# Advances in biosensing strategies for HIV-1 detection, diagnosis, and therapeutic monitoring☆

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## ARTICLE INFO

## ABSTRACT

HIV-1 is a major global epidemic that requires sophisticated clinical management. There have been remarkable efforts to develop new strategies for detecting and treating HIV-1, as it has been challenging to translate them into resource-limited settings. Significant research efforts have been recently devoted to developing point-of-care (POC) diagnostics that can monitor HIV-1 viral load with high sensitivity by leveraging micro- and nano-scale technologies. These POC devices can be applied to monitoring of antiretroviral therapy, during mother-to-child transmission, and identification of latent HIV-1 reservoirs. In this review, we discuss current challenges in HIV-1 diagnosis and therapy in resource-limited settings and present emerging technologies that aim to address these challenges using innovative solutions.

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## Contents

## 1. Introduction

HIV-1 remains a major epidemic despite significant efforts made with early diagnosis, treatment, and prevention of HIV-1. In 2014, there were 36.9 million people living with HIV-1, with 2 million new cases, and 1.2 million deaths worldwide [1]. The overall incidence of HIV-1 has decreased due to various factors including suppressive antiretroviral therapy and needle exchange programs in developed countries [2–4]. Prevalence of HIV-1 increases more slowly each year due to the expanding implementation of (ART), particularly in resource-limited settings [5,6]. As such, there is an increasing need to (i) reduce incidence of HIV-1 infection by strengthening current prevention programs, (ii) detect and diagnose HIV-1 infections as early as possible, and (iii) effectively monitor treatment efficacy, including management of secondary (non-AIDS) HIV-1-related illnesses such as cancer, cardiovascular, liver diseases, and co-infections [7–12]. However, solving these problems is expensive and challenging in resource-limited settings given the large number of HIV-1-infected individuals living in these settings.

In developed countries, nucleic acid testing (NAT)-based viral load and flow cytometry-based CD4 cell counting are routinely used to monitor ART therapy. However, these technologies are complex and costly, making them unsuitable for resource-limited settings, where there is a shortage of laboratory infrastructure and financial support. Although first-line ART drugs may be available for free or inexpensively in resource-limited settings, the expansion and universal access of ART has been significantly thwarted by the lack of appropriate diagnostic tools for detecting HIV-1 infections and initiating and monitoring ART. Creating sensitive diagnostics would allow for early identification of acute HIV-1 infection (AHI), thus helping reduce the transmission rate among high-risk populations. Such tools could also be used to identify HIV-1 infection in pregnant women to reduce the risk of mother-to-child transmission (MTCT) during childbirth; currently a major problem in resource-limited settings [13].

To address this technological gap, researchers have been developing devices for HIV-1-infected individuals in resource-limited settings that are affordable, sensitive, specific, user friendly, robust and rapid, equipment-free, and deliverable (ASSURED) [14]. Microfluidic and nanotechnologies have the potential to fulfill ASSURED criteria, because they require small sample volumes, have short assay turnaround and enable highly sensitive detection. We begin our discussion with recently developed diagnostic devices employing different micro- and nanotechnologies for clinical management of HIV-1 in resource-limited settings. These devices utilize a variety of sensing modalities including electrochemical, optical, and mechanical sensing. We then detail the latest advances in diagnostic tools currently being used for the detection of acute HIV-1 infection, CD4 cell count, viral load measurement, and latent HIV-1 reservoirs. Lastly, we provide future directions on the development of POC devices for improving HIV-1 management in resource-limited settings.

## 2. Recent advances in biosensors and chemical detection systems for HIV-1 detection

Nanotechnology has had a large impact on the field of biosensing by allowing biomedical scientists and engineers to create tools that can directly monitor biological interactions for diagnosis of diseases, including HIV-1. In this section, we introduce emerging technologies, categorized by their transducing method, which are being used to both qualitatively and quantitatively detect HIV-1 via direct (i.e., capture of intact virus), indirect (i.e., capture of virus by host antibodies), or amplification methods (i.e., polymerase chain reaction).

### 2.1. Electrochemical-based assays

Electrical sensing technologies are used to report binding and recognition events occurring on a sensing surface, including protein-antibody, nucleic acid hybridization, and enzyme-cofactor coupling [15–17]. The broad class of sensors has several advantages, including short assay times and ease-of-use. However, there are technical challenges when used as point-of-care diagnostics in resource-limited settings, such as increasing the signal-to-noise ratio and eliminating electrical interference from highly ionic biological backgrounds. In the following section, we will provide a brief overview on different electrochemical sensing modalities and their applications in HIV-1 detection.

#### 2.1.1. Electrical sensing-based platforms

Electrical-based sensors monitor electrochemical reactions, such as enzymatic conversion or capture of biological targets, through an electrode interface by monitoring changes in electrical current, resistance, impedance, or voltage signals [18]. There are different strategies to measure electrical properties on a sensor surface, including amperometric, voltammetric, potentiometric, and impedance measurements.

Amperometric/voltammetric biosensors are mainly used to measure an electrochemical reaction, which is triggered by the generation or perturbation of a redox current. Thus, these biosensors simply record current generated by direct oxidation or reduction of target molecules that are immobilized on an electrode surface [18,19]. For affinity-based sensing strategies, antigen molecules are captured by specific antibodies elements (e.g., antibodies aptamers) on an electrode surface and the resulting electrochemical reaction blocks electron transfer, thus reducing output current. The degree of output reduction provides quantitative measurements of the captured target molecules [20]. For instance, an amperometric sandwich immunoassay was developed for HIV-1 protein detection, where the electrode was modified with anti-p24 antibodies to capture HIV-1 p24 protein, followed by labeling with a horseradish peroxidase secondary antibody [20]. Signal was produced by immersing the electrode in a solution containing hydrogen peroxide and hydroquinone, which are directly catalyzed by the HRP-secondary antibody complex. Results showed a linear dynamic range spanning from 0.01 ng/mL to 100 ng/mL of HIV-1 p24 protein and a detection limit of 0.008 ng/mL was observed, which is two orders of magnitude better compared to conventional ELISA methods (~ 1 ng/mL). Amperometric sensors were also utilized to measure zidovudine (ZDV), a nucleoside analog reverse-transcriptase inhibitor that can be one of many drugs used for ART [21]. The concentrations of ZDV were measured using an amperometric sensor fabricated using silver nanofilm (Ag-NF) and multiwalled carbon nanotubes (MWCNTs) immobilized on glassy carbon electrode (GCE). This amperometric strategy under optimal conditions reported a linear detection range for ZDV concentrations spanning from 0.1 to 400 ppm (0.37 μM–

1.5 mM) with a detection limit of 0.04 ppm (0.15 μM). Further examinations indicated a 98.6% of ZDV recovery from human serum samples. Results from this approach indicated that amperometric methods could be used to monitor HIV-1-drug levels.

Potentiometric biosensors mainly measure potential or charge accumulation on an electrode surface. These biosensors consist of (i) ion-selective electrodes, where the electric potential responds selectively to the concentration of a target ion, and (ii) a reference electrode, which records charge accumulation [18,22]. Using this signal transduction strategy, HIV-1 integrase activity was measured [23]. A series of metal complexes of two ligands ($HL^1$ and $HL^2$) were designed as clinical models to investigate the metal chelating mechanism of integrase inhibitors. To explain a metal speciation model, potentiometric analyses were performed using $HL^2$ with $Mg^{2+}$, $Mn^{2+}$, $Co^{2+}$, and $Zn^{2+}$. Results indicated that metal ions played a pivotal role in HIV-1 integrase inhibition, suggesting that this strategy could be used for inhibitor design and development. Sensitive measures of active integration have the potential to explore potential sources of HIV-1 persistence by detecting the presence of low-level residual viral replication or *de novo* infection in various tissues in the setting of otherwise suppressive ART.

Impedance biosensors monitor electrical impedance of an interface in AC steady state with constant DC bias conditions [24]. This is performed by applying a minute sinusoidal voltage at a specific frequency, while monitoring the produced current response. The current–voltage ratio in this electrochemical impedance spectroscopy (EIS) provides

