance for quality assurance practices</td>
</tr>
<tr>
<td>Monitoring and evaluation</td>
<td>Checklists and assessment tools</td>
</tr>
<tr>
<td>(Essential to ensure accuracy and reliability)</td>
<td></td>
</tr>
<tr>
<td>Supply management</td>
<td>Improvement of methods to provide and maintain necessary equipment</td>
</tr>
<tr>
<td>(Timely availability of needed equipment,</td>
<td>System for acquisition of high quality reagents and supplies</td>
</tr>
<tr>
<td>supplies and reagents)</td>
<td></td>
</tr>
<tr>
<td>Data management</td>
<td>Standardized forms</td>
</tr>
</tbody>
</table>
According to these recommendations, at a minimum, viral load testing should be offered at central laboratories which include national reference laboratories, national public health laboratories, large teaching hospitals and other centrally-located laboratories. Primary care facilities should have the ability to collect and, if necessary, store specimens for transport to central laboratories for VL testing.

The scale up of access to ART provides an opportunity for nations to develop their laboratory services. If the laboratory aspects of HIV patient management are integrated into existing services, they could serve to strengthen and develop the capacity of national laboratory services. In the case of strong vertical programs, there is concern that HIV-related aspects of laboratory services will be managed by non-government organizations fragmenting services, diverting scarce resources from non-HIV tests. Partnerships between the public and private sector and strong advocacy on the part of national laboratories could minimize this and ensure resources and put to best use without creating duplication or fragmentation of laboratory services.

5.2 Difficulties associated with VL testing in resource-constrained settings

Resource-constrained settings suffer the majority of the HIV burden yet lack the ability to offer widespread VL testing. Difficulties associated with the provision of virologic testing include the cost of acquiring technology and appropriate laboratory space designated for nucleic acid based tests, the need to train scientists, the lack of access to technical support and infrequent participation in quality assurance programmes (Crowe 2003). Furthermore, these expensive tests rely on expensive and dedicated equipment specific for VL testing. Even after acquiring the equipment, laboratories with the personnel and infrastructure may lack the resources to purchase kits or supply-chain management and delays at customs agencies may delay their delivery.

Many VL technologies require physical resources that may not be available in less-developed countries. This includes access to uninterruptated electricity, air conditioning, protection from dust and access to clean water (Fiscus 2006). Within countries, resources vary and are often concentrated in urban centres and much less accessible in rural areas creating a system whereby well-defined infrastructure is required to ensure proper specimen handling and efficient results reporting.

### Table 2 Barriers to Viral Load Testing in Resource-Limited Settings

<table>
<thead>
<tr>
<th>Physical</th>
<th>Personnel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninterrupted electricity</td>
<td>Training of technicians</td>
</tr>
<tr>
<td>Air conditioning</td>
<td>Preventing emigration of trained staff</td>
</tr>
<tr>
<td>Clean water</td>
<td>Managing the supply chain</td>
</tr>
<tr>
<td></td>
<td>Collecting and transporting samples</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monetary</th>
<th>Infrastructure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of purchasing/ leasing equipment</td>
<td>Delivery of reagents</td>
</tr>
</tbody>
</table>
5.3 Advantages DBS

DBS can be used as an easy and inexpensive means for the collection and storage of specimens under field conditions. The small amount of whole blood collected can be obtained by finger or heel prick and demands no further manipulation on site. The collection of blood by finger prick is a less invasive methodology that can easily be performed with minimal training. The reduction in required materials, less biological waste production and no need of highly skilled personnel at clinic level, decreases costs considerably in comparison to standard blood collection methodologies by venipuncture. Once the DBS is dry, they can be sent to the laboratory. Transport associated risks are minimal as DBS cannot break or leak and can be sent at ambient temperatures, without the need for cold packs or expensive dry ice. DBS samples do require proper packaging, including desiccants and adherence to national and international shipment regulations of biological specimens. DBS samples can be sent by regular mail to national or international reference testing facilities, potentially without issues. Additionally, the small size of DBS - a little bit bigger than a credit card - can easily be stored and used after prolonged storage periods.

5.4 Disadvantages of DBS

Although the collection, shipment, and storage of blood samples are tremendously simplified by using DBS samples, it raises laboratory complexity and requirements. To use DBS in laboratories that do not perform PCR for early infants diagnosis, a new sample procedure needs to be developed and validated. The introduction of DBS based viral load testing in the laboratory requires punch and elution phases in sample processing. These steps, in combination with extraction methods, are essential for valid test results and therefore require extensive testing and validating. Additionally, viral load platforms are designed for plasma samples and therefore require alterations to sample input volume and viral load calculations when used with DBS. Additionally, the performance characteristics of HIV RNA assays must be determined for DBS samples, requiring sensitivity, specificity, lower limit of detection determination, and other parameters to be determined.

6.0 Description of Available Viral Load Technologies:

Commercially available VL assays differ in their sensitivity, dynamic range, target region and in the case of nucleic acid based assays in the methods of nucleic acid extraction, amplification and detection. The differences in the region targeted by the assays means that they also differ in their versatility and ability to detect and quantify different HIV subtypes.

HIV viral load technologies can be broadly categorized into Nucleic Acid Testing (NAT) and non-NAT technologies based the molecular methods used to quantify HIV virus particles circulating in the
body. NAT technologies detect and quantify viral RNA; whereas non-NAT technologies are based on the detection and quantification on HIV viral enzymes and proteins which can be used as a correlate measure of viral RNA.

<table>
<thead>
<tr>
<th>Type</th>
<th>Nucleic Acid Testing Approaches</th>
<th>Non-Nucleic Acid Testing Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>bDNA</td>
<td>VERSANT HIV-1 Quantiplex v3.0 (Siemens)</td>
<td>Reverse Transcriptase</td>
</tr>
<tr>
<td>NASBA</td>
<td>NucliSens EasyQ HIV-1 v2.0 (bioMerieux)</td>
<td>ExaVir Load (Cavidi ab)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Amplicor HIV-1 Monitor v1.5 (Roche Molecular Systems)</td>
<td>P24*</td>
</tr>
<tr>
<td></td>
<td>COBAS TaqMan (Roche Molecular Systems)</td>
<td>Ultrasensitive, Heat denatured p24</td>
</tr>
<tr>
<td></td>
<td>RealTime HIV-1 (Abbott)</td>
<td>antigen quantitation assay (Perkin</td>
</tr>
<tr>
<td></td>
<td>VERSANT HIV-1 RNA 1.0 assay (kPCR) (Siemens)</td>
<td>Elmer Life Sciences)</td>
</tr>
</tbody>
</table>

*p24 assays were not included in the literature review and technical brief. See Section 6.2.2.

6.1 Nucleic Acid Testing (NAT) Technologies:

There are three major methods employed for detecting and quantifying HIV RNA: (1) Reverse Transcription polymerase chain reaction (RT-PCR), (2) nucleic acid sequence based amplification (NASBA), and (3) branched chain DNA (bDNA). These methods all share three steps beginning with sample preparation and/or viral nucleic acid extraction, progressing onto target nucleic acid sequence amplification or the amplification of the signal generated from the detection of target viral RNA, and finishing with the detection and/or quantification of amplified products.

6.1.1 RT-PCR Technology

Polymerase Chain Reaction (PCR) artificially replicates nucleic acid sequences of the target which are then made available for detection. The amplified target sequences (amplicons) are captured and visualized through a colorimetric reaction (enzyme: substrate reaction). Detection of the amplicons can either be during the amplification process as the product accumulates in ‘real time’ or at the end of the amplification reaction, ‘endpoint.’

RT-PCR is a method of PCR using a Reverse Transcriptase (RT) enzyme to convert viral RNA into complementary DNA (cDNA). The cDNA undergoes replication and detection. RT-PCR is used to quantify HIV RNA and to determine viral load.

