Review Article

Indian J Med Res 134, December 2011, pp 823-834

Low-cost assays for monitoring HIV infected individuals in resource-limited settings

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Received September 30, 2011

Use of a combination of CD4 counts and HIV viral load testing in the management of antiretroviral therapy (ART) provides higher prognostic estimation of the risk of disease progression than does the use of either test alone. The standard methods to monitor HIV infection are flow cytometry based for CD4+ T cell count and molecular assays to quantify plasma viral load of HIV. Commercial assays have been routinely used in developed countries to monitor ART. However, these assays require expensive equipment and reagents, well trained operators, and established laboratory infrastructure. These requirements restrict their use in resource-limited settings where people are most afflicted with the HIV-1 epidemic. With the advent of low-cost and/or low-tech alternatives, the possibility of implementing CD4 count and viral load testing in the management of ART in resource-limited settings is increasing. However, an appropriate validation should have been done before putting them to use for patient testing.

Key words CD4 count - HIV monitoring - low-cost assays - plasma viral load - resource-limited settings

Introduction

The cost of antiretroviral therapy (ART) has reduced dramatically due to the production by generic manufactures and this has led to the increased usage of ART in developing countries. As ART is becoming more affordable and accessible, inexpensive laboratory tests are also needed to monitor the progression of disease in HIV infected individuals living in resource-limited settings, where HIV burden is usually high[1]. HIV treatment can be managed without routine laboratory assessment[2], but CD4+ T cell count monitoring allows the evaluation of disease progression[3], and viral load testing can increase adherence and facilitate timely switching of failing regimens, minimizing the development of resistance[4,5]. Thus, use of a combination of CD4 counts and HIV viral load testing in the management of ART provides higher prognostic estimation of the risk of disease progression than does the use of either test alone[6,7].

Historically, several candidate markers have been explored for monitoring the course of HIV infection and response to treatment. Though several new parameters are being evaluated[8,9], the levels of absolute numbers of peripheral CD4+ T cells (immunological marker) and the viral load (virological marker) have eventually become the reference markers in clinical practice for

monitoring HIV infected individuals. These indicators are used to determine disease stage and progression, and assist in making decisions regarding when to start or change ART regimens, and assess treatment responses[10]. The lack of infrastructure for laboratory monitoring of HIV disease is one of the major reasons for ineffective implementation of ART roll-out programmes in several resource-limited settings in the developing countries[1].

The reference standard for CD4+ T cell testing is enumeration of T cell subsets by flow cytometry. For plasma viral load (PVL), a number of nucleic acid test (NAT) based assays that use reverse transcriptase-polymerase chain reaction (RT-PCR), branched DNA (b-DNA), and nucleic acid sequence based amplification (NASBA) technologies are approved in many countries. Such reference assays are prohibitively expensive and also require sophisticated infrastructure facilities with expensive laboratory equipment as well as trained technical personnel, which are not widely available in resource-limited countries[11]. The high initial cost of equipment and problems associated with equipment routine maintenance, lack of technical support, and lack of access to quality assurance/control (QA/QC) programmes have limited widespread use of standard methodologies. This review addresses the importance of newer alternative methodologies, which are low-cost and low-tech for the use in resource-limited settings.

The reference standard and low-cost monitoring of CD4+ T-cell count

CD4+ T cell monitoring in conjunction with clinical status will be of benefit in terms of assessing prognosis and guiding the treatment response. Also, CD4+ T cell count allows timely administration of primary prophylaxis against specific opportunistic infections (OIs) and an associated reduction in hospital admissions, with use of affordable drugs such as trimethoprim sulphamethoxazole[12]. The precise CD4+ T cell count at which the risk of specific OIs justifies prophylaxis has been well established in the western countries[13].

