A low cost design and fabrication method for developing a leak proof paper based microfluidic device with customized test zone

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This article describes a fabrication process for the generation of a leak proof paper based microfluidic device and a new design strategy for convenient incorporation of externally prepared test zones. Briefly, a negative photolithographic method was used to prepare the device with a partial photoresist layer on the rear of the device to block the leakage of sample. Microscopy and Field Emission Scanning Electron Microscopy data validated the formation of the photoresist layer. The partial layer of photoresist on the device channel limits sample volume to $7 \pm 0.2 \mu\text{l}$ as compared to devices without the partial photoresist layer which requires a larger sample volume of $10 \pm 0.1 \mu\text{l}$. The design prototype with a customized external test zone exploits the channel protrusions on the UV exposed photoresist treated paper to bridge the externally applied test zone to the sample and absorbent zones. The partially laminated device with an external test zone has a comparatively low wicking speed of $1.8 \pm 0.9 \text{ mm/min}$ compared to the completely laminated device with an inbuilt test zone ($3.3 \pm 1.2 \text{ mm/min}$) which extends the reaction time between the analyte and reagents. The efficacy of the prepared device was studied with colorimetric assays for the non-specific detection of protein by tetrabromophenol blue, acid/base with phenolphthalein indicator, and specific detection of proteins using the HRP-DAB chemistry. The prepared device has the potential for leak proof detection of analyte, requires low sample volume, involves reduced cost of production ($\sim \$0.03$, excluding reagent and lamination cost), and enables the integration of customized test zones. © 2015 AIP Publishing LLC.

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INTRODUCTION

Improved global health in the current scenario is greatly influenced by the accessibility of standard diagnostic tests. Current laboratory-based diagnostic tests involve the use of sophisticated techniques, instruments and also depend on the availability of highly skilled operators. However, implementation of these standard procedures remains inconceivable in developing and under developed countries especially in the countryside/remote locations under emergency conditions owing to lack of regular power resource, cost effectiveness, and trained professionals.^{1–5} Alternative detection assays are available but with their own set of limitations. For example, the lateral flow immunoassays readily available over-the-counter are less cost effective and involve larger sample volumes which is of concern for sample analysis in infants. Also, these immunoassays use nitrocellulose as the substrate which has certain disadvantages: shelf life issues, low tensile strength, protein incompatibility with surfactants used during pretreatment, inconsistencies in flow characteristics due to desiccation, protein inability to bind covalently or directionally to nitrocellulose, and involvement of elevated drying temperatures.⁶

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Similarly, conventional microfluidic devices require external pumps and detectors for sample movement and detection. Circumventing these drawbacks, paper based microfluidic devices have emerged as an effective alternative to high end costly bioassays and thus qualifying for the ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and deliverable to end-users) criteria suggested by the World Health Organization (WHO).⁷ Paper as a substrate has the following advantages: (i) inexpensive, portable, and easily accessible,⁸ (ii) compatible with biological samples,⁹ (iii) can be easily modified to immobilize different biomolecules like protein, DNA, small molecules, etc.,^{10,11} (iv) ease of storage, transport, and disposal,¹² (v) paper absorbs liquids through capillary motion and evaporation, which eliminates the need for external pumps to drive fluid movement,¹² and (vi) it serves as a good medium for colorimetric tests, providing a strong white contrast against the colored substrate.¹² Paper as a substrate has been extensively used in analytical and clinical chemistry; most common examples include paper chromatographic techniques used for the separation of different biomolecules, litmus paper, and paper based diagnostic tests.^{1,6,13–27} It also finds the use as a substrate for the storage of biological samples like blood, saliva, etc.^{28–33} With the advent of advanced fabricating and patterning techniques, paper based microfluidic devices were introduced for multiplex analyte detection.^{12,34,35} Since then various patterning techniques and fabrication processes have been developed involving detection and separation of analytes.^{36–39} Two methods find special mention for their simplicity and low cost: wax printing and alkyl ketene dimer (AKD) printing. Wax printing is a nontoxic, cost effective fabrication process but the inherent spreading of wax when melted leads to low resolution of created microfluidic channels. The AKD printing process uses alkyl ketene dimer as cellulose hydrophobizing material and is a simple method retaining the flexibility of the paper substrate.^{40,41} The development achieved in this area along with new detection systems using colorimetry to electrochemical, chemiluminescence, electrochemiluminescence, fluorescence, and electrical methods has opened avenues for new sensing technologies.^{12,34,42–57}