the impedance signal and allows the precise monitoring of changes in conductivity/resistivity or charging capacity of an electrochemical reaction or interface where bio-chemical interactions occur. A micro-device was fabricated to record impedance changes for analysis of HIV-1 viral nano-lysates [25]. In this study, multiple HIV-1 subtypes were captured using magnetic beads-coated with anti-gp120 antibodies, followed by viral lysis. The nano-lysate samples were then applied to microdevices consisting of a pyrex wafer with two gold microelectrodes. EIS was then recorded from 100 Hz to 1 MHz to evaluate impedance changes between multiple HIV-1 subtypes and control (sample without HIV-1). This device demonstrated that HIV-1 samples produced distinct impedance values compared to controls. HIV-1 samples mixed with Epstein–Barr virus (EBV) were also tested and the results were not significantly different from that of HIV-1, demonstrating the specificity of HIV-1 detection. To demonstrate portability of this device, the same strategy was applied to polyester-based flexible materials (Fig. 1A) [26]. Two silver microelectrodes were printed on a flexible polyester film and integrated into a microfluidic channel. Nano-lysate of HIV-1 samples from plasma and whole blood samples were then applied into the microchannels and the impedance was recorded from 100 Hz to 1 MHz. This flexible impedance biosensor showed its potential as an inexpensive (less than $2 in material cost) and disposable assay to selectively capture and detect HIV-1 from a clinical specimen. In another study, mass-producible and flexible impedance sensors were fabricated using conductive inks [27]. Multiple HIV-1 subtypes, EBV, and Kaposi's sarcoma-associated
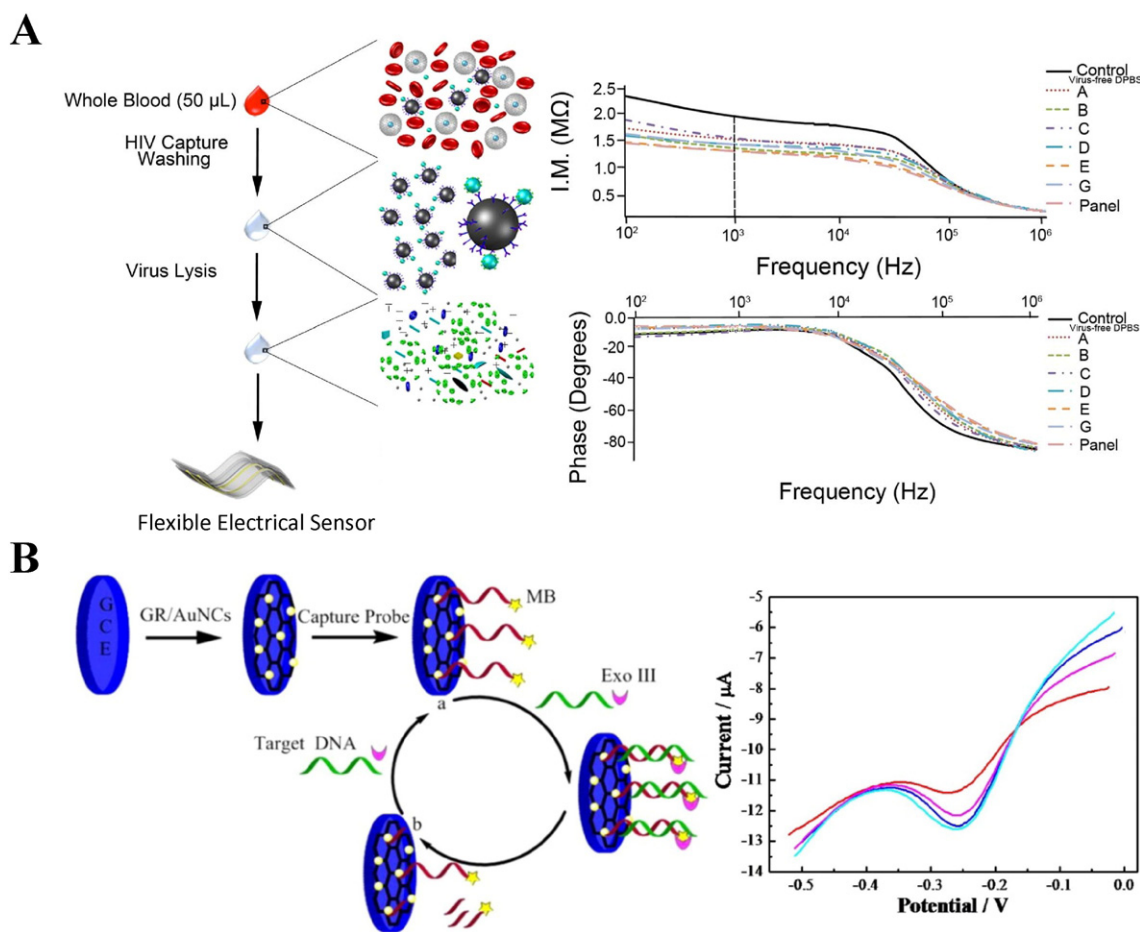


**Fig. 1. Electrochemical assays for HIV-1 detection.** (A) Two-rail microelectrodes were printed on a flexible polyester-based material for detection of HIV-1 nano-lysate. HIV-1 was captured with magnetic particles coated with anti-gp120 antibodies (off-chip), followed signal measurements by electrochemical impedance spectroscopy [26]. (B) A gold nanocluster modified graphene electrode (GR/AuNCs) was fabricated to detect HIV target sequences using an exonuclease III (Exo III)-assisted target recycling amplification method [40]. In this method, the electrode surfaces were modified with capture probes (aptamers) and labeled with methylene blue (MB). During hybridization between target and capture probes, nucleic acids folded themselves into a duplex DNA structure, followed by the digestion of the capture probe by Exo III from its 3′-end, resulting in the release of MB molecules, which were recorded by differential pulse voltammetry technique (A) was adapted with permission from [26]. (B) was adapted with permission from [40]. Copyright (2015) American Chemical Society.

herpes virus (KSHV) were detected from a fingerprick volume (50 μL) of physiological buffer, plasma, and artificial saliva samples. Further developments in reducing the number of sample processing steps will significantly improve these sensors for POC applications.

### 2.1.2. Graphene-based sensors and assays

Graphene material consists of a two-dimensional (2D), single-layer sheet of sp2-hybridized carbon atoms, which form a hexagonal lattice structure [28–30]. From a structural and functional perspective, graphene is a semiconductor material and behaves as a semimetal due to its zero-bandgap [30–32]. This material has a remarkable ambipolar electric-field effect and exhibits a large theoretical surface area (2630 m$^2$/g) with superior electrical conductance (64 mS/cm) [33,34]. In addition, graphene has a low charge-transfer resistance and a rapid electron transfer rate [30,35,36]. Due to these promising physical and electrochemical features, graphene has been broadly used as an electrode material for electrochemical sensing modalities, and been applied into a few clinical biosensing and diagnostic applications [17,37–39]. As a potential diagnostic tool, graphene-based materials have been modified with nucleic acids, aptamers, peptides, and antibodies to measure current and amperometric changes within biochemical reaction such as redox interactions. For instance, a gold nanocluster-modified graphene electrode (GR/AuNCs) was developed to measure HIV-1-originated target sequences using an exonuclease III (Exo III)-assisted target recycling amplification strategy (Fig. 1B) [40]. GR/AuNCs were decorated with capture probes (aptamers), which were then labeled with methylene blue (MB) on 3′-end and cytosine (C)-rich base on 5′-end. During hybridization between target and capture probes, nucleic acids folded themselves into a duplex DNA structure, followed by the digestion of the capture probe by Exo III from its 3′-end, resulting in the release of MB molecules. In this platform, the signal changes were monitored using differential pulse voltammetry technique and detected down to 30 aM of HIV-1 target probe with a dynamic range spanning from 0.1 fM to 100 nM. Using this platform, serum samples were examined and results showed 99.8% of a recovery rate when HIV-1 target probe was tested at 10 fM.

Flexible and wearable materials could be used to perform tasks such as continuous HIV-1 viral load monitoring, which may be useful for determining ART efficacy. Graphene-based sensors have also been decorated on flexible materials for HIV-1 diagnostics [41]. In this work, a paper microchip modified with graphene-silver electrodes was designed to capture and detect HIV-1 lysate on-chip. Geometry of electrodes and graphene notably affected the performance and sensitivity of microchips. First, HIV-1 particles were captured on-chip using streptavidin-anti-gp 120 antibody complex. Then, captured viruses were lysed on-chip and the lysate was measured using impedance/capacitance spectroscopy between 1 Hz and 10 KHz. HIV-1-free controls had different capacitance values compared to HIV-1 lysate samples.

## 2.2. Optical-based assays

Target binding-induced changes in optical signals, such as fluorescence, light absorbance and transmission, or refractive index change, have been widely used to identify or quantify biological interactions. Optical assays are highly sensitive and can potentially be used to detect captured intact HIV-1 particles by eliminating background interference signals. In this section, we will discuss different optical methods developed for detection of HIV-1.

### 2.2.1. Fluorescence-based assays

Fluorescence is the most common optical method used for disease diagnostics. Antibodies conjugated with fluorescent markers provide specificity in tagging proteins or cellular components. The current gold standard for detecting and counting cells is flow cytometry, which uses a laser beam focused on cells in stream to differentially separate and count cells based on their fluorescent marker. Early flow cytometers

were only capable of functioning in centralized labs due to portability and electrical requirements. Traditional flow cytometry is used to characterize cells but can be implemented for viral nucleic acids. In one example, anti-digoxigenin (anti-DIG) and anti-dinitrophenyl (anti-DNP)-coated microparticles were used to capture to PCR end-products. By performing flow cytometric analyses, they were able to detect different HIV-1 subtypes, including the subtypes O and N, with a detection range of 50 to 1 million copies [42].

