

Emerging Technologies for Point-of-Care Management of HIV Infection

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Keywords

CD4 cell count, viral load, diagnostics, AIDS, micro- and nanotechnology

Abstract

The global HIV/AIDS pandemic has resulted in 39 million deaths to date, and there are currently more than 35 million people living with HIV worldwide. Prevention, screening, and treatment strategies have led to major progress in addressing this disease globally. Diagnostics is critical for HIV prevention, screening and disease staging, and monitoring antiretroviral therapy (ART). Currently available diagnostic assays, which include polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and western blot (WB), are complex, expensive, and time consuming. These diagnostic technologies are ill suited for use in low- and middle-income countries, where the challenge of the HIV/AIDS pandemic is most severe. Therefore, innovative, inexpensive, disposable, and rapid diagnostic platform technologies are urgently needed. In this review, we discuss challenges associated with HIV management in resource-constrained settings and review the state-of-the-art HIV diagnostic technologies for CD4⁺ T lymphocyte count, viral load measurement, and drug resistance testing.

ASSURED:

affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users

INTRODUCTION

Antiretroviral therapy (ART) has been highly effective in reducing mortality in HIV-infected individuals (1). ART can reduce HIV transmission among people with HIV-serodiscordant status by up to 96% (2). Expanding ART in developing countries has averted ~5.5 million AIDS-related deaths (3, 4). It is estimated that 12.9 million HIV patients in low- and middle-income countries had received ART by the end of 2013, a significant increase from 5 million patients estimated at the end of 2008 (5). The United Nations Member States set a global target of 15 million people receiving ART by 2015, and access to ART continues to expand to achieve this goal. Additionally, the World Health Organization (WHO) guidelines issued in 2013 recommended earlier ART initiation when CD4⁺ T lymphocyte count falls below 500 cells/ μ l as opposed to 350 cells/ μ l as the clinical cut-off. Hence, the updated guidelines will increase the number of individuals eligible for ART by ~9.2 million relative to the number of patients eligible for ART based on the 2010 guidelines (3). The new guidelines also strongly recommend routine and targeted viral load testing to diagnose ART failure.

Mother-to-child transmission (MTCT) remains the primary cause of HIV infection in infants in developing countries (6). Timely HIV detection and ART initiation in HIV-infected mothers can effectively prevent MTCT, but only 65% of pregnant women living with HIV in 21 African priority countries (Angola, Botswana, Burundi, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of the Congo, Ethiopia, Ghana, Kenya, Lesotho, Malawi, Mozambique, Namibia, Nigeria, South Africa, Swaziland, Uganda, United Republic of Tanzania, Zambia, and Zimbabwe) had received ART for preventing MTCT by the end of 2012 (3). Many infants born to women living with HIV are undiagnosed owing to unavailability of nucleic acid amplification tests (7). Routine antibody-based screening tests are not applicable to the diagnosis of infants born to HIV-infected mothers because these infants have high levels of HIV antibodies from their mothers for the first 18 months regardless of their HIV status. Correct diagnosis of HIV infection in infants is of utmost importance to guide early ART initiation and other supportive care. Early treatment in HIV-infected infants may have significant long-term benefits, such as the potential for functional cure whereas unnecessary treatment of uninfected infants may have adverse long-term effects such as potential drug-related toxicity (8).

The expansion of access to ART has given rise to urgent challenges in early diagnosis, timely ART initiation, longitudinal disease monitoring, and MTCT prevention in resource-constrained settings, where laboratory infrastructure and skilled staff are limited (**Figure 1a**). Point-of-care (POC) diagnostics is the most feasible approach in addressing challenges with respect to early HIV diagnosis and ART expansion in the developing world, and the WHO has provided framework criteria for the development of POC diagnostic tools (**Figure 1b**) (9). These criteria dictate that POC tests should be "ASSURED": affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users (9). True POC diagnostic tools do not require sophisticated laboratory infrastructure, expensive reagents, or trained staff to provide test results, and they eliminate the logistical challenge of transporting samples to central laboratories (10).

Dipstick assays such as OraQuick[®] Rapid HIV-1/2 Test that detect HIV antibodies are simple and relatively inexpensive compared to PCR assays, and there are currently several rapid tests in the market approved by the US Food and Drug Administration (FDA) (11). However, dipsticks are qualitative assays with poor sensitivity (11). Further, they cannot detect acute HIV infection in adults prior to seroconversion or identify infants born to HIV-infected mothers (11, 12). Acute HIV-infected individuals may contribute substantially in HIV transmission as up to 50% of new HIV infections occur during the acute infection stage (13).

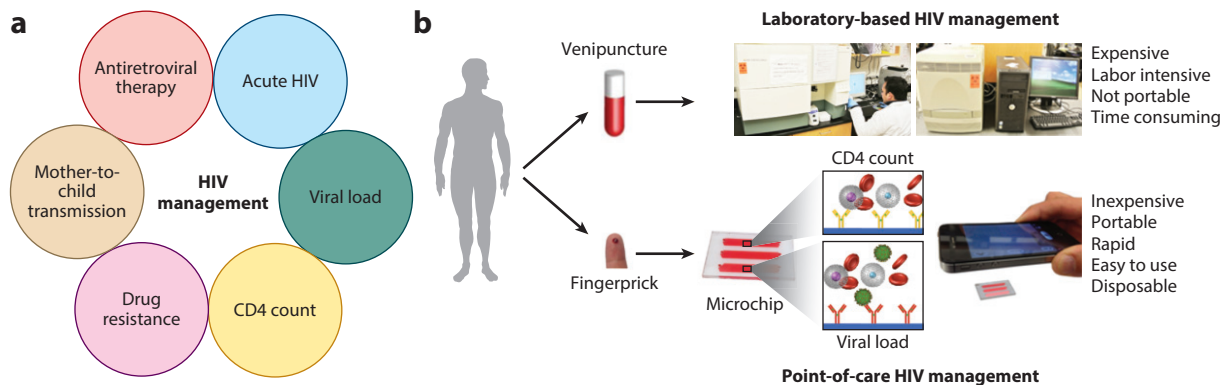


Figure 1

(a) Schematic of major concerns in HIV management and prevention. (b) Novel microchip technologies have a huge impact on developing point-of-care (POC) devices to detect HIV/AIDS and monitor treatment in the developing world.