Paper-based microfluidic systems, however, suffer from two major drawbacks: first is the leakage of sample from the rear side of the channels during sample analysis and second is the difficulty in precise incorporation of reagents in the test zone without the inherent spreading of reagents to the surrounding areas including the microchannels. Additionally, covalent immobilization of certain detection reagents (e.g., antibodies, aptamers, enzymes, etc.) in the test zone requires multiple chemical treatment cycles and washing steps, which further aggravates the fabrication handling process. To overcome these problems, various efforts have been made starting from clamping the device to plastic or glass, encasing the device in between adhesive tape, and printing toner for laminating the device on both sides. Though advantageous, these methods have drawbacks of their own. For example, the methods using certain adhesive tapes have the limitation that adhesive bond may weaken with the addition of sample and with time may wear off. Similarly, the paper printed with a layer of toner mainly avoids contamination, acts as a thermal adhesive, and helps in containing the reagents rather than acting as a mechanical support.^{37,38,58–66}

Here, we report a fabrication process and design to mitigate the problems mentioned above. We used a fabrication process using negative photoresist instead of using the simple wax or AKD printing technologies since the photolithography technique presented multiple steps wherein the extent of partial polymerization on only one surface of the paper based microfluidic devices (μ PAD) can be tuned to offer a leakproof surface. The current fabrication process (processing time of ~ 1 h) allows the partial polymerization of photoresist on the rear of the device, which prevents sample leakage during tests and thereby reduces the cost of laminating both sides of the device from \$0.036 to \$0.018 per device, thus reducing the overall device cost to \$0.048. We also report a new design and method for preparing the test zone with controlled spotting facility of reagents and treatments involving multiple washing and regeneration steps. Briefly, the design exploits the slight protrusions of channels on the UV unexposed surface of the photoresist treated paper, which act as bridges to connect the external test zone to the sample and absorbent zones. Since the device involves an externally fitted test zone, the base device can be a universal platform for different diagnostic applications. The customized

test zone with embedded reagents specific for respective target analytes can be integrated to the base device at the time of analysis.

MATERIALS AND METHODS

Chemicals and reagents

Whatman chromatography Paper No. 1 (20 × 20 cm) was obtained from GE Lifesciences. Propylene glycol monomethyl ether acetate (PGMEA), triarylsulfonium hexafluorophosphate salts (photoacid), Coomassie brilliant blue G-250, divinyl sulfone, and bovine serum albumin (BSA purity of ≥98%) were purchased from Sigma Aldrich. EPON SU-8 resin was a gift from Momentive™. Tetrabromophenol Blue (TBPB), phenolphthalein indicator, and 30% hydrogen peroxide (H₂O₂) were purchased from Merck. Acetone and isopropanol were purchased from Himedia. 3,3'-Diaminobenzidinetetrahydrochloride hydrate (DAB) was purchased from Amresco. Monoclonal rat anti-human FABP3 antibody and goat anti-rat IgG horseradish peroxidase (HRP) affinity purified polyclonal antibody were obtained from R&D systems. Recombinant heart type fatty acid binding protein (HFABP or FABP3) was purified in the lab with ≥95% purity. Transparency sheets (polyester with a thickness of 100 μm) were purchased from Oddy India Ltd. All other reagents were of analytical grade.

Designing of microfluidic channels

Design patterns were drawn with AUTOCAD (Version 2010). The designs consisted of six rows of 12 devices each totaling to 72 devices per sheet. The design of the microfluidic device consisted of a sample zone of 3 mm, an absorbent zone of 5.5 mm with connecting channels 3 mm in length and 1 mm in width. The test zone region was left empty in between the two zones. The designs were printed on a transparent sheet with a RICOH 2030 printer using a black toner cartridge (MP C2551) from RICOH.

