SUPPLEMENT ARTICLE

Quantifying HIV for Monitoring Antiretroviral Therapy in Resource-Poor Settings

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There is increasing evidence to support the inability of CD4 cell count monitoring to predict virological failure in human immunodeficiency virus–infected individuals receiving antiretroviral therapy. There is renewed interest in improving access to viral load monitoring in resource-constrained regions to monitor adherence to treatment and to switch therapy. The field is rapidly changing as new technology platforms are made available for evaluation. This article presents an up to date summary of the assays available for viral load monitoring and suggests approaches for their implementation.

Improved access to antiretroviral therapy (ART) through price reductions of patented medications, availability of generic drugs, and significant increases in donor funding have resulted in a dramatic expansion of the number of human immunodeficiency virus (HIV)–infected individuals receiving treatment in low- and middle-income countries. Recent global estimates suggest that one-third of the 7.1 million individuals who need ART are accessing it in developing countries [1]. The simplified public health approach that has been used to provide large-scale drug delivery has been found in limited studies to be cost effective [2]. The focus has thus shifted toward evaluating the feasibility and access to laboratory assays, such as measurement of CD4 cell count and viral load, for these regions to support HIV clinical management programs. One of these laboratory tools, the plasma viral load monitoring assay, has become the standard of care for monitoring patients receiving ART in the developed world [3–5], with treatment failure being defined largely on the basis of failure to suppress viral load or occurrence of viral rebound after initial suppression.

Much data are available that support the use of viral load testing for monitoring response to therapy, determining prognosis [4, 6, 7], and identifying early virological failure necessitating treatment switches. World Health Organization (WHO) guidelines published in 2004 for ART in resource-poor environments suggested that CD4 cell count measurement was desirable for initiation of treatment but that the use of viral load for monitoring was optional [8]. These guidelines have been revised to suggest that increased access to virological testing is highly desirable, particularly for clinical decision making related to switching drug regimens [8]. The appropriate threshold for switching has not been determined. Although some organizations, including the WHO, have suggested that an HIV RNA load of 10,000 copies/mL is a reasonable threshold for recommending a change in ART [9, 10], this threshold is based on a limited data set that primarily evaluates short-term clinical outcomes. It is clear that, at this level of viral replication, failing ART regimens select for resistance mutations on a cumulative basis. As a result of the original WHO guidelines, the most common method for monitoring treatment response in resource-constrained environments is measurement of CD4 cell count. Recent data from Uganda and South Africa

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have confirmed that CD4 cell count monitoring does not accurately identify patients with virological failure [2, 11, 12]. Although CD4 cell count measurements (absolute CD4 cell counts, change in CD4 cell counts, and CD4 cell count slopes) were shown to significantly correlate with HIV load measurements in cohort analyses, they were found to have a poor predictive value in identifying virologic failure in individual patients [2]. With use of WHO guidelines and definitions for treatment failure, individuals with decreases in CD4 cell count and undetectable viral loads would have changed treatment unnecessarily [2]. The argument raised by Badri et al [2] that monitoring of CD4 cell count is an inadequate alternative to monitoring of viral load and cannot be used to substitute for viral load monitoring is receiving steadily growing support.

Recent studies published from South Africa demonstrated the importance of using viral load monitoring in conjunction with targeted adherence monitoring for conserving the first-line drug regimen [13]. This is particularly important when the second-line regimen costs significantly more than first-line treatment and when available regimens are limited. With use of a combination of viral load and CD4 cell count laboratory monitoring and counselor-driven adherence interventions, it has been shown that >95% of individuals commencing ART can continue to receive a first-line treatment regimen over a 3-year period. In addition, HIV drug resistance data from several developing countries have indicated that more-complex resistance profiles may arise when treatment switches are based on clinical and immunological criteria alone [14]. Other applications cited for the viral load assay include a tool for monitoring treatment adherence, making diagnoses during early infancy, and conducting sentinel surveillance [15]. Because of the significant differences in laboratory capacity among different countries and among regions in a country, a single laboratory solution for viral load monitoring is not feasible. The scope of this discussion will include both nucleic and non-nucleic acid–based testing approaches for measuring HIV load.

