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# Nano/Microfluidics for diagnosis of infectious diseases in developing countries ☆

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ABSTRACT

Nano/Microfluidic technologies are emerging as powerful enabling tools for diagnosis and monitoring of infectious diseases in both developed and developing countries. Miniaturized nano/microfluidic platforms that precisely manipulate small fluid volumes can be used to enable medical diagnosis in a more rapid and accurate manner. In particular, these nano/microfluidic diagnostic technologies are potentially applicable to global health applications, since they are disposable, inexpensive, portable, and easy-to-use for detection of infectious diseases. In this paper, we review recent advances in nano/microfluidic technologies for clinical point-of-care applications at resource-limited settings in developing countries.



**Contents**



*Abbreviations:* AED, antiepileptic drug; AFM, atomic force microscopy; AIDS, acquired immunodeficiency syndrome; ART, antiretroviral therapy; CCD, charge-coupled device; ELISA, enzyme-linked immunosorbent assay; FLASH, fast lithographic activation of sheet; HIV, human immunodeficiency virus; LED, light-emitting diode; LOC, lab-on-a-chip; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction; PDMS, poly (dimethylsiloxane); PMMA, polymethymetacrylate; POC, point-of-care; POCKET, portable and cost-effective; QD, quantum dot; RDT, rapid diagnostic test; RF, radio frequency; SPR, surface plasmon resonance; TB, tuberculosis; WHO, World Health Organization.

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## 1. Introduction

Infectious diseases are a leading cause of death in developing countries [1,2]. Over 95% of these deaths are caused by the lack of proper diagnosis and treatment, such as difficulty in accessing adequate health care infrastructure [3]. To develop diagnostic tools for infectious diseases at resource-limited settings, the World Health Organization (WHO) has established a set of guidelines: (i) affordable, (ii) sensitive, (iii) specific, (iv) user-friendly, (v) rapid and robust, (vi) equipment-free, and (vii) delivery to those who need it, leading to the acronym "ASSURED" [2,4]. These guidelines can be used to develop more suitable diagnostic approaches for low-resource settings to enhance the overall quality of life of the global population [5–7].

Point-of-care (POC) diagnostics offer great potential to detect and monitor infectious diseases at resource-limited settings, because POC diagnostics can be taken to remote locations. Desired characteristics of POC diagnostic technologies include (i) disposability, (ii) cost-effectiveness, (iii) ease of use and (iv) portability [8]. POC diagnostics should be able to analyze small volumes of bodily fluids, e.g., blood, saliva and urine. Given the contagious nature of these samples, the devices should be disposable to protect the end-users from exposure to biohazardous waste. In addition, disposable POC devices eliminate unnecessary steps, such as washing processes between sample preparations, which can make the devices easier to use even in regions poorly supplied with water. The cost of diagnostics is also one of the important parameters for global health applications [9]. To decrease the cost of POC diagnosis, several aspects should be considered: (i) minimal use of expensive reagents, (ii) inexpensive manufacturing for mass production, (iii) quality control, and (iv) miniaturization [3]. In addition, for clinical use of medical diagnostic devices in resource-limited settings, environmental conditions, such as insufficient water, unreliable electricity, high temperatures (35–45 °C), and humidity need to be considered [10].

Nano/Microfluidic technologies are emerging as powerful methods which could address the challenges imposed by conventional diagnostic devices [11,12]. These approaches enable on-chip POC diagnosis and real-time monitoring of infectious diseases from a small volume of bodily fluids [13]. These technologies can be used to integrate various assays into a single device [14–17] and to deliver target samples to specific reaction chambers in a controlled manner [16,18–24]. Among these technologies, nanofluidics has been highlighted by the recent advent of nanoscience and nanotechnology since the rise of microfluidics in the 1990s [25]. Generally, nanofluidics can be defined as the field of study in fluid flow in and around nanoscale objects [25,26]. For its applications to POC diagnostic devices, nanofluidics can be used to enhance microfluidic functions, such as temporal and spatial control of nanosized samples (e.g., HIV virus ~100 nm) in lab-on-a-chip (LOC) devices with nanostructures and nanotech materials [27,28]. Given these features, nano/microfluidic devices have been used for sample preparation, such as continuous blood flow fractionation [29–32], nucleic acid extraction [33], and purification of small molecules [34].

The cost of micro/nanofluidic technologies can be minimized by mass production through simple plastic fabrication techniques. For example, nanostructures that can enhance capillary-driven microflow in plastic microfluidic devices can be easily scaled-up for mass production using nanomolding techniques. Thus, microfluidic devices can enable on-chip POC diagnosis of blood-related infectious diseases in a disposable and mass-producible format [35]. Nano/Microfluidic devices can also be built at a low cost by using other materials, such as paper in a process called "fast lithographic activation of sheet (FLASH)", without high-end microfabrication facilities [36]. These devices are disposable, cheap and useful for on-chip processes and involve sample pre-treatment in a rapid and high-throughput manner.