Device fabrication

The microfluidic channels were fabricated on Whatman chromatography Paper No. 1 using the FLASH technique with certain modifications.³⁴ Briefly, the photoresist (EPON SU-8 resin: 52% by mass, triarylsulfonium hexafluorophosphate salts (photoacid): 5% by mass, and PGMEA: 43% by mass, mixed overnight) was poured onto the paper, spread evenly with a glass rod, and allowed to soak the photoresist for 5 min. The photoresist impregnated paper was then baked on a hotplate set at around 130 °C for 5 min or till complete evaporation of the PGMEA. After complete cooling of the paper at room temperature, the paper was covered on one side with a black paper (with 0% transparency) and the other side with the design printed transparent sheet. The paper surface in contact with the transparency sheet was then exposed to UV light (40 W) at a distance of 3 cm for 25 min, following which the design sheet and the black paper were removed and the paper was again exposed to UV for 10–15 s on the same surface. The paper was baked at 130 °C for 5 min. The unpolymerized photoresist was removed by soaking it in acetone, followed by a rinse in acetone (1×), and finally rinsing the paper with 70% isopropanol using a squirt bottle. The rinse with isopropanol was done on the surface that was not in direct contact of the UV source. The devices were ready to use after drying at room temperature or were stored covered with aluminum foil. The circular test zone of appropriate sizes from Whatman chromatographic Paper No. 1 was prepared by using a punching machine. This untreated test zone was used to load reagents before it was placed on the microfluidic channel. It was then incorporated externally between the sample and absorbent zone partially overlapping the microfluidic channels, followed by laminating the device on one side to hold the test zone in place.

Characterization of the constructed device

The surface morphology of the photoresist modified surface and unmodified surface of the device was analyzed through Atomic force microscopy (AFM) using an ambient air scanning probe microscope (Agilent Technologies 5500, USA) and a silicon nitride probe. Images were recorded with typical contact mode using Picoscan 5 software. The scan speed was set at $4.476\text{ }\mu\text{m/s}$, I Gain-0.7, P Gain-1, and AC Drive of 1.7%. Images of cross-sections of the modified and unmodified paper were taken using a Field Emission Scanning Electron Microscope (FESEM) (Zeiss) with EHT (extra high tension voltage) 1 kV and a Nikon-SMZ800 microscope equipped with a Nikon D7000 camera.

Flow characterization

Flow rates were determined by applying a 10% Coomassie brilliant blue G-250 in methanol to the sample zone and measuring the time for the dye to flow from the sample zone to the absorbent zone. The entire flow process was video graphed using a camera, and the time required by the dye to travel from the sample zone to the tip of the absorbent zone was calculated from the video. Multiple experiments were conducted to obtain an average linear velocity.

Quantitative assay for nonspecific detection of protein in artificial urine

Artificial urine was prepared following the protocol of Brooks and Keevil.⁶⁷ Stock solutions containing BSA were prepared using this artificial urine. Desired concentrations of BSA (10–100 μM) were obtained by diluting the stock.

The external test zone was spotted with 3 μl of 125 mM citric acid buffer at pH 1.8, dried at room temperature, followed by addition of 3 μl of 10 mM TBPB in ethanol, and allowed to dry at room temperature. 5 μl of each sample was dispensed on the sample zone of the micro fluidic device and allowed to flow through the channels to the test zone and react with the reagents spotted. The reaction was allowed to proceed for 10 min after which the color development was recorded.

Quantitative detection of base

Different concentrations of sodium hydroxide (NaOH) solution were prepared in MilliQ (18 m Ω) water in the range of 1 mM to 10 mM. The test zone was spotted with a solution of phenolphthalein in ethanol and allowed to dry at room temperature. 5 μl of each sample was dispensed on the sample zone and allowed to flow through the channels to react with the reagent spotted on the test zone. The development of color was monitored immediately after migration of sample to the test zone.

