Application of microfluidic paper-based analytical device (μPAD) to detect COVID-19 in energy deprived countries

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Summary
Coronavirus disease (COVID-19) has spread all across the world. Low- and medium-income countries are more affected economically and socially compared to developed countries due to the lack of a rapid, robust, and affordable testing infrastructure. Furthermore, the high cost of real-time polymerase chain reaction (PCR) system, sophisticated user-handling procedure, and high expense of the conventional clinical tests are the root causes of the less accessibility of the testing systems to the users. In this study, a COVID-19 Point-of-Care (POC) ecosystem model is proposed for the low- and medium-income countries (or energy deprived countries) that will facilitate the technological development with locally available fabrication components. In addition, the nontechnological development phases have also been discussed, which encompasses the collaboration among academia, local as well as government bodies, and entrepreneurial ventures. In addition, a hypothetical design of a microfluidic paper-based analytical (μPADs) POC platform is proposed to detect COVID-19 analyte using unprocessed patient-derived saliva, which is a miniaturized form-factor of conventional real-time polymerase chain reaction (PCR) technique. The device contains four major reaction zones, which are sample zone, buffer zone, loop-mediated isothermal amplification (LAMP) Master Mix zone, Ethylenediamine tetraacetic acid (EDTA) zone, and sensor zone. To obtain quicker test results and easier operation, a handheld image acquisition technique is introduced in this study. It is hypothesized that in a remote setting, the proposed design could be used as an initial guideline to develop a POC COVID-19 testing system, which may be simple, easy-to-use, and cost-effective.

KEYWORDS
COVID, microfluidic, Point-of-Care

1 | INTRODUCTION

COVID-19 was first discovered in Hubei Province, China, in December 2019, which further spread globally due to human transmission. We have witnessed the rapid increase in the epidemiology of this disease across the world, which resulted in personal, social, and economic losses to individuals. One such industry that is badly affected by the novel corona virus is the airline industry as most of the aviation operation is ceased resulting in greater economic losses.¹,² According to World Health Organization (WHO), PCR is the designated clinical
platform to detect COVID-19, which has been proven as a convenient detection platform in developed countries, whereas it is an unfeasible approach for low- and middle-income countries with limited resources and high COVID-19 burden. Real-time PCR requires trained professionals to handle and 3 to 4 days of processing time to acquire results. Moreover, in remote settings, such a platform is expensive, therefore, a limited number of products could be purchased by centralized laboratories, and such an instrument is difficult to handle as well as manufacture in local settings. In addition, a lack of a centralized data acquisition system impedes the accurate tracking of the total number of infected and recovered cases as well as infected geographical regions. Therefore, the lack of a robust infrastructure and feasible detection platform spread the ignorance regarding individuals’ health conditions, which results in the spread and the rise of COVID-19 cases. We believe, a portable POC diagnostics along with convenient data acquisition and monitoring system that not only an end-user can conveniently use in an at-home setting but also healthcare providers could use in a doctor’s office or hospitals. From an engineering perspective we believe, a low-cost DIY POC diagnostics that could be manufactured using locally available materials, and easy to use without any prior training would be a feasible platform for detecting COVID-19 in remote regions.

