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REVIEW

Advances in addressing technical challenges of point-of-care diagnostics in resource-limited settings

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ABSTRACT

The striking prevalence of HIV, TB and malaria, as well as outbreaks of emerging infectious diseases, such as influenza A (H7N9), Ebola and MERS, poses great challenges for patient care in resource-limited settings (RLS). However, advanced diagnostic technologies cannot be implemented in RLS largely due to economic constraints. Simple and inexpensive point-of-care (POC) diagnostics, which rely less on environmental context and operator training, have thus been extensively studied to achieve early diagnosis and treatment monitoring in non-laboratory settings. Despite great input from material science, biomedical engineering and nanotechnology for developing POC diagnostics, significant technical challenges are yet to be overcome. Summarized here are the technical challenges associated with POC diagnostics from a RLS perspective and the latest advances in addressing these challenges are reviewed.

How to significantly improve health care and reduce disease-related mortality in resource-limited settings (RLS) remains a fundamental issue despite tremendous efforts from national and international levels. The prominent health issue in RLS centers at the prevention and control of infectious diseases such as HIV, TB and malaria, as well as emerging epidemics of influenza A (H7N9), Ebola and Middle East respiratory syndrome coronavirus (MERS-CoV). These devastating pathogens not only inflict enormous economic and disease burdens on RLS, but also increase the risks for worldwide spread of diseases. To date, it is estimated that there are approximately 40 million people who died of AIDS and 36.9 million HIV-infected individuals living across the world [1]. TB, malaria or together with co-infection of HIV, exacerbated the challenges for reducing mortality and improving clinical outcomes [2,3]. Because of the lack of functional disease surveillance system in RLS, outbreaks of influenza A (H7N9), Ebola and MERS-CoV have caused significant global concerns. From the year 2013 to 2015, a total of 681/274 (cases/deaths) of influenza A (H7N9) infection were reported from China, Hong Kong, Malaysia, Taiwan and Canada, with a death rate of 37.1% [4]. In 2014, the outbreak of Ebola virus disease (EVD) caused 4507 confirmed cases and resulted in 2296 deaths in West Africa [5]. MERS-CoV has also caused a total of 1517 cases, major in Saudi Arabia and Republic of Korea in 2014 and 2015, respectively [6]. Thus, the health care systems in RLS should be strengthened to enable better diagnosis, treatment and prevention of infectious diseases.

To improve clinical management of infectious diseases at the individual level and at the population level, accurate, cost-effective and timely diagnosis is the key [7–11]. Given sustained financial support and well-equipped laboratory infrastructure in resource-rich settings (RRS), traditional diagnostic methods such as enzyme-linked immunosorbent assay (ELISA), cell culture, flow cytometry, biopsy, polymerase chain reaction (PCR) and sequencing are widely implemented. On the contrary, clinical symptoms and signs are often used by physicians to make empirical diagnosis with minute aid of lab tests (e.g. lateral flow assays) in RLS, thus leaving infected individuals under-diagnosed and/or loss of follow-up and permitting further disease transmission in the general population. Considering the weakness of health care systems and financial status in RLS, point-of-care (POC) diagnostic technologies are preferred to aid timely diagnosis and treatment of patients in primary care settings, as they can rapidly provide laboratory evidence without reference to sophisticated laboratory infrastructure and well-trained operators [12,13]. Although tremendous efforts have been made in material science, engineering and nanotechnology towards developing POC diagnostic devices, only few of them can meet the WHO criteria for POC testing, that is, affordable, sensitive and specific, user-friendly, rapid and robust, equipment-free, and delivered to those who need it (ASSURED) [14].

From a research point of view, home-brew POC assays include paper-based assays [15–17], microfluidics devices [18–21], and a variety of biosensors have been designed to detect/quantify cells [22,23], viruses [24,25], bacteria [20,27,28], proteins [18,29], or nucleic acids [30,31] from various clinical sample matrices. Among the developed POC diagnostic technologies (Table 1), paper-based assays such as lateral flow strips and dipsticks are the most widely used assays in the clinic because of its simple working procedure, scalable

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Table 1. Brief introduction of POC testing platforms.

Platforms	Brief definition	Typical example
Dipstick	Dipsticks are traditionally designed to detect/measure targets when immersed in urine. Dipsticks can also be used to detect target from a small volume of liquid, with flow of liquid along dipsticks.	Urine test strips
Lateral flow test	A lateral flow test is known for lateral flow of liquid on an immunochromatographic device to detect a target analyte in a clinical sample matrix without the need for specialized and costly equipment.	Pregnancy test strips
Vertical flow test	A vertical flow test is commonly used as an array to detect multiple targets with liquid (including sample and reagents) flowing through the array with the aid of a vacuum or pump.	Vertical flow array
μPADs	μPADs are a new class of microfluidic devices using paper as a substrate. They contain microchannels to direct fluidic flow in a desired pattern.	μPAD-based liver function test
Microfluidics	Microfluidics literally means the study of a small volume of liquid at a scale of sub-millimeter, interfacing with physics, chemistry, nanotechnology, biotechnology and engineering. In POC testing, microfluidics devices have been used to detect biomarkers from a small volume of sample with high sensitivity and specificity.	Microfluidics devices
Nanotechnology	Nanotechnology is a broad research domain with interest particularly in matter with dimension ranging from 1 to 100 nm. In POC testing, gold nanoparticles have been widely used (e.g. lateral flow strips).	Gold nanoparticles; nanopores, nanowells, etc.