There are two main approaches to monitor ART effectiveness and HIV/AIDS progression: (a) CD4⁺ T lymphocyte count, which is an indicator for the status of a patient's immune system after HIV infection, and (b) viral load measurement as another indicator of viral replication in plasma (10, 14). In this review, we focus on emerging micro- and nanotechnologies that leverage microfluidics and electrical- and optical-based biosensing modalities for CD4⁺ T lymphocyte count and viral load measurement. Drug resistance testing is also of importance for physicians to initiate effective treatment regimens and for policy makers to monitor the prevalence of drug-resistant subtypes. We also discuss the major challenges associated with HIV early diagnosis, ART monitoring, and POC diagnostic development under the WHO criteria for HIV management in developing countries.

CHALLENGES IN THE MANAGEMENT OF HIV ANTIRETROVIRAL THERAPY

HIV diagnosis is a critical step to provide treatment to HIV-infected individuals and to prevent HIV transmission. A significant proportion of HIV-infected persons are undiagnosed and unaware of their HIV status, especially in developing countries with the highest burden of HIV infection (3). For instance, only 40% of pregnant women and 35% of the infants born to HIV-positive mothers received HIV testing and counseling in developing countries as of the end of 2012. Structural, logistical, operational, and social barriers are the major reasons for such low HIV testing coverage in resource-constrained settings (3). Linking persons diagnosed with HIV infection to care is another challenge. Individuals may not return for their HIV testing results because of logistical difficulties or social stigma. In the first few months after starting ART, mortality in resource-constrained settings is commonly higher than that in high-income countries (15), partially owing to delays in diagnosis and ART initiation (16). Rapid POC HIV testing for viral load measurement and/or CD4⁺ T cell count can facilitate timely initiation of ART and close monitoring of treatment efficacy, which in turn slows disease progression and reduces transmission to others.

Further, poor ART compliance in AIDS patients leads to viral replication and emergence of drug resistance. Therefore, regular viral load testing to effectively monitor ART and detect

virological failure is highly recommended by the WHO. Treatment failure is manifested by increasing levels of viral replication, detected as recurrent plasma viremia, which in turn leads to declining CD4⁺ T lymphocyte count and immunological failure. Timely detection of treatment failure in patients on ART is of great importance to prevent emergence of high-level drug resistance and to avert clinical disease progression. For example, one study reported that routine viral load monitoring reduced drug resistance by 80% (17). In the past, treatment failure has been detected through routine CD4⁺ T lymphocyte count testing or by monitoring for clinical symptoms where CD4⁺ T lymphocyte count testing was not available. CD4⁺ T lymphocyte testing alone, however, is inadequate to detect virological failure (18) because virological failure precedes decrease in CD4⁺ T lymphocyte counts. Routine viral load testing allows earlier detection of treatment failure for timely switching to more effective treatment, and it reduces the chances of unnecessary switches to second- and third-line drug treatments that are more expensive and complex (19). The most recent WHO guidelines specifically recommend routine viral load testing for ART monitoring in developing countries (3, 19). Although routine viral load monitoring guides efficient treatment and prevention, there is currently no commercially available affordable and sensitive POC device with high specificity for viral load testing that meets ASSURED criteria in low- and middle-income countries.

The infrastructure and operational capabilities of healthcare systems are major drivers for the introduction and scale-up of POC testing technologies. POC technologies can be a means for addressing the challenges of timely HIV detection and treatment monitoring in resource-constrained settings. These challenges include shortage of trained staff, low physician density in areas with high prevalence of disease, and the limited gross national income per capita in developing countries (20). Access to basic laboratory infrastructure such as refrigeration and instrumentation in developing countries is severely limited, which dictates a high degree of assay integration from sample collection to result reporting for POC diagnostics. In addition, lack of clean water, unreliable power supply, and poor waste management facilities create major challenges for laboratory operations in developing countries (20). Ideally, POC HIV diagnostic tools should be able to perform without clean water and should function in variable humidity and temperature conditions in the field. Therefore, reagents and biological supplies such as antibodies used in POC tests should be designed to withstand such conditions to ensure high selectivity and sensitivity (10, 21, 22). In the following sections, we discuss the POC HIV diagnostic assays for screening and ART monitoring tools that have been developed in the past decade and discussed in the UNTAID HIV/AIDS Diagnostic Technology Landscape Report (9) (Tables 1 and 2).

POINT-OF-CARE TECHNOLOGIES FOR HIV DIAGNOSTICS AND ART MONITORING

It is critical for a POC HIV test to be automated, with a minimal number of steps requiring manual operations in sample handling, testing, and data interpretation. The instrument for reading the results should be portable, affordable, sensitive, and report results rapidly. Microfluidic systems and lab-on-a-chip technologies that handle small volumes of fluids (<100 μ l) have been utilized to miniaturize diagnostic technologies and reduce the cost per assay by decreasing the amount of expensive reagent used per test (23). In addition, these technologies are advantageous because of rapid sample processing and multiplexing capability to diagnose multiple diseases simultaneously. In the following sections, we present recent advances in HIV/AIDS diagnostics at the POC for CD4⁺ T lymphocyte count and viral load measurement. We also discuss p24 assays, rapid HIV tests, and drug resistance tests, as well as their implications for improving HIV/AIDS patient care in resource-constrained settings.