There are four commercially available RT-PCR based viral load assays: (1) Amplicor HIV-1 Monitor v1.5 (Roche Molecular Systems), (2) COBAS AmpliPrep/ COBAS TaqMan v2.0 (Roche Molecular Systems), (3) RealTime HIV-1 (Abbott), and (4) VERSANT HIV-1 RNA 1.0 assay (kPCR) (Siemens).

(1) The Amplicor HIV-1 Monitor v1.5 uses endpoint PCR targeting the HIV-1 gag gene to quantify viral load. Following manual RNA extraction and transfer, the COBAS Amplicor automates the process of nucleic acid amplification and detection by photometric quantification.
(2) The COBAS TaqMan is a real-time PCR that targets both the gag and LTR regions of the HIV genome (version 2), lowering the limit of detection compared to the Amplicor HIV-1 Monitor v1.5. Coupled with the COBAS AmpliPrep, viral load quantification on the COBAS TaqMan is a fully automated process.

(3) Abbott’s RealTime HIV-1 also uses real-time PCR and targets the pol integrase region. HIV RNA quantification is automated using the m2000sp for sample preparation and nucleic acid extraction and the m2000rt for amplification and detection.

(4) VERSANT HIV-1 RNA 1.0 assay (kPCR) targets the integrase section of the HIV-1 pol gene. The assay is automated on the Sample Preparation (SP) Module and Amplification/ Detection (AD) Module.

NB: Roche Molecular Systems will phase out the Amplicor HIV-1 Monitor v1.5 and the COBAS Amplicor HIV-1 Monitor v1.5 in June 2010. The Amplicor HIV-1 Monitor v1.5 has been considered the gold standard in VL testing for several years. Reagents will be available as long as stocks permit in Europe although as the Taqman v2.0 has not yet received FDA approval, reagents will likely be available for an extended period through the American market. (Correspondence with Susanne Fiscus and Roche).

6.1.2 NASBA Technology

Nucleic Acid Sequence Based Amplification (NASBA) is an isothermal method of amplification that removes the need for the heat stable enzymes and thermocycling instruments required for performing RT-PCR. In this method, the target RNA is exponentially amplified by a three enzyme system and quantified by a competitive co-amplification of laboratory generated controls. Endpoint electro-chemiluminescence detection completes the process.

NucliSens EasyQ HIV-1 v2.0 (bioMerieux) is the latest model to use NASBA targeting the gag region for amplification and quantification of HIV RNA. Extraction can be manual or automated on the NucliSens extractor or NucliSens miniMAG.

6.1.3 bDNA Technology

Branched chain DNA signal amplification (bDNA) is a method of HIV RNA quantification whereby a signal, not the target is amplified. In the VERSANT HIV-1 Quantiplex v3.0 (Siemens), target probes bind to sequences within the pol gene of HIV-1 RNA. Unlike previously described for RT-PCR and NASBA technology, in bDNA it is signal sites that are detected following a series of hybridization reactions and through use of chemiluminescence technology. The VERSANT HIV-1 Quantiplex v3.0 is fully automated with use on the 440 Molecular system and does not require separate RNA extraction.

6.2 Non-NAT Technology

Non-NAT technologies quantify proteins and enzymes specific to HIV instead of HIV RNA as a correlate measure of viral load. These include assays measuring the level of reverse transcriptase activity and concentration of circulating p24 protein.

6.2.1 Reverse Transcriptase

The ExaVir Load (Cavidi) is a functional assay that extracts and quantifies reverse transcriptase (RT). Virus particles are separated from plasma in the first stage of the assay. This is followed by an ELISA
to quantify the RT activity. The RT activity in an unknown sample is then compared to the activity of a recombinant RT enzyme standard of a known concentration. The extrapolated result is reported as fg RT/mL of plasma and using a conversion factor, HIV-1 RNA equivalents/mL are provided by software. ExaVir Load does not target a specific nucleic acid sequence and is therefore able to quantify all HIV-1 and HIV-2 subtypes. The procedure is also simpler and less expensive than molecular methods but does require a laboratory to be equipped with standard ELISA as well as overnight incubation resulting in a total reading time of 2-3 days.

6.2.2 p24 antigen quantification assay

The Ultrasensitive, Heat denatured p24 antigen quantification assay (Perkin Elmer Life Sciences) is an enzyme immunoassay for the quantification of p24 antigen. The assay requires low to medium technical skill, training is easy, throughput is high and no separate areas are required. There are concerns over the sensitivity of the technology, the need for an external buffer not supplied by the manufacturer and unimpressive results in studies evaluating the correlation of p24 antigen and HIV RNA. Although it may be useful in early diagnosis of infants, the p24 antigen quantification assay is not a reliable correlate of HIV RNA. It is for this reason that the Ultrasensitive, Heat denatured p24 antigen quantification assay was not included in the literature review of viral load technologies.

<table>
<thead>
<tr>
<th>Disadvantages</th>
<th>Skilled</th>
<th>Dedicated</th>
<th>Dedicated</th>
<th>Skilled</th>
<th>Dedicated</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Name</td>
<td>AMPICOR HIV-1 MONITOR Test v1.5</td>
<td>COBAS AM IPO/COBAS TestMan HIV-1 Test, v1.2</td>
<td>Nicholsen HIV-1 v2.0</td>
<td>VERSANT HIV-1 RNA 3.0 Assay Dedicated equipment</td>
<td>Abbott RealTime HIV-1 v2.0</td>
<td>ExaVir Load 3.0</td>
</tr>
<tr>
<td>Advantages</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
</tr>
<tr>
<td></td>
<td>High throughput equipment</td>
<td>High throughput equipment</td>
<td>High throughput equipment</td>
<td>High throughput equipment</td>
<td>High throughput equipment</td>
<td>High throughput equipment</td>
</tr>
<tr>
<td></td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
</tr>
<tr>
<td></td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
</tr>
<tr>
<td></td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
</tr>
<tr>
<td></td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
</tr>
<tr>
<td></td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
</tr>
<tr>
<td></td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
</tr>
<tr>
<td></td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
</tr>
<tr>
<td></td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
</tr>
<tr>
<td></td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
</tr>
<tr>
<td></td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
</tr>
<tr>
<td></td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
</tr>
<tr>
<td></td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
</tr>
<tr>
<td></td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
</tr>
<tr>
<td></td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
<td>Medium technical skill</td>
</tr>
<tr>
<td></td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
<td>High throughput</td>
</tr>
<tr>
<td></td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
<td>Low cost</td>
</tr>
<tr>
<td></td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
<td>Single room required</td>
</tr>
<tr>
<td></td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
<td>Equipment can be used for other pathogens</td>
</tr>
</tbody>
</table>