μPADs: microfluidic paper-based analytical devices.

manufacturing capability and low cost [32]. In contrast, the other POC diagnostic technologies are still under development, where technical challenges are yet to be overcome, or clinical validations are needed. Here, we summarized the technical challenges that are confronted during assay development and reviewed new research approaches that may provide viable solutions to developing quality-assured POC diagnostics. Because of the unavailability of systematic reports on POC diagnostics from industry perspectives, we limited our literature search to POC diagnostics for infectious diseases in PubMed and Web of Science.

General technical challenges for developing POC diagnostics

Technological complexity as required by the assay working mechanism forms the primary barrier for developing POC diagnostics. For a POC test to be useful in the clinic, it needs to go through the following procedures: i) sample collection, ii) sample processing, iii) testing and/or instrumentation, iv) results analysis and data report, v) interpretation and vi) waste disposal. These procedures do not form a barrier for implementing POC tests in RRS, as required laboratory infrastructure and logistics support are readily available. The multiple testing

procedures, however, pose great technical challenges for POC testing in RLS, especially in rural areas, because of limited laboratory testing capability and lack of skilled operators. Further, insufficient logistical support in RLS forms the secondary barrier for POC testing. For example, there is no clean running water, or uninterrupted electricity supply in rural areas. When a clinical sample is collected, sample processing (e.g. centrifugation or nucleic acid extraction) and instrumentation for nucleic acid assays are required, but not feasible for POC testing because of cost constraints and lack of trained personnel. Some reagents need refrigeration for long-term stability, which is also challenging for POC testing in RLS. Another important technical challenge is safely disposing of biological waste, including used diagnostic devices and clinical samples. In RLS, waste disposal and biosafety are not as stringently regulated as RRS. Open air burning may be the only option for disposing clinical waste and medical devices. Some of the chemicals used in POC assays require special treatment before disposal (e.g. guanidinium thiocyanate). Therefore, technical challenges associated with technological complexity and logistical limitations in protein-, nucleic acid- and cell-based assays, need to be overcome, so that they can be widely used in RLS.

Beyond the technical challenges to perform POC testing in RLS, poor clinical performance of POC diagnostics also form a barrier to bring clinical benefit to the POC settings. In the case of TB testing at the POC, few diagnostics can meet the clinical requirement. Conventional smear microscopy, owing to its inexpensiveness and rapidity, is commonly used in RLS. However, this method is limited by low and varying sensitivity (34–80%) [33] with a poor detection limit of 5000–10000 colony forming units (CFU)/ml [34]. Also, the morphology detection of *Mycobacterium tuberculosis* (MTB) is not specific and MTB should be differentiated from other mycobacterial strains (e.g. *Mycobacterium kansasii* or *Mycobacterium marinum*). For POC testing, lateral-flow based antigen/antibody strips have also been developed for detection of TB from serum in RLS. However, these strips suffer from low accuracy for rapid diagnosis of TB [35,36]. Another type of lateral flow assay has been developed to detect lipoarabinomannan (LAM, a component of MTB cell wall) antigen from patient urine samples. Although this assay is inexpensive and simple-to-use, it has a low sensitivity of 28.2% in patients who had positive culture of TB [37]. Nevertheless, this assay had a sensitivity of 66.7% in detection of TB in AIDS patients with CD4 cell count <50 cells/μl [37], which may help the management of AIDS-TB co-infection patients in sub-Saharan countries. Similarly, poor clinical performance is also experienced when using HIV lateral flow strips for detection of acute HIV-infected individuals [38]. Considering the suboptimal clinical performance of current POC diagnostics and urgent need in RLS, the niche of their specific applications should be well selected and validated, particularly to remove any false-negative and false-positive results at the patient level.

The requirement for an instrument proves challenging for POC testing in RLS. Ideally, POC assays should not rely on the use of any equipment in RLS. Otherwise, the cost on purchasing equipment and routine maintenance, particularly for large equipment such as flow cytometers, would be inevitable, which proves to be financially unsustainable and technically

challenging for RLS. As recommended by the WHO Sexually Transmitted Diseases Diagnostics Initiative in 2004, rapid assays should be equipment-free for POC testing [14]. Nowadays, the concept of POC testing has gone beyond of the scope of rapid lateral flow assays, especially with the mainstream of developing molecular diagnostic devices with fast advances in microfluidics and nanotechnology [11,39]. The use of hand-held, lightweight, battery-operated instruments is gradually becoming acceptable for performing molecular diagnosis with basic laboratory infrastructure. These portable instruments may vary from an eggbeater [40] or a solar panel [41], to a smart phone with/without add-on modalities. Although these POC assays are not equipment-free, they can be implemented in non-laboratory settings with basic logistical support [23,41].

Another important technical challenge, which is often neglected by POC assay developers, is the user-interface. However, good the assay result is, it should be simple-to-understand and easily interpretable for less-skilled health care workers in RLS and even patients in home-settings. For instance, glucose meters are one of the representative examples of successful POC assays on the market. The user-interface of glucose meters is further improved by adding result-reading capability [42]. As a significant portion of diabetic patients are elderly and/or vision-impaired, audio reporting of test results by a speaker not only eliminates the visual difficulty of reading test results, but also increases the likelihood of patient adherence for glucose self-monitoring. Another example is a recent development of blood typing using a paper-based device, termed as a group legible immunohematology format (GLIF) card [43]. The improvement on user-interface is that the assay result is self-interpretable, showing 'A', 'B' 'O' on the device for blood type A, B or O, respectively [43]. Thus, end-users would readily understand the results without interpretation from a health care worker, permitting self-testing in RLS. However, few assays are now equipped with good assay interface for end-users with varying levels of technical training, which should be improved in up-coming POC assays.