AVAILABLE HIV QUANTIFICATION ASSAYS

The first descriptions of viral load monitoring were based on the quantification of plasma HIV RNA levels with use of a variety of nucleic acid–based amplification techniques [6]. Nucleic acid–based amplification assays are the mainstay of viral load monitoring in high-income countries. Assay design is complicated by the high level of genetic heterogeneity characteristic of HIV-1 and the emergence of recombinant strains [16, 17]. This diversity remains an ongoing challenge for assay development and necessitates constant molecular surveillance. There are also differences between assays across suppliers and in different assays provided by the same supplier, and this should be taken into account when analyzing research or patient data. Disadvantages include the need for relatively sophisticated laboratory expertise and appropriate laboratory facilities and space to avoid contamination.

NUCLEIC ACID AMPLIFICATION ASSAYS

Nucleic acid amplification tests are divided into those that are based on target amplification, which form the bulk of available assays, and those that are based on signal amplification. Detection is done using either end point polymerase chain reaction (PCR) or real-time PCR; the latter simply means that detection is done as the product accumulates during the exponential phase of the reaction. The advantages of nucleic acid testing approaches are that many of these assays have been well validated and are available in quality-assured kits, and there is clinician familiarity with interpretation of results. Assays vary considerably by sample preparation, amplification and detection methodology, region of the genome targeted, and dynamic range. Assays are summarized in Table 1.

At present, there are several commercially available Food and Drug Administration–licensed assays for viral load testing: Roche Amplicor Monitor (version 1.5), which uses 3 different formats (manual, manual extraction, and comprehensive bioanalytical system [COBAS] amplification and detection); COBAS Ampliprep/COBAS Amplicor (Roche Molecular Systems) [35, 48, 49]; the bioMérieux NucliSENS HIV-1 QT assay (bioMérieux) [48, 49]; and the Versant HIV-1 RNA assay (version 3.0; bDNA; Siemens) [50, 51]. Assays such as the Abbott LCx assay have been largely discontinued [52] and replaced with the Abbott HIV-1 RealTi*m*e assay (Abbott Diagnostics). The Roche (version 1.5) assay is a reverse-transcription PCR–based, target amplification assay targeting the *gag* p24 region of the genome, and improved performance of this assay across subtypes has been demonstrated over the original version (1.0) of the assay. The Roche assay may be conducted in a manual format or on the semi-automated COBAS Amplicor with automated amplification and detection [47] or via an automated extraction on the COBAS Ampliprep as a front end to the COBAS Amplicor. The NucliSENS HIV-1 QT assay is based on the nucleic acid sequence–based amplification [49, 53], which is an isothermal amplification technique targeting the *gag* region that has an end point electrochemiluminescence detection step. The Seimans Versant assay (version 3.0) is a nucleic acid hybridization method that depends on signal amplification performed in a 96-well format on the 440 bDNA analyzer. Comparative evaluations of these assays have shown that they are highly correlated, in addition to being sensitive and specific [49].

Increasingly, the trend in the field and in resource-poor settings is to move toward real-time technology options that are faster and have higher throughputs, larger dynamic ranges, and