Table 3. Advantages and Disadvantages of Viral Load Technologies
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Roche Diagnostics</th>
<th>Roche Diagnostics</th>
<th>Roche Diagnostics</th>
<th>bioMerieux s.a.</th>
<th>Siemens</th>
<th>Siemens</th>
<th>Abbott Molecular</th>
<th>Cavidi AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulatory Approval</td>
<td>CE-IVD, FDA</td>
<td>CE-IVD, FDA</td>
<td>CE-IVD, FDA</td>
<td>CE-FDA</td>
<td>CE IVD</td>
<td>CE IVD</td>
<td>CE IVD, FDA</td>
<td>CE IVD</td>
</tr>
<tr>
<td>Target Region</td>
<td>gag gene</td>
<td>gag gene</td>
<td>gag and 5’ LTR</td>
<td>gag gene</td>
<td>pol gene</td>
<td>pol gene</td>
<td>Integrate region of pol gene</td>
<td>Measurement of Reverse Transcriptase activity</td>
</tr>
<tr>
<td>Linear Range</td>
<td>Standard: 400 to &gt;750,000 RNA copies/mL Ultra-sensitive: 50 to &gt;100,000 RNA copies/mL</td>
<td>Standard: 400 to &gt;750,000 RNA copies/mL Ultra-sensitive: 50 to &gt;100,000 RNA copies/mL</td>
<td>20 to 10,000,000 RNA copies/mL</td>
<td>10 to 10,000,000 RNA copies/mL</td>
<td>50-500,000 copies/mL</td>
<td>37-11,000,000 copies/mL</td>
<td>200 to 600,000 copies equivalents/mL</td>
<td></td>
</tr>
<tr>
<td>HIV -1 and/or HIV-2 Subgroup</td>
<td>HIV-1 Group M (subtypes A-H)</td>
<td>HIV-1 Group M (subtypes A-H)</td>
<td>HIV-1 Group M (subtypes A-J), CRF01_AE, CRF02_AG</td>
<td>HIV-1 Group M</td>
<td>HIV-1 Groups M and O</td>
<td>All HIV-1 subgroups (including N and O) and all HIV-2 subgroups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recognition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Materials required but not</td>
<td>Standard lab consumables and small equipments for performing PCR</td>
<td>Standard lab consumables and small equipments for performing PCR</td>
<td>Consumables: Sample processing units, Sample input tubes with barcode clips, K-tips, K-tubes, Wash buffer</td>
<td>Pipette tips Lab set-up: Extractor, analyser, biohazard hoods, centrifuge</td>
<td>Refrigerated centrifuge &amp; standard lab consumables and small equipments</td>
<td>Vortex, centrifuge, pipettes/ tips</td>
<td>Standard ELISA equipment</td>
<td></td>
</tr>
<tr>
<td>supplied</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to result</td>
<td>Approx. 8 hours</td>
<td>Approx. 8 hours</td>
<td>5.8 hours</td>
<td>&lt;3 hours (inclusive of extraction)</td>
<td>25 hours</td>
<td>6.5-7 hours/ run</td>
<td>48 hours (5 hours hands on work)</td>
<td></td>
</tr>
<tr>
<td>Throughput</td>
<td></td>
<td></td>
<td>8 hrs: 168</td>
<td></td>
<td>46 samples/ run</td>
<td>12-168 samples/ run</td>
<td>89 samples/ run</td>
<td>180 samples/week</td>
</tr>
<tr>
<td>Mean time to failure</td>
<td>n/a</td>
<td></td>
<td>365 days</td>
<td></td>
<td>&gt;1 year</td>
<td>4 months</td>
<td>135 days</td>
<td>&gt;10 years (based on running 180 samples/week)</td>
</tr>
<tr>
<td>Access to QC reagent</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Compatibility with EQA</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>programme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of equipment</td>
<td>n/a</td>
<td>COBAS AMPLICOR</td>
<td>COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v1.5</td>
<td>NucliSens EasyQ HIV-1 v2.0</td>
<td>VERSANT HIV-1 RNA 3.0 Assay</td>
<td>Versant HIV-1 RNA 1.0 assay (kPCR)</td>
<td>Abbott RealTime HIV-1</td>
<td>ExaVir Load v3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Analysator: 34,877.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>COBAS AmpliPrep instrument, COBAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>POA</td>
<td>POA</td>
<td>130,000 Euros</td>
<td>POA</td>
<td>3,500 USD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TaqMan Analyser:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GBP</strong></td>
<td><strong>162,000 GBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cost per test</strong></td>
<td><strong>80 GBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>55 GBP (inc. consumables)</strong></td>
<td><strong>72.40 GBP (inc. consumables)</strong></td>
<td><strong>POA</strong></td>
<td><strong>POA</strong></td>
<td><strong>40 Euros</strong></td>
<td><strong>18-25 USD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

I don’t understand what is meant by the mean time to failure. This needs some explanation

Consider adding a row with other analytes that can be assayed on the instrument. Some also quantitate HCV and HBV viral loads
7.0 Considerations when choosing viral load technology

There are some key considerations to be made when selecting a technology for VL testing and developing the laboratory capacity of a country’s or region’s control program. If the assay is an upgrade, a comparison will have to be made between the existing and future technology’s lower limit of detection. If the new assay detects lower values than that in use at present, programme managers will have to consider the ramifications of more patients potentially being identified as having ongoing, albeit low levels, of viral replication. A clear threshold for switching therapy could help to avoid potential confusion caused by the improved detection of a new assay. If the technology is a new addition to the laboratory services, programme managers will need to ensure the infrastructure exists to safely collect and transport samples and report results in a timely fashion (Stevens 2008). Clinicians may have to be educated on how to interpret VL results.

In deciding upon a technology, both the operational and performance characteristics will require careful consideration. The essential operating characteristics for VL testing have been described for each level of the health care system in Table 4.

Prior to purchase, a comprehensive costing analysis should be performed and should include consideration of the cost of reagents, consumables, staff time, instrument maintenance, running of internal controls and EQA, resulting, sample transport and the fixed overhead (Stevens 2008). The options of leasing equipment should also be explored with manufacturers. Often schemes exist to enable a country to lease the equipment and purchase reagents saving on a capital investment and increasing the economic feasibility of introducing VL testing.

Optimum operational characteristics will differ at each level of the healthcare system, from primary level health posts to national referral centres. A tiered approach reflecting the capabilities and demands on each level of the health care system could assist in the allocation of limited resources.

7.1 Performance Characteristics (Quality of tests)

Accuracy is a measure of the degree of closeness of the reported value to the true value. Accuracy may be reported as measures of bias or misclassification and may vary with the magnitude of the result; however, it is most important to know the accuracy of a technology over the clinically relevant range.

Bias or mean difference reflects the average difference between the results of the technology under evaluation with an accepted reference standard. Misclassification reflects the percentage of results that were incorrectly identified around a given limit. For example, with viral load testing, authors may report the % misclassification above and below 400 copies/ml. The % misclassification “up “ represents the number of specimens which were incorrectly identified by the test under evaluation as being above 400 copies/ml and the % misclassification “down “represents the number of specimens incorrectly identified by the index test as being below 400 copies/ml.

The accuracy of a technology can also be evaluated by sensitivity and specificity measurements. Sensitivity is the ability of a technology to correctly identify true positive cases and specificity is the ability of a technology to correctly identify true negative cases. In the case of HIV VL testing, which is used for quantitative as well as binary measurements, sensitivity and specificity may be measured around a clinically relevant threshold. This may be set as the lower limit of detection (eg. >400
copies/ml for the standard Amplicor HIV Monitor version 1.5) or at a clinically relevant point (eg. >5,000 copies/ml). In this situation the sensitivity would represent the test’s ability to correctly identify all those with VL counts above the threshold and specificity would represent the test’s ability to correctly identify all those specimens with VL counts below the threshold.

Precision measurements answer the question ‘how close are the results of replicate testing using a single technology?’ Precision can be measured for intra-assay (within run) variability or inter-assay (between run) variability.

Intra-assay variability is a measure calculated from testing multiple replicates of the same specimen within a single assay. Inter-assay variability is calculated from testing multiple replicates of the same specimen in different assays or at different times. The two measures together describe the amount of variation that can be expected among results using the same specimen. Precision is also called reproducibility and can be thought of as the degree to which repeated measurements under unchanged conditions show the same results. Precision can also be calculated between laboratories to determine how much variation is introduced into the results by having the test performed by different technicians in different settings.

Information on precision can inform how comparable results are from the same lot of tests, different lots of tests, or even different laboratories. Data on precision are often reported as the coefficient of variation (CV) which is a measure of dispersion similar to standard deviation but is calculated with consideration for the data and can therefore be compared across studies. A lower value of CV indicates less variation and greater assay reproducibility.

Manufacturers’ publish information on their technology’s accuracy and precision; however, independent, peer-reviewed evaluations are a more reliable source of information. Evaluations of technology should include a comparison of the test under evaluation to a reference standard of acceptable standing in the scientific community.