Technical challenges associated with protein-based POC assays

Protein-based POC assays are the most common assays that have been developed and used in the clinic. In general, protein – either antigens or antibodies – can be easily immobilized on paper surface, as well as the surface of microfluidic devices, polystyrene beads and magnetic beads through a variety of surface chemistry approaches. The most well-known assay formats are lateral flow assays, agglutination assays, and dipstick assays, and they have been used to detect biomarkers of infectious diseases, cancer, and non-communicable diseases (e.g. cardiovascular diseases). Despite, wide applications in the clinic, protein-based POC assays suffer from unsatisfactory sensitivity, batch-to-batch variation, low multiplexing capability and subjective result interpretation. In addition, proper selection of antibody-antigen pair is time-consuming, labor-intensive and costly during assay development.

The detection limit of protein-based POC assays can be improved by signal amplification, protein enrichment, the use of sensitive signal agent such as a fluorescence dye, and/or a more sensitive detection mechanism. The strategy of signal amplification can be utilized through the biotin-streptavidin approach with/without enzymatic reaction. It should be cautioned that background noise may also be enhanced along with target signal, and therefore this approach does not necessarily increase the signal/noise ratio. A microtip-based system was developed to enrich the concentration of MTB through electro-osmosis. Coupled with fluorescence labeling, this method detected MTB cells as low as 8000 CFU/ml within 10 min [44]. Although this method is not sensitive enough for POC testing, this method can be coupled with other detection strategies (e.g. nucleic acid amplification methods) for further improvement. Another approach to enhancing the detection limit in lateral flow assays, is to use fluorescence detection to replace gold nanoparticle agglutination-induced visualization by the naked eye [45]. Although this approach increased the detection limit of nucleic acid by 10-fold compared with traditional lateral flow strips, a fluorescence reader was needed, making it less suitable for use in RLS. Recently, localized surface plasmon resonance (LSPR) (Figure 1A) [46,47] and giant magnetoresistive (GMR) sensors [48] have been developed to sensitively detect very low concentrations of proteins and biotargets from biological samples, indicating that ultrasensitive detection strategies may provide a solution to significantly improving the detection limit of POC assays, provided that they can be made portable and inexpensive.

To enhance the multiplexing capability of POC assays, microfluidic devices have been introduced. With multiple branches built into a single microfluidic device, the multiplexing capability has been significantly enhanced. In a recent report, ligands specific for their targets were separately immobilized in individual channels, markedly reducing the consumption of biological samples [49]. The other approach to multiplexing is to immobilize multiple reagents on different spots in a single microchannel. For example, simultaneous detection of HIV and syphilis by ELISA was achieved on a microfluidic device, termed as 'mChip' for POC testing in Rwanda (Figure 1B) [50]. Besides microfluidic devices, the multiplexing capability of POC assays has been expanded on paper, including paper microfluidics and vertical flow arrays. In the paper microfluidics-based approach, paper has been engineered to have hydrophilic channels to direct fluid flow to multiple spots where different detection ligands are immobilized [51]. Microfluidic paper-based analytical devices (µPADs) are developed by the Whitesides group to enhance the multiplexing capability at low cost [51,52]. They have demonstrated that a paper-based test can measure both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) to detect drug-related hepatotoxicity in RLS (Figure 1C) [53]. Vertically flow array is another attractive approach for multiplexing [54]. As so far demonstrated, this assay format can be used to simultaneously measure IgE reactivity against 10 allergens on an array format. Not only did this approach enhance the multiplexing capability, but also eliminated interference with detecting multiple targets on
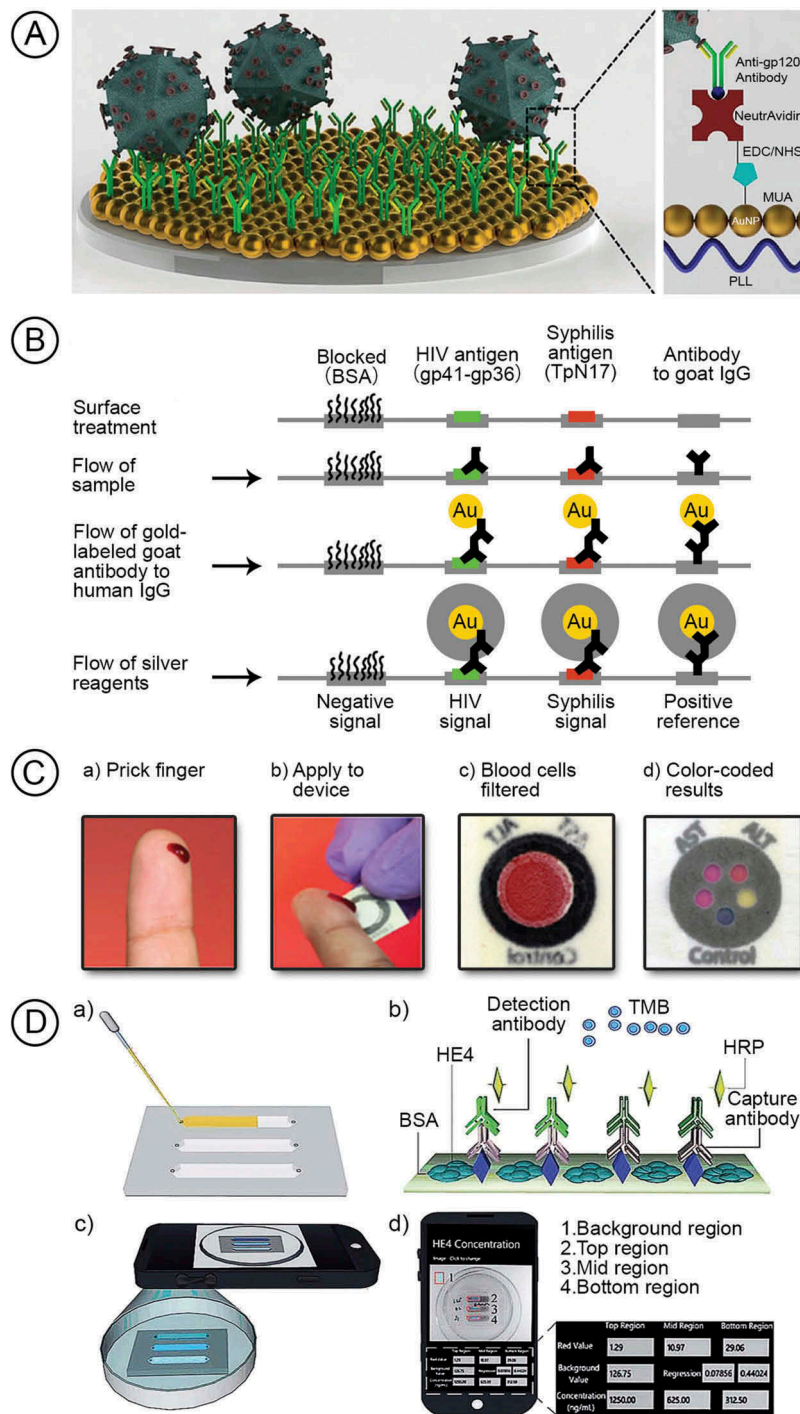
Figure 1. New approaches to addressing technical challenges associated with protein-based POC testing in resource-limited settings. (A) Ultrasensitive detection of biotargets using a nanoplasmonic detection platform [47]. (B) Simultaneous detection of HIV-1 and syphilis in a microfluidic device. Image was adapted with permission from [50]. (C) μPAD-based liver function test. Image was adapted with permission from [53]. (D) Cell phone-based colorimetrical detection of ovarian cancer from urine on-chip [18].