Table 1. Summary of Nucleic Acid Testing Assays

	bioMérieux		Siemens	
Variable	NucliSENS HIV-1QT	NucliSENS EasyQ HIV-1 (version 1.1)	VERSANT HIV-1 Quantiplex (version 3.0; bDNA)	VERSANT HIV RNA (version 1.0; kPCR)
Source(s)	[18 –20]	[22, 23] (for version 1; no source for version 2)	[24]	[25, 26]
Assay type	NASBA; isothermal amplification; electro chemiluminescence detection; manual extraction, NucliSENS extractor, or Nuclisens miniMAG	NASBA; real-time detection; molecular beacons; Nuclisens miniMAG; Nuclisens EasyMag	bDNA; sandwich nucleic acid hybridization method; signal amplification	Real-time PCR
Linear range, RNA copies/mL	51–5 million	100–3 million	50–500,000	31–11 million
Specimen type	Plasma, serum, DBS, any body fluid, EDTA, citrate, heparin	Plasma, serum, DBS, any body fluid	Plasma, EDTA, ACD	Plasma, serum, DBS, EDTA
Specimen volume, μL	200–1000	200–1000	1000	500
Area of genome targeted	Gag; target amplification	Gag; signal amplification	Pol; target amplification	Pol; target amplification
Controls	3 Internal calibrators (synthetic RNAs): Qa (high), Qb (medium) and, Qc (low); positive, negative controls not supplied with kits and left to lab decision	1 Internal calibrator; no external controls provided and inclusion reduces throughput	6–9 Standards and 3 controls (negative, low, high) per plate	Positive (high, low); negative
Subtype reactivity	Group M; not suitable for G, O [18];	Group M; not suitable for O, G	Group M	Group M (clades A–H, CRF-AE and AG); group O
Technical skill	High	High-Med, if automated	High-Med, if automated	High
Lab set-up (PCR specifications required for all) and major equipment	Extractor (or centrifuge, vortex); waterbath/heating block; biohazard hoods; reader	Extractor; analyzer; biohazard hoods; centrifuge	Siemens 340 or 440 molecular system; biohazard hoods, refrigerated centrifuge, heating block, waterbath, vacuum system	Main system VERSANT kPCR molecular system
Throughput, no. of samples per run	20–30 [46]	miniMAG (12 per run); EasyMAG (24); EasyQ analyzer (48 per run) [46]	12–168	89 (+7 calibrators and controls)
Time to result, h	3.5	2.5 (for 24 samples)	22	5–6 h
Cost per test (kit only), US$[a]	40–100	40–60	125	30–75
FDA approval	Yes	No	Yes	No
CE marking	Yes	Yes	Yes	Yes
Advantages	Isothermal; many sample types	Closed system; rapid; automated; medium technical skill	High throughput; Versant 440 system fully automated; no special laboratory set-up required; no separate extraction or amplification areas	Real time; can be fully automated; amerase to prevent contamination
Disadvantages	Contamination risk; postamplification steps required; dedicated space and equipment; high technical skill; cost; technical support required	Dedicated space and equipment; cost; technical support required; not FDA approved; significant risk of contamination in high-volume laboratories	Dedicated space and equipment; technical support required	Dedicated space and equipment; high technical skill; cost; technical support required

NOTE. Data in tables have been generated from the literature, in the absence of consultation with manufacturers. ACD, acid citrate dextrose; COBAS, comprehensive bioanalytical system; DBS, dried blood spot; FDA, Food and Drug Administration; kPCR, kinetic polymerase chain reaction; LTR, long terminal repeat; NASBA, nucleic acid sequence–based amplification; PCR, polymerase chain reaction; RT-PCR, reverse-transcriptase PCR.

fully automated extraction steps. Examples include the Roche Taqman assay (versions 1 and 2) [27, 54], NucliSENS EasyQ (versions 1.2 and 2) [21, 55], the Abbott RealTi*m*e HIV-1 assay (Abbott Molecular) [32, 41], and very recently, the K-PCR assay (Siemens). The latter assay is relatively new, with 2 peer-reviewed publications [25, 26] at the time of manuscript preparation. The Roche Taqman and Abbott RealTi*m*e assays are the only real-time assays with Food and Drug Administration approval. The Roche Taqman assay can be conducted on either the COBAS Taqman 48 or 96 format analyzers and can be automated through the COBAS Ampliprep analyzer. This is a real-time, fluorescent assay format using dual hydrolysis probes that are based on the 5′ exonuclease activity of Taq polymerase. The Roche Taqman assay (version 2) was launched in 2009 [37]. The NucliSENS assay is a nucleic acid sequence–based amplification methodology using a molecular beacon detection format that can be semi-automated with use of the manual NucliSENS miniMag extraction methodology [22] or automated using the EasyMag analyzer [55]. More recently, the Abbott RealTi*m*e HIV-1 assay was released and can be automated using 2 instruments: the m2000sp for sample preparation (largely replacing the m1000) and the m2000rt for real-time amplification and detection. The Abbott RealTi*m*e HIV-1 assay, using HIV-1 primer and probe sequences that are targeted