The reference standard for CD4+ T cell count: Multi-parameter flow cytometric quantification of CD4+ T cells with microprocessor-assisted analysis is the reference standard. A wide range of instruments are available commercially from different companies including the popular manufacturers' flow cytometry, Becton Dickinson and Beckman Coulter Corporation[14]. The cost of reagents to perform full lymphocyte subset multi-platform analysis, in which CD45+ T cells are identified by staining with 8 monoclonal antibodies (MAbs) by means of 3-colour fluorescence to identify CD3+/CD4+ and CD3+/CD8+ T cells, is also prohibitive in many countries. Among the available flow cytometers, the FACSCount (Becton Dickinson) instrument is a simpler, relatively less expensive and automated for clinical laboratories and quantifies CD4+/CD8+ T cell counts by simultaneous fluorescent MAb staining of CD3, CD4, and CD8 surface molecules by use of different fluorochromes attached to each MAb.

Low-cost alternative assays for CD4+ T cell count: Currently several modified flow cytometry and new gating approaches are available to make the assay low-cost and low-tech without compromising the quality of the test results. The assays with modified flow cytometry technologies (volumetric system) are EasyCD4 and CyFlow. Extensive evaluation studies have been carried out with these modified flow cytometry in different countries on the efficiency of the each assay in providing accurate and reproducible results[15-20]. New approaches also include primary gating, in which CD4+ T cells are defined by an autogate in a single histogram of CD4+ T cell fluorescence intensity versus light scatter by use of a single-platform volumetric flow cytometry. This is considered relatively economical compared with the use of full test panel of MAbs[21]. The summary of the different low-cost assays of CD4+ T cell count and evaluation studies data are shown in Tables I and II.

Guava EasyCD4 assay is a micro-capillary flow cytometry technology based system made by Millipore-Guava Technologies, USA. The flow cytometer is coupled with a laptop computer for analysis. The system uses a diode green laser as a light source. The reagent kit consists of two monoclonal antibodies directly conjugated to PECy5 and PE for CD3 and CD4 T-cell antigens. The procedure does not require sheath fluid and the system's sampling precision depends on the integrity of the fluid pathway. Studies[15,16,21] from other settings and at Chennai, India[18] have shown it easy to perform, and generate reliable results. Bland-Altman analysis showed close agreement with the EasyCD4 assay yielding CD4+ T-cell counts with a mean difference of -26 cells/µl higher than by flow cytometry. However, EasyCD4 is less accurate at high absolute CD4+ T-cell counts, particularly when the count is more than 500 cells/µl. This may be due to the standardization and optimization of these alternative assays to provide better results at the lower critical CD4+ T-cell counts. The technicians need substantially less training than the standard flow cytometry for this

Table 1. Summary of various CD4+ T-Cell enumeration techniques

Parameter	Dedicated technology based assay						Manual assay (microscope based)
	FACSCount	Cyflow Counter	PointCare Now	Guava-Millipore	PIMA	PLG	Dynabeads
Manufacturer	Becton Dickinson (CA, USA)	Partec GmbH (Munster, Germany)	Pointcare Technologies Inc, (MA, USA)	Guava technologies-Millipore (CA, USA)	Alere Medical Pvt. Ltd, USA	Beckman Coulter (CA, USA)	Dynal Biotech, Oslo, Norway
Platform	Dedicated CD4/ CD4% counter	Dedicated CD4 Count	Dedicated CD4 & CD4% counter	Dedicated CD4 & CD4% counter	Dedicated CD4 Count	Dedicated CD4 & CD4% counter	Magnet Hemocytometer Light or Fluorescence microscope
Principle	Flow cytometry	Flow cytometry	Flow cytometry	Micro-capillary Flow cytometry	Flow cytometry	Flow cytometry	Direct count of immunocaptured cells
Monoclonal antibodies used	Anti-CD4, Anti-CD14 & Anti-CD15	Anti-CD4, Anti-CD45, Anti-CD3, Anti-CD8	Anti-CD4 conjugated with gold nano-particles	Anti-CD4 & Anti-TLC	Anti-CD4 & Anti-CD3	Anti-CD45 & Anti-CD4	Magnetic beads conjugated to Anti-CD4 and Anti-CD14
Specimen type	Whole blood	Whole blood	Whole blood	Whole blood (venous & capillary)	Whole blood (venous & capillary)	Whole blood	Whole blood
Specimen volume (µl)	50	50	40	10	25	100	125
Report	Absolute CD4 count & CD4%	Absolute CD4 count	Haematology parameters (Hb, platelet, WBC, TLC & DC), Absolute CD4 count & CD4%	Absolute CD4 count & CD4%	Absolute CD4 count	Absolute CD4 count & CD4%	Absolute CD4 count
Approximate cost of Instrument (US$)	30,000	22,000	28,000-30,000	26,000-30,000	1,000-1,500	50,000-60,000 (If Coulter EPICS XL-MCL)	1,000-8,000 (Depending upon the type of microscope used)
Cost of reagents (US$)	6-8	2-3	10	2-3	10-12	3-4	3-5
Advantages	• Autogating • Minimal steps, less human error • Less biohazard • Single platform, CD4 count & CD4% • Quick results	• Low-cost • Quick results • EQAS available • Can be battery operated (mobile testing can be done)	• Autogating • No skill is required (test can be done in single press) • No specimen preparation	• Autogating • Low cost of reagents • Generates minimal bio-waste • Minimal specimen volume required	• Easy to use procedure • Very minimal biowaste • Specific trained personnel not required	• Simple gating strategies • Low cost of reagents • Faster reporting •	• Simple • Rapid • Absolute CD4 count