Recently, new technologies have been developed to fluorescently label and count cells directly, thus eliminating the need for specialized flow cytometer equipment. For example, CD4 cells were labeled with quantum dots, captured on bioactivated PDMS nano-bio chips, and imaged using a portable single wavelength epi-fluorescent microscope (Fig. 2A, B). The nano-bio chips were self-contained and had a waste reservoir and reagent storage on-chip (Fig. 2C). CD4$^+$ T cells were differentiated from other lymphocytes and monocytes (Fig. 2E, F) [43]. CD4$^+$ T cells can also be captured using controlled shear stress in a flow chamber and counted using fluorescently labeled antibodies [44]. This method was further improved by depleting monocytes to capture CD4$^+$ T cell at lower concentrations [45]. Another novel approach to assess HIV-1 infection and status was to count multinucleated giant cells based on induction on synctia—multi-nucleate enlarged cells formed by infected cells [46]. After infection of cell lines MT2 and SUPT1 with syncytium-inducing (SI) HIV-1 isolates, DNA staining was performed with propidium iodide and cell sizes were evaluated using phase contrast (Fig. 2F) and fluorescent microscopy (Fig. 2G–I). Results indicated that SI cells (Fig. 2I) could be distinguished from non-SI (Fig. 2G, H), suggesting that this method could be useful for assessment of HIV-1 inhibition by different antiviral therapies. However, HIV-1 that uses CCR5 (chemokine (C-C motif) receptor 5 (gene/pseudogene)) for entry tend not to produce syncycium in the above laboratory cell lines, which are the most common coreceptor-using strains isolated from patients during acute and early infection [47–49]. This assay is thus limited to HIV-1 that uses CXCR4 (C-X-C chemokine receptor type 4). Most recently, technology using HIV-1-specific transcription-mediated amplification (TMA) to cross-link probes with fluorophores onto cells to flow, characterize, and sort RNA or DNA producing cells—as infected cells are as low as one in a million. While this technique is currently expensive and not yet high throughput, it represents the current state-of-the-art for flow-based nucleic acid technologies [50].

### 2.2.2. Lens-free imaging platform for HIV-1 detection at the POC

Light microscopes are widely employed in the imaging of biomolecules and cells in clinical and research environments. However, they are bulky and costly, and thus have limited availability in resource-limited settings. Recently, a portable lens-free shadow imaging system was introduced, which incorporated a light source and an image sensor (such as CCD or CMOS) for the imaging of cells [44,51–56]. Briefly, LED light shining on a sample diffracts, creating a pattern on the surface of a sensor located below the sample. A software program deconvolves these diffraction patterns and reconstructs a "shadow" image of the actual sample. Since there is no lens, a wide field-of-view can be imaged, which is two orders magnitude greater than that of a regular light microscope. The entire platform is portable, rapid, and more practical than lens-based technologies and has recently been integrated with a smartphone [57,58]. This device has been used to accurately enumerate CD4$^+$ cells in patients recently infected with HIV-1 in resource-limited settings [51]. CD4$^+$ T cells were captured in a microfluidic channel using antibodies decorated on glass surfaces (Fig. 3A). Captured cells were then imaged with a lens-free shadow imaging system, and total cell number was calculated using computer-aided software in under one minute (Fig. 3B). The platform was demonstrated to work with HIV-1-positive blood samples collected from patients in Tanzania, and provided comparable results with conventional cell counting devices and microscopes (Fig. 3C).
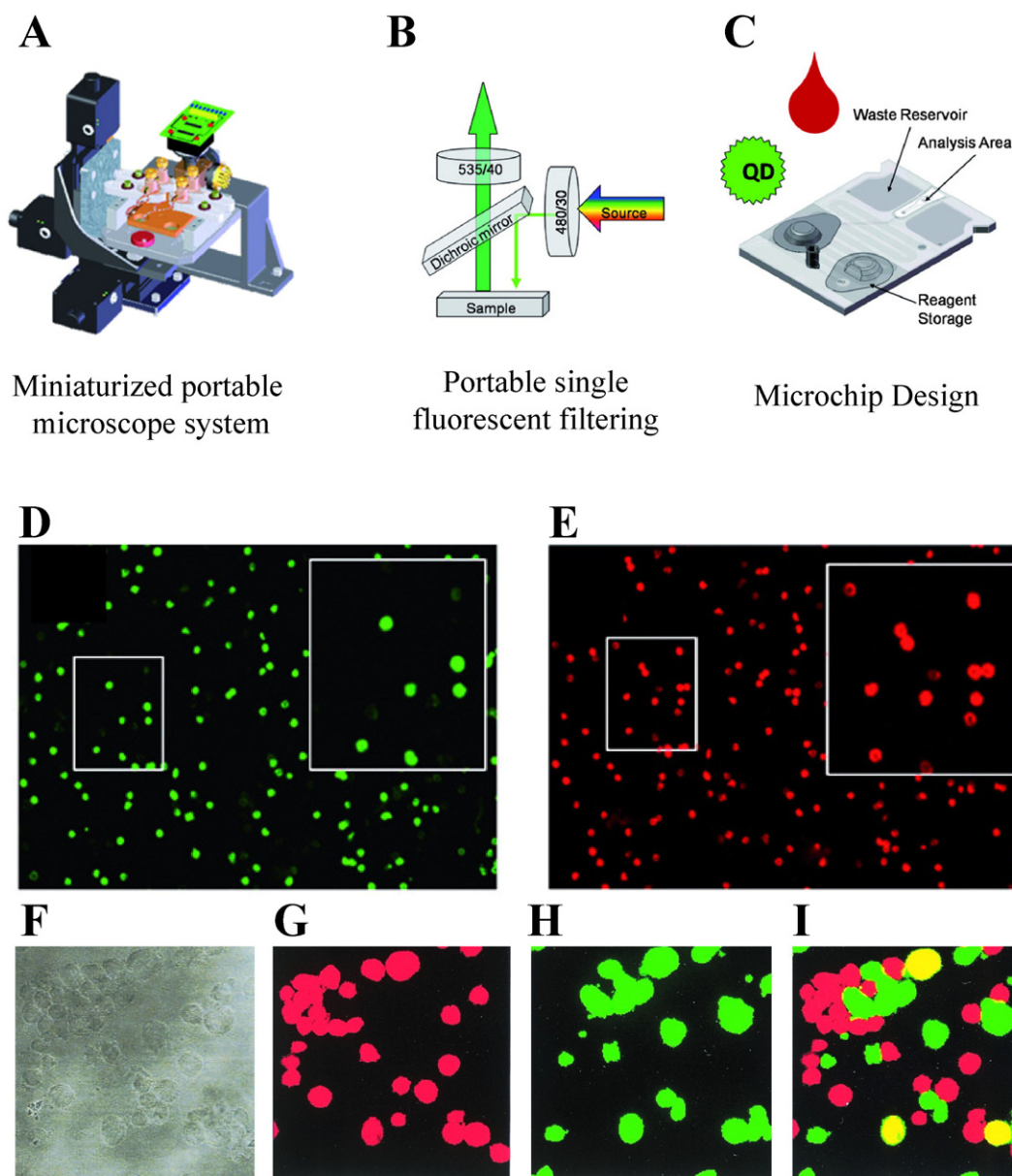
**Fig. 2. Integration of fluorescent microscopy into biochips for CD4 T-cell enumeration.** (A, B) A benchtop single wavelength epifluorescence microscope was used with nano-bio chips to count CD4 T cells [43]. (C) The design of the nano-bio chips included on-chip waste reservoir and reagent storage. Fluorescent images were taken of CD-specific antibody-labeled whole blood using green and red quantum dots as secondary labels to identify monocytes and T lymphocytes (D) and CD4$^+$ cells (E). A fluorescence-based method was developed to detect HIV-1 that induce syncytia [46]. HIV-1-infected MT2 cells were labeled with red or green fluorescent dye. Panel (F) shows stained cells from phase contrast microscopy. HIV-1-induced fusion between cells could be detected as yellow-stained syncytia (I) from a background of cells labeled with red (G) and green (H) fluorescence. (A–E) Reproduced in part from [43] with permission of Royal Society of Chemistry. (F–I) Reproduced in part from [46] with permission from the American Society for Microbiology.

## 2.2.3. Nanoplasmonic assays for HIV-1 at the POC

Nanoplasmonic detection systems have been recently developed to quantify HIV-1 and subtypes from unprocessed whole blood from patients. Nanoplasmonic systems measure the collective oscillation of electrons on metal nanoparticles by monitoring optical resonance. In plasmonics, light is trapped in between metal structures inside nanometric volumes, which leads to enhancement in near-field signals. Evanescent waves bounded by metal dielectric propagate surface plasmons, which are caused by conduction electrons oscillating in metals. There are two commonly used optical biosensors that exploit plasmonic behavior: surface plasmon resonance (SPR) based on a planar, thin film gold surface and localized surface plasmon resonance (LSPR) based on confined gold nanoparticles. Both LSPR and SPR are sensitive to the local refractive index changes when target binds to the film or nanoparticles, and are capable of detecting single molecules [59]. Binding events