Table 1 Products on the market or in the pipeline for CD4⁺ T lymphocyte count at the point of care (9, 25, 98–100)

Product	Technology	Assay time (min)	Throughput (per day)	Cost per instrument (\$)	Cost per test (\$)	Sample (whole blood)	Weight (kg)	Power
EMD Millipore®-Muse™ Auto CD4/%CD4 System	Flow cytometry	32–34	16	13,605	272	10 µl	5.94	AC/battery
Partec CyFlow® miniPOC	Flow cytometry	17	250	11,748	3.96	20 µl	6.2	AC/DC/car battery
PointCare NOW	Flow cytometry	8	50	25,000	10	40 µl	12	AC/battery/solar
Alere Pima™ CD4 Test	Image-based	20	20	6,500–12,000	6–12	25 µl	2.54	AC/battery/solar
BD FACSPresto™	Image-based	21–22	50–60	TBD	TBD	20 µl	5	AC/battery
MBio™ Diagnostics CD4 system	Image-based	23	80–100	5,000	6	15 µl	2.5	AC/battery
Daktari™ CD4 Counter	Electrical sensing	14	30–35	8,000	9	16 µl	2.5	AC/battery/solar
Visitec CD4	ELISA	40	120	3,000	5	30 µl	0.39	DC/battery
MyT4™ CD4 Test	Centrifugation	10	40	500	8	100 µl	6.5	AC/DC/battery

Table 2 POC viral load measurement products and assays in the pipeline (9, 40, 42, 43, 47)

Product	Technology	Assay time (min)	Throughput (per day)	Cost per instrument (\$)	Cost per test (\$)	Sample	Weight (kg)	Power
ExaVir™ Load	ELISA	48 hours for 30 tests	60	4,500	12–25	1 ml plasma	NA	AC
Liat™ Analyzer	RT-PCR	30–35	15	25,000	TBD	150 µl plasma or 75 µl fingerstick blood	3.75	AC/battery
Alere q HIV-1/2 Detect	NAT	60	8	TBD	TBD	25 µl whole blood	7.8	AC/battery
Wave 80	NAT	70	40	10,000	20	50 µl whole blood	1.4	Battery/solar
EOSCAPE HIV™ System	NAT	60	32–64	1,000	10	1 ml whole blood/plasma	1	AC/car battery
BART	Isothermal NAT	90–120	42	TBD	TBD	200 µl plasma or 100 µl whole blood	54.5	AC
SAMBA Analyzer	NAT	60	12	8,000	15	100 µl whole blood or plasma	0.9	Battery
Truelab™ Real Time micro PCR system	RT-PCR	60–90	13	12,000	10	150 µl whole blood	TBD	AC/DC/battery
NWGHF Savanna HIV Viral Load Test	PCR	95	397	TBD	TBD	1 ml plasma or 100 µl whole blood	11.3	AC
GeneXpert System	NAT	60	36	5,000	3–5	50–100 µl whole blood	2	AC/DC/battery

Abbreviations: NA, not available; NAT, nucleic acid–based tests; RT-PCR, reverse-transcription polymerase chain reaction; TBD, to be determined.

POINT-OF-CARE CD4⁺ T LYMPHOCYTE COUNTING TECHNOLOGIES

Several methods have been developed for counting CD4⁺ T lymphocytes at the POC, including simplified flow cytometry, electrical sensing, imaging technologies, and centrifugation. Here, we introduce products on the market and in the pipeline for CD4⁺ T lymphocyte counting at the POC. **Table 1** compares CD4⁺ T lymphocyte counting products with respect to throughput, assay time, weight, cost per instrument, cost per test, and sample volume.

Flow Cytometry

Flow cytometry, the current gold standard method for counting CD4⁺ T lymphocytes, uses a light beam focused on a stream of cells and several detectors, including a fluorescence detector, to identify cells of interest in a biological sample through capturing light scatter and fluorescent signals emitted from the fluorescence-labeled cells. Most flow cytometry-based methods utilize antibodies that bind to surface markers on cells, such as CD4, for quantifying CD4⁺ T lymphocytes. Although this technology is accurate and sensitive, currently available flow cytometers in developed-world settings are complex, time consuming, bulky, and expensive, and they require highly skilled operators and routine maintenance that cannot be implemented in resource-constrained settings (10). Therefore, several smaller flow cytometers have been developed to detect and count CD4⁺ T lymphocytes at the POC (**Table 1**). Although these flow cytometry-based POC devices are portable and less complex, with high sensitivity and specificity, they are still relatively expensive, as the minimum equipment cost is \$11,748 (**Table 1**). Their use outside of centralized laboratories in the developing world is further limited by electrical requirements and low throughput (24).