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Table 1 (Contd.). Summary of various CD4+ T-Cell enumeration techniques

Parameter	Dedicated technology based assay						Manual assay (microscope based)
	FACSCount	Cyflow Counter	PointCare Now	Guava-Millipore	PIMA	PLG	Dynabeads
	• EQAS available • Less training required • Robust		• Can be used for point-of-care testing • Additional haematology testing is also available • Can be easily operated with battery	• Single platform, CD4 count & CD4%	• Minimal specimen volume	• Single platform CD4 & CD4% • High sensitivity with delayed Specimen (72 h at RT)	
Disadvantages	• Expensive reagents	• CD4% not reported • Limited data available on validation	• Expensive reagents • Low throughput • No EQAS available • Very limited data available on validation	• No published data available	• CD4% is not available • Not much data published	• Initial cost of instrument • Requires a trained personnel	• Low-throughput • Subjectivity in visual counting • CD4% not reported • No EQAS available

Source: Ref 54

Table II. Summary of validation data of alternate low-cost assays of CD4 T cell count

Alternate method	Comparator for the validation	Results	Country	Investigators
FACSCount	FACSCount & FACSScan	CD4, r - 0.986 CD4%, r - 0.977,	Thailand	Pattanapanyasat *et al*, 2008[22]
Cyflow Counter	FACSCount	CD4, r - 0.991	Zimbabwe	Zijenah *et al,* 2006[15]
	FACSCount & FACSScan	FACSScan CD4, r - 0.96 FACSCount CD4, r - 0.97	Thailand	Pattanapanyasat *et al,* 2005[23]
Guava PCA	Conventional flow cytometry	CD4, r - 0.938	India	Thakar *et al,* 2006[24]
	FACSCount & FACSCalibur	CD4, r - >0.93	Thailand	Pattanapanyasat *et al,* 2008[25]
	FACSCount	CD4, r - 0.94	Thailand	Pattanapanyasat *et al,* 2007[26]
	FACSCount	CD4, r - 0.99	India	Balakrishnan *et al,* 2006[18]
	Coulter tetraCHROME	CD4, r - 0.947 CD4%, r - 0.950	India	Mohanakrishnan *et al,* 2011*
PIMA	FACSCalibur	CD4, P = 0.72	Zimbabwe	Mtapuri *et al,* 2010[27]
	FACSCount	CD4, r - 0.98	Senegal	Diaw *et al,* 2011[28]
	FACSScan	CD4, r - 0.95 CD4%, r - 0.96	Thailand	Pattanapanyasat *et al,* 2005[29]
Panleukogating	Coulter CYTO-STAT tetraCHROME	CD4, r - 0.95 CD4%, r - 0.98	West Indies	Sippy *et al,* 2008[30]
	FACSCount	CD4, r - 0.98 CD4%, r - 0.97	India	Mohanakrishnan *et al,* 2011*
Dynabeads	Standard flow cytometry	CD4, r = 0.89	Burkina Faso	Diagbouga *et al,* 2003[31]

*unpublished data

assay and it requires less volume of specimen (10 µl) and generates very minimal biowaste. Studies from other settings have also shown the good results[19,24,32,34].