of molecules on metallic nanoparticles cause changes in the absorbance spectrum, which can be monitored before and after sample incubation to determine the presence of target. LSPR provides broad spectral measurements with a higher sensitivity and signal-to-noise ratio than conventional SPR [60]. In a recent approach, plasmonic nanoprobes were used to detect the gag sequence HIV-1 based on hybridization between complementary target and probe DNA sequences [61]. Another recently developed platform detected clinically relevant concentrations of HIV-1 with a sensitivity as low as ~100 copies/ml across multiple HIV-1 subtypes (A, B, C, D, E, G, and subtype panel) [62]. In this method, surfaces were modified in several layers for immobilization of antibodies to capture HIV-1, consisting of a poly-L-lysine (PLL) layer, monolayer of gold, and antibody specific to the target. This platform has been validated with different infectious diseases, and has recently been developed as a portable device [63], showing its potential in resource-limited settings.
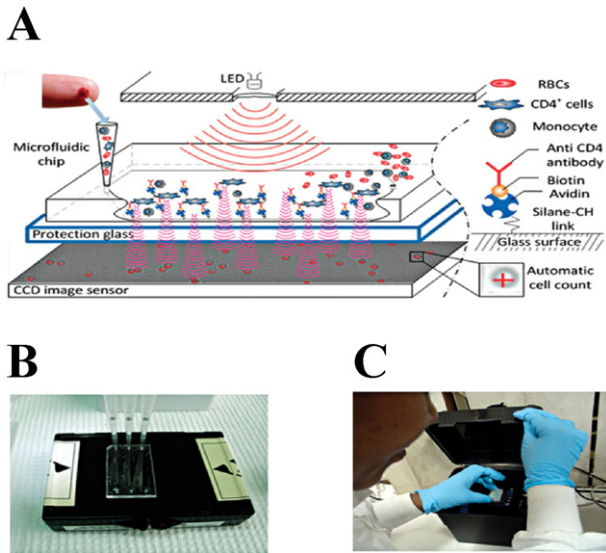
**Fig. 3. Lens-free shadow imaging system for HIV-1 diagnosis.** (A) General mechanism of lens-free shadow imaging system. Light from an LED passes through a pinhole and shines on a microfluidic channel in which CD4+ T-cells were captured. The light forms a diffraction pattern on a CCD surface. These holographic patterns reconstructed by software and cells were identified and counted. (B) Inset indicates the actual image of CCD sensor. (C) Filling the microfluidic channels with patient sample blood. (D) Portable system in actual trials in Tanzania for HIV-1 patients. Images were reproduced with permission from [51].

### 2.2.4. Photonic crystal based assays for HIV-1 at POC

Photonic crystals consist of periodic arrangement of dielectric materials that selectively reflect a tunable wavelength (resonance wavelength) when light is incident on a surface [64]. A biomolecular interaction on the PC surface produces a shift in resonance wavelength that correlates with the concentration of a target analyte [65]. PC-based biosensors have been employed for the detection of a wide array of biomolecules including proteins, cells, nucleic materials, and pathogens [66–70], such as rotavirus (Fig. 4E, F), human influenza virus (H1N1) (Fig. 4C, D), and *Legionella pneumophila* bacteria [67,71–74]. Recently, a TiO$_2$-coated one dimensional (1D) PC biosensor was utilized for capturing and quantifying HIV-1 viral load from serum, suggesting that such a device could be used to detect the virus at low concentrations [75]. In this study, a PC surface was functionalized with anti-gp120 antibodies, and used to specifically capture HIV-1 with a limit of detection in the range of $10^4$ copies/mL to $10^8$ copies/mL in serum (Fig. 4A, B). PCs have also been shown to detect a single nanoparticle [76]. Although PC biosensors hold great promise as reliable and sensitive diagnostic tools, they require expensive optical read-out elements and have yet to be made portable. Smartphone incorporated PC systems have been recently developed in an effort to create a POC device aimed for application in resource-limited settings [77,78]. Recently, there have also been efforts to reduce the size of a PC system, such as using PCs integrated within a microfluidic channel to detect biotargets [79]. These recent studies suggest that both smartphone integration and microfluidics with PCs may enable the creation of portable optical biosensors at the POC for detection of HIV-1 and other infectious diseases in developing world.
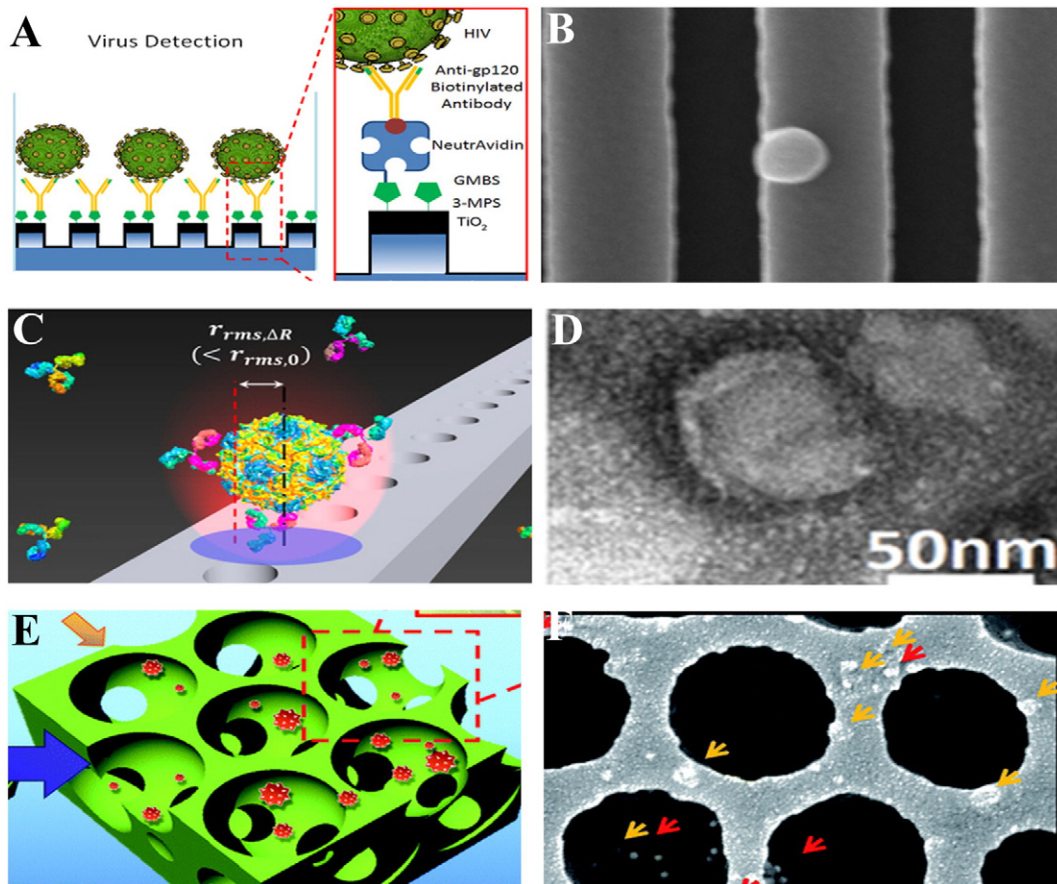


**Fig. 4. Photonic crystal (PC) biosensors for detection of HIV-1 and other viruses.** (A) General overview of one dimensional PC structure and the surface functionalization for HIV-1 capturing using anti-gp120 antibody. (B) SEM image of HIV-1 on the PC surface. (C) 2D holes fabricated on a beam for capturing influenza virus. (D) TEM image of influenza virus. (E) Schematic of inverse opals for capturing virus. (F) SEM image of captured rotaviruses. (A,B) Adapted from [75] with permission from Macmillan Publishers Ltd. Copyright (2014). (C,D) Reprinted from [80] with permission from Nature Publishing. Copyright (2015). (E,F) Reproduced from [74] with permission of The Royal Society of Chemistry.
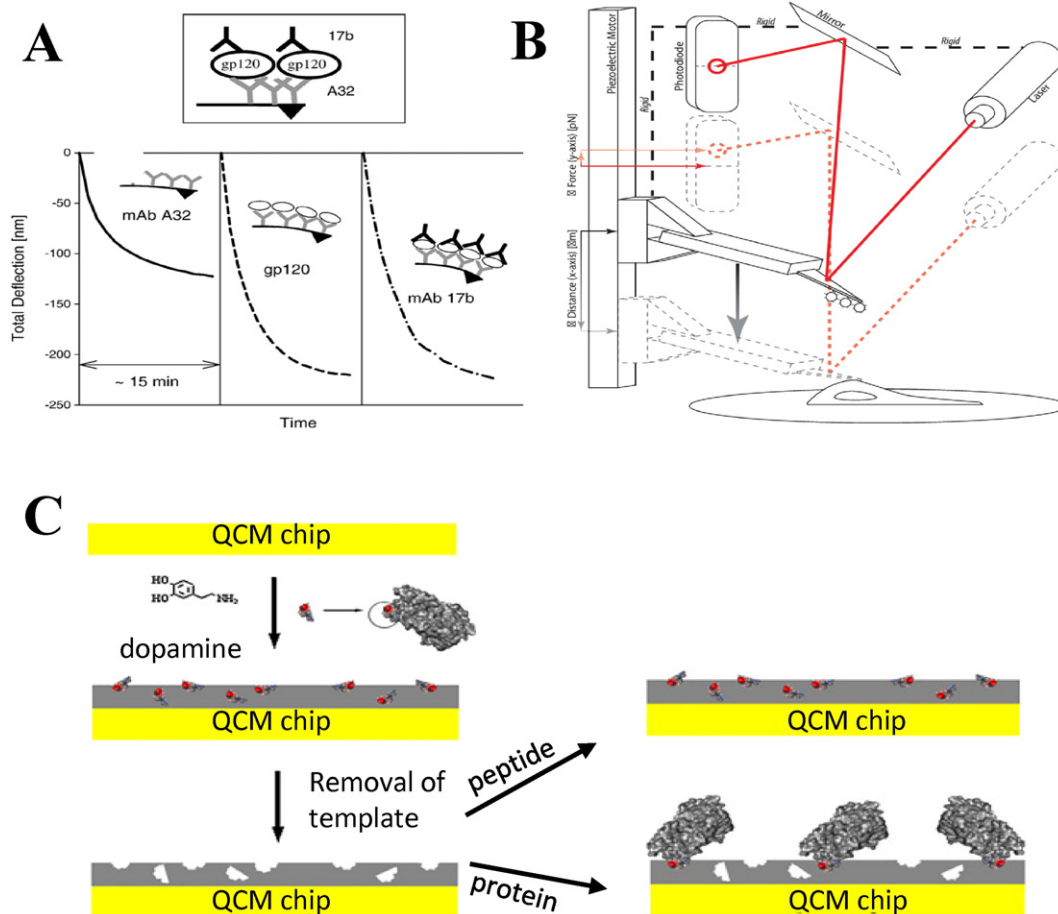
## 2.3. Mechanical assays