Several researchers are implementing microfluidics technologies to develop a reliable platform for detecting COVID-19 viruses miniaturizing the real-time PCR and incorporating continuous flow assay or antibody-based bioassay. Although such testing platforms could be reliable, these require sophisticated manufacturing techniques, expensive and cumbersome to use in countries with limited energy resources. In contrast, lateral flow-based microfluidic paper-based analytical devices (μPADs) have shown feasibility as a portable, low-cost, disposable, easy-to-use POC platforms in remote regions where energy is an issue. Lateral flow-based assay integrated with immunochromatography and nanoparticles has shown tremendous success in the past for detecting glucose level, virus, and bacteria in local settings. Therefore, in our opinion, an integration of different technical components in one platform, such as lateral flow-driven paper-based microfluidic substrate, real-time PCR technique, immune-chromatographic bioassay, nanotechnology, and rapid prototyping method will ensure achieve a reliable sensitivity and visual detection level at a low-cost. Also, interfacing such a platform with data analysis, manipulation, and acquisition techniques would take it one step further for developing a quantitative detection platform as well as an infected case tracking system. Although the advancement of cutting-edge technology allows us to develop a low-cost and feasible COVID-19 detection platform, the successful implementation is not possible without clinical utility validation, FDA approval, and entrepreneurial ventures. Therefore, a viable COVID-19 POC diagnostics platform for low-and medium-countries requires an amalgamation of all the above-mentioned technical and non-technical components.

In our perspective, an ecosystem integrating all technological and non-technical components is essential for developing a reliable COVID-19 POC diagnostic platform (Figure 1). Thus, the main objective of the current work is to describe the major components of a COVID-19 POC diagnostic ecosystem, the essential technological mechanisms from engineering aspects, and the feasibility of establishing such platforms in limited-resource settings.

The specific objectives of the study can be listed as

- Importance and significance of establishing a microfluidic COVID-19 POC diagnostic platform for low- and medium-income countries or energy deficit countries
- Rational to use different components for developing a COVID-19 testing platform
- To propose a microfluidic POC diagnostic platform, incorporating the fundamental concept of PCR, for detecting COVID-19 entities using unprocessed patient’s saliva

2 | COVID-19 POC ECOSYSTEM: TECHNOLOGY COMPONENT

A successful COVID-19 POC ecosystem requires a few crucial technology components, such as μPADs design and development, biochemistry assay establishment, analytical testing to ensure visual detection, and interfacing the test with smart devices as well as data analytics (Figure 1). In the following section, we discussed the rationale of using different components for developing a COVID-19 testing platform.

2.1 | The rationale for using different technological components for COVID-19 ecosystem

We discussed the significance and benefit of using μPADs for detecting COVID-19 in the previous section. Fabrication type, accurate biochemical process execution, and validation govern the design of a μPAD. In the following
paragraphs, we discussed the rationale for choosing the most feasible technological components.

Currently, a wide range of paper-based materials and fabrication techniques are prevailing to detect viruses in both academic and industrial settings. Such materials include printing paper, chromatography papers, Whatman FTA, different grades of Whatman filter papers, nitrocellulose membrane, parchment paper, and unconventional substrates, such as kitchen towels.13,14 Researchers capitalize on different properties of papers, such as surface characteristics, surface area, capillary flow rate, pore size, porosity, and thickness to achieve different microfluidic applications.15 It is worth mentioning that the choice of material and microfeature patterns need to be compatible with the biochemical assay needs to perform on the device. For instance, to lyse cells, to perform nucleic acid amplification, and to immobilize metallic nanoparticles-conjugated sensors at a different time point, Whatman FTA paper is a unique choice due to its proprietary blend of lytic reagents dried into the cellulose matrix, which facilitates the on-paper biochemical steps performance using microvolume of sample and a pipette.

To perform the assay and execute multiple steps in a time-dependent manner, microfeatures need to be fabricated precisely. Notably, in a resource-limited setting, using a sophisticated fabrication procedure could be challenging as well as expensive; therefore, an affordable and easy-to-use fabrication method is the most desirable. To achieve the desired microfeatures with high precision while manipulating certain properties of the paper substrate, different fabrication techniques are adopted, such as photolithography, saline and UV/O₃ patterning, PDMS printing, inkjet etching, plasma treatment, wax patterning, subtractive laser treatment, wax dipping, computer-controlled knife shaping, CO₂ laser cutting, and printed circuit technology.6 It is worth mentioning that the choice of fabrication techniques is highly governed by the choice of the paper substrate and biochemical process. To perform the assay and execute multiple steps in a time-dependent manner, microfeatures need to be designed and fabricated accurately. At low- or medium-income settings, to minimize the cost of manufacturing and ensure an easy-to-use DIY manufacturing technique, wax patterning, and CO₂ laser cutting are the best options.16,17