CyFlow is another desktop single-platform technology made by Partec, Germany. It is a volumetric software controlled absolute count system equipped with either a single 532 nm green solid-state laser used for one fluorescence parameter or two lasers with a mercury arc lamp applicable for 2 or 3-colour analyses. Data acquisition and analysis are performed in real time with FlowMax software. It also can be used as a mobile system that can run on car batteries. It works on simple no-lyse and no-wash protocol. The machine is more robust than conventional flow cytometry and this mobile CD4+ T cell testing laboratory can be established for the field level use. Investigators from several resource-limited settings have evaluated the precision of the CyFlow system and observed good correlation with the standard assay[15,16,23,34-36].

PointCare NOW system is a mobile technology developed by PointCare Technologies Inc., USA. This DualZone (ImmuZone and HemaZone) technology provides two categories of testing: CD4 and CD4 per cent, combined with haematology profile (including haemoglobin and white cell differentials). Full automation and closed-capsampling make this system easy and safe to use, with minimal training. Heat-stable reagents eliminate cold-chain shipping. This instrument operates on the QuadScatter optical system with CD4 antibodies tagged to gold nano-particles. The CD4 T-cells are identified using a PointSmart primary gating strategy. This method eliminates the high energy consumption of the lasers that are used in traditional fluorescence-based methods. A whole blood sample (2 ml) is placed in the machine and after a single button operation CD4 results are generated in about 10 min. This machine can work on a battery back-up. Although this system has several advantages, evaluation studies are not sufficient in the peer-reviewed literature[37].

Recently introduced PIMA point-of-care (POC) CD4 analyzer by Alere, USA, is becoming more attractive in resource-limited settings. It has major advantage in providing immediate clinical decision-making and fast programme delivery. This POC CD4 test uses disposable cartridges and as a battery-powered analyzer. Although it has additional advantage as use of a finger prick specimen, the performance on finger prick blood is not as good as on venous blood[28]. The

performance of the venous blood have shown to give better results[27,28,38,39].

The CD45 gating approach combined with the use of fluorescence beads in the procedure has converted the standard flow cytometry into a single-platform approach. This gating approach also eliminates the need for multiple equipment (flow cytometry and haematology) and should be less expensive than the available methods when labour, cost and inconvenience of repeat samples, and haematology costs are considered. These absolute count tubes contain a lyophilized pellet that dissolves during sample preparation, releasing a known number of fluorescent beads. By gating the bead population during analysis, absolute cell counts can be readily determined by a simple calculation. Currently, the reagents are also available in the kit format from Beckman Coulter Inc., USA. This methodology has been validated in several settings and an excellent performance has been documented with multicentric studies[30,40-43]. The major advantage with this CD45 gating approach is analyzing aged samples even beyond 3 days storage.

A newly introduced single tube reagent from Becton Dickinson for FACSCount has several advantages particularly, being low-cost and also provides both CD4+ absolute count and its percentage. However, the validation data are very limited.

Flow cytometry versus low-cost assays to enumerate CD4+T cell count: Though the flow cytometry is considered the reference standard, disadvantages include the fact that, it is expensive in terms of initial cost of equipment and an ongoing operational cost including annual maintenance contract. Highly trained personnel are generally needed to run the standard flow cytometry, which are technically complicated and very often company technical support is needed. Most of the newer low-cost methodologies are simple to operate and robust. It could provide both absolute CD4+ T cell count and CD4+ T cell percentage; and is very useful as a built-in control to determine whether the CD4+ T cell count is changing or stable, is available[44]. Also biological variables, including diurnal variation, exercise or rest, and smoking, can significantly alter absolute CD4+ T cell count[45]. Most importantly, CD4+ T cell percentage is essential for monitoring paediatric cases.