the same array; a significant problem seen with lateral flow assays because of the downstream effect [54].

In POC assays, colorimetrical detection is often used, which may result in subjective result recognition and interpretation. This issue has been recently resolved by using a portable reader or developing a mobile App to analyze results objectively [18,55,56]. In addition, the portable reader can detect 'dubious' results and can discriminate with a much higher resolution

compared with the naked eye. Recently, an integration of cell phone based colorimetrical detection with on-chip ELISA was developed for early ovarian cancer detection from urine (Figure 1D). Clinical validation showed that this assay had a sensitivity of 89.5% at a specificity of 90% [18]. Thus, the introduction of a portable reader can potentially increase the sensitivity and the accuracy for quantification or semi-quantification.

Two other technical challenges associated with protein-based POC assays are proper antigen/antibody selection and batch-to-batch variation during assay development and manufacturing. To improve sensitivity and specificity, careful asses antigen-antibody reaction is of importance. Whether there is a need for monoclonal antibody for the assay, or there is a need to evaluate different monoclonal antibodies for different epitopes should be well balanced against cost and complexity. Particularly for multiplexed testing, the cross-reaction between unpaired antigen/antibody should be avoided by stringent assessment. Despite careful selection of antigen/antibody pairs in assay development, antibodies gradually lose immuno-affinity from different passages of antibody-production cells. For large-scale production, batch-to-batch variation should be minimized with appropriate quality control and quality assurance methods.

Technical challenges associated with nucleic acid-based POC assays

Nucleic acid-based POC assays have been extensively explored in the last decade with the significant drive to detect viruses and bacteria in non-laboratory settings. One of the striking needs is to develop HIV viral load testing to directly measure the number of circulating HIV virions so as to monitor antiretroviral therapy (ART) in AIDS patients in RLS. Although several nucleic acid amplification test (NAT) approaches have been designed to aid ART monitoring, they are technologically complex and expensive, limiting their use in well-equipment laboratories by skilled operators [7,9]. Recently, there are considerable advances in developing field-implementable instruments to measure HIV viral load in RLS. However, the technical challenges in sample collection/sample processing, nucleic acid extraction, amplification and instrumentation still remain. The challenges as discussed here also occur with other NAT-based POC applications.

Sample collection/processing is the primary step for NAT, and is the bottleneck at the POC. Generally, plasma samples are needed and the presence of blood cells interferes with the downstream nucleic acid amplification. For example, it has been reported that the presence of hemin and hemoglobin in nucleic acid extract can inhibit the amplification efficiency in PCR [57]. Thus, a method to rapidly isolate plasma from unprocessed whole blood would be welcome for NAT-based POC applications. A number of studies have been reported for plasma separation based on the mechanisms of size-exclusion [58], hydrodynamic forces, or microchannel geometry [59]. Although these approaches can extract plasma from unprocessed whole blood without using a centrifuge, they still suffer from the use of an external instrument (either a pump or a vacuum), low-volume of blood processing, hemolysis, dilution of blood, or cell contamination. Alternatively, dried blood spot (DBS) is used as a substrate to collect blood samples in RLS and transfer them to well-resourced laboratories [60]. However, this approach cannot solve the sample processing issue faced in field-testing. Other types of clinical sample matrices (e.g. sputum for TB and nasopharyngeal swab samples for influenza) also present significant challenges for field sample processing prior to NAT in RLS.

