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## Progress in the development of paper-based diagnostics for low-resource point-of-care settings

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### Abstract

This Review focuses on recent work in the field of paper microfluidics that specifically addresses the goal of translating the multistep processes that are characteristic of gold-standard laboratory tests to low-resource point-of-care settings. A major challenge is to implement multistep processes with the robust fluid control required to achieve the necessary sensitivity and specificity of a given application in a user-friendly package that minimizes equipment. We review key work in the areas of fluidic controls for automation in paper-based devices, readout methods that minimize dedicated equipment, and power and heating methods that are compatible with low-resource point-of-care settings. We also highlight a focused set of recent applications and discuss future challenges.

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For accurate and reliable disease detection, many gold-standard diagnostic methods rely on multistep sample preparation and analysis techniques that require expensive laboratory equipment and trained technicians. Thus, these tests are not appropriate for point-of-care (POC) use, especially in low-resource settings, and are not available to populations who need them most. Over the last two decades, research and development in the field of microfluidics has made advances in the miniaturization, automation and cost-reduction of gold-standard laboratory-based sample processes, in order to make them appropriate for POC applications [1]. Traditionally, POC diagnostic tests were designed as single-use chips composed of plastics, polydimethylsiloxane or silicon that utilized the many advantages of microfluidics, including requiring only small sample and reagent volumes and having a rapid time to result [2]. In recent years, there has been an explosion of interest in the use of porous materials in POC diagnostic devices. The Whitesides group is responsible for generating much of this interest, starting with their demonstration in 2007 of using patterned cellulose to simultaneously detect glucose and protein in urine samples [3] and continuing with multiple contributions on detection using microfluidic paper-based analytical devices ( $\mu$ PADs) ([4–6], earlier work is reviewed in [7]). Since then, there have been many advances in the still growing subfield of ‘porous network microfluidics’ or ‘paper microfluidics’.

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Specifically, we define a ‘paper’ (the term ‘paper’ is defined broadly here to include porous membranes such as **nitrocellulose**) or ‘porous network’ as a device that exclusively uses capillary pressure to drive flow in the porous matrix of which it is composed, for the automatic pumping of fluid within the device. These networks are most often composed of the porous materials used in the conventional lateral flow industry: **cellulose** and nitrocellulose [8,9]. These porous network devices share other attributes of the conventional lateral flow test (LFT); namely they are disposable, have a rapid time to result, are composed of inexpensive materials, and can be cost effective to manufacture.

In recent years there have been multiple reviews focused on work in paper microfluidics. Ballerini *et al.* reviewed the increased functionality and variety of paper-based materials for the development of POC diagnostics [10]. Li *et al.* have described the various fabrication techniques and some applications for paper-based technologies [11], while other reviews have focused specifically on paper-based device fabrication via ink-jet printing or toner transfer methods [12]. Nie *et al.* and Maxwell *et al.* separately described electrochemical sensing techniques in paper-based devices [13,14], while Liana *et al.* took a broader perspective focusing on recent advances in all paper-based sensing technologies and the need to increase device sensitivity [15]. There have also been recent reviews on the challenges of transferring traditional plastic-based electronics to paper-based formats [16], as well as the development of LFTs for the detection of contaminated food [17]. Shah *et al.* reviewed the development of an entire paper-based analytical kit for biomarker and bacterial detection at the POC [18]. Kuo *et al.* described the emerging trend of hybrid devices that utilize the advantages of different substrates in one combined effective device [19]. Additionally, developments in materials science that are relevant to paper microfluidics have been reviewed recently [20–22], including a discussion of the advantages of paper as a material for devices [23]. Most recently, Yetisen *et al.* have reviewed multiple areas in the field of paper microfluidics, including device fabrication, device capabilities, detection techniques on paper and quantitative handheld readout systems [24]. Although this is not a comprehensive list of porous membrane-based devices reviews, it is representative of the breadth of literature surrounding paper microfluidics and indicates the rapidly growing interest in the field.

In this Review, we focus on recent work in the field that specifically addresses the goal of translating the multistep processes that are characteristic of gold-standard laboratory tests to low-resource POC settings. There are specific constraints that must be addressed when designing tests that are appropriate for use in these settings. For example, the often lengthy time to result of many laboratory-based diagnostic tests can be inappropriate in low-resource settings, where requiring patients to make at least two visits before getting diagnosed may be impractical [25]. These tests need to be affordable, specific, sensitive, user-friendly, rapid and robust, equipment-free and deliverable (or ‘ASSURED’) [26]. Thus, a major challenge is to implement multistep processes with the robust fluid control required to achieve the necessary sensitivity and specificity of a given application in a user-friendly package that minimizes equipment. We review key work in the areas of fluidic controls for automation in paper-based devices, readout methods that minimize dedicated instrumentation, and power

and heating methods which are compatible with low-resource POC settings. Finally, we highlight a focused set of recent applications and discuss future challenges.

## Fluidic controls for automation in paper-based devices

A major challenge in creating POC diagnostic tests that are appropriate for applications in low-resource settings is to achieve precise and robust automated fluidic control of the multiple fluid sources in an assay. As in conventional lateral flow assays, properties of the porous materials, including pore size, pore structure and surface treatments, affect the delivery of reagents in an assay. These considerations have been discussed previously in the context of conventional LFTs and will not be discussed here [27]. Recently, a number of tools have been developed to control fluid flow in porous network devices. These tools serve to replace the costly and often complicated valves and pump controls in conventional microfluidics. This section will discuss fluidic controls for automation in porous network devices including network topology, channel geometry for changing flow rates, on-switches or delays for flow, off-switches for flow, and spatial and temporal controls for manipulating rehydrated reagents.

### Network topology for the sequential delivery of multiple fluids

Networks with multiple inlets for each outlet have been developed to perform the automatic sequential delivery of multiple fluids to a detection region [28–31]. Fu *et al.* have demonstrated a simple three-inlet 2D paper network (2DPN), shown in Figure 1, in which fluids, after being applied simultaneously to the inlets, are staged in the main horizontal leg of the device, and then delivered sequentially to the detection region [28]. The dimensions of the network can be used to accommodate different volumes of fluids and the number of inlets increased to accommodate a greater number of reagents.

More recently, Dharmaraja *et al.* demonstrated that sequential delivery can be achieved through the use of a Pseudo-1DPN [32]. This format consists of a linear strip composed of a porous material and multiple fluid-filled wells as sources. As in the case of the 2DPN above, the contents of the sources are delivered sequentially to the detection region in order of distance from the detection region. The authors presented an electrical circuit model for explaining the sequential delivery of the multiple fluids in the strip as a balance of the capillary pressures and fluidic resistances in the system. An alternative source used in conventional LFTs that may be more appropriate than fluid-filled wells for the lowest-resource settings are porous pads. Pad sources were also used to produce clean sequential delivery when the fluidic resistances between the pads were made sufficiently large by increasing the interpad spacing.

### Channel geometry to control flow rates

A key method for controlling the flow rate in porous channels is to vary the geometry of the channel. Flow in 1D lateral flow strips is well characterized. The 1D transport of the fluid front in a porous matrix during wet-out is characterized by the Lucas–Washburn equation:

$$L^2 = \frac{\gamma D t}{4\mu} \cos\theta \quad \text{Equation 1}$$

where  $L$  is the distance moved by the fluid front,  $t$  is time,  $D$  is the average **pore diameter**,  $\gamma$  is the effective surface tension,  $\cos\theta$  is the contact angle dependence, and  $\mu$  is viscosity [33–35] (for review on the physics of paper see Alava and Niskanen [36]). Therefore, in a constant width strip:

$$L \sim \sqrt{t} \quad \text{Equation 2}$$

the length of the strip can be used to control the interaction time between reagents in an assay. Additionally, the volumetric flow rate can be adjusted by varying the width of the strip [37]. In an extension to 2D structures, Medina *et al.* investigated imbibition in triangular shapes of blotting paper and characterized the deviation from **Lucas-Washburn flow** [38]. Recently, Fu *et al.* have described the use of simple 2D geometries to control the flow rates of fluids and presented some basic design rules for transport in paper networks [37]. For example, in the case of an expansion in geometry, a transition to a greater width results in an increase in the transport time of the fluid front relative to that in a constant width strip. The complementary result for the case of a contraction, a transition to a smaller width, is that the transport time of the fluid front is decreased relative to that in a constant-width strip. Thus, a simple control parameter for regulating the transport time of the fluid front is the downstream location of the expansion or contraction. In addition, the width of the expansion or contraction can be used to further adjust the transport time.