Table 1. ***(Continued.)***

Roche Molecular Systems		Abbott	Biocentric
Amplicor HIV-1 Monitor (version 1.5)	COBAS Taqman	*Real*Time HIV-1	Biocentric Generic viral load
[24]	[27–31]	[32–34]	[42–44]
RT-PCR; end point PCR; Microwell Plate Manual and COBAS Amplicor [24] or COBAS Ampliprep/Amplicor [35, 36]	RT-PCR; real-time PCR; dual-labeled hydrolysis type probes; Armored RNA internal quantitation standard (HIV QS); 2 versions (version 1 and 2) [37]	RT-PCR; real-time PCR; partially ds real-time probe with fluorescent label; armored RNA internal standard; automated via m2000rt; automated extraction (Abbott *m*2000*sp,* previously m1000 [38]); Abbott LCx assay largely discontinued	Real-time PCR; RT-PCR
400–750,000 (standard); 50–100,000 (ultrasensitive) [36]	40–10 million for version 1; 20–10 million for version 2 [39]	40–10 million	300–50 million
Plasma, DBS	Plasma, DBS	Plasma, DBS, EDTA, ACD	Plasma, DBS [45]
200 or 500	1000	200–1000	200–500
Gag; target amplification	Gag; target amplification; LTR and gag regions targeted in version 2	Pol integrase region [24]; target amplification	LTR; target amplification
High positive; low positive; negative; internal control	High positive; low positive; negative; internal control standard	Low positive; negative; internal control	One internal calibrator; no external controls provided
Group M; not suitable for O [40]	HIV-1; group M; group O included for version 2.0	All [34]; better for CRF02-AG recombinants [41]	Group M; not suitable for O, G
High-Med, if automated	High-Med, if automated	High-Med, if automated	High-Med
For manual, thermocycler, ELISA reader, washer, and microcentrifuge; for automated, COBAS Ampliprep and COBAS Amplicor analyzers	COBAS Taqman with or without COBAS Ampliprep 48 or 96 system; biohazard hoods; centrifuges; 168/8 h day per continuous loading	Automated extraction and prep *(m*2000*sp);* amplification and detection (m2000rt); bar code reader for primary tubes; centrifuge; biohazard hood	Thermocycler; biohazard hoods; centrifuge
12–48 (9–21 per run)	48 or 96	48 (includes 3 controls)	1–96 samples/run
7	5 [24]	5 [24]	4 h
20–100	30–100	20–40	14
Yes	Yes (for version 1)	Yes	No
Yes	Yes (for version 1)	Yes	Yes
Amperase to prevent contamination; Can fully automated; medium throughput; medium to high technical skill	Amperase to prevent contamination; single tube; fully automated; Medium technical skill; wide dynamic range; Ampliprep can be docked to Taqman for automated sample transfer [47]; single room required; Amplilink software for interfacing	Closed system post-PCR [41]; wide dynamic range [33]; can be fully automated	Close system; rapid; automated; high technical skill; open system; difficult to quality control all components
Limited linear dynamic range; 2 versions; dedicated space and equipment; cost; technical support required	Dedicated space and equipment; may have docking of COBAS Ampliprep/Taqman allowing 1 room; cost; technical support required	Dedicated space and equipment; cost; technical support required	Dedicated space and equipment; high technical skill; capital equipment expensive; technical support required; not FDA approved

[a] Costs are variable, generally dependent on the region, volume of samples, and negotiation with suppliers.

to the integrase region of the *pol* gene, has been reported by some investigators to be more sensitive than Roche COBAS Taqman (version 1 and 2) and to correlate well with Roche COBAS Monitor (version 1.5) [37, 38], and other researchers have reported good concordance among all 3 assays [47]. A real-time PCR assay called the Biocentric generic viral load assay is available from Biocentric that can be placed on a variety of real-time analyzers and is referred to as an open system. Concerns have been expressed with respect to the difficulties that may arise with quality assuring this assay approach across centers

Other direct approaches have been to measure the proviral DNA load in peripheral blood mononuclear cells [56, 57] or selected CD4 cells [57], which has shown to have some correlation with monitoring efficacy of treatment in individuals with undetectable viral RNA loads. No commercial assays are currently available for measuring proviral DNA load. The literature abounds with other in-house assay options, usually real-time assays developed in many different countries in an attempt to reduce costs. These have been described as targeting different regions of the HIV genome with a host of primer and probe designs using different technology platforms [58, 59–61]. A full detailed discussion of these different approaches is beyond the scope of this manuscript, but these approaches currently have limited capacity for expansion because of the high costs of equipment and relative sophistication of assays or lack of availability in a well-validated and rigorously quality-controlled kit form.