Electrical Sensing

Electrical sensing is a powerful technology that can be used to develop portable CD4⁺ T lymphocyte counters. For example, electrical sensing of cell lysates in a microfluidic device can be effectively used to count CD4⁺ T lymphocytes in a fingerprick blood sample (16 μ l) (**Figure 2a**) (25). Environmental conditions such as temperature and pressure can affect results from this system and necessitate calibration and quality control. In other microfluidic-based approaches, electrical and mechanical signatures of cells including membrane capacitance and cell size were utilized to count CD4⁺ T lymphocytes on-chip (**Figure 2b**) (26, 27). The sensing microelectrodes at the inlet and outlet of a capture chamber count the cells that enter and exit the microfluidic device (27). Cell count is obtained by measuring the difference between the number of cells before and after capturing CD4⁺ T lymphocytes in a microchip. This method has shown a limit of detection of 9 cells/ μ l and has demonstrated a strong correlation with an automated microscope-based cell counting method ($R^2 = 0.997$) for cell concentrations of 100–700 cells/ μ l (27). These electrical sensing methods, however, require lysing red blood cells to reduce cell counting errors due to the higher concentration of erythrocytes compared to lymphocytes.

Image Processing

Various platforms depend on taking bright-field or fluorescent images of captured CD4⁺ T lymphocytes and analyzing the images for cell counting. Microfluidic devices were used to selectively capture and isolate CD4⁺ T lymphocytes from a fingerprick volume of whole blood samples

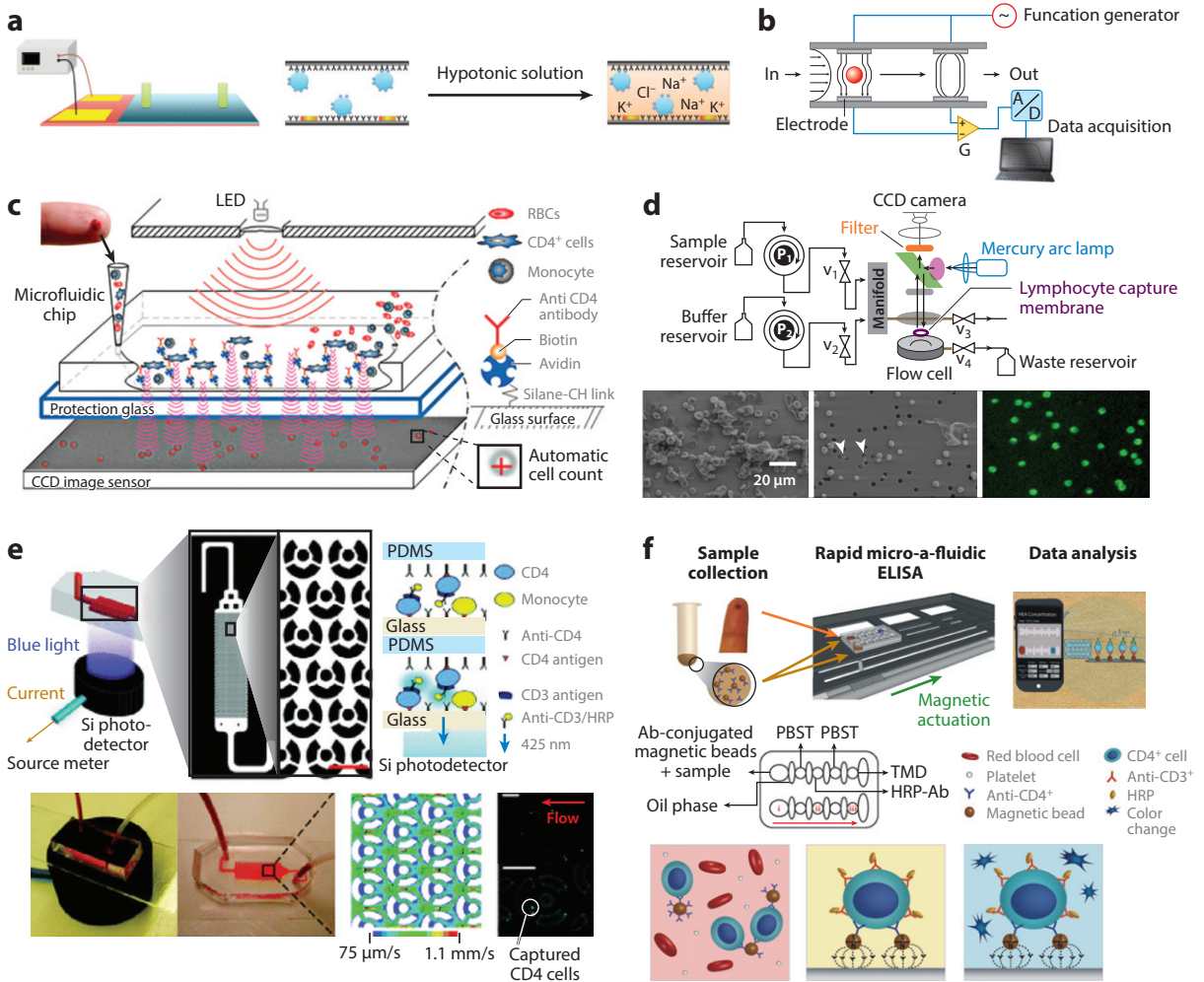


Figure 2

Microchip technologies to detect and quantify CD4⁺ T lymphocytes. (a) Cell detection by impedance spectroscopy of cell lysate on-chip. Reproduced with permission from Reference 25. (b) Parallel electrodes in microchips to detect cells by their physical and electrical properties via impedance measurement. Reproduced with permission from Reference 26. (c) Lensless Ultra wide-field Cell monitoring Array-based Shadow imaging (LUCAS). Reproduced with permission from Reference 29. (d) CD4⁺ T lymphocytes were separated using a filter membrane in a microchip and counted using a CCD (charge-coupled device) camera. Lymphocytes were selectively captured on the filter membrane in a microchip and stained for detection. Arrows show red blood cells passing through the filter membrane. Reproduced with permission from Reference 33. (e) Microfluidic device for capturing and counting CD4⁺ T lymphocytes using a chemiluminescence-based method. Reproduced with permission from Reference 36. (f) Micro-a-fluidic ELISA assay (m-ELISA) to automatically isolate and quantify CD4⁺ T lymphocytes in unprocessed whole blood samples. Abbreviations: HRP, horseradish peroxidase; PBST, phosphate buffered saline plus 0.5% tween; TMB, tetramethylbenzidine.