In this review, we provide a broad overview of recent advances in nano/microfluidic technologies for POC diagnostics targeting infectious diseases at resource-limited settings. We also highlight the applications of on-chip POC diagnostics focusing on detection, imaging, and counting techniques. Furthermore, we provide current trends and perspectives for nano/microfluidic POC applications of infectious diseases, such as (i) HIV/AIDS, (ii) malaria, and (iii) tuberculosis (TB).

## 2. Recent developments in nano/microfluidic technologies targeting infectious diseases at resource-limited settings

In this section, we will specify key examples of recent advances in nano/microfluidic technologies that can enhance the development of a platform for global POC diagnosis and monitoring of infectious diseases at resource-limited settings.

### 2.1. On-chip detection and imaging

On-chip detection and imaging techniques play an important role to detect infectious diseases in a fast and accurate manner. Among them, optical microscopy is a powerful method for detection and imaging of various diseases at molecular and cellular levels. Optical microscopy can be combined with other techniques to enhance on-chip detection of blood-related diseases by using erythrocyte deformability [37,38]. Recently the optical microscopy field has significantly evolved through emergence of optofluidic technologies which fuses optics and microfluidics [39]. The optical microfluidic technologies have been used to develop POC diagnostics with enhanced detection techniques, such as absorbance, fluorescence, chemiluminescence, surface plasmon resonance (SPR), and interferometric detection [12]. However, optical microscopy has several limitations, such as lack of portability, high costs due to use of expensive optics, and regular maintenance. To overcome these limitations, lensless and portable charge-coupled device (CCD)-based platforms have been recently developed [22,40]. This approach can be used to detect cells and particles by shadow imaging, enabling a lensless and ultra-wide field monitoring. Thus, the captured microscale targets (i.e., cells or beads) in a microfluidic device are detected and counted by their shadow images without fluorescence labeling [22,23]. This approach can be useful for developing a handheld, battery-supplied, imaging platform which will be cheap to maintain and to operate.

More recently, a new imaging platform, called the "mobile-based clinical microscopy", has been developed for an integrated and portable mobile phone microscopy system for global health [41,42]. Generally, most regions of developing countries have either insufficient infrastructural resources, such as lack of healthcare facilities and manufacturers. For the same regions, mobile phones or wireless networks are often available, where the camera-equipped mobile phones can also be used at a lower cost, compared to microscopy-related cameras. Recently the mobile phone network, especially available in developing countries, was envisioned as an alternative route to achieve diagnostic imaging and telemedicine [42]. The clinical feasibility of this technique was also demonstrated by conducting successful detection of malaria and tuberculosis by bright field and fluorescence imaging, respectively [42]. This technique is potentially useful in developing a portable platform that can be combined with disposable nano/microfluidic POC devices for global health applications (Fig. 1).

### 2.2. On-chip flow cytometry

The ability to rapidly count and analyze cells by using techniques, such as flow cytometry, is important for diagnosis of a number of infectious diseases, such as HIV [43]. Despite this need, conventional flow cytometers are often too expensive to use in resource-limited