In the case of **fully wetted flow**, when the fluid front has reached a wicking pad, an absorbent pad used to wick additional fluid through the substrate, the velocity is approximately steady and is described by Darcy's Law. In 1D, Darcy's Law reduces to:

$$Q = \frac{-\kappa A}{\mu l} \Delta P \quad \text{Equation 3}$$

where  $Q$  is the volumetric flow rate,  $\kappa$  is the permeability of the material to the fluid,  $A$  is the area of the channel perpendicular to flow,  $\mu$  is the viscosity of the fluid, and  $\Delta P$  is the pressure difference along the direction of flow over the length  $l$  [39]. An analogy to electrical circuits can be useful, where pressure difference is analogous to electrical potential difference, the volumetric flow rate is analogous to current, and the fluidic resistance depends on physical parameters of the system, including geometric factors in the paper circuit. Extension to simple 2D geometries is straightforward; fluidic resistances in series are summed while fluidic resistances in parallel can be added in reciprocals. Fu *et al.* presented a description of how one can predict and control flow rates for simple changes in geometry, such as those in Figure 2 [37]. A time series comparison of experimental and simulation results for flow in the strips of different geometries indicates good quantitative agreement [37]. Furthermore, simple manipulation of inlet leg length and width were used to control the time of reagent delivery in a 2DPN [37].

Mendez *et al.* employed complex paper shaping to adjust the flow rate in their devices [40]. Specifically, they used fan shapes located downstream of a rectangular strip, as shown in Figure 3, to achieve the quasi-stationary flow of conventional lateral flow strips that use cellulose wicking pads. Once fluid reaches the fan-shaped section, there is a sudden increase in the volume of available dry porous media, similar to the situation in a wicking pad, and the transport of the fluid front within the rectangular stem transitions from Lucas–Washburn to quasi-stationary. A significant advantage of this method is potential simplification to device fabrication and manufacturing by requiring only a single material. Additionally, quasi-stationary flow can enable approximately constant reaction time within porous membrane-based assays.

### On-switches/delays to control the arrival time of fluids

Another key fluidic control is the ability to delay the downstream arrival of a given fluid for a specific amount of time. As described above, the use of network topology and channel geometry can be used to achieve sequential delivery, but for the case of larger reagent volumes, these methods can significantly increase the footprint of the device. Thus, alternative paper fluidic tools that act as an on-switch, or a timed fluid flow delay, are critical. Several methods have been developed and will be discussed in this section. These include methods that do not require any user intervention, such as dissolvable barriers and hydrophobic surface treatments, as well as user-activated methods, including folding tabs and push buttons.

One type of on-switch or delay for fluid flow uses dissolvable barriers. Lutz *et al.* have demonstrated that a dissolvable sugar barrier will produce a delay in the transport time of fluid relative to if the barrier were absent [41]. Both the extent of the sugar barrier and the concentration of the sugar solution used to form the barrier within the porous material can be used to control the delay time.

Delays from seconds to almost an hour were achieved with measured variability in arrival times between 9 and 19% [41]. One potential issue with this method is the effect of the barrier components on downstream processes. For example, low concentrations of sugar, such as trehalose, are used for the preservation of dry reagents and are generally compatible with common signal-generation schemes for LFTs. However, the impact of higher concentrations of sugar, as employed by the barrier for longer delay times, on signal generation should be considered. Another potential issue for the use of higher sugar concentration barriers is the effect on sample and reagent fluid flow downstream of the barrier due to increased fluid viscosity. A second type of on-switch for flow is based on the use of hydrophobic and hydrophilic coatings to control the wetting properties of the substrate. Noh *et al.* controlled flow rate using a hydrophobic wax coating on the cellulose substrate to affect the wettability of the cellulose and reduce the flow rate in a wax-treated region [42]. A wide range of delays were demonstrated, from 30 s to over 1 h, with increasing time delays achieved by depositing increasing quantities of wax [42], as shown in Figure 4. The use of multiple wax-treated regions in series provided fine-level control of flow rate within a device. Chen *et al.* employed hydrophobic and hydrophilic coatings to develop a single-use fluidic diode [43]. In their diode design, a hydrophobic barrier

separates two channels with surfactant deposited on only one side of the barrier. Thus, fluid flow can be switched on in the direction in which surfactant is rehydrated before reaching the hydrophobic barrier; the rehydrated surfactant enables the fluid to cross the previously hydrophobic barrier. No flow is allowed in the other direction unless triggered, as shown in Figure 5. As in the case of the dissolvable barriers, the effect of the released chemical, in this case the bolus of surfactant, on downstream processes should be considered. A third method for creating a delay of fluid flow was demonstrated by Toley *et al.* They demonstrated delays of fluid flow in nitrocellulose channels by using a parallel piece of cellulose to wick fluid from the nitrocellulose channel. The delay time could be varied by manipulating the length and thickness of the cellulose piece. Delays of up to approximately 18 min were demonstrated with coefficients of variation in delay times of less than 10% [Toley B, McKenzie B, Liang T, Buser J, Yager P, Fu E. Tunable-delay shunts for paper microfluidic devices. Accepted to *Analytical Chemistry* (2013), Unpublished Data].

Several active methods for the automated switching on of flows have also been developed. Koo *et al.* have demonstrated electrowetting of a dielectric to control flow rate in a porous channel [44]. Specifically, they showed the ability to tune the flow rate in nitrocellulose channels by varying the applied potential between their hydrophilic and hydrophobic electrodes, with increasing potential difference producing shorter time delays [44]. A potential advantage of this method is very fine control of the flow rate. Li *et al.* have demonstrated magnetically actuated valves that are triggered using an ionic resistor to sense fluid flow in the channel [45]. A potential advantage of this method is the capability to also switch off fluid flow. A disadvantage of both of these methods is the requirement of additional instrumentation, a power source in the former case, and an electromagnet and electrical circuit in the latter case. The cost per test may still be reasonable for lower-resource settings since the instrumentation cost may be amortized over the instrument lifetime. However, the requirement for maintenance or repair on the instrumentation may not be supported in the lowest-resource environments.

In addition to the automated methods for turning on fluid flows described above, simple and user-friendly methods of turning on flows based on a mechanical step have been developed. Fu *et al.* have demonstrated a card format for simple device activation that is appropriate for use in POC settings [30]. In this method, reagents are loaded onto source pads located on one side of a folding card, while the network is located on the opposite side of the folding card, as shown in Figure 6. Folding then creates contact between the source pads and network, initiating fluid flow from all sources into the network simultaneously. Reagents can be stored dry in the source pads, so the user only adds sample and water, further simplifying user operation. While this technique requires a mechanical step, it is not a time-critical step. Use of the method does require careful design of the card such that folding produces a reproducible and reliable contact between the source pads and the network.

Another type of mechanically activated valve has been demonstrated by Li *et al.* in a paper device [46]. A foldable paper tab-based switch was designed such that the fluidic channel upstream of a reaction site is discontinuous while the tab is folded. Once the tab is unfolded, fluid can then progress to the reaction site. In this manner, multiple valves can be employed to deliver multiple reagents in a desired sequence. Similarly, Martinez *et al.* designed user-

activated single-use buttons in their 3D  $\mu$ PADs to be used in two contexts; a user initiating one or more assays from a panel of assays and running a fluidic de-multiplexer [47]. The 3D  $\mu$ PADs were fabricated such that a gap exists between two adjacent layers of paper and fluid cannot wick between the paper layers without intervention. In order to connect the paper channels, mechanical force was applied by pressing the button with a stylus to bring the layers into contact. More recently, Liu *et al.* have demonstrated the SlipChip approach in paper substrates [48]. In their SlipPAD, the ON/OFF switch of flows is accomplished through user sliding of two paper layers [49]. A significant advantage of this approach is high-throughput parallel detection in an array format. Although simple in design, a potential disadvantage of mechanically activated valves if used for a time-critical operator step, is the possibility of user error that could produce downstream variability in signal generation.

### Off-switches for volume metering

A complementary fluidic control to the on-switch is a fluid flow off-switch. A critical use of an off-switch is to automatically meter multiple fluid volumes, enabling easy-to-use devices that do not require the user to make any precise measurements of fluid volumes. One automated method to control the shut-off of multiple flows independently is the use of inlet legs that are submerged into a common well at varying distances, as demonstrated by Lutz *et al.* [50]. The level of the fluid in the well drops as fluid wicks into the paper inlets. Fluid shuts off from the inlets at different times, in order of shortest to longest submerged lengths, as shown schematically in Figure 7. Additional parameters that can be used to control the shut-off volume for an inlet include the geometry of the fluid well, the geometry and composition of an inlet leg, and the use of a regulator wick (as shown in Figure 7). This method has been characterized for reproducibility with coefficient of variation in shut-off times between 5 and 20% [50]. Automated sequential reagent delivery from a single user activation step has been demonstrated using this method in a simple 2DPN [50]. A potential disadvantage of this method is the requirement for the device to rest on a level surface in a particular orientation. An alternative method to turn off flow from multiple inlets independently, is the use of pads of varying fluid capacity that are prefilled to saturation [30]. Contact between the pads and inlets activates the multiple flows. Fu *et al.* have demonstrated that volume metering using source pads provides reproducible fluid release profiles, with coefficient of variation of less than 10% in the volumes delivered [30]. The properties of the pad, most importantly the bed volume and the surface treatment, determine the release profile of fluid from the pad to the inlet. Furthermore, the rate of fluid volume released into the paper network can be adjusted by varying the cross-sectional area of the inlet.