7.2 Quality of Testing (need elaboration)

The quality of testing is an important consideration in the selection of technologies. Even if the best test is purchased, if the test is performed badly, the results will not be useful. The selection of a VL technology needs to take into account the following:

1. Can staff be trained on the technology with ease
2. Is there access to technical support
3. Is it possible to perform regular maintenance;
4. Are materials for quality control and external quality assurance (EQA) programme available (eg. DBS and NSQAP)?
Table 5 Essential performance standards in the clinical management of patients with HIV at each level of the health care system.

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Tertiary/ Reference Laboratory</th>
<th>District Level Laboratory</th>
<th>Primary Health Care Facility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance Criteria</td>
<td>Reliably 1000 or less copies/ml or RT equivalence</td>
<td>Reliably 1000 or less copies/ml or RT equivalence</td>
<td>n/a</td>
</tr>
<tr>
<td>Method</td>
<td>PCR or RT-ELISA</td>
<td>RT-ELISA</td>
<td>Send DBS or POC when available</td>
</tr>
<tr>
<td>Specimen type</td>
<td>Whole blood (EDTA), plasma or DBS</td>
<td>Whole blood (EDTA), plasma or DBS</td>
<td>Whole blood finger or heel prick</td>
</tr>
<tr>
<td>Specimen volume:</td>
<td>Adult</td>
<td>Up to 1.2 ml plasma (depends on method)</td>
<td>1ml</td>
</tr>
<tr>
<td></td>
<td>Paediatric</td>
<td>Preferably &lt;0.5ml</td>
<td>Up to 1ml</td>
</tr>
<tr>
<td>Time to result</td>
<td>&lt; 2 weeks*</td>
<td>&lt; 2 weeks</td>
<td>3-6 weeks</td>
</tr>
<tr>
<td>Further analysis of data required?</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Throughput</td>
<td>Medium or high (&gt; 30-100 samples/ two weeks)</td>
<td>Medium or high (&gt; 30 samples/ two weeks)</td>
<td>Variable</td>
</tr>
<tr>
<td>Complexity</td>
<td>May be high</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Training required</td>
<td>Dedicated training essential</td>
<td>Dedicated training essential</td>
<td>Training essential</td>
</tr>
<tr>
<td>Environmental/ energy issues</td>
<td>Requires uninterrupted mains electricity, may require climate control</td>
<td>Requires uninterrupted mains electricity, may require climate control</td>
<td>None</td>
</tr>
<tr>
<td>Storage requirements of reagents</td>
<td>Requires freezer and refrigerator</td>
<td>Requires freezer and refrigerator</td>
<td>None</td>
</tr>
<tr>
<td>Storage requirements of specimens</td>
<td>Requires freezer and refrigerator</td>
<td>Requires freezer and refrigerator</td>
<td>Requires desiccant</td>
</tr>
<tr>
<td>Maintenance/ machine calibration</td>
<td>Essential</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Service after the sale</td>
<td>Essential</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Additional equipment/ facilities required</td>
<td>Sufficient space for PCR suite</td>
<td>ELISA reader, incubator</td>
<td>No</td>
</tr>
<tr>
<td>Availability of QC reagents</td>
<td>Must be available but not necessarily from the manufacturer</td>
<td>Must be available but not necessarily from the manufacturer</td>
<td>No</td>
</tr>
<tr>
<td>Participation in manufacturer- independent QA programme</td>
<td>Essential for all platforms</td>
<td>Essential for all platforms</td>
<td>Regular auditing</td>
</tr>
<tr>
<td>How does the test perform</td>
<td>Specimens should be processed within 24</td>
<td>Specimens should be processed within 24</td>
<td>Thoroughly dried then stored with desiccant</td>
</tr>
</tbody>
</table>
on aged specimens? (Upper limit of storage time before testing) hours then plasma may be frozen hours then plasma may be frozen prior to shipping

*In the case of PMTCT services, more urgent results might be required.
8.0.2 HIV viral load determination with DBS samples

The standard method of viral load determination requires collection, plasma processing, cold chain, and storage of specimens by trained personnel and demands complex laboratory infrastructures. The high costs and complexity related to viral load quantification renders this method unsuitable for resource-constrained settings that have been the most affected by the HIV epidemic. As cost-effective antiretroviral drugs become increasingly available in resource-limited countries and VL quantification becomes the major indicator of antiretroviral treatment success or failure, there is an increasing need to develop more cost-effective, practical, and reliable methods that are adapted to field conditions for HIV viral load quantification. The technical, practical and economic advantages of DBS over liquid plasma are numerous. DBS can be obtained by health care practitioners with little training and sent to laboratory facilities as regular non-hazardous mail at ambient temperatures. The potential of DBS to reduce the expenses in viral load quantification by simplifying the collection, storage, and transport of samples could allow viral load quantification in settings where traditional viral load methodology is not feasible.

8.1 Description of Available DBS Techniques

Filter paper (manufacturer’s specifications – awaiting response from manufacturers)

<table>
<thead>
<tr>
<th>Paper cards</th>
<th>903</th>
<th>FTA</th>
<th>FTA Elute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturing company</td>
<td>Whatman part of GE Healthcare</td>
<td>Whatman part of GE Healthcare</td>
<td>Whatman part of GE Healthcare</td>
</tr>
<tr>
<td>Material/thickness</td>
<td>903</td>
<td>FTA</td>
<td>FTA Elute</td>
</tr>
<tr>
<td>Adjustable format</td>
<td>Yes. Custom formats are available as well as standard catalogue items. This is an untreated matrix that is suitable for antigen testing as well as nucleic acid testing.</td>
<td>Yes. Custom formats are available as well as standard catalogue items. This is a treated format that is suitable for nucleic acid testing.</td>
<td>Yes. Custom formats are available as well as standard catalogue items. This is a treated format that is suitable for nucleic acid testing.</td>
</tr>
<tr>
<td># of spots</td>
<td>Standard format has 5 circles, 12-13mm ID. Custom formats can have as many circles as needed</td>
<td>A few standard formats are available, 1 – 4 circles, 1” in ID. Custom formats can have as many circles as needed</td>
<td>Standard format has 4 circles, 12-13mm ID. Custom formats can have as many circles as needed</td>
</tr>
<tr>
<td>Quantity of whole blood per spot</td>
<td>75 – 80ul per circle</td>
<td>100 – 125ul per circle</td>
<td>70 – 80ul per circle</td>
</tr>
<tr>
<td>Recommended drying time</td>
<td>3 hours</td>
<td>3 hours</td>
<td>3 hours</td>
</tr>
<tr>
<td>Robustness to heat, humidity, etc.</td>
<td>Stability of &gt; 3 months for viral RNA has been documented in reference papers</td>
<td>Stability of &gt; 3 months for viral RNA has been documented in reference papers. DNA is stable at room temperature for more than 10 years.</td>
<td>DNA is stable at room temperature for more than 10 years.</td>
</tr>
<tr>
<td>Lot variability</td>
<td>Not documented for HIV testing</td>
<td>Not documented for HIV testing</td>
<td>Not documented for HIV testing</td>
</tr>
<tr>
<td>Cost per piece</td>
<td>Depends on format and quantity</td>
<td>Depends on format and quantity</td>
<td>Depends on format and quantity</td>
</tr>
<tr>
<td>Pack quantity</td>
<td>100 per pack</td>
<td>100 per pack</td>
<td>100 per pack</td>
</tr>
<tr>
<td>FDA/ CE approved</td>
<td>Class 2 medical device and CE marked as a sample</td>
<td>For research use only</td>
<td>CE marked as a sample collection device for blood</td>
</tr>
</tbody>
</table>
**transport of blood for diagnostics**