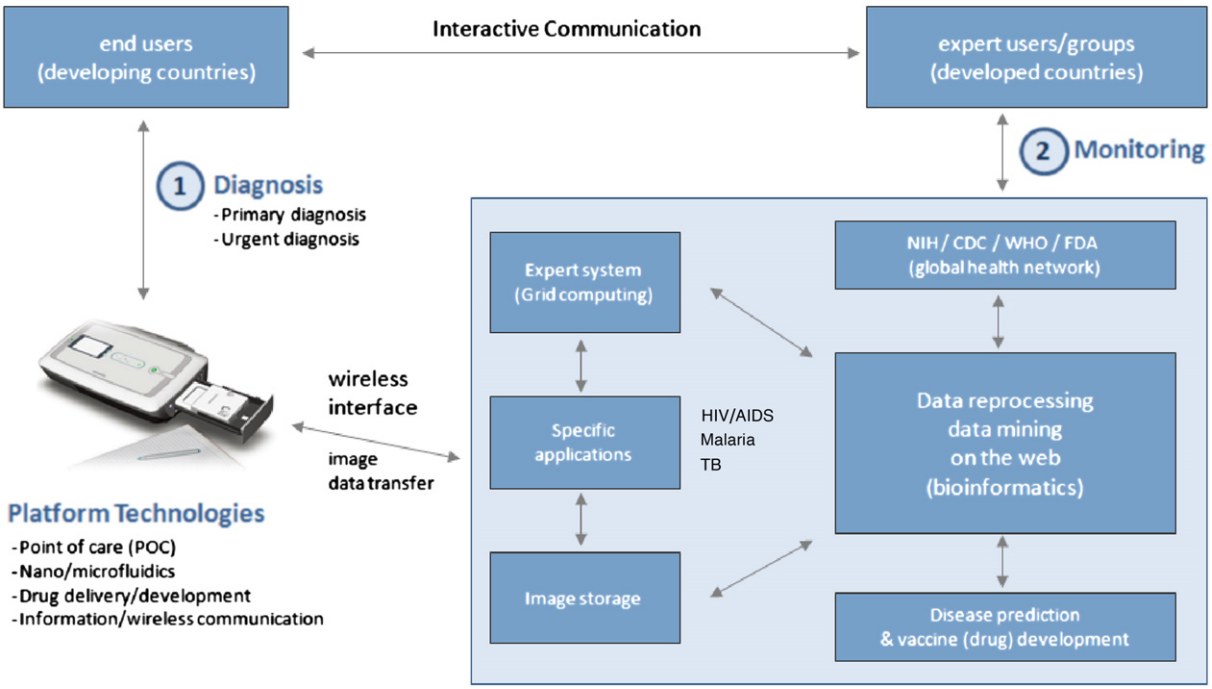


**Fig. 1.** A schematic of a platform for POC diagnostics in developing and developed countries. The device shown above was selected as a representative example of a wireless data-reading platform for global health.
The image of the device was reprinted from Ref. [3] by permission of Annual Reviews.

settings. Thus, for the past decade, there has been interest to miniaturize the flow cytometry instruments [44,45]. To develop miniaturized flow cytometers, several desired features include: (i) label-free detection, (ii) lensless imaging or minimal use of expensive optics, (iii) simple channel geometry, and (iv) sheath-free focusing.

Recently, a microfluidic device was developed to perform simple, rapid, and affordable CD T-lymphocyte counts, especially for HIV monitoring in resource-limited settings [46]. This approach enabled capture and imaging of CD4 cells with a miniaturized flow chamber by microscope optics and digital camera technology. More recently, a label-free detection method was also developed for counting label-free $CD4^+$ T-lymphocytes in resource-limited settings [30,31]. In this approach, 10 μL of whole blood was injected into a poly(dimethylsiloxane) (PDMS)-based microfluidic chip that was functionalized with antibodies to capture $CD4^+$ cells. Another approach that eliminates the need for expensive optics by using a portable CCD platform has also been used for lensless imaging of $CD4^+$ T-lymphocytes (Fig. 2a–c) [22]. We describe this concept in detail in the HIV/AIDS section.

The microfluidic channel geometry is also important for controlling detection sensitivity. For instance, a flow focusing channel was used to decrease the complexity of channel geometries, resulting in high detection sensitivity and throughput [47] (Fig. 2c). This microchannel had a width of 100 μm at the inlet and 500 μm at the detection region. The fluorescently-labeled samples were hydrodynamically focused at the entrance of the focusing channel and passed through the expansion channel at the detection region. Since the flow velocity of the samples was decreased at the detection zone inside the expansion channel, the detection sensitivity was enhanced an order of magnitude, compared to normal channels, while preserving the detection throughput since the flow rate remained the same. For the clinical use of the concept, this geometry can be modified with sheathless focusing techniques associated with fluorescent nanoparticles [48]. All these approaches are potentially useful to overcome the optical and geometrical constraints of on-chip flow cytometers.

### 2.3. On-chip immunoassay

Nano/Microfluidic technologies enable the generation of on-chip immunoassays for POC diagnostic applications. The conventional enzyme-linked immunosorbent assay (ELISA) approach has a number of limitations for its use in resource-limited settings, because it requires long assay times, cumbersome liquid handling, and large amounts of expensive reagents and equipment [24]. To address these limitations, recently Kitamori *et al.* developed a practical micro-ELISA system for sensitive and rapid diagnosis. This approach showed potential for development of a POC platform that enables the use of small samples (10 times smaller) and fast analysis (20 times faster) compared to conventional ELISA method [49]. For immunoassay-based diagnosis, rapid diagnostic tests (RDTs) have been developed for detection of various entities. For example, immunoassays can be performed by lateral flow methods to provide rapid results at low cost, similar to pregnancy tests [50]. To enhance the quantitative results of these RDTs, the immunoassay can be combined with optical, electrical, and mechanical transducers in an integrative format [50–54].

Microfluidics-based immunoassays are potentially beneficial to maximize sensitivity, minimize sample volume requirements, and produce fast and accurate results [55–57]. For example, Yager *et al.* developed an on-chip diffusion immunoassay to measure the concentration of small molecules within microfluidic channels [58]. This assay is based on characterizing the distribution of a labeled probe molecule after it diffuses from one region into another with antigen-specific antibodies in the microfluidic T-sensor. Thus, microfluidic diffusion-based studies can be suitable for high-throughput screening and analyzing blood samples. Recently, the same research group developed a microfluidic immunoassay with on-card dry reagent storage for malaria detection, showing possibility of quantitative analysis of multi-analytes such as human blood samples [54]. A dry cantilever assay has also been developed to increase the sensitivity of immunoassays. This assay with its data tracking and recording functions may be potentially useful for POC-based immunoassay applications [59].