Nucleic acid extraction is another significant challenge for performing NAT at the POC. The gold standard method for nucleic acid extraction was developed by Boom et al. in 1990 using chaotropic agents [61]. This approach required multiple loading steps of reagents, a centrifuge for the spin column and subsequent washing steps. Although several types of automated nucleic acid extraction instruments are commercially available, it is challenging to adapt these instruments for POC testing in a cost-effective manner. A new method for nucleic acid extraction is creating a temporary paper fluidic circuit where complex chemicals are sequentially processed through folding and unfolding of 2D surfaces [62]. Although this microfluidic origami device is rapid (<30 min), this method may not be applicable when heating is needed for nucleic acid extraction. In addition, the efficiency for extracting target nucleic acid compared with the gold standard still requires further verification.

Another paper-based nucleic acid extraction approach was recently reported for detection of influenza A (H1N1) from human nasopharyngeal specimens [63]. In this method, a piece of poly(ether sulfone) (PES) filter paper was attached to a cellulose absorbent pad for capillary force movement of sample (Figure 2A). For nucleic acid extraction, a nasopharyngeal specimen was lysed and then added to the PES filter paper. Influenza A (H1N1) RNA was isolated on the PES filter paper, and subsequently purified by rinsing with ethanol. This extraction method is easy and rapid, providing a centrifugation-free method for nucleic acid extraction outside of laboratory settings, which, as demonstrated, can be coupled with loop-mediated isothermal amplification (LAMP) and lateral flow strips for amplification and detection, respectively.

Nucleic acid can be amplified with certain enzyme or a set of enzymes isothermally (e.g. nucleic acid sequence based amplification (NASBA), recombinase polymerase amplification (RPA), LAMP or by temperature cycling (i.e. PCR). Either way, temperature control is critical to ensure amplification efficiency and accuracy. However, controlling temperature to such a precise degree proves to be challenging in RLS, given the lack of stable electricity supply. Therefore, isothermal amplification is more often used than PCR for performing NAT at the POC to eliminate rapid cycling of heating and cooling and to reduce/eliminate power consumption associated with thermal cycling. For isothermal amplification, a chemical heating approach has been developed [64,65]. In this approach, an exothermic reaction of calcium oxide and water was used to generate heat (Figure 2B), while engineered phase change materials were used to buffer the temperature to approximately 65 °C for isothermal amplification of HIV by RT-LAMP [65]. This non-instrumented nucleic acid (NINA) heater was further developed by adding thermally insulated, stainless-steel canisters and plastic screw-top lids to allow three amplification reactions to run simultaneously [64]. With this simple electricity-free heating instrument, amplification of HIV RNA was made possible, facilitating HIV-1 RNA testing in RLS. Considering the lack of stable power supply in RLS, this electricity-free approach may provide a generic and practical temperature control for isothermal amplification and microfluidic PCR with continuous flow design.

Recently, a solar heating system was developed to provide electricity for PCR amplification, which is generally energy-intensive because of rapid thermal cycling [40]. In this method,
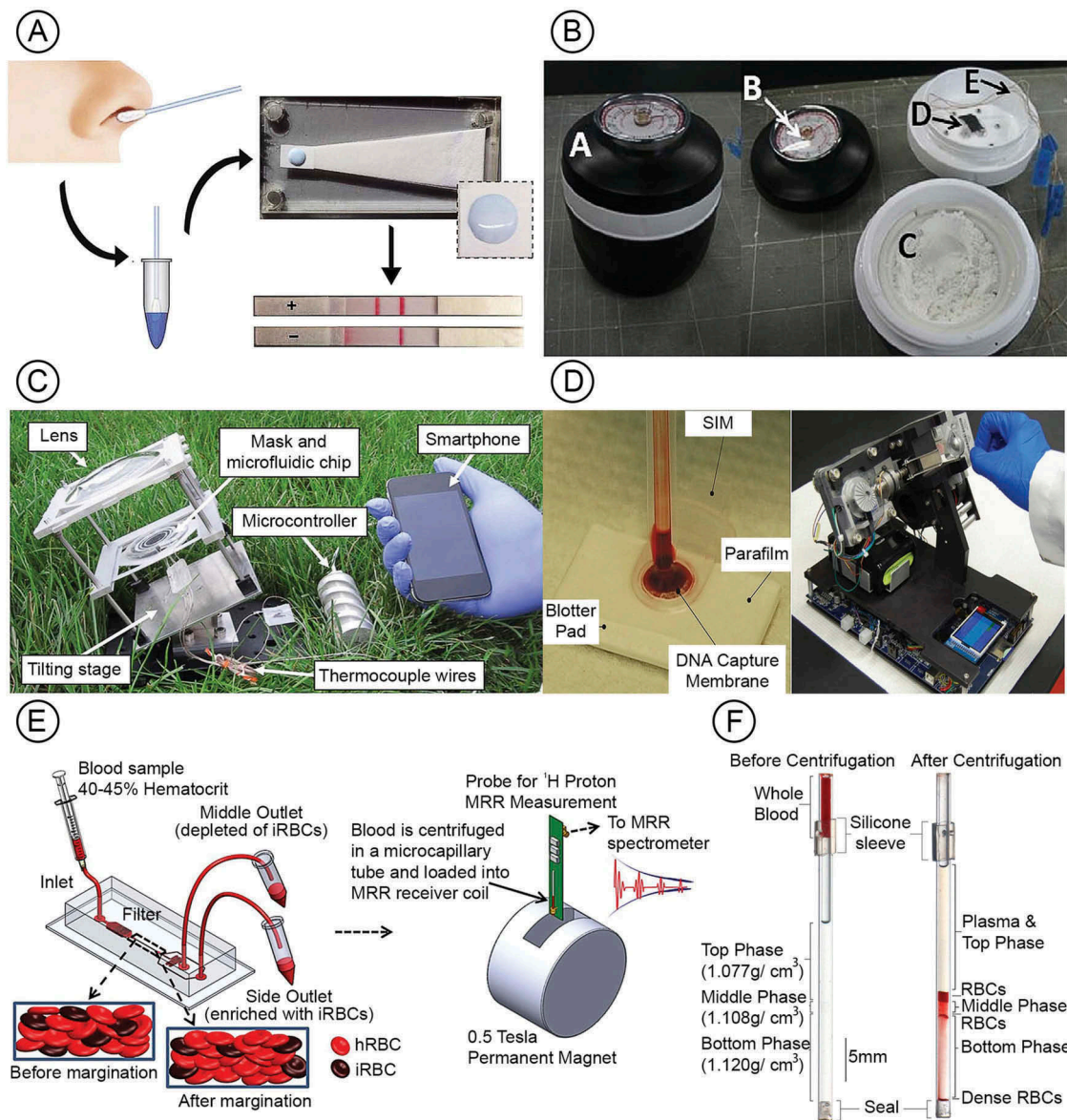
Figure 2. New approaches to addressing technical challenges associated with nucleic acid-based and cell-based POC testing in resource-limited settings. (A) Paper-based extraction of influenza A (H1N1) RNA for POC testing. Image was adapted with permission from Rodriguez NM, Linnes JC, Fan A, *et al.*, Paper-based RNA extraction, *in situ* isothermal amplification, and lateral flow detection for low-cost, rapid diagnosis of influenza A (H1N1) from clinical specimens. Anal. Chem., 2015. 87(15): 7872-9. Copyright 2015 American Chemical Society [63]. (B) An exothermic chemical reaction-based electricity-free heater for isothermal nucleic acid amplification [65]. (C) A solar thermal PCR system for detection of KSHV/HHV-8 at the POC [41]. (D) Miniaturized qPCR with a certain degree of automation to detect HIV-1 DNA from whole blood. Image was adapted with permission from [67]. (E) Microfluidics-based cell enrichment coupled with magnetic resonance relaxometry for sensitive detection of malaria parasites [74]. (F) Identification of sickle cell disease using a density-based separation device [75].