<table>
<thead>
<tr>
<th>Recommended storage time and conditions</th>
<th>Room temperature storage for DNA, +4C for RNA</th>
<th>Room temperature storage for DNA, +4C for RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>For nucleic acids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-term: +4℃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-term: -20℃</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggestions and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>903 has been used since the 1990’s, first with antigen testing and later HIV-1 and viral load tests</td>
</tr>
<tr>
<td>Many supporting references on the use of 903 for HIV testing</td>
</tr>
</tbody>
</table>

| FTA offers the advantage of pathogen inactivation, safer for handling and shipping |
|Many supporting references on the use of FTA to detect HIV-1 DNA and RNA|

### 8.2 General instructions for DBS specimen collection

- Clearly label card with appropriate instruction number
- Do not touch the filter paper circles before or after blood collection
- Clean the puncture site with 70% isopropanol or 70% ethanol
- Use sterile, disposable lancet
- Keep the puncture site below the heart level
- Wipe away the first drop of blood
- Apply the second drop of blood to the surface of the filter paper circle by gently pressing the paper to puncture site or use sterile micropipette
- If not completely covered, apply immediately a second drop of blood to the circle
- Fill all the circles only on one side of the filter paper
- Dry the specimen at ambient temperature for 3-4 hours in horizontal position, out of direct sunlight and protected from insects or rodents
- Avoid touching or smearing the blood spots
- Pack filter paper with desiccants and humidity indicator in a gas impermeable zip lock bag
- Insert DBS bags into rip-resistant envelope
- Include documentation and place in large envelope for transport

### 8.3 Storage of DBS

For the correct storage and transport of DBS samples, humidity control is essential. When stored in humid environments, bacterial and fungal growth leading to sample degradation could occur and should be prevented. It is advisable to regulate temperature from the time of specimen collection until arrival at the laboratory facility. To prevent exposure to high temperatures, samples should be kept out of direct sunlight and should not be left in a hot room or car. Although results have shown minimal degradation of HIV RNA in DBS samples stored for prolonged periods (up to years) at various temperatures, it is recommended to regularly mail DBS samples to the central laboratory to reduce potential environmental effects on RNA integrity.

### 8.4 Punch method (manufacturer’s specifications – awaiting responds)

<table>
<thead>
<tr>
<th>Punch methods</th>
<th>Scissor</th>
<th>Office hole punch</th>
<th>Harris-unicone and multicore</th>
<th>DBS Puncher (1296-071)</th>
<th>MultiPuncher (1296-081)</th>
<th>AutoPuncher (1296-091)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>-</td>
<td>-</td>
<td>PartnerTech</td>
<td>BSD Robotics</td>
<td>BSD Robotics</td>
<td>BSD Robotics</td>
</tr>
</tbody>
</table>
When implementing DBS specimens in the laboratory, risks of contamination should be identified and prevented as soon as possible. The process of DBS handling could harbour contamination risks when punching, sample elution, and RNA extraction are processed manually. By automated processing of punching and extracting RNA from DBS, many of these contamination risks could potentially be eliminated. A study investigated potential contamination risks of using manual (with three cleaning methods) and an automated punching method (Wallac autopuncher). Both manual and automated showed little to no contamination risks when analysing HIV DNA by PCR. In fact, the manual method with no cleaning procedure at all showed no false positive results. The automated punching device caused minor false positive HIV DNA results.
8.5 Elution and extraction method

Although the extraction of RNA is essential for successful viral load quantification, little is known regarding the efficiency of methodologies isolating RNA from DBS. The isolation of RNA from DBS is more complex and demanding in comparison to plasma or serum. The RNA isolation from liquid plasma shows little to no variability, while DBS shows large variability between commercially available extraction methods in viral load quantification. Besides the quantity of isolated RNA, the ability to sequence (related to the quality of RNA isolates) varies between isolation methods as well. This indicates that the complexity and importance of specifically developed filter paper isolation methodologies are essential for valid viral load results. Although not thoroughly tested or fully validated, various manufacturers offer DNA and/or RNA DBS isolation protocols for the existing HIV nucleic acid platforms. BioMerieux was the first manufacturer to offer CE-IVD marking for DBS extraction and detection with the EasyMAG and the EasyQ V2.0 platform for HIV viral load quantification.

8.6 Detection Platform

To use the existing viral load platforms for DBS samples, alterations to the viral load calculations are necessary to compensate for the small sample volume. Most platforms are designed for 1 mL serum or plasma input volume and thus providing incorrect viral load results when based on 50 µL blood. The presence of red blood cells (haematocrit levels) in DBS samples also requires correction in order to calculate the exact plasma quantity. As noted by Leelawiwat 200-, the uncorrected mean difference was 0.4, as the haematocrit corrected viral load difference was 0.283 or even 0.127 log c/mL when corrected for standard haematocrit levels. The exact haematocrit levels can only be calculated when liquid whole blood is available and therefore this calculation depends on standard haematocrit levels when implemented in routine use. Some studies used one spot (50µL) or two spots (100µL) as input volume.

9.0 Summary of Viral Load systematic review

9.1 Quality Assessment

- Number of studies defining population characteristics (age, sex, ART status)
- Number of studies reporting training of technicians
- Number of studies reporting basic summary statistics describing data (mean and SD, range)
- Number of studies reporting appropriate accuracy measurements (pearson’s correlation, bias, limits of agreement, sensitivity and specificity as measured at a clinical cut-off)
- Number of studies reporting precision measurements (%CV and the number of replicates/ sample, number of samples, mean VL of samples used)

9.2 Accuracy Measurements

- Sensitivity, specificity, %CV (intra- and inter- assay variation) and % of results differing by >0.5log are most relevant accuracy measurements in decision making if a new test is going to be introduced
- Bias is highly relevant if technology is going to be replaced or upgraded in a laboratory
  - Need to understand how the new assay will affect the VL levels reported and determine if this will have an impact on clinical decision making

9.2.1 Bias (mean difference)

Thirty studies calculated bias (mean difference) according to the Bland and Altman method for comparing an index test to the gold standard, Amplicor Monitor v1.5.

Bias was also reported by a number of studies whereby the assay under evaluation (index test) was compared to an assay other than the Amplicor Monitor v1.5 (see below).
9.2.2 Correlation

- Comparisons made to the Amplicor Monitor v1.5
- Comparison made between index test under evaluation and assays other than the Amplicor Monitor v1.5

9.2.3 Sensitivity and Specificity

- Summarize Sensitivity Data in Table Format → Relevance if multiple cut-offs are used?

Specificity was reported in 17 (50%) of publications; however, only 3 (10%) used HIV-1 positive samples and evaluated the specificity of the assay at a determined cut-off point (Table X). Reports of specificity from the remaining 14 publications involved the use of HIV-1 negative samples and do not represent the likelihood of a given technology misclassifying a specimen at a clinically relevant VL level.

<table>
<thead>
<tr>
<th>VL Assay</th>
<th>Cut-off (VL copies/ml)</th>
<th>Specificity (%)</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>ExaVir Load V2</td>
<td>400</td>
<td>98.2</td>
<td>Mine 2009</td>
</tr>
</tbody>
</table>
9.3 Precision Measurements

- Intra-assay %CV
- Inter-assay %CV

10.0 Summary of DBS Systematic Review

Eleven studies evaluated the viral load measurement performances of commercially available quantitative assays with DBS samples in correlation with plasma results. All studies used Whatman filter paper 903 (GE Healthcare, USA) for the DBS samples [26, 40-41, 45-50, 52, 58, 77]. Various detection platforms were used for the analysis of DBS viral loads correlated to plasma performances. The Roche Amplicor HIV-1 monitor v1.5 [26, 48, 52], Roche COBAS Taqman RT analyzer [41, 52], BioMerieux Nuclisense EasyQ v1.1 or v1.2 [47, 49-50, 58], BioMerieux Nuclisense EasQ v2.0 [77] or the Abbott M2000 system [40, 45-46] were used. The DBS viral load performances were compared to plasma by using the same platform [26, 40-41, 45-46, 48-50, 58, 77] or plasma and DBS viral loads were determined by different platforms [47, 52] or at different locations (check). One study performed plasma and DBS analysis within one run, therefore potentially reducing intra-assay variances when correlating DBS and plasma viral load results [48].