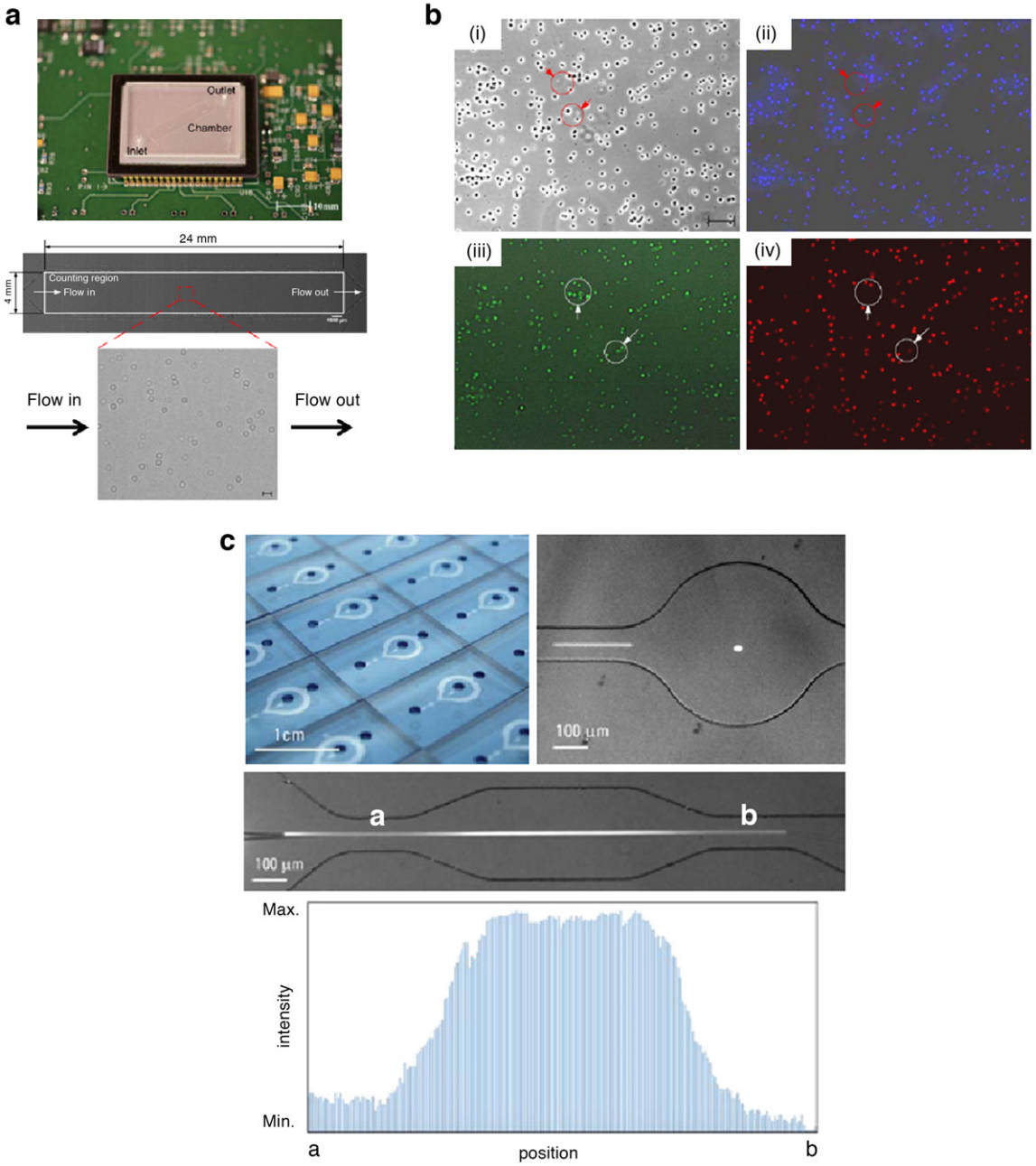


**Fig. 2.** On-chip microfluidic approaches. (a) CCD-based lensless imaging platform with a disposable microfluidic device (top) and the CCD shadow image of $CD4^+$ T-lymphocytes captured in a microfluidic device (bottom). Scale bar is 100 μm. (b) Phase contrast and fluorescent images to identify captured cells: (i) phase contrast image of cells, (ii) DAPI stained cells, (iii) $CD4^+$/AF488 stained cells, and (iv) $CD3^+$/AF647 stained cells. (c) Photograph of a mass-producible device and a bright field image of the expansion channel at the detection zone (top and middle) and intensity profile of fluorescence of particles flowing through the expansion geometry (bottom).
Panels a and b are modified from Ref. [22] with permission from Elsevier. Panel c is reprinted from Ref. [47] with permission from the Royal Society of Chemistry.