using either antibodies or filter membranes (28–33). The isolated cells were then detected utilizing lensless cell shadow imaging (Figure 2c) (29–31), quantum dots (32), or fluorescent antibodies (Figure 2d) (33). With the current advances in telecommunication technologies in the developing world, cell phones can facilitate image analysis for counting CD4⁺ T lymphocytes captured and isolated in microchips either near patients or transferred to a central laboratory (34, 35). The

chemiluminescence detection method is another image-based technology that has been used to count captured CD4⁺ T lymphocytes in a small volume (3 μ l) of unprocessed whole blood on a microchip (**Figure 2e**) (36).

ELISA-based and Centrifuge-based Technologies

Enzyme-linked immunosorbent assay (ELISA) is a colorimetric test that employs the capture and detection of antibodies to quantify the concentration of a target bioagent such as molecules or cells in a solution. An ELISA-based semiquantitative test called Visitect[®] CD4 was developed to measure CD4 protein on T cells. This device-free method can visually determine if the finger-prick blood sample has CD4⁺ T lymphocytes above or below a set threshold (e.g., 350 cells/ μ l). This device has shown a sensitivity of 97% for samples with CD4⁺ T lymphocyte count below 350 cells/ μ l and specificity of 80% for samples with CD4⁺ T lymphocyte count above 350 cells/ μ l compared to flow cytometry (9). An automated reader for the device has also been developed that eliminates the need for visual interpretation of the test results.

An automated ELISA-based platform that utilizes a cell phone imaging system was developed to count CD4⁺ T lymphocytes in unprocessed whole blood samples (**Figure 2f**) (37). This platform is referred to as micro-a-fluidic (m-ELISA). m-ELISA eliminates the operational fluid flow in traditional microfluidic ELISA methods by transferring magnetic beads with captured analytes through multiple reservoirs containing ELISA reagents, which are separated by oil on-chip. The process of m-ELISA was automated with the aid of a single-axis motorized stage, and colorimetric readouts were measured using a cell phone-based imaging and analytical approach. This assay demonstrated an accuracy of 97% for CD4⁺ T lymphocyte count among 35 unprocessed whole blood samples using a clinical cutoff of 350 cells/ μ l (37).

The MyT4TM CD4 Test is a centrifuge-based method that integrates a mixer with a spinner to count CD4⁺ T lymphocytes by means of heavy microparticles conjugated with anti-CD4 antibodies. Only the complex of microparticles and captured cells can penetrate into a high-density solution in a microcapillary chamber. The pellet of the beads and cells can be seen using a lens on a microcapillary column (9). The height of the pellet in the capillary microchamber is a function of the CD4⁺ T cell count in the sample that can be seen visually.

POINT-OF-CARE VIRAL LOAD AND PCR-BASED MICROCHIP TECHNOLOGIES

Currently, no commercially available POC viral load measurement device fits the ASSURED requirements in resource-constrained settings. Therefore, POC devices to quantify viral load in biological samples based on the WHO definition of treatment failure (viral load >1,000 copies/ml) (38) as well as the Department of Health and Human Services and AIDS Clinical Trials Group definitions of treatment failure (viral load > 200 copies/ml) (39–41) are urgently needed to effectively and sensitively detect HIV and to monitor ART at the POC. Here, we introduce platforms in the pipeline of development that can potentially measure HIV viral load in biological samples at the POC. **Table 2** compares these platforms with respect to assay time, throughput, cost per machine, cost per test, sample volume per test, and weight of instrument.

ELISA

ELISA principles have been adopted to develop various laboratory-based immunoassays over the past two decades. However, the laboratory-based ELISA assays available in the developed world

RT-qPCR:

reverse-transcription
quantitative
polymerase chain
reaction

are complex and require highly skilled technicians to operate in a clinical laboratory. Therefore, researchers have developed automated and less sophisticated ELISA-based platforms that can be used for viral load measurement in resource-constrained settings. For example, the ExaVirTM assay can detect viral load as low as 200 RNA copies/ml (42) and is less complex than commercial RNA viral load assays with lower demands on laboratory infrastructure. However, this platform has limitations, including the requirement of large sample volume (1 ml), long assay time (48 h), and low throughput (180 per week) (43).

PCR-based Methods and POC Nucleic Acid-based Tests

Reverse-transcription polymerase chain reaction (RT-PCR) has been used in commercial viral load assays, and several attempts have been reported to develop inexpensive POC RT-PCR (44, 45). LiatTM Analyzer is a portable RT-PCR platform that automatically extracts and amplifies HIV RNA from whole blood with a limit of detection of 57 copies/ml for multiple HIV-1 subtypes, including clades A–H and group O, as well as HIV-2 (40). This assay offers sample-to-result automation with a minimal training requirement for the operator. Portable cartridges with a dipstick-based nucleic acid detector have also been developed, which include reagents for HIV-1 RNA amplification using various amplification methods (46, 47). Although nucleic acid tests are highly sensitive for viral load measurement, they are low throughput and require sample preprocessing such as centrifugation and amplification.