### Temporal & spatial control of rehydrated reagents

Complementary to the control of fluid transport using the tools described above is the ability to manipulate the spatial and temporal concentration profiles of rehydrated reagents within the porous channels. For example, Fridley *et al.* have demonstrated that the amount of reagent delivered in a pulse can be tuned by varying the amount of reagent printed in each spot and the number of spots arrayed across the channel [51]. Furthermore, the duration of the reagent pulse can be adjusted by varying the amount of sugar patterned with the reagent. Patterning is a serial process, so the time for patterning reagents onto substrates must be

factored into the time for device fabrication. However, a significant advantage of patterning directly onto the substrate is that a complete assay can be fabricated using a single material; this can translate to a reduced number of pieces that must be assembled to create the final device and lower manufacturing costs.

We have examined a variety of tools for the control of fluid flow and reagent delivery in porous materials. These constitute a set of fluidic tools that can be used alone or in combination to create automated integrated paper microfluidic assays. Examples are included in the applications section below.

## Readout methods compatible with low-resource POC settings

It is important that the users of POC diagnostic devices in low-resource settings are able to easily read and interpret assay results with minimal instrumentation. High-performance tests often rely on methods that require expensive dedicated instrumentation for data acquisition, analysis and interpretation. However, the added cost and requirement for maintenance then makes these tests unsuitable for many POC settings [26]. LFTs are a longstanding example of a POC immunoassay that produces a visible signal that can be simply read by eye [27]. For example, the pregnancy dipstick is a well-known example of a LFT, which detects human chorionic gonadotropin. Current disadvantages of many commercially available instrument-free LFTs are that the signal is restricted to being qualitative and that sensitivity is too low to have clinical significance for a number of analytes of interest [52]. Introduction of a dedicated reader to enable more sensitive or quantitative readout of LFTs has been a successful approach for higher resource POC settings [27] (commercially available dedicated readers including smartphone-based readers have been reviewed by Yetisen *et al.* [24]). However, this approach leads to a greater cost of the final device and a requirement for maintenance that may not be appropriate for lower-resource settings. Here we focus on several promising methods that have been developed to enable quantitative or higher sensitivity readouts that are easy to use, low cost and have minimal dedicated instrumentation. These include methods for instrument-free semi-quantitative visual readout and adapters for use with nondedicated mobile phones for extended assay readout capabilities.

### Quantitative visual readout

A noninstrumented approach has been developed to provide semi-quantitative readout of LFTs, called a ladder-bar assay [53–56]. The ladder-bar assay is based on the stepwise generation of signal (e.g., due to binding of analyte and antibody conjugated to label) at patterned stripes of reacting species in the detection region (e.g., immobilized capture antibody) to produce a readout in which the number of colored lines is an indication of analyte concentration. Fung *et al.* adapted the ladder-bar method to demonstrate an enzyme-based assay for the quantification of hydrogen peroxide, the product of glucose and glucose oxidase [53], as shown in Figure 8. The assay response can be tuned through the manipulation of the number and spacing of capture stripes, the density of capture molecules, and the velocity of fluid flow through the detection region. According to the Lucas–Washburn relationship, the fluid front velocity decreases with the distance traveled. Therefore, the time of interaction between analyte and surface-bound antibodies increases at



each subsequent test line. This complication should be taken into consideration during the development of a ladder-bar assay. Recently, a similar readout strategy was demonstrated by Lewis *et al.*, using a device to quantify the level of hydrogen peroxide in a sample [57]. In this method, oxidative cleavage of a hydrophobic compound by hydrogen peroxide yields hydrophilic compounds, which modify the wettability of the channel and the rate at which the sample containing hydrogen peroxide wicks through the membrane. The number of colored radial bars, read at a specific time point, determines the sample concentration. Higher quantitative resolution is achieved without instrumentation through this method; however, the requirement for readout at a specific time may not be compatible with user needs in lower-resource settings and has the potential for signal variation due to user error in time of readout. A complementary approach is distance-based readout. Zuk *et al.* and Chen *et al.* have demonstrated an enzyme-based immunoassay for theophylline with coefficients of variation of less than 10% [58, 59]. Most recently, Cate *et al.* have demonstrated distance-based readout using multiple detection schemes, including an enzyme-based system for glucose detection [60]. They obtained a comparable limit of detection to, and a dynamic range approximately half that of a commercial system [60].

### **Nondedicated mobile phone reader model for expanded assay readout capabilities**

The nondedicated mobile phone reader model is promising for applications in low-resource POC settings that require an expanded assay readout, for example, fully quantitative output, over what is possible with simple visible readout by the user. In recent years, multiple reports have testified that mobile phones hold the power to transform global health and diagnostic technologies [61,62]. This is due to their ease of use and highly ubiquitous dissemination, with recent estimates of at least 5 billion devices worldwide [101]. Camera-equipped mobile phones have permeated throughout even the least developed countries. They are lightweight, portable, increasingly affordable, and can provide high resolution images (>2 megapixels) [63].

Martinez *et al.* first outlined a process for the quantification of diagnostic assays by remote analysis of image data taken by a mobile phone camera [63]. In their demonstration, the camera on a mobile phone was used to image the colorimetric results of a bplexed glucose and protein assay. The image was then sent to a remote computer through a wireless communication system, and there the image was processed to output analyte concentration. In their use scenario, the quantitative results could then be reported back to the user's mobile phone along with a prescribed treatment. A complementary approach to extract quantitative kinetic data using smartphones was presented by Stevens and Yager [64]. They demonstrated that the rate of intensity generation extracted from smart-phone-acquired image data could be correlated with the concentration of the target analyte in a flow-through membrane-based immunoassay. Potential advantages they cite are a shorter time to result and a larger dynamic range compared with end-point measurements. In an extension of this work, Dell *et al.* demonstrated the ability to process the kinetic data in real time using an Android mobile phone [65].

Further utilizing the ubiquity of camera-equipped mobile phones, the Ozcan laboratory (MA, USA) has developed adapters that can physically attach to a nondedicated mobile

phone for use in multiple contexts. Zhu *et al.* have demonstrated wide-field fluorescent and darkfield imaging using an adapter composed of compact, lightweight and cost-effective optical components, including a simple lens, a plastic color filter, LEDs and a battery [66]. A spatial resolution of 10  $\mu\text{m}$  was achieved with a field of view of 81  $\text{mm}^2$ , allowing for the possibility of a high level of multiplexing [66]. Considering the high-sensitivity of fluorescence detection, this approach is especially promising for applications that require a very low limit of detection. More recently, Mudanyali *et al.* extended this work to perform imaging and analysis of commercially available LFTs in reflection and transmission mode using the smartphone plus adapter system demonstrated in Figure 9. In this demonstration, raw images were acquired and digitally processed by an application on the smartphone, increasing robustness of the results, especially at lower concentrations that approached the limit of detection [67]. As a further extension of this work, a personalized food allergen test was demonstrated [68]. Compared with visual inspection, the optical readout was rapid (image was processed within 1 s) and allowed for a more sensitive quantitative result (between 1 and 25 ppm). The smartphone plus adapter approach is promising, directly addressing issues such as variability of ambient lighting conditions and variability of user positioning of the smartphone.

## Power & heating compatible with low-resource POC settings

Many laboratory-based assays utilize mains electricity for operation. For example, power is required to generate the excitation source for fluorescence-based assays. Examples of processes that require heating are nucleic acid amplification reactions and cell lysis protocols. However, the requirement for mains electricity is not compatible with low-resource POC settings in which electricity is either unreliable or unavailable. Recently, several groups have developed novel techniques to deliver consistent power or heat to assays without this requirement.