NONNUCLEIC ACID TESTING APPROACHES

The cost of capital equipment to conduct nucleic acid testing, together with the high costs of reagents and consumables (US $15–$100 per test, depending on the region) [15, 62], prohibits implementation in many resource-poor environments. In the absence of the sample volumes required to facilitate negotiation of instrument leasing and reduction of costs, alternatives need to be sought.

Indirect measures of viral load that require less equipment and skill have thus been evaluated for resource-poor settings [62] and include the ultrasensitive, heat-denatured p24 antigen quantification assay (Perkin Elmer Life Sciences) [63], which is no longer being developed for viral load monitoring, and the ExaVir Load (Cavidi AB) reverse-transcriptase assay [64–66]. More recently, other approaches have included the evaluation of flow cytometry–based markers of activation, such as the quantification of bright CD38 expression on CD8 cells [67, 68]. These have been advocated as screening tools facilitating a reduction in the number of viral load assays that need to be performed. Assays are summarized in more detail in Table 2.

REVERSE TRANSCRIPTASE ACTIVITY ASSAY

The measurement of reverse transcriptase activity as an alternative to RNA quantification is favored as an approach because of the lack of reliance on subtype sequence [64, 74, 75] and the relative lack of requirement for sophisticated laboratory facilities and scientific expertise, compared with nucleic acid–based technologies. The ExaVir Load assay measures the activity of virus-encoded, reverse transcriptase, which is packaged together with the viral RNA in the HIV particle, in an enzyme-linked immunoassay format. Initial versions of the assay were compromised by the susceptibility of the enzyme to inhibitory antibodies and other interfering molecules [76]. In subsequent versions, virions are first separated from plasma by passage through a gel column that removes interfering substances; then, the DNA produced as a result of the reverse transcriptase activity is measured. Work in South Africa and Australia demonstrated good correlation between earlier versions of the assay and the Roche RNA assay in longitudinal follow-up of patients receiving ART [64, 65]. The current assay, ExaVir Load (version 3), is more sensitive than version 2 and has a lower limit of ~200 copies/mL. In a recent study, 95% of samples with an HIV RNA level >400 copies/mL were detectable with ExaVir Load, and there was similar hands-on time (12 min/sample vs 11.4 min/sample), compared with the Roche COBAS Amplicor Monitor (version 1.5) [77].

ULTRASENSITIVE, HEAT-DENATURED P24 ANTIGEN QUANTIFICATION ASSAY

Numerous reports in the literature describe the performance of the quantitative p24 antigen assay (Perkin Elmer/NEN HIV-1 ELISA p24 antigen kit) with a heat denaturation step for dissociating immune complexes for monitoring virological response [78–80]. Schupbach et al [81] further improved assay sensitivity by using an external buffer (not provided by the manufacturer) that improves dissociation. The assay is now used widely as an alternative to nucleic acid testing for diagnosis of HIV infection during early infancy; this use appears to be the focus of the supplier [82, 83]. Several publications support the use of the assay for monitoring ART [63, 65, 79, 84–86]; however, some describe less successful outcomes [87], and the assay is no longer being developed for viral load monitoring. The sensitivity of the assay is reported to be 10,000–30,000 RNA copies/mL, which is not adequate for facilitation of treatment changes in any setting.

FLOW CYTOMETRIC–BASED ASSAYS

Centralized testing for CD4 cell count has been established in many resource-limited settings; thus, a significant amount of interest has been expressed in developing a flow cytometric–based direct or indirect marker of viral load. A few publications cite the possibility of using measurements of the chronic activation of $CD8^+$ T cells with use of activation markers, such as CD38 [67, 88] or the programmed death-1 molecule [88]. In a recent study in South Africa involving a cohort receiving ART, the CD38 marker was added to the CD4 cell count assay [67]. This was introduced at baseline before the start of ART, and changes in $CD38^+$ mean fluorescent intensity on $CD8^+$ T cells were regularly assessed during follow-up and compared with viral load values. Results revealed a gradual decrease in CD38 mean fluorescent intensity with time in patients who responded to treatment and developed undetectable viral loads. Increases in CD38 mean fluorescent intensity were associated with or preceded an increase in viral load, and this approach may potentially be used to reduce the number of viral load tests conducted after further standardization and investigation.