### *2.4. Nanosensors*

Today's POC diagnostics can be improved by modifying conventional detection techniques associated with nano/microfluidic interfaces using lateral flow [60] and diffusion [61], such as surface plasmon resonance (SPR) and atomic force microscopy (AFM). For instance, Yager *et al.* developed a portable SPR system for label-free POC diagnostics [62]. This prototype diagnostic chip consists of a near-infrared light-emitting diode (LED) source and stationary wide field imaging optics containing a compact liquid crystal polarizer that can be used to electronically switch the source polarization of images. Given the powerful SPR imaging-based reader, this device can be used for rapid detection of the antiepileptic drug (AED) phenytoin in saliva. This system can also be easily combined with micro/nanofluidic channels [63,64], enabling real-time label-free protein detection and separation. However, for the portable POC diagnostic applications, miniaturization of the optics and electronics still remains a challenge for SPR systems.

Nanosensors, such as nanotubes, nanowires, and cantilevers can improve sensitivity, reduce production costs, and characterize various analytes that have previously been difficult to detect by conventional technologies. For example, carbon nanotube-based sensors for monitoring respiratory gas and biomolecules, such as DNA, protein, and glucose, have been developed [65,66]. These sensors, which detect picomolar concentrations of label-free DNA, show a high sensitivity of molecular detection. In addition, an AFM-based approach was used to

detect viruses for diagnosis of viral infections. This platform consists of a silicon chip functionalized with a microarray of antibodies and an AFM-based detector [67]. This technique was used to detect and identify nanoparticles that interacted with the tip of the AFM as the tips scanned the microarrays [68]. To detect virus particles, a ViriChip assay integrated with microarray, antibody capture, and label-free readout systems was used. The viruses captured on antibody coated surface were analyzed by AFM. Thus, the glass-based ViriChip can directly detect infectious viral antibody-specific particles within 30 min. In addition, nanosensor technologies hold great potential to develop clinically relevant platforms with high detection sensitivity. These technologies are potentially beneficial to develop nano/microfluidic POC devices that enable label-free detection and identification of actual virus particles in resource-limited settings [69].

## 3. Current challenges and future perspectives

In this section, we will discuss current challenges and perspectives of nano/microfluidic technologies for POC diagnosis and monitoring of infectious diseases, such as HIV/AIDS, malaria, and tuberculosis.

### 3.1. HIV/AIDS

The World Health Organization emphasizes the need to develop POC devices for HIV/AIDS diagnosis and monitoring in resource-limited settings [70]. These POC devices can be developed as an accurate, inexpensive, and disposable platform which enables the enumeration of $CD4^+$ T-lymphocytes and HIV viral load [71–73]. For clinical applications, the POC device needs to detect around 200 $CD4^+$ cells $\mu L^{-1}$ and 400 copies $mL^{-1}$ of HIV from whole blood [74]. Antiretroviral therapy (ART) is targeted at repressing virus replication suppressing the progression of the HIV infection toward the disease (AIDS), resulting in gradual increases in the number of $CD4^+$ T-lymphocytes [75,76]. Therefore, $CD4^+$ T-lymphocyte counting and viral load quantification have been used to monitor HIV. However, the conventional methods, such as expensive flow cytometry and quantitative PCR, still have several limitations such as a long diagnostic time and the need for a well-trained technician [77].

The number of $CD4^+$ T-lymphocytes per microliter of HIV-infected blood has prognostic and therapeutic indications and it has been critically used to monitor disease states and to decide ART treatment [76]. $CD4^+$ T-lymphocytes act as host cells [78]. HIV binds to the CD4 receptor via HIV gp120 envelop glycoprotein, which allows the virus to infect and damage the cells during the process. Currently, the $CD4^+$ T-lymphocyte count has been performed four times per year in developed countries, but only twice a year in developing countries using conventional flow cytometers [79].

For global health applications, a number of studies have been conducted to miniaturize the flow cytometers as a portable POC device [80]. However, a number of challenges, such as the cost and complexity have to be addressed in resource-limited settings. Therefore, simple and mass-producible microfluidic devices can be useful for POC diagnostic applications for HIV/AIDS. For instance, recently a simple method for quantifying anti-HIV-1 antibodies in the sera of HIV-1 infected patients was developed using the microfluidic immunoassay, called “POCKET (portable and cost-effective)” [51]. In this approach, HIV-enveloped antigen was patterned on a polystyrene surface as a stripe and a slab of PDMS with microchannels was placed orthogonally to the stripe. The HIV-1 infected patient sera were directly introduced into the microchannels to quantify anti-HIV-1 antibodies. Although the microfluidic device allows detecting easily the highly increased anti-HIV-1 antibodies in patient sera, it was insufficient to make a correlation with HIV disease states.