### Fluidic batteries

Various groups have developed fluidic or paper-based batteries in order to avoid the expense and hazardous waste associated with traditional lithium ion batteries. These fluidic batteries can be powered by electrochemical reactions and are fully disposable. Hilder *et al.* have characterized the open-circuit voltage and discharge current – up to 1.2 V and 500  $\mu\text{A}$ , respectively – of screen-printing zinc/carbon–poly(3,4-ethylene-dioxythiophene) (PEDOT)/air batteries on various paper substrates [69]. Liu *et al.* further developed this method by integrating a paper-battery into an electrochemical sensing platform [70] as shown in Figure 10. Their integrated battery powered an electrochemical reaction and detection system to detect glucose and hydrogen peroxide in artificial urine samples. Thom *et al.* designed a paper-battery capable of powering a UV light-emitting diode, and demonstrated it in an on-paper fluorescence assay [71]. Their assay detected  $\beta$ -D-galactosidase in a sample in less than 20 min. Furthermore, Zheng *et al.* reviewed conductive papers and energy storage mechanisms for paper-based devices [21].

### Smartphones as a power source

To date, the majority of smartphone-based technologies have been used for sensing and detection techniques rather than for powering diagnostic devices [72,73]. The one major exception has been MobiSante's US FDA-approved ultrasound imaging system that is powered by a dedicated smartphone-based reader [102]. Smartphones have the capability to provide reliable power directly at the POC. For example, the iPhone can output 3.3 V using commercially available pinout connectors such as the PodSocket Breakout from Spark Fun Electronics (CO, USA) [103]. This inexpensive (US\$15 retail), reusable device plugs directly into the charging port of an iPhone and can easily create a POC power source.

### Heaters

To provide consistent heat to a system, there are two well-developed options suitable for low-resource POC settings. The first is an inexpensive, single-use, flexible thin film heater, shown in Figure 11, which has been demonstrated to heat disposable plastic cassettes for nucleic acid amplification reactions for up to 1 h with minimal power requirements by Liu *et al.* [74]. As an example, a temperature of 40°C requires only 0.15 W of power (~0.82 V from a thin film heater, Minco Products, Inc., MN, USA). This voltage could easily be supplied with a mobile phone or fluidic battery as described above. The second option eliminates the need for any electrical power by utilizing heat supplied by an exothermic chemical reaction. LaBarre *et al.* have described the use of exothermic chemical reactions activated by the addition of aqueous buffer as the heat-generating mechanism for a POC device, achieving constant temperature profiles at 38°C, 55°C and 63°C ( $\pm 1^\circ\text{C}$ ). Critical to achieving a robust and stable heating system is coupling the heat-generating reaction to a phase-change material, as shown in Figure 12. They demonstrated the use of their heating mechanism to power a nucleic acid isothermal amplification reaction (Loop-mediated isothermal amplification) in a tube. This reaction ran at  $63 \pm 1^\circ\text{C}$  for 38 min with detectable signal down to one genome copy per microliter [75].

### Selected applications for low-resource POC settings

Using several of the above-described tools, multiple groups have developed paper-based devices for low-resource POC applications. Devices have been developed to perform multistep sample processing for sample preparation, amplification and high-sensitivity detection in a package suitable for use at the POC.

### Sample preparation

One of the most challenging aspects of device development for the POC is sample preparation. Govindarajan *et al.* demonstrated the extraction of bacterial DNA using multiple folding steps in a paper origami device [76]. This device utilized dried lysis buffer that was rehydrated upon assay activation. Osborn *et al.* demonstrated multiple mechanisms for paper-based dilution, mixing and sample separation based on the diffusion of particles through nitrocellulose membranes [77]. Most recently, Abbas *et al.* have used a combination of polyelectrolyte coatings and the shape of the paper substrate for analyte separation and concentration [78]. Separations were achieved, using devices such as shown in Figure 13, via differential electrostatic interactions of the species of interest in the sample with the

charged paper substrate. Transport of the analyte into the very small diameter tip, approximately 20  $\mu\text{m}$ , of a star geometry was accomplished through capillary flow and evaporation at the tip, producing a reported concentration factor of many orders of magnitude [78].

### Multistep high-sensitivity assays

There is a need for assays with improved limits of detection in a package suitable for use at the POC. Fu *et al.* have developed a signal-amplified 2DPN folding card assay for human chorionic gonadotropin detection [29] that relied on a single time-critical user step. In an extension of this work, they eliminated the time-critical user step by fully automating the sequential delivery of reagents in a signal amplified 2DPN for the detection of the malaria protein *Pf*HRP2 [30]. The assay achieved a fourfold improvement in the limit of detection compared with the assay without the signal amplification and rinse steps. In this case, a commercially available gold-enhancement reagent was used; further gains in sensitivity should be possible using alternate signal-amplification methods such as enzyme-based systems. Lewis *et al.* have developed a novel signal amplification method in a time measurement-based paper assay [79]. Their method for the detection of hydrogen peroxide uses the reaction between hydrogen peroxide and an initially water insoluble oligomer to produce a water-soluble oligomer and allows the flow of sample through the device [79]. The transport time for the sample decreases with increasing concentration of analyte [79]. A second approach that can deliver high sensitivity is nucleic acid amplification. Rohrman *et al.* have published results demonstrating isothermal amplification (recombinase polymerase amplification) in glass fiber at ten copy sensitivity using lateral flow detection [80]. Nucleic acid amplification in a porous matrix coupled with the advances described above in equipment-free heating have the potential to bring high sensitivity nucleic acid amplification assays to low-resource settings.

A third approach to achieve high-sensitivity is to use noncolorimetric detection methods in paper-based assays. Ge *et al.* have developed an origami-based paper immunoassay with chemiluminescence detection [81]. The multistep assay was implemented through a sequence of appropriate user folds. Using this system, they demonstrated detection of cancer markers from whole blood with comparable performance to a commercial assay [81]. In a complementary demonstration, Li *et al.* demonstrated a paper origami device based on electrochemiluminescence detection [82]. Wan g *et al.* extended this work for the detection of a cancer marker in clinical samples; their device is shown schematically in Figure 14 [83].

### Challenges

Moving forward, a big challenge to the creation of paper-based diagnostic devices for use at the POC is integration to create standalone automated sample-in to result-out devices. As discussed earlier in this article, there has been much activity and progress to date on creating tools for flow control, power and heating methods compatible with low-resource settings, extended readout capabilities with minimal instrumentation, and discrete modules for specific analytical processes. A fully integrated device will include all processes that are required to achieve the performance specifications of the intended application: sample

transfer, sample preparation, capture and labeling of the target analyte, detection and transmission of results. The integration issues relevant to the creation of paper-based diagnostic devices are analogous to the issues in conventional microfluidics-based diagnostic device development. Successful device integration can be achieved by a two-part approach composed of a system-level view of the whole device and individual module development [84].

This approach enables balancing issues of connectivity of the modules and the cost and ease of overall device manufacturing, with potential trade-offs in the performance of the integrated modules, compared with the optimized individual modules; all with the goal of meeting the performance specifications of the intended application and the design constraints of the intended setting.

For example, issues relevant to device integration need to be considered when making a choice of final device material(s). The best performance for the individual modules may be produced when utilizing a different material in each module. This is because the individual modules may have different functional requirements that translate to a need for different material characteristics for properties such as flow rate, compatibility with specific surface treatments, and propensity for surface adsorption. However, choosing to include multiple materials in the final integrated device requires that the chosen materials be compatible on several levels. First, the material used in a downstream module must be compatible with the upstream processes (e.g., resistant to a solvent used in an upstream process that then flows downstream). Second, the materials need to be compatible with respect to the creation of robust fluidic connections between the materials in different modules. Third, the disparate materials must be compatible in terms of the existence of a straightforward fabrication process that has the potential to be scaled up. Additionally, the added cost to device manufacturing when using disparate materials should be considered and determined to be reasonable for the intended application, if the device is to have a chance at translation to a commercial product [85]. Down-selection to one material may result in a decrease in performance in one or more modules, but this may be acceptable, if performance specifications for the intended application are still met. Thus, there are multiple trade-offs involved in the final material(s) choice. A thorough understanding of the cost constraints and performance requirements of the end-use application will be needed to optimally balance the issues involved in creating a fully integrated device.

A second big challenge is to fulfill the promise of nondedicated mobile phone use in a POC diagnostic system. Recently there has been much discussion of the expanded use of mobile phones in POC diagnostics; multiple potential functions include: directing test operation, analyzing test results, interpreting test results and transmitting test results to a healthcare provider and instructions back to the patient. A critical requirement for realizing the full potential of mobile phone use in POC diagnostics is infrastructure to interface with the existing healthcare system. For example, protocols for the mode of patient interaction with the healthcare provider need to be developed. Specifically, protocols for the use of synchronous or asynchronous communications, procedures for prioritization of these communications based on the nature of patient test results, and mechanisms for ensuring appropriately timely communications between the patient and healthcare provider after the

transmission of test results must be developed, tested and implemented. A further requirement will be to gain acceptance of these within existing medical practice. Future development of POC diagnostics to be compatible with nondedicated mobile phones will require that additional technological and regulatory issues be addressed. For example, given the numerous mobile phones available to the consumer, defining the subset of mobile phones that adhere to a common standard and can be used with a given diagnostic test will be required. A significant issue for the nondedicated mobile phone reader model is to implement a mechanism for keeping abreast of mobile phone manufacturer updates to relevant phone models for retesting of compatibility with the diagnostic test. Issues associated with secure transmission of patient information [86] will also have to be addressed by the diagnostic device companies.