DBS samples were prepared with EDTA-blood, finger prick [58] or heel prick blood [26]. The specimen input volumes were 50 µl [26, 49], 75 µl [41], 100 µl [40-41, 46, 50, 77] blood or unspecified [47, 52]. Sample storage conditions varied, ranging from -70°C [26] to ambient temperatures [45-46, 52, 58, 77] for several weeks. Samples were either humidity controlled [26, 41, 46-47, 58, 77] or unspecified [40, 48-50, 52, 58]. Plasma input volumes for the reference viral loads were 100 µl [50], 500 µl [41], 600 µl [45] or unspecified [26, 40, 46-49, 52, 58, 77]. Plasma samples were stored at -80°C [45-46, 77], -70°C [26, 50], -20°C [47-49] or not specified [40, 52, 77].

For viral load measurements, the extraction method is essential for consistent viral load results. The various manual or automated BioMerieux extraction methods were used [26, 41, 45, 47, 49-50, 77], the M sample preparation system (Abbott) [40, 45-46], manual extraction [48] or the applied methods were unspecified [52, 58]. Andreotti et al began the extraction of DBS samples with the Roche viral isolation method but continued to use the NucliSENS isolation due to incorrect isolation of RNA specimens [41].
• The variances found with the Abbott RealTime between studies indicate that RNA extraction and other factors are (more) essential rather than the type of platform.

• Haematocrit corrections, sample input volume corrections are one of the major causes for the varieties found. (main cause of variance found in Realtime platform)

The mean VL found with DBS samples was 0.01 [40] – 1.6 log/ml[26] lower compared to the mean VL found in plasma (see table x). Collectively, the Pearson correlations found for viral loads in DBS and paired plasma samples ranged from \( R = 0.72 \) to 0.955 [40, 46, 48, 50, 52] and \( R^2 = 0.70 \) to 0.998 [47,
Van Deursen et al determined the lower detection limit (95% detection rate) of the Abbott platform and were established at 800 c/ml [77]. Although not specifically determined, lower detection limits by other studies was roughly estimated to be around 1000 -2000 c/mL, except one study that found excellent correlation (r=0.99) starting with values of over 400 c/mL [46].

One study analysed the performance of NucliSENS and m2000rt systems, providing plasma VL correlation of 0.960 and DBS correlation of 0.845 between the two methods [49]. In general, 51.9% to 100% [26, 40, 45, 49, 77] of the DBS samples analysed had <0.5 log variance in comparison to plasma and 94.4 to 100% [40, 45, 77] were within <1 log variance, if indicated.

![sensitivity for various (log) viral loads](image)

- The overall sensitivity of DBS is over 80%, independent on type of platform
- This graph shows the gradual increase in sensitivity when viral load increases (almost 100%)
- 3.48log =3000 copies/ml, indicates that it could be used for therapy failure (5.000 copies/mL) - ?
Correlation coefficient for the various platforms detected by the reviewed articles.

A complete DBS circle can contain up to 50 µl of whole blood which is roughly 20-25 µl of plasma. The HIV quantitative assays are developed and validated for large plasma sample volumes, creating the necessity to alter the DBS viral loads for the smaller input volume. The DBS viral loads determined by the platforms can be recalculated to HIV copies per millilitre by applying the difference in plasma and DBS sample volume as a factor. Additionally, haematocrit values were calculated to correct the DBS viral load results \[26, 47, 58\]. The haematocrit values are applied in the VL calculations by determining the correct estimate of plasma in 50 µl of whole blood samples. One study found that haematocrit correction reduced the correlation difference by 0.238 log, to -0.127 log difference \[26\]. An alternative is to alter the reference sample volume to allow the VL of DBS and plasma to be easily correlated \[50\]. Abbott determined the correction factor for DBS viral load calculations and found 1.94 ± 0.06 log copies/ml difference \[40\]. A m2000rt protocol is now available to reduce additional manual data correction.

Although not supported by everyone \[41\], the possible contribution of DNA and intracellular virus particles can contribute to overestimated viral loads or positive DBS results in samples while viraemia was undetectable in plasma \[45, 52\].

### 11.0 Considerations for Future Evaluations

The present literature review highlights the need for rigorous evaluations of viral load quantification technologies particularly as new versions of current assays and POC tests are introduced on the market. A major drawback of the current literature review is the lack of available information on the most recent versions of Viral Load assays. Meticulous assessment is needed prior to dissemination of new technologies for efficient use of health care resources. Methodological standards should direct future evaluations of VL tests to improve the quality of studies and the comparability of results.

An early review of the use of methodological standards (Carrington Reid et al, 1995), assessed evaluations of diagnostic tests published over a 16 year period according to their use of seven
standards (Table X). Despite the financial costs and risks associated with disseminating tests of uncertain efficacy, few evaluations were compliant with the methodological standards. These standards are still relevant today and should be a cornerstone in the planning of all diagnostic evaluations.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spectrum Composition</td>
<td>At least three of the following four descriptors are provided: age distribution, sex distribution, summary of presenting clinical symptoms and/or disease stage, eligibility criteria for study subjects.</td>
</tr>
<tr>
<td>2. Analysis of Pertinent Subgroups</td>
<td>Results for indexes of accuracy were cited for any pertinent demographic or clinical subgroup of the investigated population.</td>
</tr>
<tr>
<td>3. Avoidance of Workup Bias</td>
<td>Cohort studies: all subjects were assigned to receive both diagnostic testing and gold standard verification, either by direct procedure or by suitable clinical follow-up. Case-control studies: if diagnostic test preceded gold standard procedure, disease verification was obtained for a consecutive series of study subjects regardless of their diagnostic test result; if diagnostic test followed, test results were stratified according to the clinical factor (s) that evoked the gold standard procedure.</td>
</tr>
<tr>
<td>4. Avoidance of Review Bias</td>
<td>Prospective cohort studies: patients always receive the diagnostic test first, gold standard procedures were evaluated independently and a statement about independence in interpreting both the test and the gold standard procedure. If the gold standard procedure was done before the diagnostic test, a statement about independence in interpreting the test and the gold standard procedure is required. Case-control studies: if the diagnostic test followed disease verification, a statement is required to indicate an independent evaluation of the diagnostic test.</td>
</tr>
<tr>
<td>5. Precision of Results for Test Accuracy</td>
<td>SEs or CIs were reported for test sensitivity and specificity or likelihood ratios.</td>
</tr>
<tr>
<td>6. Presentation of Indeterminate Test Results</td>
<td>Report (1) all the appropriate positive, negative, and indeterminate results generated during evaluation of the diagnostic test and (2) whether indeterminate results had been included or excluded when indexes of accuracy were calculated.</td>
</tr>
<tr>
<td>7. Test Reproducibility</td>
<td>Summary measures of instrument variability.</td>
</tr>
</tbody>
</table>

In the design of evaluations specific to viral load technologies, standard 3, Avoidance of Workup Bias, and standard 4, Avoidance of Review Bias, are less important given the nature of the technology. Clinical specimens can be obtained from consenting patients and the tests run simultaneously, by technicians blinded to the results or the second results. Technicians should be provided with training to reduce the likelihood of inter-observer variability influencing test results.

For the results of an evaluation to have clinical relevance, the index test needs to be compared to a standardized panel containing specimens of clinically relevant viral load concentrations or an acceptable reference standard. The current reference standard, Amplicor Monitor v1.5, will be phased out and it is important its replacement has undergone rigorous evaluation. Studies comparing two technologies without the incorporation of a reference standard provide information on the comparability of results obtained from the two systems; however, incorporating a standardized panel or reference test would make the results more relevant in the context of existing scientific literature.
The use of an acceptable reference standard will be a critical factor in assessing the accuracy and precision of the point-of-care tests under development. Although these tests have great potential to improve access to VL testing, it is important that the performance characteristics are well documented prior to their dissemination.