Colorimetric visual detection is the most convenient readout for POC diagnostics, which helps individuals decide a remote-settings.18-20 Therefore, using a metallic nanoparticles-based sensor that provides a robust color-dependent signal in the presence of a target analyte is crucial.21,22 Although visual detection is desirable to make a YES/NO decision, it gives only qualitative or semi-quantitative results. To obtain a quantitative result and to perceive the severity of illness index, different analytical tools are required. In remote settings, the most accessible system could be an automated software application integrated into a mobile phone or a portable device that can quickly quantify the result and indicate the severity of the illness index.

Also, collecting result data from each test and use them for further analysis, such as identifying the epidemiology and trend of the disease, are equally important. Therefore, interfacing the data analysis method with a cloud-based data repository system is necessary. Such data could be retrieved, manipulated, or used for retrospective analysis.
2.2 COVID-19 POC ecosystem: Nontechnology component

Technology components play a crucial role in COVID-19 POC product development; however, nontechnology features, such as clinical validation, receiving regulatory approval, initiating entrepreneurial ventures, and ensuring the practical applications are essential parts of a POC ecosystem. In this section, we discussed these four critical components of the COVID-19 POC ecosystem for bringing the platform from the bench-level to the user-level.

For clinical validation and regulatory approval of a COVID-19 POC diagnostics, the platform should detect, quantify, and predict the concept of interest to an adequate level. Platform potential can be predicted by using patient-derived samples, such as saliva samples which will be used for clinical validation at phases 1 and II. Clinically equivalent biochemical solution quantity should be used as a response to predict the magnitude of the virus DNA and thus could be used to predict the severity of the disease. The obtained data will further be processed by using automated image techniques. The predictive potential of the platform can be evaluated by comparing the obtained response data between patients and patient-derived samples. To find the minimum patient-derived sample size for the platform, statistical analysis should be performed by using retrospective response data attained from previously found results. The predictive potential of the platform involves critical parameters of the chemical solutions, including the volume, concentration, and pH. Based on the obtained predictive potential, the clinical cutoff of the chemical solutions should be selected. Furthermore, to achieve clinical validation, several reproducible tests should be performed across several laboratories and results should be cross-verified. Finally, the platform is used to demonstrate the ability to predict the endpoints (i.e., sensitivity, selectivity, positive predictive value [PPV], and negative predictive value [NPV]) and clinical safety and efficacy of the platform.

To develop and translate a COVID-19 POC diagnostic for clinical use, support from industry to the academic is needed. Though, there exists a gap in the area of diagnostic tool development. However, in near future once such clinical predictive model becomes more robust and reproducible, it may attract more funding and can give better clinical outcomes.

2.3 A prospective COVID-19 microfluidic diagnostic

Herein, we proposed a microfluidic POC diagnostic platform, incorporating the fundamental concept of PCR, for detecting COVID-19 entities using unprocessed patient’s saliva. The hypothetical design concept of this platform will be easy to manufacture in low- to medium-income countries using limited resources as well as easy to use.

We hypothesized a Whatman FTA-based paper substrate will be an ideal paper-based platform for developing a COVID-19 diagnostic device. Such a paper substrate could be backed-up by an absorption pad and a laminating pad (Figure 2A,B). The microfluidic features could be patterned with a wax pen or wax printer to develop a non-penetrable wax barrier whereas the intermediate barriers would be patterned with a dissolvable trehalose barrier that would delay the transport of the reagents. To identify the presence or absence of the target virus we are proposing a red-colored gold nanoparticles sensor that will shift the red color from red to blue in the presence of microliter-sized sample volume. The color shift will indicate the presence or absence of the target virus, which could be further used for quantitative analysis.