Quantitative assay for specific detection of protein

For the quantitative detection of protein (anti-heart type fatty acid binding protein antibody), the test zone was covalently modified through DVS (divinylsulfone) chemistry to immobilize recombinant human HFABP (acted as capture molecule) following the protocol of Yu *et al.*⁶⁸ The homobifunctional DVS molecule contains electrophilic vinyl groups which exhibit cross-linking activity with nucleophiles. The chemical strategy involves the addition of one of the vinyl groups to the hydroxyl groups on cellulose, while the remaining vinyl groups covalently attach to the nucleophile bearing biomolecules. Briefly, $12.0 \times 9.0\text{ cm}$ sheets of chromatography paper 1 were immersed in 20 ml 10% DVS solution (v/v, 0.1M sodium carbonate, pH 11), incubated in separate 400 ml-capacity plastic zip bags, and agitated for 2 h on a rocking shaker. Following incubation, the DVS-activated (DVS+) paper was removed from the bags and rinsed in a plastic tray with 100 ml MilliQ water for three times. These membranes were dried for 2 h in ambient conditions and then punched with a punching machine to obtain circular test zones each of diameter 4 mm. The HFAB protein prepared in PBS (phosphate buffer saline, pH 7.4) was then spotted on the test zone and allowed to react under ambient conditions

overnight. This was followed by three washing steps with PBS to remove any unbound protein. After drying the zone, it was spotted with BSA to block any exposed site. This was followed by three washing steps to remove excess BSA and subsequent drying under normal room conditions. The sample zone was spotted with $5\text{ }\mu\text{M}$ HRP conjugated anti-rat antibodies (detector molecule) and allowed to dry overnight at 4°C . Different concentrations of rat anti-HFABP antibodies ($0\text{--}3.4\text{ }\mu\text{M}$) were prepared in PBS and applied to the sample zone. The sample was allowed to migrate to the test zone, dried for 5 min followed by 5 applications of PBS buffer ($20\text{ }\mu\text{l}$ each) at the sample zone to allow unbound antibodies to migrate to the absorbent zone. This was followed by application of DAB substrate ($10\text{ ml } 0.05\% \text{ DAB in PBS with } 10\text{ }\mu\text{l } 30\% \text{ H}_2\text{O}_2$). In the presence of H_2O_2 , DAB is converted to an insoluble brown reaction product and water by the enzyme HRP. Color development after 5 min was monitored to determine the concentration of analyte present in the sample.

Method for digitization of obtained results

To quantify the obtained results, the images of the devices were acquired using an Epson image scanner. The color images were then transferred to a computer, converted to 8-bit gray scale using Adobe Photoshop, and the mean pixel intensities noted corresponding to each analyte concentration. To obtain background corrected data, the mean pixel intensity of each device was subtracted from the intensity of a control device not exposed to any analyte. A similar method was used for the detection of protein with TBPB, where instead of converting the image to gray-scale, the cyan channel in the CMYK (cyan, magenta, yellow, and key [black]) format was chosen to quantify the colorimetric response.⁴⁴

RESULTS AND DISCUSSION

Device fabrication process

Detection of different analytes implicates the use of different reagents which in traditional μPADs are spotted on the test zones. However, it requires an extremely controlled process to spot since erroneous handling can allow the reagents to cross to the other zones. Furthermore, detection strategies involving the use of sandwich assays demand covalent immobilization of capture molecules on the test zone itself. This adds multiple treatment steps of the test zone, which in the case of traditionally designed μPADs is not feasible. In addition to the above shortcomings, μPADs also suffer from another disadvantage, that is, the leakage of sample from the back side of the device. As a means of overcoming these drawbacks, we designed a device consisting of a sample, absorbent, and externally inserted test zone, as well as evolved a fabrication method which adds a leak proof layer at the back side of the device during the fabrication step itself.

The device design and fabrication process are illustrated in scheme as Fig. 1 (see supplementary material, Figs. S1 and S2⁷¹). For this concept, we used the paper surface that was not directly exposed to the UV source. The slight projection of the channels on this side of the patterned paper acted as bridges to connect the test zone to the sample and absorbent zones. The

FIG. 1. Schematic representation of fabrication process used to develop a leakproof device with prefabricated test zone.

channels were designed in a way that 1 mm length of the channel overlapped with the test zone to facilitate effective movement of fluid to and from the channels to the test zone. The incorporation of the test zone between the sample and absorbent zones is a simple but critical step and can be manually performed without much difficulty. The different zones varied in size connected by channels: size gradually increased from the sample zone to the absorbent zone which helped to increase the wicking of fluid from the sample zone to the absorbent zone in a controlled manner. The addition of the absorbent zone to the design setup helped to absorb the unbound detector molecules and reagents, resulting in less nonspecific detection signals. The optimized size ratio of the sample, test, and absorbent zone was 1.83:1.33:1.00 (supplementary material, Table S1⁷¹).