the solar panel converts sunlight to heat and provides the energy to maintain three temperature zones of 95 °C (denaturation), 60 °C (annealing) and 72 °C (extension). In the design, sunlight is focused through a lens, passes through a ring-shaped mask, and is then converted into heat on an absorber layer (Figure 2C). A continuous-flow microfluidic PCR chip is designed to pass a sample through three temperature zones for 35 cycles. To precisely control the desired temperatures on the chip, three thermocouples are connected to a microcontroller and a mobile app is developed. In this report, the authors demonstrated that the solar PCR amplified Kaposi's sarcoma herpes virus (KSHV/HHV-8) DNA within 30 min with power consumption reduced by 100-fold compared with traditional thermal cyclers. Since sunlight is ubiquitous, the solar PCR

system shows great potential for performing molecular diagnosis where electricity is limited.

An important challenge with developing nucleic acid-based POC assays is the integration and automation of different assay modules in a stand-alone system. There have been some attempts to develop a fully integrated system for molecular diagnosis at the POC [64,65]. For example, a fluorescence-based sample-to-answer quantitative PCR (qPCR) system (Figure 2D) was developed to detect HIV-1 proviral DNA in infants at the POC, aiming for early diagnosis [65]. All the required reagents were freeze-dried and stored on a disposable card. To facilitate PCR, a battery-operated bench-top PCR instrument was developed containing three modules for Electromechanical Reagent Delivery (ERD), Thermal Cycling,

and Fluorescence Detection. The proof-of-concept system detected HIV proviral DNA at the level of 5000 copies per reaction, but still needs further improvement for better sensitivity. In addition, this system could only test a single sample at once and suffers through low throughput, making it impractical to use in clinics. Although this system is battery-operated and portable, it is still technologically complex and cannot be used for POC testing in RLS.

A significant improvement in molecular diagnostic technologies is the development of GeneXpert platform from Cepheid [68]. This platform is able to automate the entire process required for sample processing, nucleic acid extraction, amplification and detection, offering a 'sample-in and answer-out' solution, which is ideal for end-users. As demonstrated so far, this platform can detect MTB and resistance to rifampin from untreated sputum samples in <2 h. For detection of MTB in culture-positive patients, this platform has a sensitivity of 92.2% and a specificity of 99.2%; for detection of rifampin resistance, this platform has a sensitivity of 97.6% compared with phenotypic drug-susceptibility testing. However, this system has not been widely adopted for POC testing in RLS, as it is still relatively expensive (instrument, disposable devices and maintenance), and it requires stable power consumption and relatively long turnaround time. Thus, developing an ideal POC NAT platform yet remains an unaddressed technical challenge.

Technical challenges associated with cell-based POC assays

Cell-based assays, because of their complexity, are not generally used for POC testing. However, a few examples of cell-based POC assays have been designed and developed to tackle global health challenges (e.g. CD4 cell count and malaria detection). CD4 cell count is important to initiate ART in AIDS patients. Within the global context of expanding ART in RLS, a variety of portable CD4 cell count approaches have been developed based on simplified flow cytometry, fluorescence staining, electrical sensing, lensless imaging, centrifugation-induced condensing and micro-a-fluidics ELISA [9]. Simplified flow cytometers such as FACSCount (Becton Dickinson, Franklin Lakes, NJ, USA), EasyCD4 (Guava, San Francisco, CA, USA), CyFlow (Partec, Jettingen-Scheppach, Bavaria, Germany), and Pan-leucogating (Beckman Coulter, Kraember Blvd Brea, CA, USA) can provide reliable results compared with FACSCalibur, a gold standard method for measuring CD4 cell count. However, they still need relatively expensive equipment, instrument maintenance, and adequate operation training, which limit their widespread use in RLS. PIMA, a portable, fluorescence CD4 cell count from Alere, has been tested at multi-sites. A WHO multicenter evaluation showed that the PIMA CD4 is more suitable for identifying adult AIDS patients eligible for ART than monitoring efficacy [69]. Further, more negative underestimation was seen with fingerprick versus venous blood when used in the PIMA CD4 [70]. The need for basic laboratory infrastructure to run fluorescence imaging precludes PIMA CD4 from being used at lower tiered laboratories in RLS. In addition, it has been noted that the accuracy of PIMA CD4 is affected by a critical but variable step of capillary sampling [71].