The conceptual design of the device will facilitate the sample flow, biochemical reactions within the device, interaction among analytes, and the detecting sensor to acquire a qualitative readout that could be further used for obtaining a quantitative result. According to Figure 2A, the entire microfluidic pattern has five zones on the device layer, which are sample zone, buffer zone, loop-mediated isothermal amplification (LAMP) Master Mix zone, Ethylenediamine tetraacetic acid (EDTA) zone, and sensor zone. The workflow will start with dispensing the patient’s saliva sample in the sample zone that will wick through the porous structure of the substrate and will gradually dissolve the adjacent trehalose barrier. The required time to dissolve the barrier will delay the sample flow to the following buffer zone that will provide a user the required timeframe to dispense the buffer in the buffer zone. The buffer zone and the Master Mix zone will be separated by another dissolvable trehalose barrier, which also will delay the flow of the sample-buffer solution so that the user can dispense the Master Mix within a required timeframe. The sample-buffer-Master Mix will be incubated for 1 hour at 60°C in a simple egg incubator. Therefore, the thickness of the trehalose barrier between the Master Mix zone and EDTA zone will be optimized so that it could retain the reagent mixture for 1 hour. After the incubation, EDTA will be dispensed in the EDTA zone and will react with the amplified sample. Finally, the resultant mixture will wick in the sensor zone to interact with the red-colored functionalized gold nanoparticles sensor. In the sensor zone, in presence of the target virus, the red color of the sensor will shift from red to blue or grey (Figure 2D, left), otherwise, in absence of the target virus, it will retain the red color (Figure 2D, right).
The entire process of developing a COVID-19 POC diagnostic assay and device has been described in Figure 2C,D. To ensure the feasibility of the proposed device design, the concentration and dimension of the trehalose barrier, dimension of the wax barrier, and the sensor zone, as well as the concentration and the volume of the reagents, have to be optimized.

We also hypothesized that such a device could be further interfaced with a mobile phone camera and mobile phone data analysis application or handheld data analysis device. Such a mobile phone application or the in-built data analysis software would be designed to analyze the colorimetric changed in the images of a tested sensor zone. Based on the shift of color such a platform will be able to provide us information regarding the presence or absence of COVID-19 in a patient’s sample. Furthermore, this software could be interfaced with the local public health database so that all test results could be recorded. Such an approach would certainly help a local setting to track the current statistics of the infected patients.

3 CONCLUSIONS

Herein, we discussed the importance and significance of establishing a microfluidic COVID-19 POC diagnostic platform for low- and medium-income countries. We also discussed all technological and nontechnological components of the platform, which are essential for materializing such a platform. Finally, we proposed a simple, easy-to-use, and low-cost POC diagnostic platform, a μPAD, to detect COVID-19 using unprocessed patient’s saliva samples. The manufacturing components and process requires a small capital and minimum training to execute, which makes the proposed technique feasible and economic for low- and medium-income countries (or energy deprived countries), which have limited resources. Furthermore, the proposed scientific approach is convincing due to two reasons, such as the biochemical process is a miniaturized version of the conventional PCR-RT technology, and gold nanoparticles sensor has been proved as a promising analyte-sensing technique. Furthermore, interfacing the testing system with an image acquisition and analysis technology will help users to identify whether an individual carries COVID-19 or not. Finally, the nontechnology components are essential for validating and commercializing such platforms. We envision that the proposed microfluidic COVID-19 POC diagnostic ecosystem will help the low- or medium-income countries to manufacture the platform locally and will facilitate the testing in a local setting. Such ecosystems will be a pragmatic approach for rapid testing of
COVID-19 and to curb down the number of the affected people in a remote setting.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Chowdury MA, Khalid F. Application of microfluidic paper-based analytical device (μPAD) to detect COVID-19 in energy deprived countries. Int J Energy Res. 2021; 1–6. https://doi.org/10.1002/er.6958