Standards 5 and 7, Precision of Results for Test Accuracy and Test Reproducibility respectively, ensure clinically relevant data are presented in the final publication. Reporting correlation alone is an insufficient parameter from which to draw conclusions concerning the assay’s accuracy. Correlation does not address the number of samples or specimens that are not quantified by one system and with regard to VL monitoring, this could have important implications on treatment regimens. The inability of a test to detect VL is a missed opportunity to detect early virologic failure prior to immunologic failure and the appearance of clinical symptoms (Gueudin et al.). Correlation coefficients also do not account for one assay consistently providing higher values in comparison with the reference standard. The correlation of a test consistently reporting values twice as high as the reference technology will be 1 despite the obvious difference in results. Bland-Altman plots display the bias, or mean difference, between two tests under comparison and provide details on the magnitude and direction of the difference in measures.

A number of studies also report the percentage of samples with results differing by greater than 0.5log₁₀. This has been considered the clinically relevant cut-off whereby increases greater than 0.5log₁₀ are due to true increases in plasma viral load after accounting for the natural variability of VL and the inherent variability of VL assays (Saag et al., 1996). When comparing two technologies; however, reporting the percentage of samples with differences greater than this cut-off sheds no light on the direction of the difference or the maximum or mean difference between the two technologies. Reported alongside bias measurements to provide directionality to the value.

Sensitivity and specificity are also two valuable measures of test accuracy. With a quantitative assay these parameters should be measured at a clinically significant cut-off. These measures should be determined in a sample representative of the target population, for example HIV+ individuals receiving therapy. Sensitivity will be greater if measured among individuals who are treatment naive or if virologic failure is expected as their VL will be higher and specificity will be greater if measured among individuals who are HIV-. Using HIV- individuals infected with a different retrovirus could rule out the likelihood of cross-reactivity; however, this should be detailed alongside sensitivity and specificity measures obtained from a representative sample of HIV+ individuals.

Precision or reproducibility should also be detailed with a clear description of how the measures were obtained. This includes information on the number of samples, the number of replicates per sample and a descriptive summary of the characteristics of the samples used including mean VL (+/- SD) and range.

It is not only the data presented but also the units that are selected for measuring results that require consideration. The objective of an evaluation is to establish technologic capability and determine the range of possible uses, diagnostic accuracy, impact on the health care provider, therapeutic impact and impact on patient outcome (Guyatt et al., 1986). Although the measure of International Units (IU) improves the comparability of findings, it is limited in its clinical relevance. Viral load is measured as RNA copies/ml (or is converted into RNA copy equivalents/ml in the case of
Covid ExaVir Load which quantifies the level of HIV RT activity as a correlate. Converting copies/mL into IU removes the clinical relevance of the findings and limits the impact of an evaluation.

Systematic evaluations of diagnostic tests can provide several benefits: elimination of poor or useless tests before they become widely applied, improved quality of diagnostic test information, reduced health care costs, and improved patient care (Carrington Reid et al, 1995). The contributions of future evaluations of VL assays can be maximized by considering the standards, ensuring high quality, and reporting the appropriate and clinically relevant measures of accuracy and precision.

12.0 Technologies on the Horizon

There have been several advances in Viral Load Technologies targeted at overcoming the limitations of current laboratory capacities in regions of the world most in need of VL monitoring. These include the quantification of surrogate markers for HIV RNA, the standardization of in-house PCR protocols, and the development POC viral load assays.

The strong correlation between RT activity and VL led to the development of the Cavidi ExaVir Load assay which, as already described, is a simpler and less costly alternative to traditional RNA quantification. The Perkin Elmer Life Sciences Ultrasensitive, Heat denatured p24 antigen quantitation assay is an enzyme immunoassay for quantitation of p24 antigen. The correlation of p24 and HIV RNA is not sufficiently support in the literature to advocate using p24 as a surrogate marker for VL; however, the assay has been used for early infant diagnosis of HIV (Stevens 2008). The linear range of the p24 assay is 10,000 to 30,000 RNA copy equivalents/mL and therefore may be of use in low-income countries to identify patients with virologic failure (Stevens 2008). The utility of the assay is limited in its ability to detect successful suppression of viral replication or early virologic failure given its high lower limit of detection.

The recent developments of in-house reverse transcriptase assays have the potential to be used for HIV viral load measurements. Even though most assays are not validated or FDA /CE approved, the cost reduction and applicability of performing this assay on any RT-PCR assay, makes it valuable. Guidance in sample extraction and data analysis are issues that would require careful consideration when implemented. The CDC has designed and tested HIV-1 taqman based primers and probes for real time RT-PCR that can be performed on various platforms [1, 2].

Point of Care Viral Load Assays would enable testing to be performed in primary care facilities by health providers with limited laboratory training. This would address the problems with transporting specimens and training highly skilled laboratory staff. A number of manufacturers are working towards the production of an accurate and reproducible POC.

Point-of-Care VL Assays under Development

Microconics PanNat System (www.micronics.net) is a low-cost, operator friendly, molecular diagnostic test platform designed for use in low resource settings. The instrument can be powered by battery for up to eight hours of testing and re-charged from power mains, car battery or solar power cells. PanNAT can also be powered by AC. It is a portable instrument that utilizes a novel thermal cycling method with low power requirements and a unique fluorescent detector. The disposable cartridges house all reagents and waste in a low volume liquid or stable dried format eliminating user reagent preparation and providing an ambient temperature for cartridge storage.
The cartridge incorporates Molecular Beacon probes, primers, enzymes and controls for amplification and detection. The PanNAT closed system cartridge prohibits PCR product cross-contamination and provides sample in/ result out operation in less than 30 minutes. As of March 2010, Micronics anticipates the development and release of the VL quantification product by 2013.

Biohelix is developing a POC HIV quantitative assay based on Helicase Dependent Amplification (HDA) chemistry coupled with a disposable lateral flow cassette for end-point detection. HDA is an isothermal DNA amplification system that uses a helicase enzyme to unwind double stranded DNA into single strands. The advantages of HDA include (1) low cost for instrumentation because the use of helicase to separate DNA eliminates the need for a thermocycler, (2) easy-to-use for assay development (uses only two primers), (3) versatile platform that can amplify both DNA and RNA (with RT) and (4) high sensitivity and specificity (www.biohelix.com).

Genefluidics (http://www.genefluidics.com/index.php) has developed a module for the direct detection of biomolecules in raw samples. Enzymes are immobilized onto the sensors through an anchor probe/target/signal probe hybrid, a substrate solution is introduced, and a control bias potential applied at the sensor surface. The short diffusion length between immobilized enzymes and the sensor enables a signal to be measured from the electron turnover of the corresponding redox reaction. The system uses only electronic circuitry and the sensor chips use only plastic substrates and other low cost materials. This allows for a high production volume and low costs for the end-user.

Inverness Medical Innovations (http://www.invernessmedical.com/) is developing a POC assay based on microarray technology and realtime detection. It is anticipated that a model will be available for outside testing and evaluations by the end of 2010 or early 2011 (Personal Correspondence with Susan Fiscus).

The Liat HIV-1 Quant Assay (IQuum, www.iquum.com) detects and quantitates HIV-1 RNA in a three step process. (1) 200 ul of plasma are loaded into a Liat HIV-1 Quant Assay tube, (2) the tube barcode is scanned and (3) the tube is inserted into the Liat Analyzer which automatically extracts and purifies nucleic acids from the sample. Reverse transcription, PCR amplification, and real-time detection quantitate HIV-1 RNA against an internal competitive control. Results are available 1 hour from sample input. The assay has so far been shown to quantitate HIV-1 group M (subgroups A-H) and Group O over a linear range of 100 to 10,000,000 copies/mL and with a lower limit of detection of 78 copies/ml. The assay is currently being tested outside the laboratory and is the most advanced in development.