Three proof-of-concept cell-based POC assays have been developed aiming to provide a simple, inexpensive and rapid solution to initiating and monitoring CD4 cell count in AIDS patients in RLS [21,22,72]. First, a lensless imaging technique coupled with microfluidics showed the feasibility to count CD4 cells after immune-capture from unprocessed whole blood through immune-affinity by anti-CD4 antibodies. The image setup was simple using only an inexpensive CCD, and the shadow images of captured CD4 cells were processed using ImageJ to rapidly provide CD4 cell count [22]. The field-testing showed that this method was easy-to-use and it was performed by local health care workers with minimum training [23]. Second, a micro-a-fluidic device was developed with magnetic beads on which CD4 cells were captured through specific antibodies. The entire testing process was automated by a portable, lightweight and inexpensive instrument [21]. Third, a centrifuge-based approach was developed to condense CD4 cells labeled with heavy microparticles in a micro-capillary column [9,72]. The height of labeled cells in the column correlated with the number of CD4 cells from whole blood and provided an attractive method for guiding ART in RLS. However, these three methods need further multisite clinical trials for assessment of sensitivity, specificity, accuracy and precision.

Recently, the magnetic susceptibility of cells was utilized to achieve cell-based testing at the POC [73]. It is known that *Plasmodium* spp.-infected red blood cells (RBCs) have an increased paramagnetic susceptibility, as malaria parasites can convert hemoglobin into hemozoin crystallites. An inexpensive benchtop MRR system was developed to detect hemozoin particles in RBCs, with a detection limit of <10 parasites/µl in a volume of 10 ml of whole blood within a few minutes [73]. Further, the authors overcame the fluctuation of MRR baseline among individuals by establishing a baseline of MRR. Together with a microfluidic-based cell enrichment approach (Figure 2E), the authors detected as low as 0.0005% of ring stage parasites [74]. This method requires minimal sample processing steps and no chemical or immunolabeling, making it attractive for rapid malaria diagnosis in RLS [73,74]. A density-based device was developed to detect sickle cell disease (SCD) with using an aqueous polymer mixture in which dense cells can be visualized after centrifugation [75] (Figure 2F). Although this method is sensitive and inexpensive, this method may suffer from the lack of a generic cell density for confirming SCD. Another approach, termed 'magnetic levitation', was recently reported for detection of cancer cells from whole blood [76] and for differentiating sickle RBCs from healthy RBCs [77]. Because of a unique levitation profile for different cell types, the authors differentiated breast, esophageal, colorectal and non-small cell lung cancer cell lines spiked in whole blood. This rapid method requires no electricity, and is label-free, inexpensive, and easy-to-use, showing great promise for cancer detection at the POC. Nevertheless, all these magnetic susceptibility-based approaches need further clinical trials for validation and are not currently approved for use in RLS.

Conclusion

POC diagnostics play a central role in confirming clinical diagnosis, monitoring treatment, and predicting prognosis in the clinic, as well as disease surveillance on a large population level. For RLS, POC assay are critical as it may be the only diagnostic option on-site to help physicians for patient care and disease management. However, there is no single POC technology so far that can detect biomarkers (nucleic acid, antigen, antibody, etc.) efficiently and specifically without any clinical constraints. Nevertheless, multiple approaches have been developed to specifically tackle the technical challenges associated with protein-, nucleic acid- and cell-based POC assays. Therefore, we envision that with joint efforts from multi-disciplinary fields by clinicians, biologists and engineers, fully integrated assays would eventually appear to enable much needed POC testing particularly for RLS.

Expert commentary

Developing POC assays for RLS would seem to be a simple task using low-cost materials and low-end technologies. But it is practically the opposite! Completing this task requires not only multi-disciplinary expertise from material science, hardware and software engineering, immunology, molecular biology and medicine, but also appreciation of regulation, culture and policy in RLS. This task is daunting, because what is needed is the development of high-end, clinical diagnostic assays on a limited budget with strict operational requirements in suboptimal conditions. Therefore, it is much more challenging to develop a POC assay for RLS rather than establish a sophisticated protocol for high-end diagnostic technologies for RRS. Working through these challenges would be highly rewarding; imagine delivering practical POC assays to save lives, treat patients and prevent diseases in RLS, where it is most needed because of the much higher disease burden in these locations.