Lee *et al.* have also developed a disposable polymer-based RT-PCR chip containing pinched microvalves for POC diagnostics [81]. For early diagnosis of HIV infection, this device has been used to detect the HIV p24 and gp120 that are major capsids and envelop proteins encoded by the HIV gag and envelop gene. For practical POC applications, however, this approach requires optimization for HIV-infected whole blood processing and simplified POC operation at resource-limited settings.

For the clinical use of CD4 POC devices in resource-limited settings, a number of challenges should be addressed, such as (i) $CD4^+$ T-lymphocytes captured from unprocessed HIV-infected whole blood and (ii) simple, rapid, and accurate counting of the captured cells. Previously, the devices that enable label-free $CD4^+$ T-lymphocyte capture and imaging have been developed [30,31]. Although these devices are disposable, they still require expensive optical microscopes to count the captured $CD4^+$ T-lymphocytes. To overcome this limitation, Demirci *et al.* have recently developed a lensless portable CCD-based microfluidic platform for HIV monitoring [22]. The microfluidic $CD4^+$ T-lymphocyte counting devices were fabricated out of poly(methylmethacrylate) (PMMA), glass slides and double-sided adhesive film without using high-cost equipment (Fig. 2a). Therefore, the device material and production costs were significantly decreased for use in resource-limited settings. In addition, the captured label-free $CD4^+$ T-lymphocytes from fingerprick whole blood were detected by CCD sensor by lensless shadow imaging techniques and were counted by automatic cell counting software in a few seconds, without any fluorescent labeling or an optical microscope [22,40]. This approach enables the microscale miniaturization of handheld devices which uses fingerprick whole blood (10 μL) for $CD4^+$ T-lymphocyte counts. Furthermore, this device can be of great interest for performing amplification-free HIV viral load quantification. Most recently, a microfluidic device was developed for HIV capture and imaging by quantum dots (QDs) from an HIV-infected patient whole blood [69]. Quantum dots with two different colors were used to detect the captured HIV by dual-staining of the envelope gp120 glycoprotein and its high-mannose glycans. HIVs were successfully captured from unprocessed HIV-infected patient whole blood onto microfluidic devices and were directly imaged. However, a successful nanofluidic device for an ultimate HIV viral load monitoring platform will be critical to increase the accuracy and possibility that the nanoscale virus particles (~120 nm) bind onto the chamber surface and to simultaneously discard micrometer sized-cells from HIV-infected patient whole blood.

### 3.2. Malaria

Rapid detection and treatment plays an important role in preventing the spread of malaria. In addition, improper diagnosis and treatment may also result in drug abuse or unexpected side effects [82]. Therefore, simple yet accurate diagnostic devices are required to minimize improper use of anti-malarial drugs or antibiotics. There are several approaches to diagnose malaria infection in resource-limited settings, such as lateral flow RDTs [83,84]. The first observation of malaria infection in human blood was in the 1880s [85] and microscopy has been considered as the gold standard for diagnosis of malaria. This method to detect malaria infection and parasites in resource settings requires well-trained researchers [86]. To overcome the need for well-trained experts and equipment, nano/microfluidic technologies can be used to develop POC devices for fast and accurate malaria diagnosis (Fig. 3). Fig. 3 shows the schematic of the lateral flow strip chip for diagnosis of malaria infection [87]. For example, the RDT strip chip can detect proteins derived from the blood of malaria parasites in a microfluidic format and can also be realized as commercialized products. This chip enables the generation of a series of visible lines to indicate the presence of specific antigens in blood that are clearly visible to the naked eye when antibody is accumulated at the test line.

Rathod et al. developed microfluidic channels to study malaria pathogenesis related complex interactions between host cell ligands