PROPOSED VIRAL LOAD TESTING ALGORITHM FOR RESOURCE-POOR ENVIRONMENTS

In high-income countries, it is recommended that viral load testing be conducted at baseline; 2–8 weeks after initiation of ART, to assess early virological response; and then every 3–4 months [5]. In general, treatment is considered to be successful if viral load decreases by >1 log by 8 weeks and is undetectable at 16–24 weeks. This algorithm may need to be revised in resource-poor countries, with viral load testing conducted less

Table 2. Nonnucleic Acid–Based Testing Strategies

Variable	Assay	
	Ultrasensitive, heat-denatured p24 antigen quantitation assay (Perkin Elmer Life Sciences)	ExaVir load (version 3; Cavidi AB)
Assay type	Enzyme immunoassay for quantitation of p24 antigen [69, 70]; separate external buffer required	Enzyme immunoassay for quantitation of reverse transcriptase activity [64, 70, 71]; colorimetric reading
Detection	Colorimetric or fluorimetric; Quanti-kin detection software	Colorimetric; detection software provided by company with kit start up package
Dynamic range	Clinical cutoff: 10,000–30,000 RNA copies/mL; reported as fg/mL: 1–3000 fg/mL	RNA copies/mL equivalents; version 3: 200 to >600,000 RNA copies/mL equivalents
Specimen type	Plasma (ACD, CDPA-1, EDTA, sodium citrate, or heparin), serum, or cell culture supernatant	Plasma
Specimen volume	200–450	1–1000
Controls	5 Concentrations (VQA serum quality controls and diluents correction controls not provided in kit)	Serially diluted standard and recommended plasma pool for control
Subtype reactivity	HIV-1 (all subtypes); HIV-2 data [72]	HIV-1
Time to result	2.5 h	2.5 days (colorimetric); 1.5 days (fluorimetric)
Tech skill	Low to medium	Low to medium
Laboratory set-up (PCR specifications required for all) and major equipment	Simple ELISA plate washer and reader; incubator; refrigerator and/or freezer; dry heat block	Incubator; freezer to store samples; ELISA plate washer and reader
Throughput	High; 96 samples per run	32 samples per run
FDA approval	No	No
CE marking	No	Yes
Advantages	Easy training; simple ELISA plate washer and reader; no separate areas required; high throughput; can be used for infant diagnosis of HIV infection [73]	Easy training; simple ELISA plate washer and reader; no separate areas required; can be used for NNRTI resistance monitoring
Disadvantages	Not sensitive enough to facilitate treatment changes in any setting; inconsistent supply; external buffer required	Controls not supplied
Cost	$20–$30[a]	$15–$25[b]

NOTE. ACD, acid citrate dextrose; CDPA-1, citrate phosphate dextrose adenine; ELISA, enzyme-linked immunosorbent assay; NNRTI, nonucleotide reverse-transcriptase inhibitor; PCR, polymerase chain reaction.

[a] Variable: costs dependent on region.

[b] Variable: costs depend on volume.

frequently or with higher thresholds being used to determine virological failure necessitating therapy switch. Nucleic acid technologies should be limited to those laboratories where there is appropriate space and separation of the various steps in the PCR process, to limit contamination. Many larger reference laboratories may satisfactorily meet these criteria. Nonnucleic acid technologies, such as the ExaVir Load assay, may be appropriate for district-level laboratories (eg, where enzyme-linked immunosorbent assay methods are already being used) and in smaller reference laboratories where there is no separate PCR suite in the laboratory; technology changes now allow PCR to be performed in 1 room for many assays.