We must divide the context of POC diagnostics into different testing scenarios and conquer their specific challenges during assay development. As discussed earlier, there are many technical challenges need to be addressed to successfully develop a POC assay for RLS. However, we need to divide the technical challenges and associate them with different tier or level of POC scenarios (Table 2). In RLS, there are considerable variations in terms of diagnostic capabilities for patient care [13]. In RLS, the first tier, national reference laboratories, have state-of-the-art diagnostic technologies, well-trained technicians, and sophisticated laboratory infrastructure and have the capacity to perform ELISA, PCR, flow cytometry, cell culture and DNA sequencing. In the second-tier, regional laboratories, may have limited access to diagnostic capability, less-trained personnel and financial support. They are generally equipped with a light microscope, spectrophotometer and basic laboratory infrastructure such as a benchtop centrifuge, a heater, or a refrigerator. They also have limited capability to perform immunoassays, blood chemistry and bacterial culture. The third-tier, district laboratories, may exist in community-based hospitals and generally do not have the diagnostic capability except for lateral flow assays or agglutination assays. In the rural areas, there is no hospital, laboratory, clean water or even

Table 2. Technical challenges for POC testing in different lab settings in RLS.

Lab settings	Instrument/device	Operator	Logistics
First tier	Bulky, expensive instruments; appropriate quality control	Sufficient staff training and quality assurance measures	Clean water, stable power supply, dedicated lab space, cold storage, appropriate waste disposal
Second tier	Relative small and affordable instruments; basic quality control	Basic staff training; basic quality assurance measures	Varying conditions for water/power supply, may have limited laboratory space and cold storage facility, basic waste disposal
Third tier	Lightweight, portable instruments; or stand-alone devices, basic or no quality control	Less-trained technicians; basic or no quality assurance	No dedicated space for specific testing; basic or no contamination control; no reliable water and power supply, varying humid and temperature conditions, no appropriate waste disposal
Rural areas	Battery-operated instruments; stand-alone devices, basic or no quality control	Physicians who have less technical training	No laboratory space; no reliable water and power supply, varying humid and temperature conditions, no appropriate waste disposal

a reliable power supply. Patients are seen by health care workers with little formal medical training or laboratory training. Training thousands of front line health workers to use a POC device is not a trivial task either. Clearly, there is no single test that can meet distinct levels of clinical need in different POC scenarios with varying diagnostic capacities. Therefore, we need to divide and define the technical challenges associated with POC assay development and conquer them in the right context.

To overcome the technical challenges, other barriers also need to be smoothly removed. These barriers include limited transcontinental collaboration, conflict of intellectual property sharing, and lack of funding mechanisms as identified in an international workshop of next generation POC diagnostics [78]. In addition, POC assay developers need to appreciate the technical challenges on the ground. For example, in most academic laboratories, students and postdocs, who work on POC assay development, have little idea of real situation in RLS. The use of instrumentation, fluorescence or other sophisticated optic detection mechanisms, harsh chemicals or reagents with short shelf-life, would not be a fit for POC testing in RLS. Furthermore, strong collaboration between academic and industry is needed to place diagnostic products in the pipeline of POC assay development. In academic laboratories, there is a lack of quality control and quality assurance when developing proof-of-concept diagnostic technologies. As a result, the reproducibility, accuracy and precision of the developed diagnostic technology during field-testing are often compromised. Through strong collaboration with industry/company partners, and appreciation of the needs on the ground, academic laboratories would be equipped with the capability to tackle many

technical challenges so that POC diagnostic technologies can bypass the 'valley of death' and eventually reach the market.

Mobile-based POC diagnostics (mHealth) have been rapidly explored to provide health care monitoring. As developed, smartphones, owing to their portability, built-in sensors, and data transfer/processing capabilities, have been used to measure heart rate, breathing rate, and blood pressure, as well as to disseminate health information. Additionally, smartphones have been coupled with miniaturized microfluidics devices for rapid data collection, result analysis, and *in vitro* diagnosis. Further, wearable devices can be wirelessly connected to smartphones for real-time monitoring of health conditions and treatment efficacy. With decreasing prices for smartphones, these mHealth-based approaches can significantly reduce the equipment requirement for implementing POC assays in RLS.

Five-year view

In the next 5 years, more innovations in technology will be made to address challenges associated with POC diagnostics in RLS. For protein-based POC diagnostics, new POC diagnostics designed under the frame of the WHO ASSURED guidelines would appear. Particularly, the user-interface between diagnostics and end-users will be significantly simplified to appreciate the testing results and thus to enhance the acceptability of protein-based POC diagnostics. Also, low sensitivity and limited multiplex capability of protein-based POC diagnostics will be improved to the level of benchmark assays that are typically performed in hospital settings. For nucleic acid-based and cell-based POC assays, hand-held, battery-operated, inexpensive instruments would be accepted for POC testing in RLS. Because of the nature of technological complexity and

multi-step procedures for cell- and nucleic acid-based POC assays, full integration from sample collection to data report would be realized and the entire process would be automated to the maximum degree while having little hands-on requirement from end-users. Cost would be still an issue to debate for developing POC assays, but it would not be the primary constraint from technology innovation as witnessed by the significant cost reduction in sequencing technologies. By then, an ideal POC assay, regardless of detection of protein, nucleic acid, cells, would appear to practically aid the control and prevention of infectious diseases on-site in RLS by 2020.

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Key issues

- The technical challenges of POC diagnostics need to be divided and defined according to the specific requirements for clinical needs and operation conditions so that they can be addressed in the right context.
- Low sensitivity and limited multiplexing capability of protein-based POC diagnostic technologies should be improved to accommodate clinical needs in RLS under the WHO ASSURED guidelines.
- Sample-to-answer capability should be achieved for nucleic acid- and cell-based POC assays with the aid of inexpensive, portable and battery-operated instrument to streamline their translation to RLS.
- Joint efforts from interdisciplinary research from material science, biomedical engineering and computer science, as well as collaboration with industry are urgently needed to synergistically speed up the development of POC diagnostics.
- Cell phone should be increasingly integrated with POC diagnostics during assay development for data collection, analysis, reporting and documentation because of its widespread use in RLS.

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