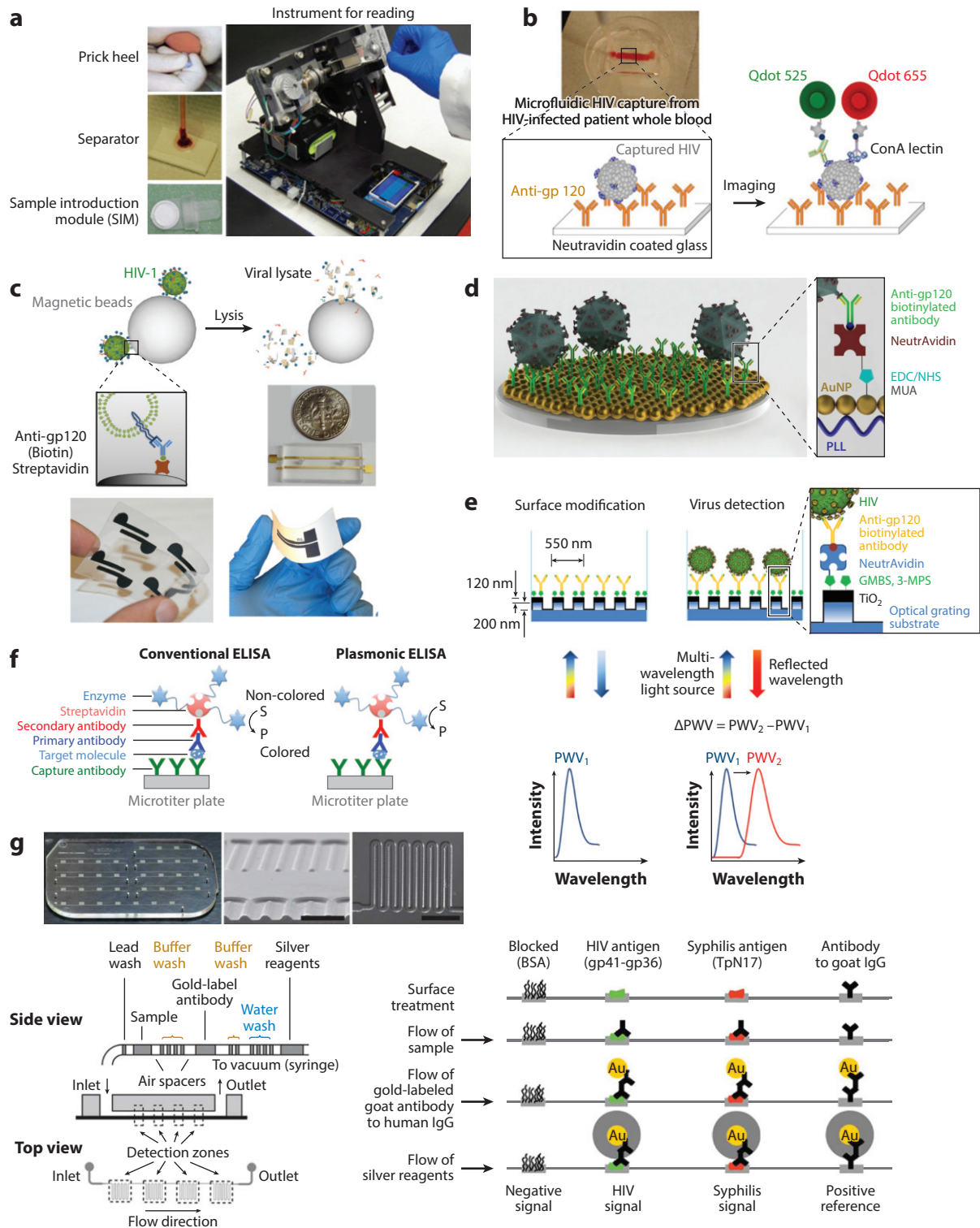
Microfluidics has been utilized to develop an automated and battery-powered miniaturized quantitative PCR chip that requires a small volume of a whole blood sample (100 μ l) (**Figure 3a**) (48, 49). This PCR-based method detects DNA without need for refrigeration. However, this device has a suboptimal limit of detection (5,000 copies/ml), which is much higher than the detection limit of standard RT-qPCR assays (20–50 copies/ μ l). This platform can be potentially used for diagnosis of HIV-infected infants having high viral load (48).

Viral Load Assays Using Intact Virus Capture and Detection

Compared to PCR and other nucleic acid-based viral load assays, intact virus detection methods have the advantage of simplifying sample preparation, which remains challenging for POC testing using nucleic acid amplification-based assays. Also, antibody-antigen complex disassociation is not required in intact HIV detection technologies, as the antibodies against the envelope surface antigens on HIV in AIDS patient samples do not fully neutralize the viruses (50, 51). Several reports

Figure 3

Microchip technologies to detect and quantify viruses and antibodies for HIV detection. (a) Miniaturized PCR to detect HIV-1 DNA using heelprick blood sample. Reproduced with permission from Reference 48. (b) Dual-fluorescent quantum dots (Qdots) in microfluidic devices to detect captured HIV-1 using anti-gp120 antibodies. Reproduced with permission from Reference 53. (c) Electrical sensing of viral lysate on-chip for HIV-1 detection and viral load measurement at the point of care. Reproduced with permission from Reference 54. (d) Nano-plasmonic platform for viral load measurement. Reproduced with permission from Reference 58. (e) Schematic of photonic crystal-based biosensor for intact virus detection and quantification in plasma samples. HIV-1 spiked in plasma samples captured on the surface of functionalized nano-structured photonic crystals, which alters the peak wavelength value (Δ PWV) shift of the reflected light. The Δ PWV is directly correlated with the viral load of the samples. (f) Ultrasensitive detection of p24 biomarker with naked eye utilizing plasmonic ELISA method. Reproduced with permission from Reference 68. (g) ELISA-like-based HIV-1 detection on a microfluidic device. This method specifically detects anti-gp41 and anti-gp36 antibodies using an ELISA method. Reproduced with permission from Reference 76.



have demonstrated the use of unprocessed whole blood samples for capturing and detecting intact HIV in microfluidic devices.