## Future perspective

The overall challenge in POC diagnostics development continues to be to create high-performance assays that are appropriate for the various settings relevant for global health applications. For the lower-resource settings, the requirements for high performance with a rapid, maintenance-free, easy-to-use, and very low cost device brings specific design and implementation challenges. Paper-based microfluidics is especially well suited to addressing these challenges. The development of precise and robust fluidic controls coupled with advances to develop electrical power-free methods and readout with minimal instrumentation underlie the great potential for creating sophisticated assays that are appropriate for low-resource settings.

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## Key Terms

<b>Nitrocellulose -</b>	Flammable membrane used often in lateral flow tests
<b>Cellulose -</b>	Material that is often used to make paper
<b>Pore diameter -</b>	Increases in this parameter results in decreases in both the viscous resistance and the capillary pressure
<b>Lucas–Washburn flow -</b>	Flow under which the viscous resistance is proportional to velocity with a coefficient that increases as the fluid column lengthens
<b>Fully wetted flow -</b>	Flow that is characterized by a quasi-steady velocity

## Executive summary

### Background

- Gold-standard diagnostic methods rely on multistep sample processing protocols that require a high level of resources and are not appropriate for use in low-resource point-of-care (POC) settings.
- Paper microfluidics is a growing subfield in which capillary pressure is used to automatically drive flow in the porous material of which the device is composed.
- Porous network devices, such as conventional lateral flow tests, are disposable, have a rapid time to result and are cost effective to manufacture.
- A major challenge, and the focus of this Review, is to implement multistep processes with the robust fluid control required to achieve the necessary sensitivity and specificity of a given application in a user-friendly package that minimizes equipment.

### Fluidic controls for automation in paper-based devices

- Critical to the development of high-impact paper microfluidic devices is the ability to achieve precise and robust automated fluidic control of the multiple fluid sources in an assay.
- The network topology of the paper device, that is, multiple inlets for each outlet, can be used to automate sequential delivery of multiple fluids to a detection region.
- Channel geometry can be used to control flow rates in a paper network.
- On-switches to delay the downstream arrival of a given fluid for a specific amount of time have been developed. Methods include dissolvable barriers, the use of hydrophobic coatings, absorbent pad delays and user-activated mechanical valves.
- Complementary off-switches to automatically meter multiple fluid volumes in the paper network have been developed. Methods include fluidic disconnects and pad-based volume control.
- Patterning reagents onto a substrate can be used to control the spatial and temporal concentration profiles of the reagents when rehydrated.

### Readout methods compatible with low-resource POC settings

- The added cost and requirement for maintenance of the dedicated instrumentation often associated with high-performance tests make these tests unsuitable for many low-resource POC settings.
- Methods for quantitative visual readout include the stepwise or continuous generation of signal in the detection region that can be read by the number of

bars in which the signal was generated or the distance over which the signal was generated, respectively.

- A nondedicated mobile phone reader model is promising for applications in low-resource POC settings that require fully quantitative readout.
- The mobile phone reader plus inexpensive adapter approach is promising, directly addressing issues such as variability of ambient lighting conditions and variability of user positioning of the mobile phone.

#### **Power & heating compatible with low-resource POC settings**

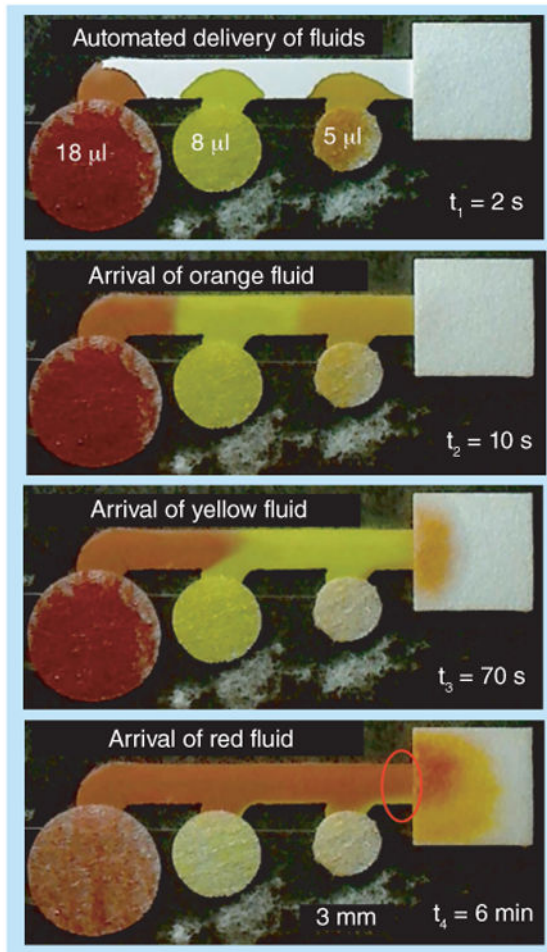
- Paper-based fluidic batteries, powered by electrochemical reactions and fully disposable, have been developed in order to avoid the expense and hazardous waste associated with traditional lithium ion batteries.
- Smartphones have the capability to provide reliable power directly at the POC.
- Single-use, flexible, thin-film heaters and chemical heating with phase change materials are promising heating methods for use in paper-based devices for low-resource POC settings.

#### **Selected applications for low-resource POC settings**

- Paper-based devices for sample preparation have been developed to perform nucleic acid extraction and perform the separation and preconcentration of analyte.
- An automated signal-amplified assay was demonstrated using network topology and pad-based volume metering.
- Nucleic acid amplification in a porous matrix coupled with advances in equipment-free heating, have the potential to bring high-sensitivity nucleic acid amplification assays to low-resource POC settings.
- High-sensitivity signal-generation methods have been demonstrated in paper-based assays using origami to manipulate fluids.

#### **Challenges**

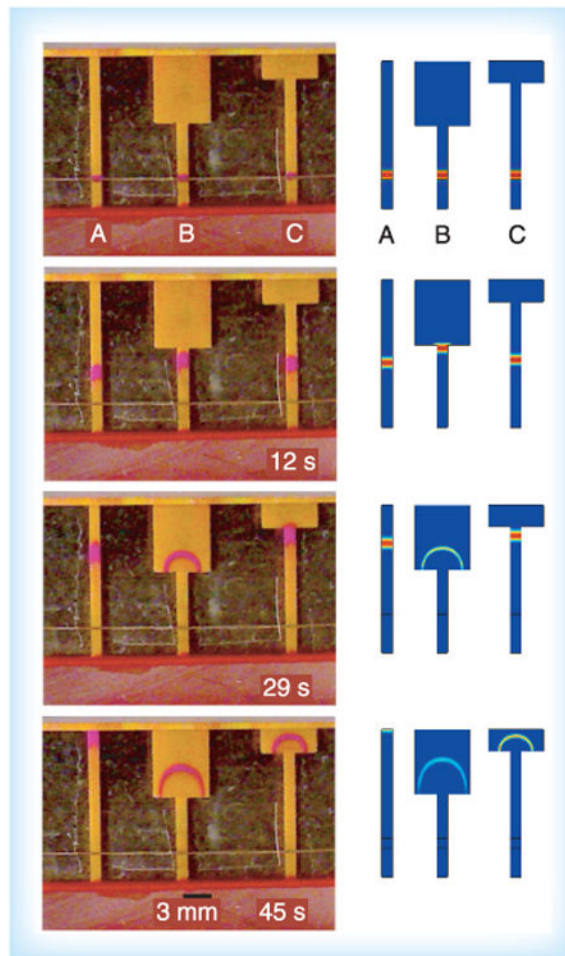
- One main challenge to the creation of paper-based diagnostic devices for use at the POC is integration to create standalone automated sample-in to result-out devices.
- A second main challenge is to fulfill the promise of nondedicated mobile phone use in a POC diagnostic system by implementing multiple potential functions that include: directing test operation, analyzing test results, interpreting test results, and transmitting test results to a healthcare provider and instructions back to the patient.
- The overall challenge in POC diagnostics development continues to be to create high-performance assays that are appropriate for the various settings relevant for global health applications.



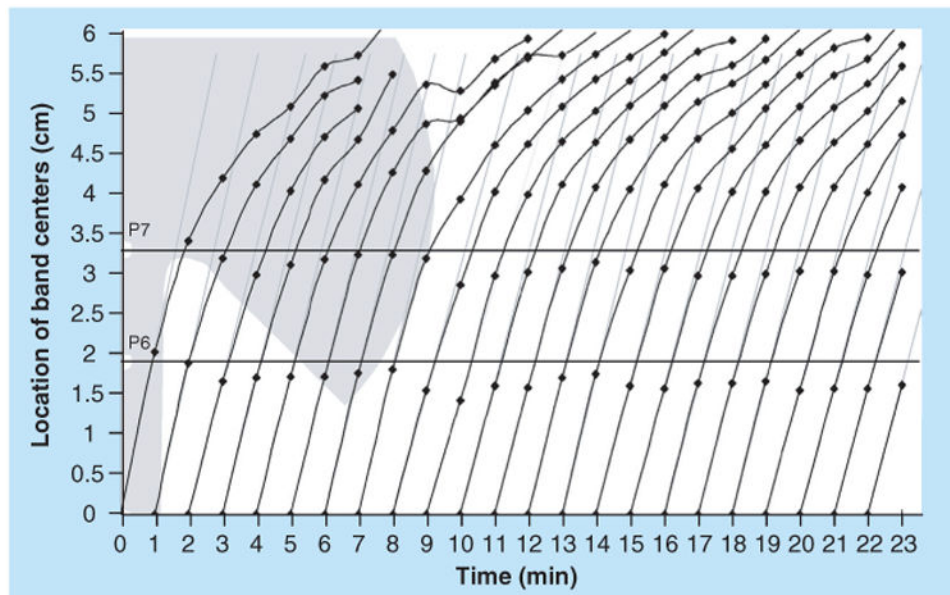
**Figure 1. 2D paper networks with multiple inlets per detection region for automated multistep sample processing**  
The sequential delivery of multiple fluid volumes has been pre-programmed into the configuration of the network.

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**Figure 2. Experimental (left) and model (right) results for fully wetted flow into a wicking pad show good agreement**  
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**Figure 3.** Velocity data from a complex shape used by Mendez *et al.* to adjust the flow rate in their nitrocellulose devices  
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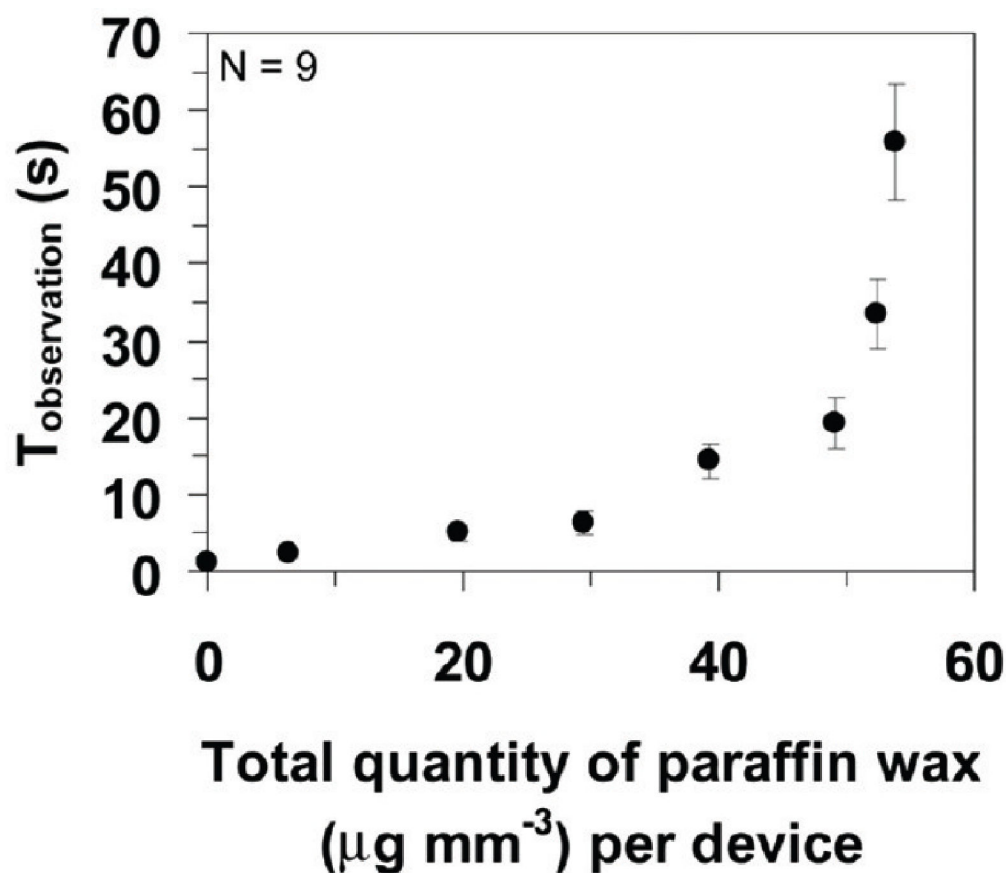
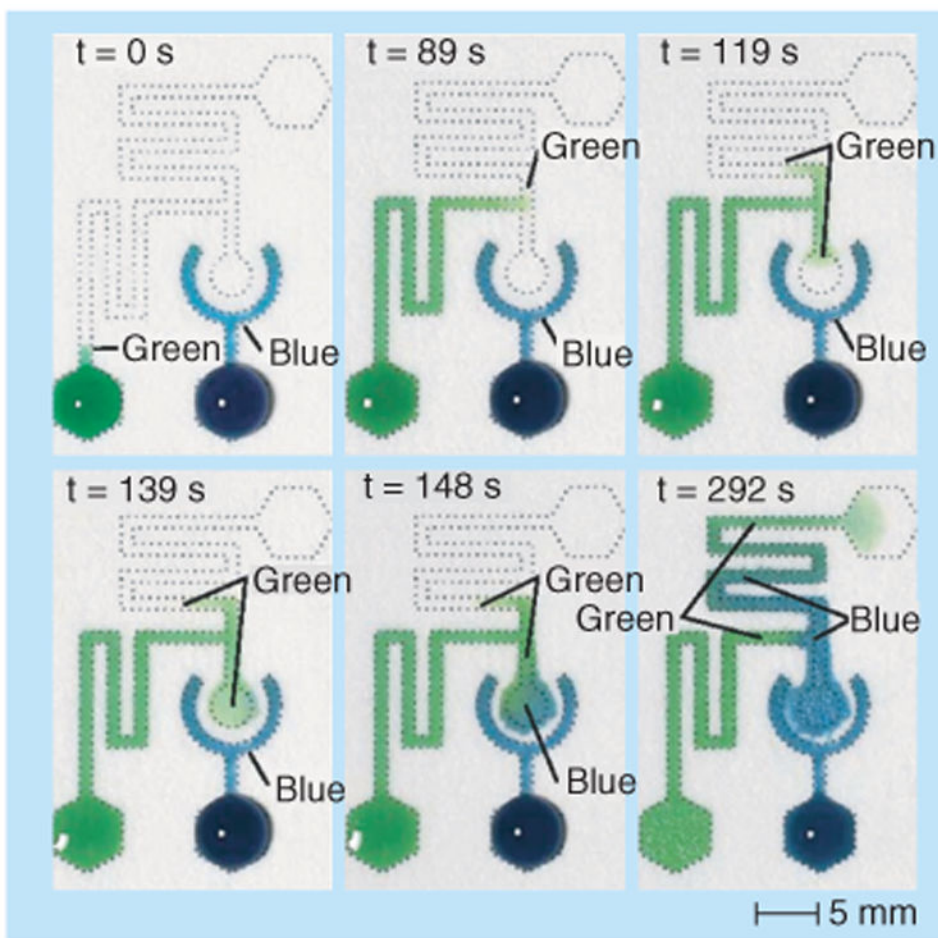
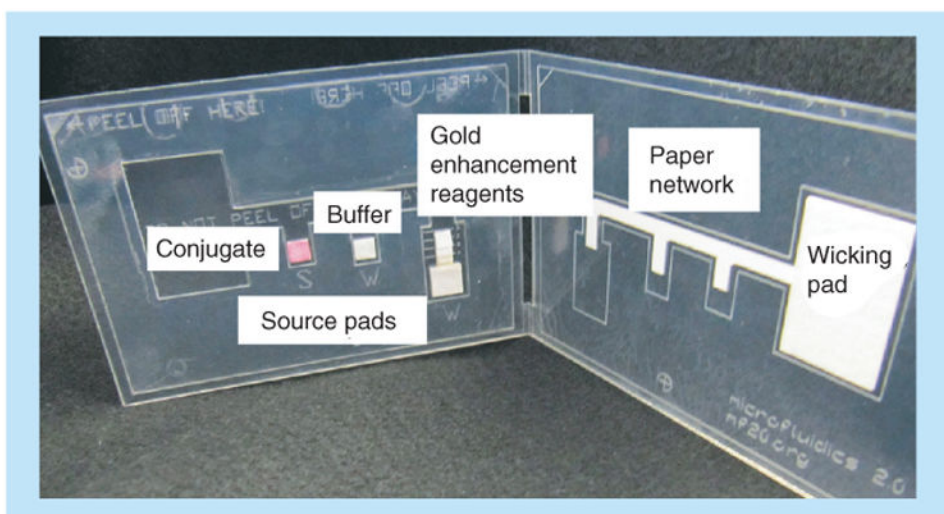


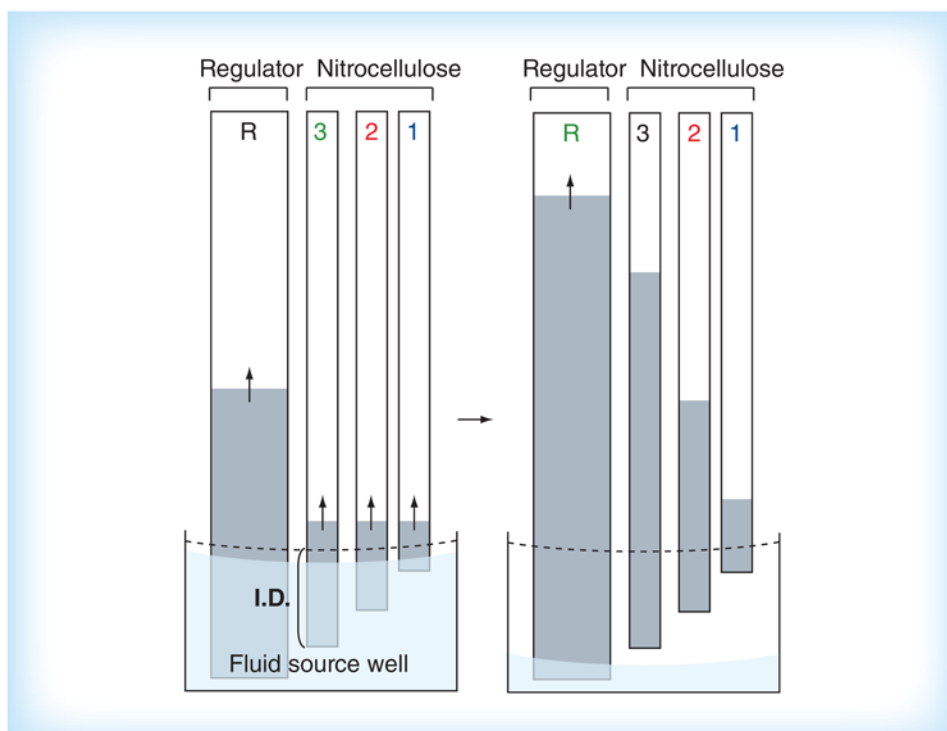
Figure 4. Plot demonstrates the ability to vary the delay time through the amount of wax deposited onto the substrate  
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**Figure 5. The use of a diode valve to program timed release of the blue fluid by the green fluid**  
Reproduced with permission from [43] © The Royal Society of Chemistry (2012).  
Please see colour figure at [www.Futuremedicine.com/doi/suppl/10.4155/Bio.13.243](http://www.Futuremedicine.com/doi/suppl/10.4155/Bio.13.243).

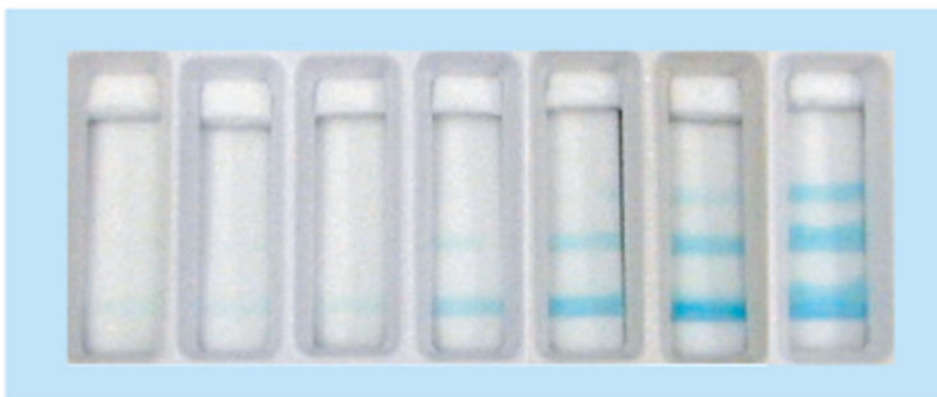


**Figure 6. Automated amplified 2D paper network assay developed by Fu *et al***  
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**Figure 7. A volume metering method in which fluid flows shut off from the inlets in the order of shortest to longest submerged length**  
I.D.: Immersion depth.

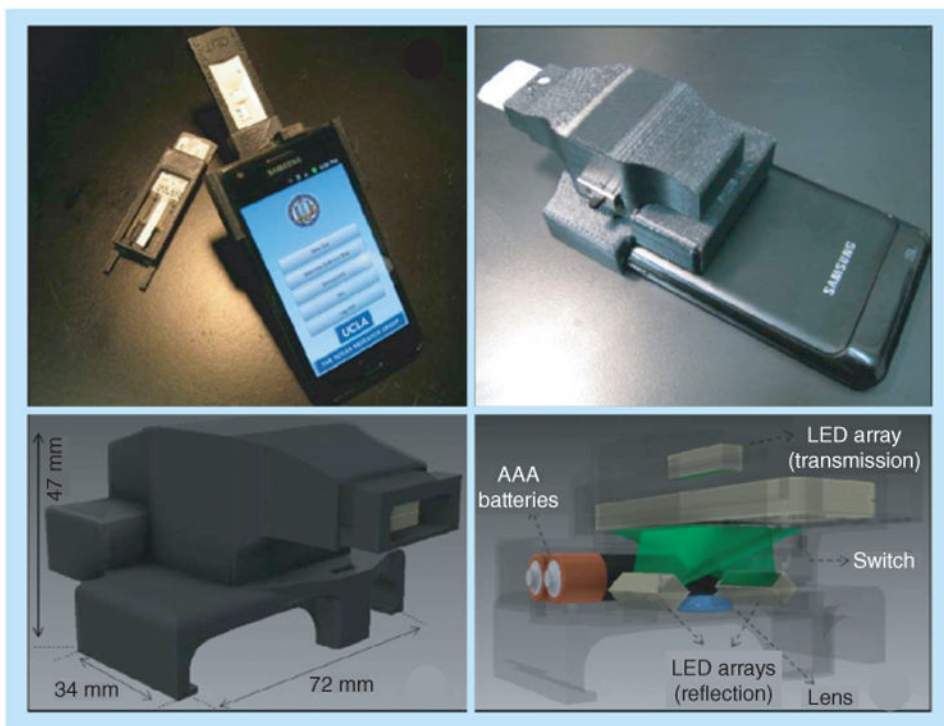
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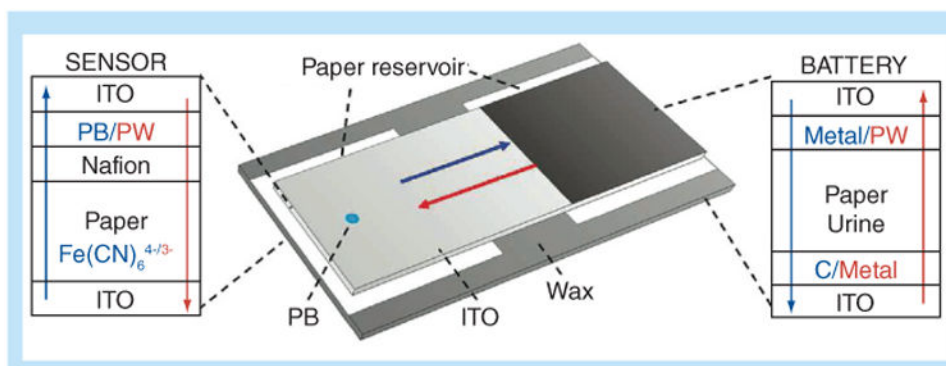
**Figure 8. Ladder-bar readout for a glucose assay**

A sample consisting of 0, 1, 5, 25, 50, 100 and 200  $\mu\text{m}$  glucose was added to the strip, from left to right, respectively.

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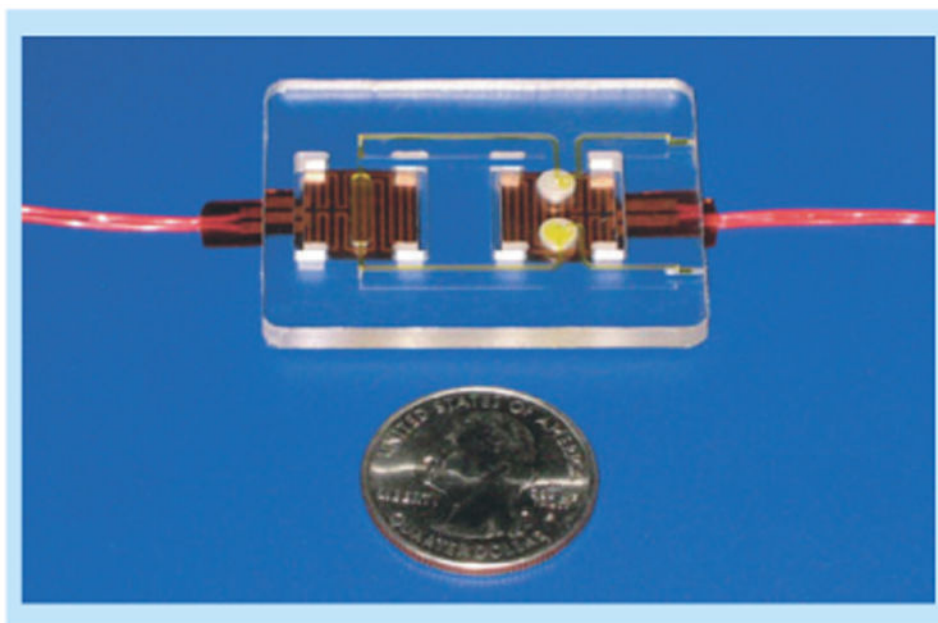
**Figure 9. Adapter that interfaces with an Android phone to perform imaging and analysis of commercially available lateral flow tests in reflection and transmission mode by Mudanyali *et al***  
Reproduced with permission from [67] © The Royal Society of Chemistry (2012).



**Figure 10. Paper battery for the detection of glucose and hydrogen peroxide in artificial urine samples**

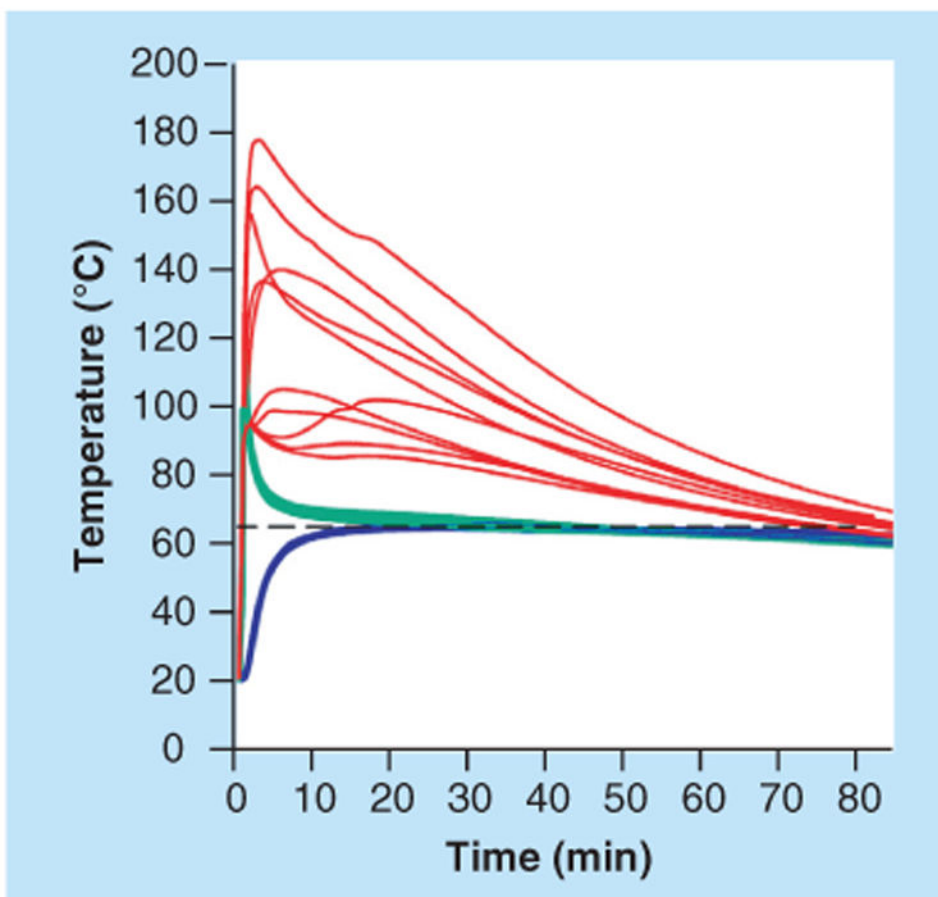
ITO: Indium-doped tin oxide; PB: Prussian blue; PW: Prussian white.

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**Figure 11.** Thin-film heater used to power nucleic acid amplification reactions for up to 1 h  
Reproduced with permission from [74] © Springer Science + Business Media (2011).

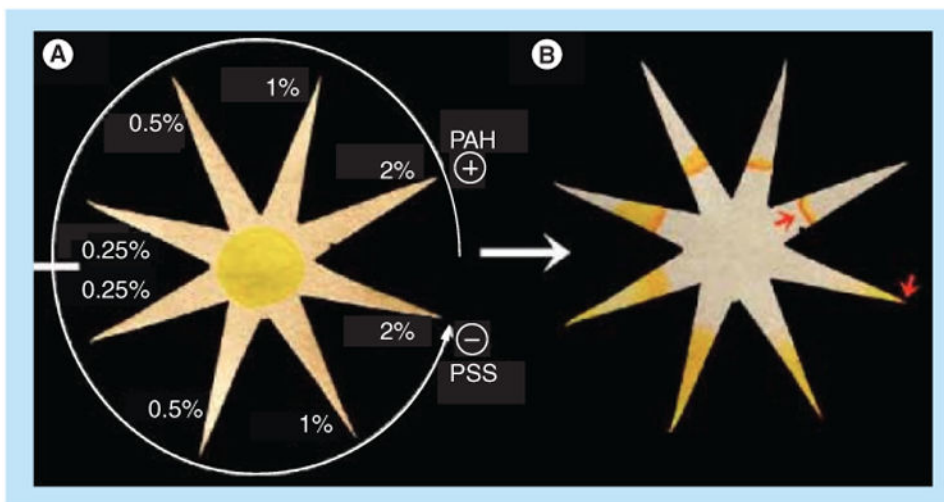




**Figure 12.** Temperature versus time of multiple runs ( $n = 10$ ) measured at the exothermic reaction (red), interface with the phase change material (green), and at the nucleic acid amplification reaction (blue)

Reproduced from [75].

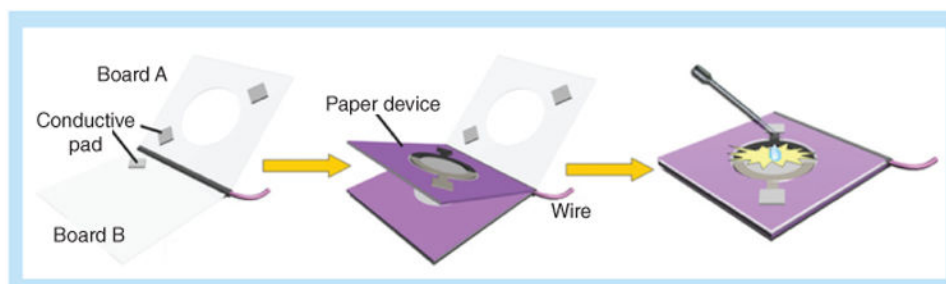
Please see colour figure at [www.Futuremedicine.com/doi/suppl/10.4155/Bio.13.243](http://www.Futuremedicine.com/doi/suppl/10.4155/Bio.13.243)



**Figure 13. Device that uses polyelectrolyte coatings and the shape of the paper for analyte separation and concentration**  
The surface charge in a given prong of the star, controlled via the concentration of polyelectrolytes as in the example in (A), affects the transport of model analyte fluorescein into that prong and can be tuned to produce highly concentrated fluorescein in a very small diameter tip, as shown by the rightmost arrow in (B).

PAH: Poly(allylamine hydrochloride); PSS: Poly(styrene sulfonate).

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**Figure 14. Schematic of a paper-based origami device to detect cancer markers in clinical samples from Wang *et al.***  
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