Although manual CD4 count method using microbead system (Dynal Biotech, Oslo, Norway) has been shown to be good[46,47], there are issues with the implementation in resource-limited settings such as labour intensive, low-throughput and requirement of qualified personnel for making accurate measurements. The POC testing device such as LabNow Inc., USA[48] or dipstick technology (personnel communication from Prof. Suzanne Crowe, Head, Pathogenesis and Clinical Research Program, Macfarlane Burnet Institute for Medical Research and Public Health, Melbourne, Australia) with a visual read-out format are currently under field level evaluation.

The reference standard and low-cost methods for monitoring of viral load

Viral load testing is the only definitive method for early detection of ART failure[49,50]. Assessment of viral load is one of the best predictors of HIV disease progression, as well as the main parameter to assess ART response. Multi-center AIDS Cohort Study (MACS) demonstrated that plasma HIV-RNA load was a better predictor of progression to AIDS and death than $CD4^+$ T-cell counts[6,51]. But, the viral load testing has not been routinely implemented in resource-limited settings due to cost, complexity, availability and accessibility.

Commercial RNA viral load assays have been widely used as the 'gold standard' to quantify HIV-1 RNA in plasma[11]. Three major diagnostic companies, Roche (Roche Amplicor), bioMerieux (NASBA) and Siemens (b-DNA) have developed HIV-1 viral load assays. Compared to previous end-point detection methodologies, recently companies (Roche TaqMan and Abbott m2000rt) have adapted the real-time technologies based on fluorescence to achieve simultaneous amplification and detection instead of end-point detection[52]. The real-time technologies offer more accurate quantification, a wider dynamic range, and a higher throughput. In addition, these companies have included automated sample preparation to reduce manual time and prevent carry-over contamination[53]. Most importantly, these assays extend the subtype coverage to detect increasing numbers of non-B strains, which are prevalent in most affected countries. However, these standard viral load assays are prohibitively expensive for the use in resource-limited settings and require very sophisticated infrastructure facilities.

Monitoring HIV-1 viral load in resource-limited settings faces multiple challenges particularly; *(i)*

viral load monitoring is only available in reference laboratories, where various clinical trials are financially supported by national and/or international funding, *(ii)* hospital facilities lack basic laboratory infrastructure such as reliable power and water supply and air conditioning, *(iii)* personnel with lack of training on technologically complex molecular assays, and *(iv)* not being robust methods.

To provide viral load monitoring in resource-limited settings, inexpensive alternative methodologies have been developed by different manufacturers and some of these have been validated in the recent years (Tables III & IV), such as the Cavidi ExaVir™ RT assay[55-59], the PerkinElmer Ultrasensitive p24 assay[56,60-62] and homebrew RT-qPCR[63]. The ExaVir™ assay and the Ultrasensitive p24 assay are based on ELISA method, measuring the activity of reverse transcriptase (RT) enzyme and the concentration of p24 antigen, respectively. These alternative methods have significantly reduced cost ($15-20), compared with commercial viral load assays ($70-100)[54]. Moreover, the ExaVir™ assay and the Ultrasensitive p24 assay are technologically simpler than standard viral load assays, and thus do not require highly skilled operators and advanced laboratory facilities. But these assay are time-consuming and labour intensive procedures. Although homebrew RT-qPCR methods are highly correlated with standard viral load assays, these still need expensive PCR thermal cyclers and highly trained operators, which restrict their application in decentralized laboratories[63].