Measuring mechanical cues generated on the cellular scale, such as displacement and mass changes, provides fundamental information about biological systems at the micro- and nanoscale including adhesion, transport, and binding affinity parameters of biochemical reactions and cellular processes [81,82]. Mechanical sensors have been broadly used in binding and biosensing studies, including measuring antigen–antibody interactions, monitoring drug binding, and measuring virus-cell surface and virus-antibody interactions. In the following section we present various mechanical sensing strategies using cantilever-based and quartz crystal microbalance (QCM) based sensors with applications for detecting HIV-1 and related diseases.

### 2.3.1. Cantilever-based tests

A microcantilever beam is an element that mechanically senses the presence of target molecules by measuring its deflection or dynamic frequency resonance on a micrometer sized suspended beam. When a biomolecule binds to a cantilever via electrostatic repulsion/attraction, hydration effects, entropic effects, or steric interactions, a deflection of the cantilever is measured by using either a reflecting laser beam or electrical readout [83–87]. A dynamic cantilever mode can also be used to determine the frequency response of the cantilever [88,89]. When a biomolecule binds to the surface, the resonant frequency is altered. One of the key benefits to using a driven cantilever is that they can be used in various environments (e.g., humid, fluid, and vacuum environments) and in different modes (e.g., suspended microchannel resonator) [81,90].

In HIV-1 detection strategies, cantilever-based arrays and sensors are mainly decorated with specific antibodies that capture HIV-1 antigens. For instance, a cantilever-based modality was designed to detect HIV-1 envelope glycoprotein (Env) gp120 from physiological buffers [91]. In this work, a cantilever surface was decorated with monoclonal anti-A32 antibodies (primary antibody) via self-assembled monolayers to capture gp120 proteins, followed by capture of a secondary antibody (monoclonal anti-17b antibodies) (Fig. 5A). In each stage, cumulative deflections were observed. In this study, the limit of detection was observed to be 400 ng/mL. Although this detection limit is the range for in vitro cultures, it is still unsatisfactory for monitoring gp120 in vivo validation (0.2–100 ng/mL) [92,93]. Such a strategy would work best for capturing free circulating virus, as cells express very low amounts of gp120 on their surface. In another study, membrane receptors were immobilized onto nanomechanical cantilevers without requiring passivation of the underlying surface [94]. By using equilibrium theory, quantitative mechanical responses were recorded for vancomycin, HIV-1 antigens, and coagulation factor VIII captured on the sensor surface. A cantilever was then used for HIV-1 experiments, where an N-terminal fragment (VHH) of llama single chain antibodies (~15 kDa)



Fig. 5. Emerging technologies incorporating mechanical assays. (A) A microcantilever was demonstrated to detect HIV-1 gp120 protein using a sandwich assay with anti-A32 and anti-17b antibodies. In this study, deflection versus time curves were collected for each step [91]. (B) A single-molecule force spectroscope (SMFS) capable of single-molecule resolution was developed to determine viral adhesion and fusion events to a cell surface. The cantilever surface was functionalized with whole viruses to contact with cell surfaces expressing viral surface receptors. Displacement values of the cantilever were recorded to monitor molecular interactions [97]. (C) A quartz crystal microbalance surface was decorated with molecularly imprinting polymer to mimic biomolecular confirmation of gp41 epitope of HIV-1. In this study, dopamine was used as the functional monomer, having analog residues with HIV-1 transmembrane protein (gp41). Then, gp41 proteins were applied to these sensor surfaces and captured on the grafted surfaces [104]. (A) Reprinted from [91], Copyright (2006), with permission from Elsevier. (B) Adapted from [97] with permission from Macmillan Publishers Ltd. Copyright (2015). (C) Reprinted from [104], Copyright (2012), with permission from Elsevier.

against HIV-1 trimeric envelope glycoprotein (gp140) was immobilized on the cantilever surface, which had been previous shown to have high sensitivity and specificity against gp140 protein [95]. A response was observed after 80%–90% of the surface was coated with gp140. Results demonstrated a dependence between molecular footprint of the target and the induced stress. Further experiments were performed to detect gp140 antigens from HIV-1 B/C subtypes, with a limit of detection down to 500 fM, which is orders of magnitude lower than the previous study [96]. Cantilever sensors can also be used to differentiate between fusion and binding events of viral particles and living cells. For example, a single-molecule force spectroscope (SMFS) was employed to monitor the strength and lifetime of molecular bonds with a single-molecule resolution [97]. In this study, viruses were first immobilized on a cantilever surface of an atomic force microscope (AFM) and then operated in contact mode with a living cell (Fig. 5B) Force–time curves were collected to distinguish between adhesion and fusion when viruses interacted with cell surfaces. Results indicated that cantilever-based sensors could be potentially used for monitoring small molecular interactions by small deflections (pN/s levels).

Overall, cantilever sensor-based approaches can provide new insights of cellular mechanisms by monitoring mechanical responses to biochemical interactions and also be potentially used to detect ultralow concentrations of biomarkers, making these sensors and assays an ideal candidate for use in the POC diagnostic settings [97,98].

### 2.3.2. QCM-based tests

Quartz crystal microbalance (QCM)-based sensors are centimeter-sized mechanical sensors that contain a piezoelectric material coated with a metal film, such as gold or silver films. QCM sensors are used to measure masses of small analytes in fluid, vacuum, or gas environments by real-time monitoring the changes in oscillation frequency [99–102]. These sensors are commercially available and can be purchased with temperature and oscillation modules that enable detection of sub-nanogram mass loads [103]. QCM surfaces are often modified through self-assembled monolayer, polymeric materials, or molecular imprinting approaches. A molecular imprinting polymer was applied in QCM sensing platforms to mimic biomolecular confirmation of gp41 epitope of HIV-1 [104] (Fig. 5C). In this study, dopamine was utilized as functional monomer with 35 amino acid residues, which were analogs to residues 579–613 of HIV-1 transmembrane protein (gp41) due to its high abundance in AIDS patients (98%). Thus, a grafted MIP surface was designed to capture gp41 proteins. Kinematic parameters for this system indicated a binding strength of 3.17 nM, which is similar to using monoclonal antibodies. A detection limit was observed to be 2 ng/mL, which was comparable to ELISA. The platform was further validated with gp41 in human urine samples, and between 86.5% and 94.1% recovery rate was observed.

## 2.4. Miniaturizing conventional assays

The need for diagnostic devices to detect and monitor HIV-1 viral load, CD4$^+$ T cell number, and therapeutic monitoring before and during antiretroviral therapy (ART) in resource-limited settings has not been yet been satisfied. Although many testing platforms exist in the developed world, which have a low unit price per test for a system, they still require centralized laboratories and specialized devices, which makes them inadequate as ASSURED POC tools in resource-limited settings. The focus of this section is to discuss the recent research efforts toward miniaturizing conventional assays by incorporating microfluidics and nanotechnologies to reduce the size and complexity of many commercially successful assays and adapting them in resource-limited settings applications.