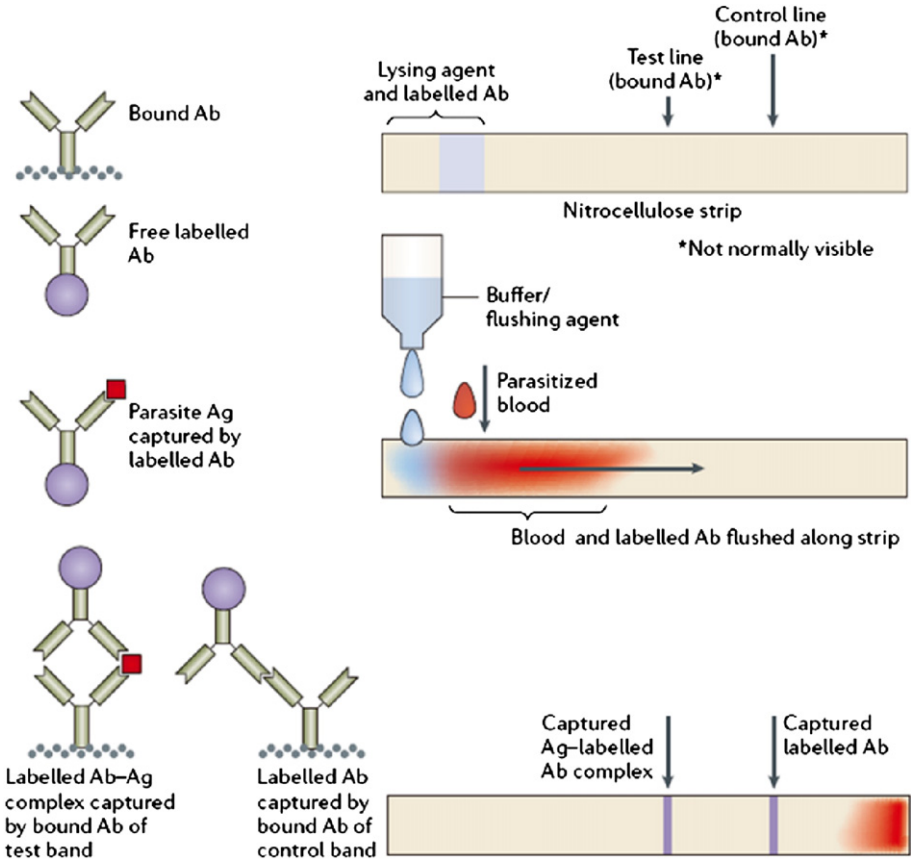


**Fig. 3.** Schematic of the lateral flow strip chip to diagnose malaria. (Top) Device preparation with nitrocellulose strip, (middle) operation principle of a lateral flow strip chip, and (bottom) expected results of a lateral flow strip.
Reprinted from Ref. [87] with permission from Nature Publishing.

and parasitized erythrocytes [88]. Since the microfluidic channels successfully mimic the sizes and shapes of capillary blood vessels, they could observe host–parasite interaction and malaria-infected red blood cells in capillary environment. The malaria diagnostic device is inexpensive and handheld for on-site analysis of patient samples and only requires microliter sample volume. Therefore, it has the potential to be widely used at field sites for more accurate malaria diagnosis.

### 3.3. Tuberculosis (TB)

TB is one of the main fatal infectious diseases to the HIV-infected patients, increasing death rate and drug resistance of immune suppressed patients [89,90]. Sputum smear microscopy has been used for TB diagnosis with relatively lower sensitivity in resource-limited settings [91,92]. Sheehan et al. reported a polymerase chain reaction (PCR)-based *Mycobacterium tuberculosis* diagnostic device designed as a microfluidic device for DNA amplification [93]. They described a noncontact thermo-cycling approach using a low power halogen lamp for DNA amplification from the pathogen. In addition, sample and reagent consumption is reduced and the lower thermal mass decreases the required time for PCR from hours to minutes. Because of the microfluidic device layout, low cost heat source and low power consumption of the system present a possibility to develop a portable field device. More recently, Lee *et al.* have developed a portable microfluidic nuclear magnetic resonance (NMR) biosensor for rapid, quantitative, and multiplexed detection of biological targets, such as bacteria, cancer biomarkers, and TB [94] (Fig. 4). The biosensor containing PMMA-based microfluidic mixers, microcoil arrays, printed circuit board, and a permanent dipole magnet was fabricated by photolithography and electroplating techniques. This sensor is based on a self-amplifying proximity assay and magnetic nanoparticles. In this system, magnetic nanoparticles served as a proximity sensor that bound to target biomolecules and subsequently formed soluble nanoscale clusters, which led to NMR signal changes. The data can be electronically obtained without bulky and expensive optical components. This rapid, simple, and high-throughput device is particularly useful in resource-limited settings. They also demonstrated the use of this system for the detection and characterization of infectious agents, such as bacteria, viruses, fungi, and parasites.

In summary, this review focused on recent advances of nano/microfluidic POC devices and its clinical applications at resource-limited settings. Most of them showed great potential to meet clinical and technical requirements for global health care. However, one major challenge still remains to network these nano/microfluidic POC diagnostics effectively with an interactive feedback for improved global health services. As described before, current mobile phone networks can be potentially beneficial for developing such a network-based platform that can improve nano/microfluidic POC devices consistently. Another major challenge may be establishing proper regulations and standards to evaluate nano/microfluidic POC diagnostics for clinical use [95]. The evaluation criteria can include several processes, especially for developing diagnostic tests, such as identification of the diagnostic target, optimization of test reagents, and development of a prototype. As shown in the literature, nano/microfluidic technologies hold great promise to achieve standard detection sensitivity levels for POC through quantitative, proof-of-principle studies in a fast, controlled, and high-throughput manner. Furthermore, to get approval for clinical use of POC devices (e.g., regulatory demands by FDA or NIH), nano/microfluidic technologies should also be capable of satisfying critical evaluation criteria, such as