HIV Capture in Microfluidics

Microfluidic-based devices have been developed to selectively capture and isolate intact HIV-1 in biological samples using antibodies against HIV-1 surface envelope epitopes (52–56). The virus capture efficiency and selectivity of these microfluidic platforms have shown great promise for viral load measurement. These methods target intact HIV for detection and eliminate the need for nucleic acid amplification (30, 57). Optical and electrical sensing mechanisms have been developed utilizing quantum dots (**Figure 3b**) (53), impedance spectroscopy (**Figure 3c**) (54), and surface plasmon resonance (**Figure 3d**) (58) for detecting captured HIV-1 in microchips.

Electrical Sensing

Electrical sensing in microfluidic devices is one of the detection strategies used in biosensors (59–63). For example, electrical sensing of viral lysate on-chip offers a reliable and repeatable sensing mechanism for detecting HIV-1 at the early stage when antibody concentration is low and not detectable with the current rapid POC HIV tests such as OraQuick® Rapid HIV-1/2 Test and Unigold (**Figure 3c**) (54). This technology can also be integrated with paper-based microfluidics to create mass-producible, inexpensive, and disposable microchips for HIV-1 detection at the POC (**Figure 3c**). However, this electrical sensing platform needs further development to reach levels of sensitivity required for clinical applications.

Nanoplasmonic Resonance Detection

Nanoplasmonic resonance, the collective oscillation of electrons on metal nanoparticles stimulated by transmission light, has been utilized in diagnostic applications by monitoring changes in plasmonic parameters such as wavelength and extinction intensity (55, 64). Recently, a nanoplasmonic detection platform was developed to capture and quantify multiple HIV subtypes from small volumes of unprocessed whole blood (100 μ l) as well as patient blood samples (58) (**Figure 3d**). This platform offers a repeatable method of viral load measurement with a detection limit as low as \sim 100 copies/ml (HIV-1 subtype D). Although this platform provides sensitive virus detection with no sample pre-processing, the platform requires a portable system for POC applications.

Nanostructured Photonic Crystals

Label-free optical sensing photonic crystal biosensors are periodic arrangements of optical nanostructures that offer sensitive, rapid, and reliable detection of a variety of targets such as cells, proteins, and viruses by monitoring the changes in dielectric permittivity at the interface of biosensing surface and sample (65). A 3D nanostructured photonic crystal biosensing platform was developed to selectively and repeatably capture and quantify HIV-1 in plasma samples (**Figure 3e**) (66). This platform was evaluated using HIV-spiked plasma samples with viral loads ranging from 10^4 to 10^7 copies/ml. This technology can also be integrated with a cell phone imaging system for viral load measurement (34). This assay, however, needs further optimization to enhance the sensitivity to 1,000 copies/ml.

P24 ANTIGEN ASSAYS

P24 antigen appears in the bloodstream at the early stage of HIV infection, when it reaches its peak concentration. The utility of p24 assays for ART monitoring is limited, although advanced ultrasensitive technologies such as gold nanoparticles (**Figure 3f**) (67) and plasmonic ELISA (68) have been developed for p24 detection. The utility of p24 assays needs further investigation and clinical evaluation (69). The technical challenges are the presence of an immune complex, which requires disassociation from p24 antibody, and the varying concentration of p24 antigen in a clinical sample with a given viral load (70).

RAPID TESTS

Rapid diagnostic tests have gained popularity in a variety of venues, such as public clinics (71) and delivery rooms for HIV testing during labor (72) in resource-rich countries. Rapid HIV tests, in particular, are faster, simpler, and more cost-effective than standard RT-qPCR, ELISA, or western blot (73). They all detect antibody rather than viral nucleic acid or virus particles. They are particularly useful in voluntary HIV counseling and disease surveillance in both resource-rich and -poor settings (74). There are currently several rapid POC HIV diagnostic tests with FDA approval claiming sensitivity and specificity equivalent to standard ELISA assays (74). Critical disadvantages of rapid HIV diagnostic tests are (a) their poor sensitivity to detect HIV infection in the acute phase before antibody production or when diagnosing newly infected infants where there are maternal antibodies and (b) they are prone to subjective interpretation of the positive results (11, 12, 75).

Recently, a rapid test referred to as mChip that does not require user interpretation of the output signal was developed. mChip is a microfluidic-based ELISA assay that detects HIV antibody against gp41 and gp36 at the POC (**Figure 3g**) (76). The microchips were fabricated using injection molding as a mass-fabrication technology with a throughput of 1 chip per 40 seconds with material cost of \$0.10 per chip. In this microchip platform, multiple reagents required for ELISA are injected into a tube divided by air spacers. This bubble-based method of reagent handling on-chip enables simple automated reagent delivery to the microchip that requires no moving parts or electricity (76, 77). Unlike common ELISA, which uses enzyme-mediated signal amplification, this assay uses reduction of silver ions on gold nanoparticles for signal amplification. This assay utilizes envelope antigens (gp41-gp36) to capture HIV-specific antibodies. The microchannel design used in this microchip helps convert a signal to a millimeter-sized scale, enabling detection without sophisticated optical instruments. This assay was evaluated in Rwanda using 70 patient samples with known HIV status and showed sensitivity and specificity of 100% and 96%, respectively, which are comparable to the sensitivity and specificity of the current commercially available ELISA tests (76). However, this method cannot be used when direct nucleic acid quantitation or detection is required, such as for ART monitoring or acute HIV detection, respectively.