### 2.4.1. ELISA incorporating microfluidics

Enzyme-linked immunosorbent assays (ELISA) are colorimetric and fluorometric based tests that are composed of an antibody/antigen interaction detected via a secondary reporter conjugated to an antibody (i.e., horseradish peroxidase, fluorescent molecules, quantum dots). ELISA tests have had remarkable success with detecting p24 antigens as shown by a recent publication, which used an inexpensive approach to achieve an attomolar limit of detection [105]. ELISA-based tests have also been used to detect T cell CD4 proteins collected from a fingerpick blood sample, and have a 97% sensitivity when the CD4$^+$ T lymphocyte count is as low as 350 cells/μL, suggesting that it could be used to quantify response to ART in resource-limited settings. Visitect® CD4 is a commercially available semi-quantitative POC test for the enumeration of CD4$^+$ T lymphocytes in whole blood within 40 min using an automated reader that eliminates visual, potentially biased interpretations [106]. Another recent technology is an automated cell phone-based imaging system called a micro-a-fluidic ELISA (m-ELISA) [107]. This was developed to detect and quantify CD4$^+$ T lymphocytes from unprocessed whole blood using magnetic beads to capture analytes from multi-channel reservoirs. The method has an accuracy of 97% for capturing and quantifying CD4$^+$ T lymphocyte from 35 blood samples with a cutoff of 350 cells/μL. A platform, named MyT4, was also developed for the detection of CD4$^+$ cells, where microcapillary columns were used to collect microparticles conjugated with CD4$^+$ antibodies and CD4$^+$ T lymphocyte cells. After centrifuging the particles and cells, the height of the pellet was measured for the quantification of CD4$^+$ T cells. This technique had a sensitivity of 95% and specificity of 87% at the threshold of 350 cells/μL [108]. There is also a microfluidics-based mChip technology (mobile microfluidic chip for immunoassay on protein markers) that has been used to detect HIV-1 and only requires 1 μl of unprocessed whole blood [109]. Another ELISA-based assay has been recently developed which combines microfluidics with a smartphone to multiplex immunoassays in a single test obtained within 15 min with a sensitivity of 92% and specificity of 79% [110].

### 2.4.2. Microfluidic-PCR assays

Reverse-transcription polymerase chain reaction (RT-PCR) is a commonly employed viral load assay. Several research groups have attempted to lower the cost of RT-PCR, so that it can be made portable for resource-limited settings [111,112]. One of these technologies is a Liat™ analyzer, which extracts and amplifies HIV-1 RNA from whole blood with a detection limit of 57 copies/mL. The system is user-friendly, automated, and requires minimum operator involvement, which makes this technology ideal for resource-limited settings [113]. Nucleic acid-based tests are sensitive, but require multiple reactions during sample processing, thereby lowering its throughput. Portable cartridges have been developed with the use of a dipstick-based nucleic acid detector for HIV-1 RNA detection with a sensitivity of 75% and a detection limit of 50 copies of HIV-1 RNA [114]. Another recent portable PCR approach has been developed to integrate microfluidics with a battery-powered quantitative PCR platform. Results using this integrated device have shown a limit of detection of 5 copies/μL, which is superior to the current gold standard RT-qPCR method, with a detection limit of 20 copies/μL [115,116]. In an effort to lower the equipment cost and size for resource-limited settings, it may be beneficial to use super helicases, which are faster, more efficient and can replace the automated heating and cooling steps [117].

### 2.4.3. Digital droplet-based PCR

Instead of relying on large volumes for PCR, microfluidic channels can be used to create small droplets that encapsulate cells or genetic material to quickly analyze nucleic acids in less than a minute, referred to as digital droplet-based PCR (ddPCR) [118]. Due to high sensitivity, ddPCR has been used to measure the number of CD4$^+$ T lymphocytes or peripheral blood mononuclear cell (PBMC)-associated genomic HIV-1 DNA or PBMCs and episomal HIV-1 DNA in these cells. With this technology, cellular DNA extracts are portioned into picoliters in a single copy level in thousands of droplets, and these droplets are then individually amplified. If a droplet contains HIV-1 DNA, it will generate

fluorescent signals and is deemed positive. The number of positive droplets is enumerated to determine the copies of HIV-1 DNA. HIV-1 DNA and 2-LTR was successfully detected from patients with suppressive ART from PBMCs using a ddPCR system from Bio-Rad [118]. Another study subsequently showed that ddPCR had improved precision for quantification of HIV-1 pol gene and 2-LTR by 5-fold and 20-fold, respectively [119]. Other potential advantages of ddPCR methods include the minimization of the detrimental effects of primer or probe-sequence mismatches and efficiency on quantitation, and the potential to reduce PCR inhibitors by partitioning assays into micro- or nanoscale reaction droplets.

### 2.4.4. Paper microfluidics

Paper-based detection systems are one of the earliest devices developed for resource-limited settings. Paper is abundant, it can be mass-produced and incinerated when disposed, reducing the cost per test. Applications for paper-based technologies were first developed as cheap pregnancy tests, but later developed into more sophisticated devices such as anti-HIV-1 antibody [120] and HIV-1 p24 antigen tests [121]. Paper has also been used for a broad range of biofluids in both home and clinical settings. Recent studies have focused on lateral flow assays that give quantitative, rather than qualitative "yes/no" results [120]. Microfluidic paper-based analytical devices (μPads) use different patterning technologies depending on their application [122,123]. They are compatible with optical, electrochemical, chemiluminescence, and microelectromechanical methods, and use small sample volumes and are easier to read compared to lateral flow immunoassays [124]. A magnetic immuno-chromatographic test (MICT) method was implemented for the detection of HIV-1 p24 within a 40 min window. This system had a limit of detection of 30 pg/mL for spiked HIV-1 p24 antigen in buffer and spiked into 50% plasma [121]. Lateral flow assay materials and lyophilized enzymes were employed on paper that could amplify and detect 10 template copies of HIV-1 DNA in 15 min [120]. The device

performed polymerase recombinase amplification using a paper-based plastic layer, acetate layer, two small pads for soaking the "master mix" and magnesium acetate buffer, and a sample wicking pad (Fig. 6A). A rectangular window was prepared on the paper, which covered the plastic base for enzyme pellet (Fig. 6B). A master mix pad placed on to enzyme pellet and a sample wicking strip were placed on the right side of the device (Fig. 6C). The protective paper was peeled from all surfaces (Fig. 6D). Acetate layer was placed on the right-hand side of the device covering sample wicking strip and master mix pad. (Fig. 6E). Magnesium acetate pad was placed on the window prepared on the acetate layer (Fig. 6F). Master mix and magnesium acetate were soaked to the membranes (Fig. 6G-H). Sample was introduced to the sample wicking strip (Fig. 6I). Sample wicking strip was folded to match the strip and master mix pad (Fig. 6J). The left-hand side of the device were folded from the midline to match magnesium acetate pad to the other components as enzyme pellet, master mix and sample (Fig. 6K). Results of the amplification reaction were generated on paper substrates, and had a limit of detection of 10 template copies of HIV-1 DNA (Fig. 6L). Paper-based assays are one of the best-studied detection platforms for resource-limited settings because of their low cost components and well-established production platforms.

### 2.5. Technology comparison

Below, Table 1 compares a select set of technologies and platforms discussed in the previous subsections. A comparison was made between detection modality (type), relevant HIV-1 detection target, target matrix, and our estimated cost of the method including disposable instruments where applicable. We have also listed some advantages and disadvantages to each technology to give the reader an idea of the current state of the art. Much of the information that is included was taken directly from the references. However, estimated cost was determined based on our own evaluation of the Methods sections from within
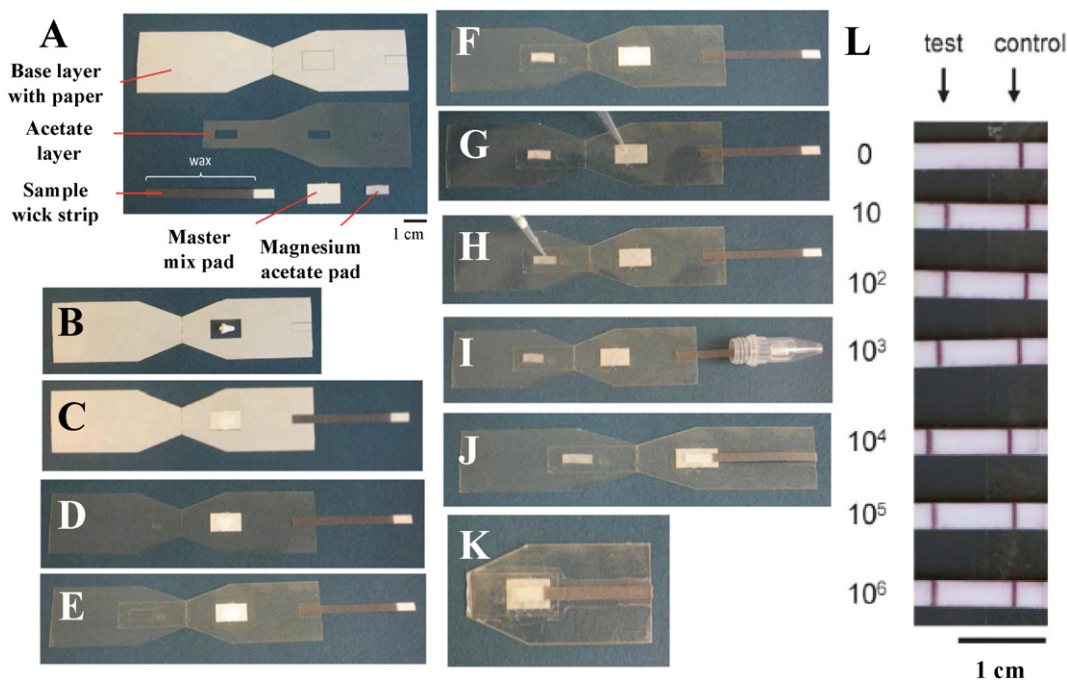


**Fig. 6. Paper-based viral load detection system to perform recombinase polymerase amplification of HIV-1 DNA, system assembly, and operation.** (A) Components of the device. (B) An enzyme pellet is placed on prepared adhesive window. (C) Pad for master mix and sample strip is placed. (D) The remaining paper is peeled to uncover all adhesive surfaces. (E) Acetate layer is fixed to the right side of the system. (F) Magnesium acetate pad is placed on the window of acetate layer. (G-H) Master mix and magnesium acetate are soaked to pads. (I) Sample is applied through sample wick strip. (J) Sample wick is folded on to the master mix pad. (K) Device is folded through the midline allowing interacting master mix, sample, and magnesium acetate. (L) Number of HIV-1 DNA copies introduced to system before recombinase polymerase amplification. This figure was reproduced from [125] with permission of Royal Society of Chemistry.