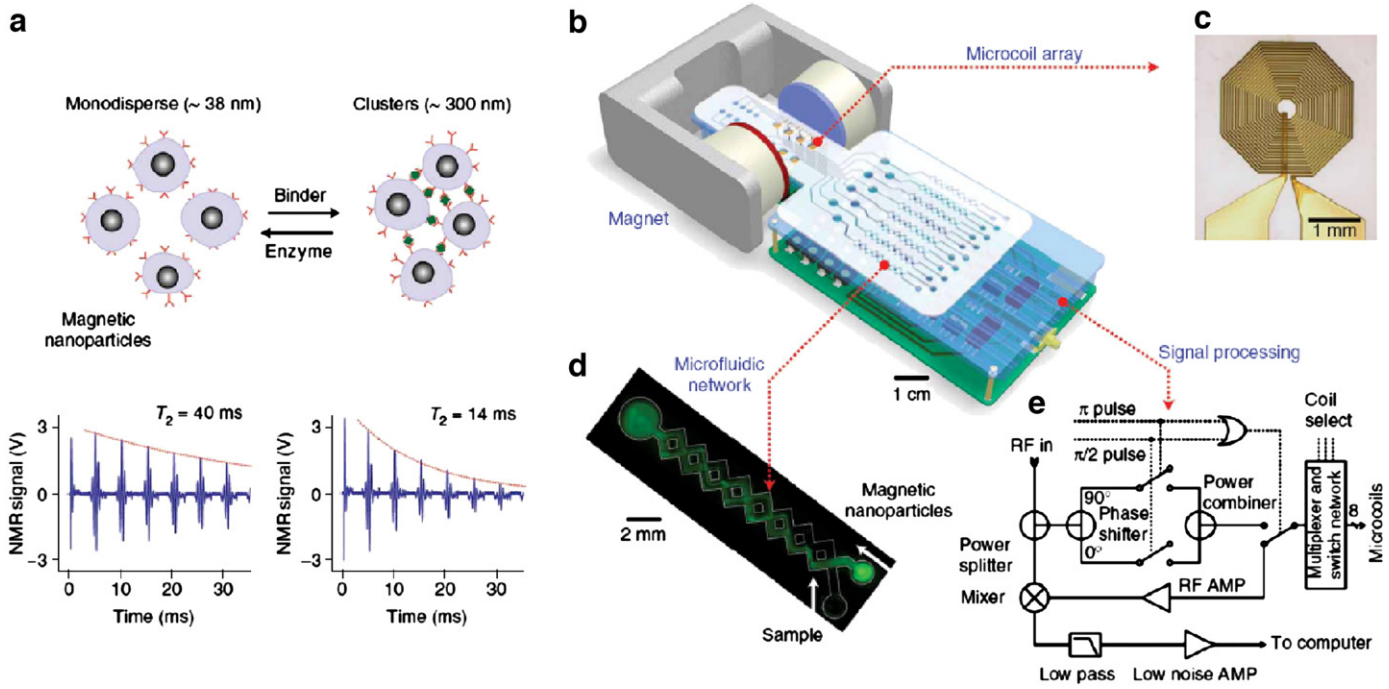


**Fig. 4.** Microfluidic NMR biosensor combined with magnetic nanoparticles for potential applications of TB testing in resource-limited settings. (a) Principle of proximity assay using magnetic particles (top) and signal detection (bottom). (b) A schematic diagram of the device. (c) A photograph of an actual microcoil which generates radio frequency (RF) magnetic fields to excite samples and receives the resulting NMR signal. (d) An image of a microfluidic network. (e) A schematic of the NMR electronics. Reprinted from Ref. [94] by permission of Nature Publishing Group.

testing characteristics and factors: (i) test performance (sensitivity and specificity), (ii) ease of use, (iii) conditions of use and storage, and (iv) shelf life [95,96]. Specifically, for commercialization, the efforts in this field should be made towards minimally instrumented POC diagnostics by developing platforms that can function without any peripherals [97].

## 4. Concluding remarks

Nano/microfluidic technologies have been successfully integrated with current POC devices for on-chip diagnosis and monitoring of infectious diseases at resource-limited settings. Nano/Microfluidic POC diagnostics have significant advantages over conventional diagnostics such as reducing costs and increasing portability and disposability. Future trends in diagnostics focus on extending availability to decentralized hospitals and rural areas more effectively where wireless networks are available. In this review, we provided an overview of recent advances in nano/microfluidic technologies for POC diagnostics. Here, we specified key requirements, such as disposability, cost-effectiveness, ease of use, and portability that can improve and further advance POC diagnostics for a wider clinical use at resource-limited settings. We also discussed current challenges and perspectives of nano/microfluidic POC devices for clinical applications, such as HIV/AIDS, malaria, and tuberculosis. Much progress, however, still remains to be made to further reduce costs and establish standard criteria that can evaluate nano/microfluidic POC devices for use at resource-limited settings.

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