SAMPLE COLLECTION AND TRANSPORTATION STRATEGIES

Sample collection and transportation remain huge stumbling blocks for the operational implementation of nucleic acid viral load testing assays in resource-limited settings. Most assays require centrifugation of samples on site and shipment of plasma within 24 h at room temperature or on dry ice if a longer delay is anticipated. Courier networks are often not available or poorly developed in these countries. Access to facilities producing dry ice is limited, and temperatures en route regularly exceed 40°C. To address these issues, alternative means of transporting samples have been evaluated, including use of (1) plasma preparation tubes that allow overnight ambient transportation without the need for aliquoting of samples [89], (2) dried fluid spots (either plasma or whole blood), and (3) collection devices, such as the SampleTanker (Research Think Tank). The use of Vacutainer plasma preparation tubes (EDTA anticoagulant) may be a solution for certain scenarios in which phlebotomy skills are available and clinic access to centrifugation is feasible. However, concerns have been expressed with respect to the ongoing biohazard risk associated with transporting tubes and because several studies have reported elevated levels of viral loads at lower limits of dynamic range, compared with conventional EDTA collection tubes [89].

Dried fluid spots have been evaluated for a number of HIV-related diagnostic and monitoring assays, including diagnostic HIV serologic testing, p24 antigen quantitation [90], HIV DNA PCR for early infant diagnosis [91, 92], and more recently, viral load determination [93] and resistance genotyping [94–96]. The use of dried blood spots for early infant diagnosis of HIV infection is already widely practiced in many resource-poor settings. Viral load monitoring with use of dried plasma spots and dried blood spots has had only limited evaluation, largely for the Roche Monitor assay (version 1.5) [97, 98] and for the bioMérieux NucliSENS HIV-1 assays [93] . The study by Brambilla et al [97] (at 11 laboratory centers) revealed a lower limit of HIV RNA load of ~4000 copies/mL, greater sensitivity for the Roche Monitor assay, and stability of results for up to 1 year when samples were stored at −70°C. A similar study evaluating the NucliSENS EasyQ/EasyMAG combination that compared dried plasma spots and dried blood spots with liquid plasma revealed comparable results down to viral loads of 3.6 log copies/mL [93]. In addition, this study suggested that samples were stable without cold storage for up to 3–6 weeks. Dried blood spot samples are easier to collect in the field than are dried plasma spots and provide a feasible option for improving access to HIV load monitoring in resource-limited settings. The sensitivity is adequate if therapy switch is only initiated above this level. Dried blood spots and dried plasma spots have also been analyzed for HIV genotype analysis and demonstrated high concordance with predicate assays [96]. Standardized extraction protocols need to be developed and published for large-scale implementation of viral load assays using dried blood spots.

APPROPRIATE ASSAY SELECTION

Assistance with making informed decisions with respect to selection of appropriate technology for monitoring of ART programs is essential in resource-poor settings. The wide array of technologies available for viral load monitoring in particular pose significant dilemmas for laboratories that are initiating such programs. The steps involved in introducing a viral load technology in a laboratory in a resource-limited setting are not dissimilar to those involving establishment of validated CD4 cell count testing [99].

Comprehensive cost analysis is essential and needs to factor in the cost of reagents and consumables, staff time used to conduct the assay, cost of instrument maintenance, cost of running internal controls and external quality assurance, costs associated with returning the results to the clinician (eg, by fax or courier), sample transportation, and any additional fixed overhead costs. A tiered laboratory approach is probably the most practical for rapid implementation and affordability, with primary centers preparing and referring more specialized testing to secondary or tertiary testing facilities. At the primary health care level, a point-of-care viral load test remains the first prize for those conducting research and for development in the HIV diagnostics field [100]. This would facilitate immediate management decisions and remove all the difficulties related to transportation and reporting of results to clinicians. Several investigators are pursuing the rapid viral load testing strategy using formats, such as various dipstick [101], molecular zipper [102], oligonucleotides on gold nanoparticles [103], and chip approaches. Secondary care centers may develop the capacity to perform less sophisticated testing, using simple systems, such as reverse transcriptase activity assays or simple automated nucleic acid testing systems. If no separate PCR suite is available, vigilance is required at these laboratories to ensure that contamination is not occurring in PCR-based assays. Tertiary ref-

erence laboratories generally have the capability to conduct nucleic acid amplification techniques for viral load testing. Constant molecular surveillance is needed in a region to ensure that the viral load assay selected (if nucleic acid based) remains relevant in terms of detecting and quantifying HIV subtypes in the particular patient population being monitored.

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