**Table 1**
Comparison of technologies and platforms discussed in Section 2. Some words were abbreviated to simplify the table—LSPR (localized surface plasmon resonance), ELISA (enzyme linked immunosorbent assay), PCR (polymerase chain reaction), RT-PCR (real-time polymerase chain reaction), MICT (magnetic immunochromatography).

| Type | Target | Matrix | Estimated cost | Advantages | Disadvantages | Ref. |
|---|---|---|---|---|---|---|
| Amperometric | p24 protein | Serum, physiological buffers | >$1000 per instrument | High sensitivity | Bulky, requires multiple wash steps | [20] |
| Potentiometric | HIV-1 integrase activity | Reaction buffer | >$1000 per instrument | High sensitivity | Sensitive to changes in temperature and ionic buffer strength | [23] |
| Impedance | Intact HIV-1 | 1% Triton-X 100 in water | <$10 per chip, >$1000 per instrument | Highly specific measurements and can be multiplexed | Bulky, requires multiple wash steps | [25] |
| Impedance | Intact HIV-1 | Physiological buffers, plasma, artificial saliva | <$2 per chip, >$1000 per instrument | Highly specific measurements and can be multiplexed | Requires multiple wash steps | [26,27] |
| Voltammetric | HIV-1 complementary target DNA | Human serum | >$1000 per instrument | High sensitivity, good selectivity | Bulky and expensive | [40] |
| Impedance | Intact HIV-1 | Serum | <$2 per chip, >$1000 per instrument | Flexible substrate | High Limit of Detection | [41] |
| Fluorescence | CD4+ T cells | Blood | >$1000 per instrument | Highly specific cell capture | Bulky and expensive | [45] |
| Lens-free Imaging | CD4+ T cells | Blood | <$2 per chip, <$1000 per instrument | Portable and disposable | Chips require storage | [51] |
| LSPR | Intact HIV-1 | Blood | >$1000 per instrument | Highly sensitive | Bulky, expensive | [62,63] |
| Photonic crystal | HIV-1 | Serum | >$1000 per instrument | Highly specific direct virus capture | Bulky and expensive | [75] |
| Mechanical | gp120 protein | Physiological buffers | >$100 per chip, >$1000 per instrument | Highly sensitive to changes on virus surface | Bulky, high LoD | [91] |
| Mechanical | gp120 protein | Physiological buffers | > $100 per chip, >$1000 per instrument | Highly sensitive to changes on virus surface | Bulky, expensive, and requires trained staff | [94] |
| Mechanical | gp41 protein | Urine | > $100 per chip, >$1000 per instrument | Antibody-free | Bulky, expensive, and requires trained staff | [104] |
| ELISA | CD4+ T cells | Blood | <$2 per chip, <$1000 per instrument | Highly sensitive and specific | Bulky, expensive, and requires trained staff | [108] |
| RT-PCR | HIV-1 RNA | Blood | <$1000 per plate, >$1000 per instrument | Highly sensitive | Multiple reaction steps | [113] |
| PCR | HIV-1 RNA | Blood | <$100 per plate | Highly specific | Expensive and requires trained staff | [115,116] |
| MICT (magnetic) | HIV-1 p24 | Plasma | <$10 per chip | Short assay time | Low sensitivity | [121] |
| lateral flow assay | HIV-1 DNA | Buffer | <$10 per strip | Short assay time, no refrigeration required | Electric heater required | [125] |

each reference to obtain an order of magnitude cost for the chips and instruments (where applicable).

## 3. HIV-1 management using emerging technologies

One of the gold-standard technologies for HIV-1 detection is nucleic acid amplification. This method has been implemented in many different ways to help solve important and challenging problems with HIV-1 management including the detection of acute HIV-1 infection, mother-to-child transmission, and HIV-1 reservoir quantification. Fig. 7 below illustrates the many ways nucleic acid amplification is currently being used.

Due to the large success with implementing these strategies in developed countries, significant efforts have been made to further develop these technologies to meet ASSURED criteria so that these advancements can be implemented in resource-limited settings. The following
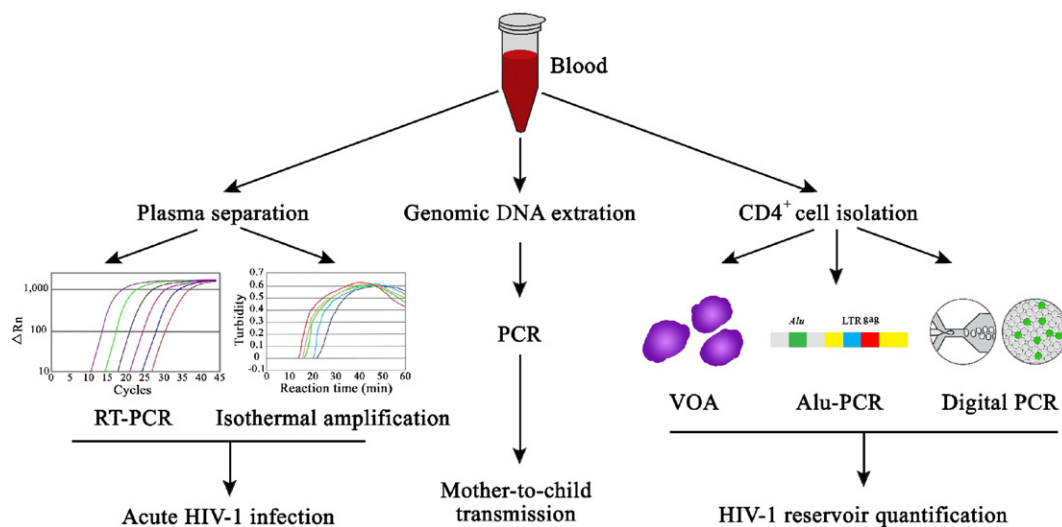


**Fig. 7. Nucleic acid amplification strategies for HIV-1 management.** Nucleic acid amplification strategies are currently being used for challenging HIV$^{-1}$ management problems such as acute HIV-1 infection (via reverse transcription PCR and isothermal amplification), mother-to-child transmission, and HIV-1 reservoir quantification (via viral outgrowth assay (VOA), Alu-PCR, and digital PCR).

section describes current challenges with HIV-1 clinical management in resource-limited-settings and discusses emerging technologies that may be useful in solving them.

### 3.1. Detection and diagnosis of acute HIV-1 infection

Early or acute HIV-1 infection (AHI) has become more important in HIV-1 management for both prevention of HIV-1 transmission and improved ART efficacy [126–128]. As acute retroviral syndromes present non-specifically, individuals with AHI may not be aware of their HIV-1 status and may continue high-risk behaviors such as unprotected sex, intravenous drug use, or even blood donation. Moreover, AHI is associated with a high level of viremia, which renders them highly contagious and leads to further HIV-1 transmission. Thus, it is essential to detect AHI as early as possible and to provide appropriate clinical management. However, AHI cannot be detected using traditional immunoassays due to having a long window period, during which HIV-1-specific antibodies are not yet generated in AHI patients. The window period varies from a few weeks to 6 months among individuals following AHI, posing a great challenge for the prevention and control of HIV-1 [127,128]. AHI is also of importance for clinical treatment [126,129]. During the early stage of HIV-1 infection, HIV-1 rapidly establishes its reservoir in infected individuals, preventing complete eradication or functional cure with current ART regimens [130,131]. Early diagnosis of AHI followed by timely treatment can reduce the magnitude of HIV-1 reservoir and may improve the prognosis. Thus, early diagnosis of AHI followed by prompt ART initiation provides an invaluable opportunity for both HIV-1 prevention and treatment.

A diagnosis of AHI is generally made using nucleic acid amplification technology to detect high levels of HIV-1 RNA in plasma, in combination with traditional immunoassays (rapid lateral flow assays, ELISA, and Western blot) to confirm the absence of HIV-1-specific antibodies. [132]. Although the multi-testing algorithm can be established in developed countries, it presents a significant challenge for POC testing in resource-limited settings due to cost and differences in infrastructure. Additionally, in some resource-limited settings, clinical management is based completely on CD4 counts rather than viral load, which may be particularly troublesome since current medical practice is to treat immediately upon diagnosis [127]. Thus, rapid, inexpensive, and simple-to-use nucleic acid assays have been developed. For example, a miniaturized SAMBA device with pre-loaded freeze-drying reagents can amplify HIV-1 RNA with a detection limit down to 78 copies/mL [133]. In comparison with other laboratory-based NAT assays such as Abbott Realtime, bioMerieux NucliSENS, and Roche COBAS, SAMBA obtained comparable sensitivity but also eliminated the need for refrigerated transportation and storage, increasing the feasibility of POC testing in resource-limited settings. In another study, the Ismagilov group developed a "SlipChip" that can perform digital reverse-transcription loop-temperature mediated amplification (RT-LAMP) for robust detection of HIV-1 RNA using a mobile phone [134]. As demonstrated, this SlipChip can differentiate $1 \times 10^5$ and $2 \times 10^5$ copies/mL with a temperature variation of 6 °C. Taken together, these two technologies represent advances in rapid detection of HIV-1 RNA in a miniaturized microfluidic devices for identifying individuals with AHI in resource-limited settings.