Modification of the FLASH technique was carried out at multiple steps for obtaining a device that evades the need for lamination on the rear side to prevent the leakage of sample. The brief exposure of 10–15 s on the rear side of the device during fabrication allowed partial polymerization of the photoresist, thus making the back side of the device hydrophobic. The absence of isopropanol washing on the UV exposed side of the paper prevented leaching of the partial photoresist layer from that side of the paper, thereby preventing sample leakage from the rear side (Fig. 2 and Movie S1⁷¹).

Furthermore, the external placement of the test zone on the hydrophobic base of the prepared device (that is, the paper platform consisting of the sample and absorbent zones) enhances the leak proof property of the device. The partial layer of photoresist on the device also limits the sample volume to $7 \pm 0.2 \mu\text{l}$ as compared to devices without the partial photoresist layer which requires a larger sample volume of $10 \pm 0.1 \mu\text{l}$. The partial photoresist reduces the overall volume of the microfluidic channels which leads to the observed reduction in sample volumes. However, the minor variations in sample volume depend on the level of moisture content of the paper.

Characterization of the constructed device

AFM images of the photoresist modified surface and unmodified surface of the device revealed a rough surface with uneven morphology for the unmodified surface, whereas the treated surface appeared to be smoother (Fig. 3(A)). The root mean square roughness factor for the photoresist modified and unmodified surface was calculated as 44.4×10^{-6} and 76.9×10^{-6} , respectively. The unmodified surface retained the fibrous structure of the paper displaying a rough contour, whereas the surface with polymerized photoresist was smooth as the photoresist impregnated the fibrous structure of the paper. Cross sectional images of Whatman chromatography Paper No.

FIG. 2. Sample device prepared by the conventional (control) and modified (sample) FLASH techniques were compared to demonstrate the impediment of leakage of sample from the rear of the sample device, which is shown in supplementary Movie S1.⁷¹

FIG. 3. (A) AFM images of (I) unmodified surface and (II) photoresist modified surface of the device. (B) FESEM images of Whatman chromatography Paper No. 1 with (I) complete photoresist and (II) partial photoresist. (C) Cross-sectional view of the control (I) and sample (II) device showing the presence of the partial photoresist layer (white layer) in the sample device and the lack of the layer in the control device. The blue layer with dye corresponds to lack of photoresist. Pictures were taken using a Nikon-SMZ800 microscope equipped with a Nikon D7000 camera.

1 with photoresist and with a partial layer of photoresist were analyzed by FESEM (Fig. 3(B)). The images revealed a change in the thickness of the papers following photoresist treatment. The thickness of the paper with complete photoresist was an average of $271.8 \mu\text{m}$, which was greater compared to the untreated ($180 \mu\text{m}$) and partially layered ($200.7 \mu\text{m}$) paper. The increase in thickness of the partially layered paper in comparison to the untreated paper indicated the presence of photoresist in its paper structure.

Cross-sectional view of the control and sample device manifests the presence of the partial photoresist layer (white layer) in the sample device as can be seen in Fig. 3(C) (II) and the lack of the layer in the control device [Fig. 3(C) (I)]. The blue layer with dye corresponds to lack of photoresist. The device fabrication process was able to confine the aqueous blue dye preventing it from penetrating through the entire thickness of the paper.