MANAGEMENT OF DRUG RESISTANCE

Drug resistance testing has become an integral part of HIV clinical management to increase ART efficacy and reduce transmission of drug-resistant variants. Two strategies have been utilized for drug resistance testing: phenotypic assays and genotypic assays (78–80). Phenotypic assays evaluate the drug susceptibility of laboratory-derived viruses containing HIV genes of interest, such as those encoding protease or reverse transcriptase, in the presence or absence of a given drug compared to a laboratory reference strain (81). Genotypic resistance assays are quicker and more affordable owing to advances in DNA sequencing technologies. The latter focus on the detection of specific

genes encoding protease, reverse transcriptase, and integrase with mutations associated with drug resistance (82, 83). Two assays, TRUEGENE HIV-1 Genotyping Kit (Siemens Medical Solutions, Inc., USA, Malvern, PA) and ViroSeq HIV-1 Genotyping System (Celera Diagnostics, Alameda, CA) have been approved by the FDA and are commonly used in developed countries. Although the commercial assays streamline the testing with high reproducibility, the inherent variation in HIV-1 derived from clinical samples adds to the complexity of interpreting test results. Despite the advances in HIV drug resistance testing over the past two decades, phenotypic and genotypic assays cannot be performed outside the central laboratories as they are expensive and require sophisticated infrastructure and trained operators. Although there is no report of POC testing on HIV drug resistance, some emerging biomedical approaches hold the potential of creating POC drug resistance tests. One promising technology is cell-based array, which has been commonly used to screen for drug candidates and test cytotoxicity (84, 85).

FUTURE DIRECTIONS

Technological advances in telecommunications offer a promising opportunity for developing POC HIV diagnostic tests for resource-constrained settings. In the past decade, cell phones have rapidly entered the market in developing countries, with more than 4.8 billion subscribers and 620 million cell phone users in Africa as of 2011 (86). The data-sharing capability of cell phones can be effectively utilized in the development of inexpensive and rapid POC tests for resource-constrained settings (34, 35, 87). Cell phones have sensors and displays built in and are capable of performing powerful computations as well as data storage. They can be further empowered by the addition of simple and inexpensive accessories for biosensing. High-resolution cell-phone cameras have been used to image fluorescently labeled target cells or molecules in biological samples, such as in lateral-flow immunoassays (88), quantum dot-based fluorescence detection (34), and fluorescence microscopy (89). By adding inexpensive and portable optical components, a cell phone can be used as a spectrometer (90). Utilizing cell phones to create biosensing tools will potentially lead to affordable, portable, inexpensive, and easy-to-use biosensing assays that are currently only available using sophisticated, expensive, laboratory-based instruments. Such advances can play a vital role in the development of new categories of biosensors for the detection and treatment monitoring of HIV/AIDS in resource-constrained settings.

The development of paper-based platform technologies represents another promising opportunity for rapid POC tests (91, 92). Paper-based platforms offer several advantages, including sample handling without the need for a fluidic control pump and disposability due to paper's environmental sustainability. Paper-based platforms are inexpensive and can be fabricated using simple and high-throughput printing technologies allowing mass production. Several detection methods have been integrated in paper-based platforms: colorimetric detection (91), electrochemical sensing (93), nanoparticle-based detection (94), electrochemiluminescence (95), chemiluminescence (96), and fluorescence (97). Although paper-based microfluidic technologies are still in an early stage of development, they offer promising approaches to develop printable, affordable, mass-producible, and disposable POC HIV diagnostic tools. Developing paper-based platform technologies to count CD4⁺ T lymphocytes and viruses in whole blood samples at the POC will potentially be an attractive approach toward HIV management in resource-constrained settings.

CONCLUSION

Despite the remarkable breakthrough of ART, HIV control and management remain challenging in resource-constrained settings. The success of expanding access to ART is highly dependent

on simple, rapid, inexpensive, sensitive, and reliable POC diagnostic tools appropriate for the conditions in resource-constrained settings. POC HIV diagnostic assays can facilitate early HIV detection, regular viral load monitoring, CD4⁺ T lymphocyte counting, and drug resistance testing that are essential in effective HIV management in developing countries. Although nucleic acid-based technologies for sensitive and specific viral load measurement are commercially available, the instruments are bulky and costly and are not yet available for POC applications that meet the ASSURED criteria for resource-constrained settings. Rapid lateral-flow tests to detect antibodies are the most commonly used POC HIV diagnostic assays because of their simple testing procedure, inexpensive materials, and short turnaround time. However, these rapid tests cannot be used in early diagnosis of HIV-infected infants or acute HIV infection in adults owing to the presence of maternal antibody or absence of host antibody, respectively. Therefore, there is an urgent need to support technological innovation in the field of POC HIV diagnostics. Innovative and cost-effective POC HIV diagnostic assays may help shift the paradigm in HIV management in developing countries by allowing earlier detection of HIV infection and early ART initiation in both adults and infants, reducing the time between infection and treatment, and providing inexpensive and more convenient ART monitoring. Such POC assays would significantly improve HIV management and mitigate the global HIV pandemic through a sustainable impact on the health care of developing countries.

DISCLOSURE STATEMENT

Dr. Utkan Demirci is a founder of, and has an equity interest in, DxNow, a company that is developing microfluidic and imaging technologies for point-of-care diagnostic solutions. Dr. Demirci's interests were reviewed and managed by the Brigham and Women's Hospital and Partners Health-Care in accordance with their conflict of interest policies.

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Errata

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