### 3.2. Mother-to-child transmission

MTCT is another major area for HIV-1 prevention and control. As reported, the rate of mother-to-child transmission can be reduced to 0.7% with a combined measure including ART in HIV-1-infected mothers, C-section, and formula feeding [13]. Thus, two aspects need to be focused on; one is the viral load (HIV-1 RNA in plasma) in HIV-1-infected mothers, and the other is early detection of HIV-1 DNA in infants for timely care. Viral load in mothers can be measured using RNA amplification technologies with either a bulky instrument or a miniature device. For detection of HIV-1 in infants, traditional immunoassays

cannot be used since passive transfer of specific antibodies from HIV-1-infected mothers interferes with the diagnosis of infected infants up to 18 months. Instead, amplification of HIV-1 DNA from peripheral blood mononuclear cells (PBMCs) is used to achieve early diagnosis of HIV-1-infected infants. PCR [115], loop-mediated isothermal amplification (LAMP) [135], and recombinase polymerase amplification (RPA) [125] have also been employed to detect HIV-1 DNA. Based on LAMP technology, a microfluidic biomolecular amplification reader (µBAR) was developed, which integrated molecular biology, microfluidics, optics, and electronics. However, this system could only detect the HIV-1 integrase gene at a level of $10^6$ copies/mL [135]. In addition, this system did not solve one of the most common bottlenecks with molecular diagnostics: many sample preparation steps at the POC. RPA is another isothermal amplification technology developed by TwistDX. The RPA technology was miniaturized into a paper and plastic device [125]. As demonstrated, this lightweight device amplified HIV-1-DNA by RPA and detected the amplicons down to 10 copies by a lateral flow strip in 15 min. However, this system was designed to amplify HIV-1 DNA after nucleic acid extraction from dried-blood spots. Recently, a fully integrated HIV-1 DNA PCR system was developed to detect HIV-1-infected infants in resource-limited settings, though this method could only detect HIV-1 DNA when using more than 5000 cells per reaction. Thus, a fully integrated nucleic acid amplification system is preferred to facilitate the diagnosis of HIV-1-infected infants in resource-limited settings.

### 3.3. HIV-1 eradication

Either achieving HIV-1 eradication or a functional cure is the next big challenge [129,136]. Curing HIV-1 can occur either by: (1) a sterilizing cure, in which HIV-1-infected cells that harbor replication-competent virus are eliminated from infected individuals; or (2) a functional cure such as ART-free remission, in which ART can be stopped with zero or low detectable viral loads without viremia rebound [137]. Strategies for achieving an HIV-1 cure include reducing HIV-1 reservoirs with simultaneously enhancing antiviral immunity or inducing cellular resistance to de novo HIV-1 infection [138–140]. The persistent nature of HIV-1 arises from its ability to integrate into the host genomes of cells in a variety of tissues, creating a long-lived viral reservoir that is relatively untouched by long-term ART [141,142]. As a result, the main challenge in achieving a "cure" for HIV-1 is the persistence of these viral reservoirs.

Reservoir studies of HIV-1 infection in the setting of ART intensification show that HIV-1 can persist indefinitely [142,143]. HIV-1 can infect both activated and resting cells, followed by integration of viral genomes into host cell chromosomes. Latently infected, memory CD4+ T-cells are the best characterized reservoir for HIV-1, which can exist in very low numbers (e.g. as low as one integrated and replication-competent proviral infected cell per million resting CD4+ T cells) [142–144]. Interestingly, a majority of integrated HIV-1 DNA does not code for replication-competent virus, and does not contribute to long-term HIV-1 persistence despite being detected by quantitative PCR-based assays [145]. Furthermore, latently infected cells are able to evade normal innate and HIV-1-specific immune responses regardless if integrated proviruses are capable of producing replication-competent HIV-1. Proliferation of these immune quiescent cells likely plays an important role in the maintenance of the long-lived viral reservoir [146,147]. After treatment initiation, the number of infected cells decreases initially, but reaches a low but stable level after several months. In the setting of ART, the resting latent reservoir decays very slowly with an estimated half-life of over 40 months [142].

Some studies suggest that HIV-1 replication may continue in certain tissues despite the use of otherwise fully suppressive ART [148–150]. Historical data suggest, however, that significantly impairing viral replication with ART is not sufficient to significantly reduce viral reservoirs [142,151]. Very early initiation of ART or the prompt initiation of ART in infants born to infected mothers has been shown to lead to smaller

HIV-1 reservoirs, which may be due, in part, to the limited reservoir seeding of long-lived memory CD4$^+$ T cells [152]. However, allogeneic stem cell transplantation of uninfected donor cells under the protection of ART or genetic modifications that prohibit HIV-1 entry is one of the few strategies that has been shown to lead to significant reductions in the viral reservoir size in individuals with established reservoirs who started ART during chronic infection [152–154]. Unfortunately, mathematical modeling studies have predicted and treatment interruptions studies have shown that HIV-1 may rebound months to years after stopping ART, even after several total body log reductions in infected cells and no detectable infected cells in peripheral blood [155].

A majority of the HIV-1 reservoir exists in tissues, such as gut-associated lymphoid tissue (GALT) and the lymphoreticular systems (lymph nodes, spleen, etc.), which is more difficult to access than peripheral blood, and hence, is less understood [156]. Tissue-derived cells may act quite differently than those collected from the peripheral blood. For example, B cell follicles appear to be an important sanctuary for SIV-infected CD4$^+$ T lymphocytes [157]. Follicular helper (T$_{FH}$) cells located in these follicles are particularly enriched in cell-associated HIV-1 DNA and RNA and support high levels of replication *in vitro* [157,158]. Other infected cell types, such as macrophages or neuroresident glial cells, may also play a role in the persistence of HIV-1 on therapy, although their precise contribution to the long-term reservoir is still under investigation [156,159,160].

Quantitative co-culture assays (qVOAs) have been developed to quantify the functional component of the reservoir. However, these platforms rely on large numbers of resting CD4$^+$ T cells (hundreds of millions) cultured in static culture-plate formats over a period of weeks [144,161,162]. As a result, they are time-consuming and labor-intensive, and have not been adapted for use in various infected tissues outside of PBMCs. Although there have been recent important improvements in the workflow of such assays [163], miniaturization and automation may allow for increased assay sensitivity and throughput. Integrated HIV-1 DNA has also been used to quantify HIV-1 reservoir by Alu-PCR [164]. However, this method cannot indicate whether infected cells with integrated HIV-1 DNA can produce infectious HIV-1 virions. Recently, digital PCR or RT-PCR has been increasingly used to characterize HIV-1 reservoirs. Since there is much interest in studying HIV-1 reservoirs in resource-limited settings where the burden of persistent disease is greatest, development of platforms involving microfluidic chip-based or other technologies that may be deployed efficiently and cost-effectively in RLS are urgently needed.

## 4. Future directions

Rapid, inexpensive and simple-to-use HIV-1 diagnostics are urgently needed for early diagnosis and ART monitoring in resource-limited settings. To address these challenges, researchers have been developing systems to detect HIV-1 using portable and inexpensive nanotechnologies integrated with microfluidics. Such devices may one day be capable of routinely monitoring ART or identifying latent HIV-1 reservoirs. Smartphones utilizing mobile applications have been integrated with microfluidics and paper-based portable platforms for the quantification of target biomolecules [77,110,165–168], and we envision that future POC technologies will likely incorporate mobile technologies since they are portable, inexpensive, and have high processing capabilities. Another technology that is likely to become important in resource-limited settings is the creation of wearable/implantable sensors, which can be used for patient health status monitoring including blood parameters, body temperature, pressure, and pH of the bio-environment [169–174]. Integration of flexible materials with mobile systems will also allow for the transmission of information. In addition, wearable biosensors can be manufactured by printing technologies on flexible materials to be potentially utilized as skin patches [175–177]. Although wearable/implantable sensors are at an early stage of development, they represent one of the promising areas for developing POC diagnostic

tools for rapid HIV-1 diagnoses and potentially monitoring ART in resource-limited settings. Since finding a cure for HIV-1 infection is the ultimate goal in the clinic, significant research efforts have been dedicated to identifying and eradicating HIV-1 reservoirs. A portable or wearable device that can perform this task would be ideal for informing physicians when to stop ART. We believe that the convergence of emerging technologies targeting unmet clinical challenges in HIV-1 management will bring hope to eventually curing the HIV-1 pandemic.

## Disclosure statement

## Acknowledgments

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