Dr. Helen Lee is an Associate Professor and Reader in Medical Biotechnology in the Diagnostics Development Unit of the Department of Haematology at the University of Cambridge (http://www.haem.cam.ac.uk/ddu/) . The Diagnostics Development Unit has been developing technologies for simplified sample extraction and rapid and sensitive detection of biomarkers of disease (DNA, RNA, antigen or antibodies). The Simple Amplification Based Assay (SAMBA) platform was developed for qualitative or semi-quantitative detection of nucleic acid. The visual, point-of-care platform would aid in the diagnosis and monitoring of HIV infection, especially in babies, and antiretroviral therapy monitoring.
Using digital microfluidics, Advanced Liquid Logic: (http://www.liquid-logic.com/index.html) is in the process of developing a POC viral load assay. The technology manipulates discrete droplets of fluid electrically using electrodes to control each droplet. This approach will allow systems to be smaller, less expensive and more flexible with a single platform performing immunoassays, PCR, clinical chemistry, and sample preparation. An immunoassay analyzer is currently under evaluation and other assay formats and a portable analyzer are under development.

Wave80 (http://www.wave80.com/pages/wave80_main.html) is developing a family of high-performance, compact-form-factor systems for measuring biomarkers in peripheral blood. EOSCAPE uses enclosed cartridges and an open-well system to amplify signals from nucleic acid. The molecular diagnostics system designed to operate in a variety of environmental conditions to make it accessible to health facilities serving remote areas with limited laboratory services.

Advantages of POC technology

As part of treatment programs, POC VL technologies could be used to identify early failure, target non-adherents early, identify late failure (arising from either adherence or genotypic failure), and measure programme performance and site specific performance. There exists a major cost-saving opportunity by targeting non-adherents early as first line drug regimens are much less expensive than second and third line alternatives. POC technologies could also be used as part of HIV control programs to aid in the early diagnosis of acute infection and infant diagnosis of HIV.

POC tests should provide results within the time-frame of a consultation, require minimal training and be easy to perform, physically should be a hand held detection device or strip, require no specialised laboratory set-up, be stable and temperature independent, and affordable. Once tests have been developed and undergone manufacture and independent evaluation, there are still several challenges facing the implementation of new technology.

Determining a Clinical VL Threshold

Identifying a threshold representing virologic failure would aid in developing treatment algorithms; however, deciding on when to switch therapeutic regimens will depend on the availability of second and third line drugs, the potential for developing future resistance, toxicity, and cost. These guidelines will need to be developed by control program managers in accordance with national resources and infrastructure. In the developed world, consistent viremia above 50 copies/mL is indicative of virologic failure. This measure will depend on the quality of the test used and the quality of testing. It also requires several consecutive results above 50 copies/mL, which may not always be feasible in developing countries where the distance to health facilities and laboratories can often deter patients from returning for repeat testing or test results. A single reading above 5,000 copies/ml should initiate an investigation into adherence and the possibility of virologic failure.

Looking forward (DBS)

- CD4 count with DBS (genomic DNA quantification)
- Standard manufacturer protocols for DBS elution and extraction procedures
- Analysis incorporated in automatic software analysis
- Roche develops a specific apparatus for DBS extraction
13.0 Conclusions

14.0 Viral Load Search Strategy (2-10-2009)

- To be updated

Search Medline:

1. ‘HIV-1’ or ‘HIV-2’ or ‘HIV’ or ‘human immunodeficiency virus’ or ‘HIV type 1’ or ‘HIV type 2’ or ‘human immunodeficiency virus type 1’ or ‘human immunodeficiency virus type 2’ or ‘exp HIV/’: 205064
2. ‘Viral Load’ or ‘rna/ or rna, viral’ or ‘viral load’: 132286
3. ‘compar*’ or ‘eval*’: 4322458
4. ‘measure*’ or ‘quant*’ or ‘technol*’ or ‘test’: 2849330
5. ‘accuracy’ or ‘performance’ or ‘precision’ or ‘sensitivity’ or ‘specificity’ or ‘sensitivity and specificity/’: 1390923
6. 1 and 2 and 3 and 4 and 5: 680
7. 6 and ‘HIV Infections’.sa_suba: 480
8. 6 and ‘Viral Load’.sa_suba: 343

Search Embase:

1. ‘HIV-1’ or ‘HIV-2’ or ‘HIV’ or ‘human immunodeficiency virus’ or ‘HIV type 1’ or ‘HIV type 2’ or ‘human immunodeficiency virus type 1’ or ‘human immunodeficiency virus type 2’ or ‘exp HIV/’: 187703
2. ‘Viral Load’ or ‘rna/ or rna, viral’ or ‘virus load’: 115212
3. ‘compar*’ or ‘eval*’: 3671665
4. ‘measure*’ or ‘quant*’ or ‘technol*’ or ‘test’: 3107878
5. ‘accuracy’ or ‘performance’ or ‘precision’ or ‘sensitivity’ or ‘specificity’ or ‘sensitivity and specificity/’: 1368293
6. 1 and 2 and 3 and 4 and 5: 651
7. 6 and ‘Human immunodeficiency virus infection’: 436
8. 6 and ‘virus load’: 426

EndNote: Results were exported into EndNote to create a database totalling 1685 references. 708 duplicates were removed and an additional 144 non-identical duplicates were manually excluded leaving 833 references in the database for further review. Following exclusion of 568 references not relevant to the study question based on their titles, 265 relevant abstracts were reviewed. Of these, 9 were excluded for not meeting the English language criteria, 13 excluded for only sampling a paediatric population, 35 excluded for using non-plasma specimens for viral load quantification and seven were excluded for evaluating the use of dried blood spots (DBS) or dried plasma spots (DPS) as a method of obtaining samples for viral load testing. The latter exclusion is based on a complementary review ongoing alongside the present search to assess the utility and value of DBS/DPS in monitoring viral load.
In addition to the 201 full copies retrieved, 11 studies were identified through contact with experts in the field and a second search run by a member of the Advisory Group, Susan Fiscus. Of the 212 publications, only 37 met the inclusion criteria. The primary reason for exclusion was an evaluation of a viral load assay not currently commercially available \( (n=133) \). This included assessments of in-house techniques, modifications of commercial assays, or the use of retired models. Seven of the 133 publications did not mention the version of the technology used in the evaluation and were excluded for the absence of this information. Another eight papers were excluded because their primary objective was an assessment of the performance assays designed to quantify p24. Evaluations of p24 assays were not included because this technology quantifies a circulating HIV protein which is used as a correlate value of viral load. A total of 34 publications were deemed to have met the inclusion criteria and were listed for data extraction and quality assessment.

**Algorithm for Inclusion:**
14.1 Publications meeting inclusion criteria


15.0 Dried Blood Spots Viral Load Search Strategy (15-02-2010)

Search Embase:

Search medline:

1. ((dried or dry) and blood and spot*) = 1220
2. (dbs or "filter paper" or "guthrie card" or "903 paper") = 7438
3. (hiv* OR "human immunodeficiency virus*" OR "human immune deficiency virus") = 192889
4. 1 OR 2 and 3 = 244
5. limit 5 to yr="1998 -Current" = 175 results

Total: 367 articles found.

EndNote: Results were exported into EndNote to create a database totaling 367 references. 29 duplicates were removed and an additional 73 non-identical duplicates were manually excluded leaving 265 references in the database for further review. Following exclusion of 114 references not relevant to the study question based on their titles, 71 relevant abstracts were reviewed. Of these, 23 were excluded for not related to study question, 11 excluded for only focusing on DNA, 4 excluded for only focusing on drug resistance, 6 excluded for using only ELISA, 3 excluded for only using dried plasma spots (DPS), 2 excluded for qualitative RNA, and 2 excluded for using in-house PCR or not commercially available methods.

15.1 Publications Meeting Inclusion Criteria:


