Low-Cost Optical Assays for Point-of-Care Diagnosis in Resource-Limited Settings

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ABSTRACT: Readily deployable, low-cost point-of-care medical devices such as lateral flow assays (LFAs), microfluidic paper-based analytical devices (μPADs), and microfluidic thread-based analytical devices (μTADs) are urgently needed in resource-poor settings. Governed by the ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverability) set by the World Health Organization, these reliable platforms can screen a myriad of chemical and biological analytes including viruses, bacteria, proteins, electrolytes, and narcotics. The Ebola epidemic in 2014 and the ongoing pandemic of SARS-CoV-2 have exemplified the ever-increasing importance of timely diagnostics to limit the spread of diseases. This review provides a comprehensive survey of LFAs, μPADs, and μTADs that can be deployed in resource-limited settings. The subsequent commercialization of these technologies will benefit the public health, especially in areas where access to healthcare is limited.

KEYWORDS: lateral flow assays, low-cost, paper-based sensors, paper-based analytical devices, resource-limited settings, SARS-CoV-2, on-site testing, thread-based sensors, point-of-care medical devices

In resource-limited areas, especially low- and middle-income countries (LMICs), people still face limitations to access high-quality medical treatment and devices. Affordability of local medical production is essential to avoid importing high-cost medical devices from developed countries. Timely and adequately accurate screening of health conditions in their premature stages is crucial for successful and effective medical outcomes. Protocols requiring specialized laboratory equipment prolong the processing of samples and overall diagnostic time, especially in a resource-limited environment. It is of utmost importance to ensure that the next generation of medical devices provide economical, reliable, and rapid screening of medical conditions outside of the laboratory environment. The development of point-of-care (POC) diagnostic devices for tuberculosis, bacterial pneumonia, malaria, and syphilis could potentially prevent the death of 1.2 million people worldwide annually.1−3 The World Health Organization (WHO) has set the criteria for evaluating POC devices for specific diseases (e.g., human immunodeficiency virus- HIV), but are broadly applicable to other POC diagnostic platforms, intended for monitoring medical conditions in resource-limited settings. These instruments should contain the following characteristics: affordability, sensitivity, specificity, user-friendliness, rapidity/robustness, equipment-free (portability), and deliverability to end-users.

The WHO has given these characteristics the acronym, ASSURED. The WHO has also recommended that the devices should be workable in resource-limited settings, such that they could be operated by personnel with minimal training under nonsterile conditions without access to reliable electrical power (Figure 1).4−8

In this review, we surveyed the recent advances in lateral flow assays (LFAs), microfluidic paper-based analytical devices (μPADs), and microfluidic thread-based analytical devices (μTADs) as POC medical devices. These analytical platforms are based on colorimetric transduction, which facilitates transportation and access to poor areas of the world. Readouts are generally achieved through reference cards which can yield semiquantitative results or through a portable color analyzer, which can yield results with an ever-increasing degree of sensitivity and specificity. We have limited the scope of the review to POC devices without external reader (i.e., equipment-free) when possible or with accessible reading devices.

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LFDAs are among the most widely used devices because they are rapid, simple-to-use, and amenable for POC.6,9,10 Commercial LFDAs are performed over a strip composed of a range of materials overlapped onto one another and mounted onto a backing card. The biorecognition and further signal transduction have been traditionally performed by specific antibodies tagged with colloidal gold or color-generating particles. The measurement for LFDAs is low-cost since the presence of lines on the device can be detected by the naked eye without needing sophisticated laboratory equipment or a portable device (such as smartphones) and trained staff. Thus, the LFDAs are powerful candidates for on-site diagnostics in LMICs.

μPADs are by far the most studied type of microfluidic analytical devices. This can be partially attributed to several advantages of utilizing paper as a basis for a de novo low-cost microfluidic device.11 The cost of the paper-based device is around only 30–50 cents,12 and each measurement costs only 2–3 US dollars. Conventional μPADs are based on a lateral flow test that guides liquid samples from an inlet to desired outlets, where chemical or biochemical reactions take place by imbibition. It is flexible, economical, easy to use, disposable, portable, and compatible with various chemical and biochemical reagents. It is convenient for use with small volumetric measurements. It relies on capillary action as a basis for mass transfer and does not necessarily require external power. Moreover, it controls fluid flows with relative ease, relying on hydrophilic channels and hydrophobic barriers, as well as tunable paper properties such as hydrophilicity, reactivity, permeability, etc. The first recognizable construction of a μPAD device was accomplished by Müller and Chegg in 1949 by integrating paraffin onto a filter paper with the desired pattern.13 A mixture of pigments was then eluted through the μPAD. Upon the monochromatic light shining through the piece of paper, transmittance could quantitatively measure the back-calculated concentration. However, Müller and Chegg’s publication did not lead to much follow-up research. It was only in 2007 that the reintroduction of a multiplexed μPAD by Whitesides’ group for the simultaneous quantification of glucose and protein in urine lead to a renewed interest and continued innovation in the field up to the present day.14 Manufacture of μPADs is often relatively simple, since various methods of fabrication have been studied and achieved widespread use in analyzing various reagents. μPAD can be constructed using inkjet etching,43 inkjet printing,52 pen plotting,27,28 PDMS printing,29 photolithography,30–39 wax-dripping,40,41 computer-assisted cutting,15,17–21,42 flexographic printing,43 stamping,44–46 wax printing,47–52 screen printing,53,54 plasma treatment,55–57 handheld corona treatment,58 wet-etching,59 and vapor-phase deposition,50–62.

Instead of paper, μTADs utilize thread as a baseline since thread is a low-cost, lightweight solution with tunable wetting characteristics.63–65 Threads comprise thin strands of cotton, nylon, and other fibers that are fixed to a supporting film where liquid samples are capillary-wicked through the threads by their intrinsic hydrophilicity without the need of an external pumping system. Besides, threads show a higher mechanical strength than paper under wet conditions and small channel width because of the better confinement of fluids.66 Therefore, μTADs are a promising option to implement mass healthcare testing in resource-limited areas. Although there are many good reviews on LFDAs, μPADs, and μTADs for equipment-free POC tests,6,66,68 a systematic focus on utilization of low-cost microfluidic devices in resource-limited areas is rarely reported. In this review, we summarize commercially used and recently developed POC medical devices in LMICs. We have selected several biomarkers and types of samples that could be used in these devices and are of high interest for resource-limited areas. Moreover, mandatory regulations requiring placement of a POC device into the market were also summarized. Finally, we discuss future directions of the POC devices.

### BIOMARKER SCREENING

This section evaluates the suitability of various low-cost diagnostic devices for their application at the point-of-care in LMICs countries and how close they are to implementation in the field. The devices are classified according to the type of targets, covering the most severe diseases currently in need of an urgent diagnostic address.

**Virus.** Viral infection presents a significant risk all around the world but especially in areas with limited screening abilities. The 2014–2016 Ebola outbreak in West Africa, which led to an estimated death toll of 11,000 people, emphasized the importance of timely screening of viral infections in resource-poor regions of the world.69 To effectively screen for viral infections, a sensitive, specific, and quick methodology has to be developed without the requirement of expensive equipment or well-trained personnel. This is not possible when standard molecular diagnostic methods, such as reverse transcription...
polymerase chain reaction (RT-PCR), the gold standard for many viral infection screening including Ebola, are utilized. The development of isothermal amplification, in conjunction with real-time recombinase polymerase amplification (RT-RPA), allows for viral screening within a piece of paper as it does not rely on high temperature nor cycle control. To this end, a μPAD employing nucleic acid amplification test (NAAT), was developed to provide timely screening of the Ebola virus. Successfully field-tested, this device provides a template for the development of other μPADs for screening viral infections in resource-limited parts of the world. This μPAD was constructed by wax printing a piece of paper with three rectangular sections containing the positive, negative, and sample areas (Figure 2A). These areas were imprinted with the necessary components for RT-RPA analysis. The RNA matrix for the Ebola virus was imprinted additionally on the positive control section of the μPAD. The device was then freeze-dried and was ready for utilization. During use, the device was rehydrated using buffer for the control sections, while the sampling section was rehydrated using the RNA extracted from the patients’ blood plasma. To assess, the device was placed on a simple heater at ~40 °C for 20 min, and fluorescence signals were recorded using equipment that was transportable in a carry-on suitcase. The devices were stored in small, black packages with desiccants for shipment to ensure minimal exposure to light and humidity. A picture of the actual device is shown in Figure 2B. In assessing the device’s shelf life, the μPADs were stored at room temperature in an environment low in light and humidity and tested at 2, 6, 30, and 60 days. Compared to freshly made reagents, the fluorescence intensity was reduced gradually over time; however, its sensitivity was maintained after 30 days. At 60 days, only 20% of the device remained operational (Figure 2C). For the field testing, the μPADs were sent to West Africa with no additional preparation for the method described above while storing the device in an air-conditioned lab for 1 month before testing. The device was able to attain 90% sensitivity (true positive rate) but only 60.8% selectivity (true negative rate), compared to the RT-PCR test conducted on the same samples. The limit of detection (LOD) of the device was 10⁷ RNA copies per μL. This is higher than running a similar protocol within a test tube (<10⁵ copies per μL). The time required to achieve the final fluorescence signal for assessment was 20 min, which is faster compared to the commercially available RT-RPA kit provided by RealStar that requires 100 min to conduct. Due to difficulties associated with field-testing in West Africa, a truly blind assessment of the μPAD was not conducted. It is therefore important to note that the protocol carried out should only be considered as a proof-of-concept for the μPAD rather than a comprehensive verification of the device.

HIV. Recent progress in microfluidic technologies has made possible low-cost miniaturized devices for detection and quantification of HIV at the point of care. HIV is still a major global public health concern with an estimated 38 million people living with HIV at the end of 2019. More than two-thirds of them reside in developing countries, where rapid self-testing has significantly facilitated diagnosis and continues being crucial in the fight against the disease. Rapid diagnostic tests for HIV infection are based on the detection of HIV-1/2 antibodies and/or HIV-1 p24 antigen by LFA (e.g., OraQuick ADVANCE HIV 1/2, SD BIOLINE HIV-1/2 3.0, ABON HIV 1/2/O Tri-Line Rapid test) and flow-through (e.g., INSTI HIV-1/HIV-2 Antibody Test, Reveal G4 Rapid

Figure 2. μPAD developed to screen for Ebola in Guinea. (A) Illustration of the μPAD, showing the position of reagent deposition and its corresponding label. (B) Actual photograph of the Ebola virus-positive (bottom) and -negative (top) μPADs under fluorescence. (C) Amplification curve of the negative patient samples (left), and that of positive patient samples (right), compared to the positive control (black triangle), and negative control (black cross). Reproduced with permission from ref 71. Copyright [2017] Springer Nature.
HIV-1 Antibody Test) tests. However, molecular techniques involving nucleic acid amplification are still required to quantitatively monitor the progression of the infection and the response to anti-retroviral therapy (ART). Recently, some approaches have been reported to adapt this technology to paper substrate.

Phillips et al. reported an autonomous, fully integrated platform microfluidic rapid and autonomous analytical device (microRAAD) for the specific detection of HIV-1 virus in human whole blood samples. The device comprised a temperature control circuit and a heating element, both reusable, and a disposable laminated µPAD including a LFA strip for visualization. The assay time from sample addition was 90 min, and it showed a sensitivity of $2.3 \times 10^7$ virus copies per mL of whole blood, which is at the high end of the peak infection range. The reagents were dried and stored within the device for 3 weeks at room temperature, which demonstrated the viability of microRAAD for resource-limited areas. Moreover, the total cost of the reusable components was $\$70$, and the consumables were below $\$3$, making it suitable for mass testing in low-income areas.

**Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2)**: The ongoing pandemic of SARS-CoV-2, which caused the novel coronavirus disease (COVID-19), spread all over the world. It was deemed imperative to slow down the spread of the virus, and effective screening is key. However, due to its rate of spread, and the novelty of the virus, diagnostic capabilities were limited. Its initial symptoms are indistinguishable from other respiratory viral infections. Fever, cough, myalgia, and fatigue are indicative of many viral infections, including influenza. RT-PCR is being used as the primary tool, and gold standard, for screening for SARS-CoV-2, in addition to CT imaging and hematological markers. However, the limitations of the RT-PCR tests in diagnosis should not be underestimated. First, due to its long turnaround times, the measurement requires a lot of time for diagnosis. As a whole, these are not quick to implement, requiring 2 to 3 h, on average, to produce results. Second, the operation of the test requires complicated procedure, well-trained personnel, and expensive equipment. This limits the capability for population screening, as virology laboratories are not equipped for the scale of viral infection that is ongoing. Areas with limited access to resources are especially affected. Therefore, the RT-PCR is not suitable for rapid and simple diagnosis of SARS-CoV-2. Another interesting approach, a qualitative LFA, was recently developed by Li et al. to recognize COVID-19 immunoglobulin M (IgM) and IgG in blood samples, using colloidal gold nanoparticles as a basis for colorimetric chromatographic detection. This method is widely employed in many SARS-CoV-2 commercial screening products (Table 1). As IgM provides the first line of defense during infections, the high-affinity IgG response is important to detect IgG and IgM antibodies against SARS-CoV-2. The measurement of IgM antibody demonstrates recent exposure to SARS-CoV-2, while the detection of COVID-19 IgG antibody shows virus exposure some time ago. LFAs that could detect both of IgM and IgG in 15 min are suitable for rapid diagnosis of COVID-19 infection.

The LFA consists of five distinct portions, the sample pad, the conjugation pad, the nitrocellulose membrane (NCM), the absorption pad, and the adhesive card. The NCM contains the IgM and the immunoglobulin G (IgG) detection site, labeled M-line and G-line respectively, as well as the control line. Anti-

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Human IgM and Anti-Human IgG were chemically immobilized onto the NCM on the M-line and G-line, respectively. SARS-CoV-2 antigen conjugated gold colloids (40 nm diameter) as well as the rabbit IgG conjugated gold colloids were sprayed onto the conjugation pad. The five portions of the LFA were then put together in the configuration shown in Figure 3. As the sample is transported via capillary flow through the device, given that there are anti-SARS-CoV-2 IgGs and IgMs in the sample, the antibodies will bind with the gold colloid conjugated SARS-CoV-2 at the conjugation pad. Proceeding down the NCM, label conjugated samples in flow are then bound to anti-IgG and anti-IgM antibodies at their respective detection line. At the end of the NCM, the gold colloid conjugated rabbit IgG will bond to its respective antibody, indicating that capillary flow reaches the end of the NCM. The anti-SARS-CoV-2 IgM and IgG will show up on their respective region as red/pink lines. If there are no anti-SARS-CoV-2 IgG and IgM in the sample, gold colloids will not be retained at the detection line, and no color will be present.

Assessing the LFA with clinically confirmed SARS-CoV-2 cases, containing 397 positive and 128 negative samples of blood, yielded 88.7% sensitivity and 90.6% specificity. POC immunoassays to detect specific diseases antigens and antibodies are frequently used in low-resource settings. However, the need of samples from the local population to include the study of its coendemic diseases in the clinical validation often delay the implementation of new diagnostics tools in LMICs. The initial shortage of biological reagents and disposables for the pandemic was a magnified difficulty for low-resource areas, limiting penetration of diagnostic tests. Despite the support provided by the WHO and regional centers for disease control, only limited development of local test or tool production has been achieved, and remote areas still lack appropriate testing provisions. An additional risk recently observed in low-resource settings is the decrease in testing for HIV, malaria, and tuberculosis as funds are redirected to tackle the COVID-19 pandemic. This might lead to a considerable increase in deaths over the next five years and some initiatives to mitigate the damage at an early stage have been implemented. Thus, organizations such as Africa Centres for Disease Control and Prevention (Africa CDC), the Bill & Melinda Gates Foundation, the Clinton Health Access Initiative (CHAI), the Foundation for
Innovative New Diagnostics (FIND), the Global Fund, Unitaid, and the WHO have recently supported agreements to provide affordable, high-quality COVID-19 antigen rapid tests to LMICs. The initial agreements executed with rapid diagnostic test producers Abbott and SD Biosensor implied the deployment of 120 million antigen tests, with a maximum price of $5 per unit, to countries with limited access to PCR test.  

Additionally, Broughton et al. developed a clustered regularly interspaced short palindromic repeats (CRISPR) associated protein 12 (Cas-12) based method for detection of SARS-CoV-2, using LFA as a visual readout device. This method requires 30 min to run, exhibiting 95% sensitivity and 100% specificity, with a LOD of 10 copies per μL. It could be an alternative halfway between PCR and other low-cost detection methods, as it provides high sensitivity but still requires small pieces of equipment (Eppendorf tubes with reagents, heat blocks or water bath (37 and 62 °C), nuclease-free water, pipettes, and tips) for RNA extraction and amplification.

Dengue Virus (DENV). Dengue fever, caused by the DENV, carried within mosquitoes, endemic to tropical regions of the world, is estimated to infect 200 million people per year and causes 150,000 deaths annually. Its symptoms are similar to other mosquito-borne viral infections, i.e., fever, rashies, and musculoskeletal pain, making it difficult for timely diagnosis, essential for effective treatment, and preventing clinical complications due to the progression of the disease.  

There are numerous protocols for detecting DENV (e.g., plaque reduction neutralization test, hemagglutination-inhibitor test, molecular assays, enzyme-linked immunosorbent assay (ELISA)), all of which are unsuited for use in DENV endemic areas due to lack of trained personnel, costs, and time constraints from disease progression. Utilizing gold colloids as a chromogenic agent, Prabowo et al. recently developed a μPAD to detect nonstructural protein 1 (NS1) of DENV, derived from the pediatric serum of the patients, by sandwich immunoassay.

The μPAD was constructed on cellulose paper using wax printing and subsequently heated to pattern the desired hydrophobic barrier. The pattern compartmentalized five different sections, the sampling conjugation test control, and the adsorbent areas. Goat anti-mouse IgG antibody and anti-NS1-antibody were immobilized onto the control and test areas, respectively. Gold colloid (40 nm) conjugated anti-NS1-antibody was dropped onto the conjugation area. After treating the device with buffer and drying, a plastic card was attached to the treated paper for structural support, and a plastic film with overlaid on top, exposing only the sampling area. The μPAD was then ready for use. As the NS1 in the sampled serum was wicked across the conjugation area, the gold-conjugated anti-NS1-antibody was attached to the protein and proceeded to wick down to the test area. In the test area, the labeled complex was attached to the cellulose-bonded anti-NS1-antibody and prevented further elution downstream. The excess gold-conjugated anti-NS1-antibody subsequently traveled along the μPAD and becomes immobilized by the cellulose-bonded IgG antibody. This last step acted as a control to ensure that the serum was eluted through the μPAD. The results can be analyzed as binary characterization of DENV visually, as well as quantification through a scanner or a
Bacteria continue to be a big problem in the 21st century due to ever-increasing drug resistance. Developing regions of the world are especially at risk due to their limited screening capability. Out of six regions of the globe, divided by the WHO, five had more than 50% resistance to third-generation antibiotics. As with viral infection, bacterial infection was also successfully screened using μPADs.

Salmonella. Salmonella is one of the leading causes of foodborne illness in the world. Often, bacterial infections are screened by culturing the bacteria in a Petri dish. This is a prolonged and laborious test, requiring days to complete. In addition, it is not feasible for field implementation, often requiring laboratories with expensive equipment. PCR was able to reduce the time requirement for bacterial screening down to 24 h. This is a significant improvement on the timelines of screening but still requires expensive equipment not available in resource-limited areas. Motivated by this, Srisa-Art et al. developed a timely and economical μPAD, employing immunogenic separation (IMS) for colorimetric detection of Salmonella.

A distance-based μPAD used for the detection of Salmonella was developed and tested. The devices were constructed by wax printing the desired hydrophobic barrier onto filter papers and heat-treating it at 175 °C for 50 s to incorporate the wax into the interior of the paper. Lastly, cellophane tape was attached to the bottom of the device to form a barrier against leakage. The protocol required two incubation steps. The detection process is summarized in Figure 4A and B. The geometry of the μPADs is shown in Figure 4C. The magnetic Dynabeads used in the IMS process were synthesized by covalently bonding the beads to Salmonella antibodies based on the procedure recommended by the manufacturer Invitrogen. The anti-Salmonella magnetic beads were used to screen out Salmonella bacteria during the IMS process to form the Salmonella anti-Salmonella magnetic beads (SaSB). Subsequently, anti-Salmonella biotin-conjugated antibody was bonded to the SaSB to provide chemical compatibility with streptavidin-β-galactosidase to facilitate the chromogenic detection of the bacteria-bead complex using Chlorophenyl red galactopyranoside (CPRG).

After optimizing the incubation time and accounting for the capture and retention efficiency of the magnetic beads, the LOD of the assay was determined to be 100 colony forming units (CFU) per milliliter. Employing the distance-based measurement, a linear range of 10^2−10^5 CFU mL^{-1} was determined (Figure 4D). The time required to complete the assay was no more than 90 min, with 75 min taken to incubate the device, while the remaining time was accounted for washing. This is relatively quick in comparison to the “conventional quick method” of PCR. Moreover, by employing distance-based measurement chemometers (Figure 4C), the amount of Salmonella in the sample was directly evaluated without image analysis. The instrument-free detection is more convenient for users and ideal for in-field diagnosis in LMICs.
Tuberculosis (TB). TB is one of the top 10 causes of death in the world with 1.4 million deaths in 2019. This infectious disease caused by various strains of mycobacteria (normally M. tuberculosis in humans) is still one of the main global public health problems. Moreover, TB is the most common illness among people with HIV, and its early detection is crucial to prevent further fatalities. Despite the acute necessity, low-cost and rapid POC devices for TB remain a challenge. Kaur et al. recently reported a paper- and plastic-based POC detection platform for TB based on loop-mediated isothermal amplification (LAMP) for nucleic acid amplification. The device showed a high specificity (68.75%) for M. tuberculosis genomic DNA and sensitivity (100%) when compared to smear microscopy using 30 clinical (sputum) samples. Despite its low cost (approximately $1.5), a laboratory incubator for DNA amplification and a UV torch for endpoint fluorescence detection using a mobile phone are still required.

Blood Cells. Blood is one of the most important biological media for medical analysis. To accomplish this, blood is centrifuged to remove the blood cells, leaving behind plasma which is rich in biomedical information. This plasma can then be separated into its constituent components using various equipment and subsequently analyzed. This process is greatly hampered in many resource-poor areas of the globe with little to no specialized equipment available. As a result, POC health services involving real-time blood sensing cannot be accomplished. It is by this motivation that Ulum et al. developed a simple and economic ethylenediaminetetraacetic acid (EDTA) adsorbed cotton thread for blood plasma separation and demonstrated its usability by performing an albumin assay on the resulting separation (Figure 5A). The construction of the microfluidic thread-based analytical device (µTAD) is straightforward and simple. Commercial cotton threads were soaked in a solution of Na₂CO₃ (10 mg mL⁻¹) for 5 min at 100 °C to remove wax and other additives on the surface of the cotton. They are then placed on top of glass slides along with supporting paper on each end, all adhered together via commercial double-sided adhesive tapes. Six protocols were conducted on the constructed µTAD to assess the ideal device configuration for blood separation. Only protocol 6 yielded the best blood plasma separation boundary from the blood cells, with clear distinctions between the red (blood cells), yellow (plasma), and white zone (untouched cotton), illustrated in Figure 5B. Smearing the used µTAD prepared via protocol 6 revealed that there were no red blood cell present in the yellow zone. It is important to note that the smear test done by the investigator was qualitative and involved classifying the smears into three categories (high amount of red blood cell, medium amount of red blood, low amount of red blood, and none).

Furthermore, to assess the µTAD’s effectiveness, a semi-quantitative albumin assay was conducted on the cotton threads prepared using protocol 6. Bromocresol green was immobilized along the cotton thread (50 mm starting from inlet) by placing the solution at the outlet and allowing it to wick from the outlet toward the inlet (Figure 5C). The µTAD was then dried in a refrigerator for 1 h. Because this was done for comparison purposes with respect to the traditional method involving centrifuging the blood to separate the plasma, followed by adding bromocresol green for colorimetric, no calibration relating the exact colorimetric intensity to albumin concentration was constructed. The µTAD produced a linear response identical with that of the centrifugal method.
Figure 6. μTAD developed to screen for potassium in blood plasma. (A) Detection mechanism of the μTAD device for potassium quantification. Nile blue, shown at the bottom, is converted to Nile red in the presence of hydroxide ion, generated via the reaction between the crown ether and the potassium ion. (B) Illustration of the μTAD device for potassium quantification. (C) Picture of the constructed μTAD, with scale bar for reference. Scale bar: 5 mm. (D) Calibration function of the potassium assay. The blue line indicates the experimental fit, while the red line indicates the confidence limit of the regression. Reproduced from ref 122. Copyright [2016] American Chemical Society.

with the same normalized intensity. Its linear response portion has an $R^2$ value of 0.991, while that of the centrifugal method was 0.995. Its LOD was 114 mg L$^{-1}$ compared to 133 mg L$^{-1}$ of the centrifugal method. Overall, the assay showed excellent performance, producing the same results as the traditional assay while saving ∼10 min in time required for centrifugation (Figure 5D).

All in all, not only was it demonstrated that cotton thread, treated with EDTA, can separate blood plasma with clear and distinct boundary, but it was also demonstrated that simple colorimetric assays could be done directly on the cotton thread. This foundation can be extended to other colorimetric assays such as that of glucose and narcotics screening. Further development of this device into a ready-to-use POC could allow areas with little to no access to laboratories to analyze blood samples simply.113

Electrolytes. Electrolytes are also important biomarkers, indicative of physiological status.114 Salts can indicate cardiovascular, renal, and liver function.115–117 The rapid urbanization in LMICs and the adoption of US/Western-style food habits have led to an increase in hypertension and cardiovascular diseases in those areas.118,119 Abnormal levels of potassium in the blood might be related to heart problems, high blood pressure, kidney disease, or metabolic acidosis.120 An increase in child mortality has been observed in developing countries when severe hypokalemia (low potassium levels in blood) is associated with diarrhea and severe malnutrition. Therefore, the development of rapid, low-cost metabolite tests is an emerging necessity in low-resource areas.

Erenas et al. have developed a cotton thread-based microfluidic device for the selective quantification of potassium. Coordination chemistry in conjunction with a color recognition software was employed to quantitatively measure the amount of potassium in an aqueous medium.104 In the construction of the device, the wax layer of the commercial cotton thread had to first be removed to facilitate flow via capillary wicking. This was done by boiling the threads in a solution (10 mg mL$^{-1}$) of Na$_2$CO$_3$ for 5 min. The threads were then washed with deionized water until the pH of the wastewater was neutral, and subsequently dried for 2 h. To allow for adequate imaging of the thread, the threads were sewn into an ethylene−vinyl acetate (EVA) foam (1.0 cm × 1.5 cm). There are 2 sampling portions of the thread, 1 mm long, for redundancy. The ligand responsible for the ion exchange reaction is dibenzo-18-crown-6-ether (DB18C6), which preferentially coordinates with the potassium ion. o-Nitrophenyloctylether (NPOE) acts as a plasticizer for the analysis solution and tetras(4-chlorophenyl) borate (TCPB) as the phase transfer agent for the reaction. Nile blue is the basis for the chromo-sensing ability. The mechanism behind the detection is outlined in Figure 6A. As the aqueous potassium ion enters the microfluidic channel, the potassium is picked up by the anionic TCPB which transfers it to DB18C6. This ligand exchange reaction recreates the anionic TCPB, which generates the hydroxide ion from the surrounding water. The hydroxide ion then reacts with Nile blue, generating Nile red as well as ammonia as a side product. Figure 6B shows an illustration of the device while Figure 6C illustrates the actual device constructed.

The linear dynamic range of the device was between 2.4 × 10$^{-5}$ and 0.1 M and the calculated LOD was 2.4 × 10$^{-5}$ M (Figure 6D). The mean standard deviation attained was 1.34%, which is significantly lower than that of other thread-based procedures aimed at screening for proteins and nucleic acids.121

Narcotics. The source of manufacture for narcotics is in the developing regions of the world. Narcotic abuse disproportionately affects regions of the world with lower-than-average economic position. These regions do not possess the necessary resources to effectively enforce policies aimed to stop the production of these narcotics. It then follows that these parts of the world lack the tools to properly screen for these abuses, i.e., medical laboratories and well-trained technicians.123 N-Methyl-D-aspartate (ketamine) is an anesthetic pharmaceutical product
that yields dissociative, analgesic, and psychedelic properties to its users.\textsuperscript{124} It is one of the most commonly abused drugs in the world, especially in Asia. The conventional method for ketamine screening requires pretreatment of the biological sample followed by instrumental analysis. A $\mu$PAD was constructed to determine the concentration of ketamine from a 3 $\mu$L sample of saliva using competitive ELISA (c-ELISA) (Figure 7A).\textsuperscript{7} This was done by printing 4-mm-diameter circles using solid wax on a 4 cm $\times$ 3 cm piece of paper to form a hydrophobic barrier that contained the analysis wells (Figure 7B). The printed paper was then heated to 120 °C for 10 min to allow for wax to penetrate the interior of the paper. The basis for detection utilizes ketamine antibody (K-Ab) and ketamine conjugated horseradish peroxidase, with 3,3',5,5'-tetramethylbenzidine (TMB) as the chromogenic substrate. The $\mu$PAD consisted of three reference wells, containing 44.5, 88.9, and 177.8 $\mu$L of K-Ab, for direct chromatographic comparison with the sampling well. After loading, and subsequent washing/drying, the $\mu$PAD device was ready for analysis.

Utilizing a color analysis software, this method for c-ELISA yielded a linear range for analysis between 1 ng mL$^{-1}$ and 1 $\mu$g mL$^{-1}$ of ketamine. LOD was calculated to be 0.3 ng mL$^{-1}$ using the “three-times greater than noise” method.\textsuperscript{7} The time required to run the analysis was less than 5 min. Clinical trials yielded 90% sensitivity and 92% selectivity (Figure 7C). Additionally, this $\mu$PAD can also be analyzed visually with reference to the standard wells for field administration.

### Commercialized POC Devices and the Regulations

Commercial POC devices have been commonly used in a variety of applications. The commercialization success of POC devices reporting the presence or absence of biomarkers in the sample by “yes/no” based on the presence or absence of the test lines is higher than their counterparts providing qualitative results. The most well-known examples are produced for pregnancy testing, influenza, and infective disease diagnosis (Table 2). While type A virus is associated with more serious influenza epidemics than type B, rapid diagnosis of influenza A and B is important to aid effective antiviral therapy. Alere BinaxNOW Influenza A & B Card is an in vitro immunochromatographic assay for the rapid detection of influenza A and B via their nucleoprotein antigens. The monoclonal antibodies are immobilized onto a membrane substrate as three lines and functionalized with other reagents to produce the test strip. By collecting nasal samples, this commercial strip can interpret the result in 15 min through the presence or absence of pink-to-purple colored sample lines. Although this device provides a simple method to diagnose

![Figure 7. $\mu$PAD developed to screen for ketamine from saliva. (A) Illustration of c-ELISA used in the $\mu$PAD, highlighting the inverse relationship between red hue and ketamine concentrations. (B) Picture of the actual $\mu$PAD constructed, scale bar: 3 cm. (C) Resulting calibration curve, relating the ketamine concentration to the hue ratio. Reproduced with permission from ref 7. Copyright [2019] Elsevier.](https://doi.org/10.1021/acssensors.1c00669)

### Table 2. Selected Commercialized POC Devices

<table>
<thead>
<tr>
<th>product name</th>
<th>manufacturer</th>
<th>analyte</th>
<th>detection time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novaplus HCG Pregnancy Test Cassette</td>
<td>Eksa Corporation</td>
<td>hCG</td>
<td>1–2</td>
</tr>
<tr>
<td>Alere Binaxnow Influenza A&amp;B Card</td>
<td>Alere Scarborough, Inc.</td>
<td>influenza A and B nucleoprotein antigens</td>
<td>15</td>
</tr>
<tr>
<td>MQuant Glucose Test strips</td>
<td>MilliporeSigma</td>
<td>glucose</td>
<td>1</td>
</tr>
<tr>
<td>Quick Vue RSV Test</td>
<td>Quidel Corp.</td>
<td>respiratory syncytial virus (RSV) Ag</td>
<td>15</td>
</tr>
<tr>
<td>Alere Determine HIV-1/2 Ag/Ab Combo</td>
<td>Alere Scarborough, Inc.</td>
<td>HIV-1/2 antibodies and free HIV-1 p24 Ag</td>
<td>15</td>
</tr>
<tr>
<td>CrAg LFA</td>
<td>IMMY</td>
<td>cryptococcal meningitis</td>
<td>10</td>
</tr>
</tbody>
</table>
influenza A and B, it suffers from complicated sample collection and additional sample treatment that hinder further applications of self-diagnosis at home or in resource-limited areas. Moreover, the complexity of sample collection results in low accuracy and low sensitivity, which cannot be correlated with lab-based measurement. Commercial chromatographic lateral flow assay (such as Nova test human chorionic gonadotropin (hCG) Pregnancy test strip) is designed for a pregnancy check by the detection of hCG in urine. Measurement of the commercial device is performed by simply dipping into untreated sample, and the cost is only $0.01–1.2 US that can be accessible for home-based or resource-limited use. These assays provide rapid test results (usually several minutes) by using low-cost materials and minimized reagents. The challenge for these devices is that they can only be used for an initial screening and fail to provide a more accurate quantitative measurement to evaluate the hCG level compared to lab-based measurements.

Although μTADs offer low-cost, portable, and accessible solutions for POC diagnostics, a successful translation of μTADs to the market has not been achieved. To survive in the market, the μTADs should fulfill the following three requirements: (i) reproducible mass production, (ii) controllable flow rate on the thread materials, and (iii) improved

Figure 8. Flowchart for the process required to market novel medical devices by the (A) FDA, (B) EMA, and (C) NMPA. Abbreviations: EC, European Committee; CE, Conformité Européenne; IDE, Investigational Device Exemption; FFDCA, Federal Food, Drug, and Cosmetic Act; PMN, Premarket notification; PMA, Premarket approval.
sensitivity compared to the μPAD. Recent pandemics (e.g., COVID-19, Ebola) have exposed the necessity of rapid testing to tackle diseases that otherwise might generate an enormous global health burden. However, the implementation of rapid POC devices is not a straightforward task in LMICs. Low-temperature storage to provide higher test stability, access to electrical supply, availability of trained staff, local epidemiology, and the lack of assay reagents and supplies at the local industry are challenges that prevent the deployment of mass testing in resource-limited settings. Additionally, during the release to the market, any developed POC device should comply with the diagnostic clinical specifications and manufacturing regulations.

Regulations are of concern in the design/manufacturing, evidence collection, as well as post-market vigilance. Overcoming this hurdle is difficult; most innovative medical devices do not attain clinical approval. The two main regulating bodies are the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). In classifying medical devices for regulatory measures, all three agencies divide them into classes of increasing risk to the user. In most cases, LFAs are low-risk medical devices, with limited possibility of injuring causing illness to the users because they are intended for screening medical conditions in vitro. As such, only general controls are imposed on these devices. The FDA classifies medical devices into three categories with increasing risk (i.e., Class 1–3). All classes are subject to general controls. Most LFAs will be classified as Class 1 or Class 2. If an exemption is granted, then the device would only require registration. If not, the device would require registration as well as submission of a premarket notification (PMN) application, also known as $10(k). The PMN encompasses 12 main aspects, ranging from descriptions of the device and clinical data to proposed packaging/labeling and biocompatibility. Upon submission of the PMN, the relevant Center for Device and Radiological Health (CDRH) division will review the application. Notable exemption to the FDA medical device clinical trial includes the Humanitarian Device exemption (i.e., exemption on devices intended for use with conditions that affect fewer than 8000 people annually in the US). This process is summarized in Figure 8A.

The EMA requires a similar process to that of the FDA. The agency would classify most LFAs as Class 1S, noninvasive devices that do not interact with the body or Class 2A in wearable LFA devices. The processes involving Class 1S as well as Class 2A devices are similar: the manufacturer undergoes self-declaration or self-certification, ensuring and formally declaring, in writing, that the product meets the criteria provided by the EC Declaration of Conformity (Annex 8); in addition, the manufacturer has a choice of four different regulations to follow (e.g., Annex 2, 4, 5, or 6). Subsequently, the manufacturer can then apply for the Conformité Européenne (CE) marking which would allow for the device to be marketed. Alternatively, for Class 2A devices, the manufacturer may forego Annex 8 altogether and opt to undergo full quality assessment (Annex 2), the successful outcome of which would allow for the application for CE marking and subsequent marketing of the device (Figure 8B).

Similarly, NMPA would also categorize LFAs as Class 1 for stand-alone LFAs and Class 2 for wearable LFAs. Depending on whether the device was imported or domestically made, different levels of NMPA will handle the approval process. China NMPA handles all classes of imported medical devices as well as Class 3 domestic devices; the provincial and local NMPA handles Class 1 domestic and Class 2 domestic medical devices, respectively. Class 1 medical devices do not need to go through the approval process; only document filing is enough. Class 2 medical devices may be exempted from the clinical trial process, depending on the judgment of the NMPA. Once the clinical trial concludes successfully, the manufacturer may declare conformation and proceed to market the device. This process is summarized in Figure 8C.

In contrast with EMA that requires the POC medical devices to acquire UL approval and CE Mark certification, FDA requires a more expensive and long-term regulatory process due to the unique requirement of evaluating efficacy. Each POC medical device with a regulatory approval does not guarantee its feasibility in resource-limited or resource-rich countries. POC medical devices in resource-limited settings require the devices to meet the standard of high quality, low cost, and special design for low-income countries. To fulfill this requirement, WHO Medical Device Technical Series help with designing, procuring, and writing regulations for resource-limited settings. In addition, the WHO Compendium of Innovative Health Technologies for Low Resource Settings could provide context-appropriate solutions to find suitable POC medical devices for resource-limited areas. Therefore, the regulatory process for POC devices is required to be developed step-by-step based on the context of a country. In resource-limited countries, it is recommended that the basic regulatory programs should be initially implemented. The resource-limited countries are also suggested to establish their own appropriate regulatory system leveraging the existing international experience. To prevent importing substandard POC devices, the resource-limited countries should take measures to strictly control the acceptance criteria in accordance with their regulatory process. Manufacturers normally push themselves above and beyond the classic regulations in the international standards to deliver POC medical devices that meet higher standards for resource-limited regions.

**DISCUSSION**

With the increasing worldwide use of digital technologies in health as recommended by the WHO in 2018, a global effort has been observed to address the challenges related to the establishment of eHealth systems. Most of the infrastructure, i.e., mobile connectivity, high-quality conference, and data analysis and storage, is already available. However, the technology for home-based medical testing is still underdeveloped. Another important milestone is the utilization of LFAs in the wearable format which consequently (i) improves the assay performance and (ii) reduces the sampling limitations. Wearable medical devices (WMDs) are poised to become revolutionary platforms for clinical use. Microfluidic quantification of biomarkers is becoming more relied on as a clinical method for recognition of illnesses as well as for personalized treatment. This is especially true pertaining to biomarkers found in limited amounts in vivo. POCs are resurging due to the increasing popularity of wearable technologies in medicine, enabling them to be incorporated into “the internet of things.” In designing/manufacturing the medical device, three key aspects should be considered to overall minimize user risk. The design should reduce, as much as possible, external contamination to exposed human tissue, radiation emitted from electronics, and leakage of the content in processing/packing. Design and manufacture should comply
with regulations imposed by the FDA, the EMA, or the NMA. Sufficient evidence should be gathered in clinical trials to ensure that the device performs as intended (i.e., demonstration safety, reliability, and accuracy) and any possible device failures are identified. This will be evaluated in the conformity assessments for the respective regulatory agencies. Once registered and commercialized, post-market surveillance must be conducted to ensure that the medical device does not deviate from its intended quality and efficacy. Design, technical, and performance concerns should be continuously assessed through feedback from patients and medical professionals. The regulatory process for POC devices is time-consuming. In resource-limited regions, donated medical devices can be of great importance to solve these problems; however, this is not always efficient or “low-cost”. An attempt in development of POC devices is made to improve the reusability of the POC devices, in the aspects of materials, chemical reagents, sampling, and reading. In the US, the reprocessing of a single-use medical device is subjected by the FDA to the same regulatory process as the original device. Low-cost lateral-flow colorimetric-based analytical assays exhibit great potential for fulfilling increasingly acute demand for POC devices that are rapidly deployable and easily implemented in areas of limited resources. These devices provide a robust platform to screen a myriad of biological, chemical agents, such as viruses, bacteria, proteins, electrolytes, and narcotics. Even though the use of smartphones and other portable scanners as readout tools for the analysis of lateral flow devices has increased the rapidity of POC screening greatly, these devices should be equipment-free. Utilizing external scanning devices provides greater sensitivity, as well as specificity, but imposes greater logistical requirements in delivering these devices to resource-limited areas. The growing availability of camera phones in resource-limited parts of the world partially mitigates this shortcoming. However, camera resolution, image-quality standardization, and robustness of smartphones still pose a challenge for telemedicine. Among the challenges requiring further research and development are limited reproducibility, relatively low sensitivity, modest specificity, limited shelf life, multiplexing possibility, and, in some cases, subjective data interpretation. Many of these devices address these limitations, but only a few of them fulfill the ASSURED criteria at the same time. Depending on the nature and unique requirements of the problem at hand, some of these constraints become more prominent than others. For example, sensitivity has the utmost importance, when the precise concentration of an analyte is needed to be monitored continuously in a dynamically changing environment (glucose in sweat). These limitations could be bypassed for answering qualitative yes/no questions or measuring an analyte whose concentration in the measured medium is relatively high. Moreover, “affordability” is also another issue that must be addressed in low-cost medical devices. Although the current LFAs are based on low-cost paper or thread, the chemical reagents to functionalize sensing regions are still at high cost, thus limiting further applications in resource-limited areas where the average public annual expenditure on health is $40.

The total LFA market is valued at $5.6 billion US in 2020, with an estimated compound annual growth rate of 4% through 2026. The estimated value of the lateral flow market in 2026 is $7.1 billion US. This market includes clinical testing, veterinary diagnostic, food safety, and environmental testing, as well as pharmaceutical quality control devices. The largest market share belongs to clinical testing, comprising around

Figure 9. Graphical insight from G01N33/558 patent search. (A) Amount of patents filed between 2015 and 2020 to the EPO, CNIPA, and USPTO. (B) Annual patents filed globally vs patents granted between 2015 and 2020. (C) Assignee currently holding the most patents globally by percentage. (D) Jurisdictions holding the most patents by percentage.
27% of the total market value. Significant aspects fueling the growth of the LFA market are the increase in demand for POC testing, as well as the increasing instances of infectious diseases, such as malaria and tuberculosis.\(^\text{147}\)

Under the International Patent Classification (IPC), the largest subset of LFA devices is categorized as G01N33/558. In both applications and granted patents, patent filing peaked in 2017 and has been declining since then (Figure 9A and B). The top assignee of the G01N33/558 patents is Abbott Laboratories, followed closely by Inverness Medical, each holding about 11% of all patents. Cliag, Kimberly Clark, Quidel, and Alere Switzerland each holds 9%, 8%, 6%, and 6%, respectively (Figure 9C). In the past 5 years, the organizations that saw the most applications were the Chinese National Intellectual Property Administration (CNIPA), the United States Patent and Trademark Office (USPTO), and the European Patent Office (EPO), comprising 39%, 15%, and 8% of all registered G01N33/558 patents, respectively (Figure 9D).

LFAs have seen a lot of progress the past 10 years, becoming a more reliable part of the medical and clinical tool kit. This is exemplified by the adoption of LFAs for mass testing of SARS-CoV-2 by many countries around the world in the fight against the ongoing pandemic, as well as the epidemic of Ebola in West Africa in 2014. The development of isothermal PCR, in the form of RT-RPA, was the first time for both bacterial and viral screening implemented within an LFA, allowing for NAAT to be undertaken in a portable and self-contained device. Additionally, for the first time, CBDs were first utilized for the capture of bacteria within LFAs, providing greater results when compared to the antibody-based methods. There is also progress being made in the market as well as in public research institutions. This synergy provides an optimistic outlook for the development of increasingly robust and field-ready devices looking forward. The research and development, as well as subsequent commercialization of these devices, will not only benefit the public health of countries that host the development of said devices but also, and more importantly, benefit the public health of resource-limited regions around the world.

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