The Cavidi ExaVir™ RT assay is an ELISA-based assay to measure the RT activity, which in turn correlates well with the standard viral load assays. The equipment needed (which are provided by the company as a start-up kit at free of cost) is mainly a vacuum pump, waste bottle, some rubber tubing and an ELISA reader or a fluorometer and some buckets for the wash procedures. The procedure has only 2 major steps (purification of virions and recovery of RT, including removal of potential inhibitors, followed by quantification of RT activity in a microtitre plate format). In brief, HIV-1 virions are first purified from plasma specimens using a solid phase extraction manifold to remove contaminants and inhibitors. Purified HIV-1 virions are then lysed to release RT enzyme. The released RT is added to a microtitre plate, which is coated with poly-A oligonucleotides. RT incorporates BrdUTP into cDNA, using the poly-A oligonucleotides as templates. After incubation at

Table III. Comparison of alternate assays for monitoring HIV-1 viral load

Assay name and detection	Equipment requirement	Analytical sensitivity (copies/ml)	Specimen type	Specimen volume (ml)	Subtypes detection	TAT (h)	Linearity range (copies/ml)	Number of specimen per run	Cost per test (US$)
Cavidi ExaVir Load Assay Reverse transcriptase enzyme activity	Incubator (33°C), freezer, ELISA reader	200	Plasma	1	All subtype and CRFs	72	200 upwards	30	15-20
Perkin Elmer - ELAST amplification system HIV-1 p24 Antigen Ultrasenstive	Incubator, ELISA reader, refrigerator	400	Plasma or serum	100	All subtype and CRFs	6	5,000 upwards	96	10
Homebrew PCR RT-qPCR	RealTime PCR	40	Plasma	0.6	All subtype and CRFs	5-6	40 to 10,000,000	96	5

TAT, turn around time; CRF, circulating recombinant forms
Source : Ref 54

Table IV. Summary of validation data of CAVIDI ExaVir Load assay

Alternate method	Comparator for the validation	Sample size (N)	Results	Country	Investigators/Refs
Cavidi ExaVir Load Assay	COBAS TaqMan	215	Versions 2, r = 0.92, $P < 0.001$ Versions 3, r = 0.95, $P < 0.001$	China	Huang *et al* 2010[55]
	Abbott M2000rt RealTime HIV-1 assay	119	version 3, r= 0.94	UK	Labbett *et al*, 2009[57]
	COBAS Amplicor HIV-1 Monitor assay	144	Versions 2, r=0.91	Australia	Greengrass *et al*, 2009[58]
	COBAS Amplicor HIV-1 Monitor assay	82	Version 2, r=0.8554	Botswana	Mine *et al*, 2009[64]
	Amplicor HIV-1 Monitor	108	Versions 3, r= 0.83	USA	Napravnik *et al* 2010[77]
	COBAS Amplicor HIV-1 Monitor assay	202	Version 3, r = 0.85	Australia	Greengrass *et al*, 2009[78]
	COBAS Amplicor HIV-1 Monitor assay	262	Version 2, r=0.90	Sweden	Malmsten *et al*, 2005[79]
	COBAS Amplicor HIV-1 Monitor assay	85	Version 2, r=0.89	Australia	Greengrass *et al*, 2005[80]
	Amplicor HIV-1 Monitor	117	Version 2, 0.81	South Africa	Stevens, *et al*, 2005[81]
	COBAS Amplicor HIV-1 Monitor assay	390	Version 2, r = 0.90	Sweden	Malmsten, *et al*, 2003[82]

33°C for 40 h, an alkaline phosphatase-conjugated anti-BrdU antibody and a substrate are sequentially added to achieve colorimetric detection. The colour intensity measured at a wavelength of 405 nm correlates with the RT activity. The RT activity of unknown samples is compared to those obtained from quantification standards, reporting the viral load as fg/RT/ml or as HIV-1 RNA equivalents/ml.

The ExaVir™ RT assay is not affected by the presence of RT inhibitors (drugs) in plasma of HIV patients. Some of the challenges with this assay are requirement of 1 ml of plasma, lengthy testing procedure, and processing limited number of samples per operator per day. The performance of the assay has been documented from different parts of the world including validation at our Centre[59]. However, with improved performance, the recent ExaVir™ version 3.0, has a detection limit of as low as 200 RNA copies/ml, shortened turnaround time (48-72 h) and increased throughput (120 to 180 samples per week per operator). This RT assay was strongly correlated with the Roche Amplicor (r=0.8554), leading to implementation at four district hospital laboratories in Botswana[64]. However, it should be noted that the ExaVir™ RT assay is a functional assay; heavily mutated RT or co-infection with other retroviruses (*e.g.*, HTLV-I) may interfere with its performance.

The PerkinElmer Ultrasensitive p24 assay is also based on ELISA to measure the concentration of HIV-1 p24 antigen, which has been shown to be strongly correlated with standard viral load assay[61]. Prior to the capture of p24 by a specific antibody-coated 96-well plate, specimens are lysed by a special kit buffer and incubated at 100°C for 5 min to release p24 molecules to a maximum degree from HIV-1 virions and from the p24/antibody complex in plasma. The captured p24 antigen is recognized by a biotinylated anti-p24 antibody, followed by the colorimetric detection step using a streptavidin–horseradish peroxidase conjugate. The emitted colour intensity of each sample is compared to intensities emitted from external standards of known concentrations, determining the level of p24. Although the Ultrasensitive p24 assay has shown a good correlation with standard viral load assays in some settings[56,65], discordant results have also been observed in a study from Spain[66]. One study observed that the Ultrasensitive p24 assay detected 66.7 per cent of the specimens with viral load less than 10,000 copies/ml, and 87 per cent of the specimens with viral load of between 10,000 and 100,000 copies/ml[67]. Lombart *et al*[68] has shown that only 27 per cent of the specimens were detected with viral load less than 1,000 copies/ml.

Homebrew RT-qPCR assays target a highly sequence-conserved region within the LTR to maximize the subtype coverage, which are more diverse in resource-limited settings than in developed countries. Rouet *et al*[63] have shown that homebrew RT-qPCR correlates well with the standard assays. Although the test cost of the homebrew assay is significantly reduced compared to commercial viral load assays, it still needs high-cost equipment, laboratory infrastructure and well-trained operators, restricting their use to district laboratories in rural areas.

The use of dried blood spot (DBS) is an interesting alternative to make the shipment of blood samples easier to the testing laboratories for performing HIV viral load test. Several studies have demonstrated that DBS are convenient for viral load measurements[69-72], although the sensitivity range and correlation with plasma values need to be determined for each commercial assay. Considering that the WHO guidelines[73] recommend switching therapy with viral loads above 5,000 copies/ml, the sensitivity obtained with DBS seems to be adequate at this time.

Recently several new approaches have been adopted to reduce the cost of viral load testing and also to ship the sample for viral testing. A new hypothesis was that pooled NAT (mini pools of 5 or 10 samples) could reduce the number of viral load assays needed to screen a population of patients on ART for virologic failure, and this costs lesser than individual viral load testing, which could make virologic monitoring feasible in resource-limited settings[74]. This method was found to be more than 40 per cent more efficient than individual viral load testing while maintaining excellent accuracy in a population with a prevalence of ART failure of 23 per cent[75]. However, this method need wider evaluation in a resource-limited setting before they could potentially be used as low cost method to make it for routine use for virologic monitoring purpose. SampleTanker[76] is another novel dried specimen technology and transport medium developed by Research Think Tank (RTT) for the collection and transportation of biological specimens for HIV-1 plasma RNA quantification and genotypic drug resistance testing in a dry state. This technology has been developed to allow for the biological testing of air-dried samples without the need for refrigeration or frozen shipping and storage. This technology has the potential to significantly reduce the costs of shipping infectious materials worldwide.

Conclusion

Every effort should be made to introduce appropriate laboratory monitoring assays in resource-limited settings, since the benefit of the scaling-up of ART seems to be largely dependent on adequate laboratory monitoring. Sustainability of assays [recognizing issues such as ongoing costs, delivery of reagents and kits, kit shelf-life, access to technical support and external quality assurance schemes (EQAS) programmes] must also be considered at the time of implementation. Importantly, the newer assays should be validated sufficiently to ensure that the results are accurate and reproducible. Further, much simpler tests, based on dip-stick technology or other POC assays, are needed and some are in the process of development or under field evaluation.

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