Flow characterization

Comparison of fluid flow rates through the constructed channels on both the devices with externally fabricated and inbuilt test zone was carried out by applying a dye on the sample

zone of each device and then recording the time needed for the dye front to reach the end of the absorbent zone. The device with inbuilt test zone had a greater flow rate of 3.3 ± 1.2 mm/min compared to 1.8 ± 0.9 mm/min of the device with externally fabricated test zone. The decreased flow rate of device with prefabricated test zone can be ascribed to the overlapping design and less area of contact between the test zone and the connecting microchannels. The noticeable decline in the flow rate could be advantageous as it would allow increased time for the reagents to interact with the analyte thereby presenting a more sensitive detection signal.

Quantitative assay for nonspecific detection of protein in artificial urine, base, and specific detection of protein (anti-HFABP antibody)

Three assay methods were chosen to validate the feasibility of the device design. The nonspecific detection of protein and base was based on direct color change on interaction of the analyte with the specific reagents. Whereas, the specific detection of protein was based on a variation of the sandwich assay involving the use of a capture and detector molecule. Fig. 4 shows the correlation of obtained signals to the corresponding analyte concentrations. All datum

FIG. 4. Representative images of BSA, NaOH, and anti-HFABP antibody detection are shown on the head of each graph, respectively. The BSA, NaOH, and anti-HFABP antibody concentrations were in the range of 0–100 μM , 0–10 mM, and 0–3.4 μM , respectively. Calibration plots for each analyte are shown. The mean intensity for each data point was obtained from the histogram in Adobe Photoshop. Each datum is the mean of 5 assays and the error bars represent the relative standard deviations. (A) Calibration curve for different concentrations of BSA in artificial urine. The linear region of the data was fit in a linear equation (shown in inset); the slope (m), intercept (b), and R^2 values are as follows: 1.71, 0.9572, and 0.98, respectively. (B) Calibration plot for different concentrations of NaOH. The linear range of the data when fit into a linear equation (shown in inset) yielded a slope (m): 3.72, intercept (b): 26.0882, and R^2 value: 0.98. (C) Calibration plot for different concentrations of anti-HFABP. The linear region of the data (shown in inset) had a slope (m), intercept (b), and R^2 value of 5.4063, 126.25, and 0.98, respectively.

points are mean intensities of five measurements of each concentration using independent devices, while the error bars represent the relative standard deviation. For nonspecific detection of protein in artificial urine, BSA was used as the analyte in the range of 10–100 μM . Quadratic least squares fitting of the data yielded a coefficient of determination (R^2) of 0.99. The responses were linear in the range of 10–40 μM and started deviating from linearity, reaching a plateau at higher concentrations (Fig. 4(A), supplementary material Fig. S3(a) and Movie S2)⁷¹). The device was able to detect protein as low as 2.5 μM to as high as 40 μM . This renders the device useful as protein excretion during proteinuria falls in the range of 2.5–4.5 μM .

Determination of base using the phenolphthalein indicator based detection was carried out in the range of 1–10 mM NaOH. Quadratic least square fitting of the data yielded an R^2 value of 0.97. The graph followed a linear pattern in the range of 1–4 mM (Fig. 4(B), supplementary material Fig. S3(b) and Movie S3)⁷¹). The limit of detection was found to be 1 mM.

Specific detection of anti-HFABP antibody used the HRP catalyzed conversion of DAB into a brown precipitate. The assay was carried out with an analyte concentration range of 0–3.4 μM . Quadratic least square fitting of the data yielded an R^2 value of 0.97. The response was linear in the range of 0–0.8 μM , beyond which the signal was saturated (Fig. 4(C)). The detection limit for quantifying anti-HFABP antibody was found to be 0.05 μM . This model assay for indirect ELISA (enzyme-linked immunosorbent assay) on the designed μPAD reaffirms the feasibility of the device for detecting various antigens and antibodies present in biological fluids during infections, autoimmune diseases, etc. The results obtained confirmed the usability of the designed device for simple detection assays as well for complex assays involving antibodies.

In the current work, image capture was performed with an image scanner, which, however, may be replaced with a cellphone equipped with suitable software. The preference for cellphones is greater as it has many advantages like portability, easy accessibility, low cost, etc. However, suitable softwares are required for increasing inter and intra phone reproducibility.^{69,70}

CONCLUSION

We report here a fabrication method for the development of paper based microfluidic device. The fabrication process allows the partial polymerization of photoresist on the rear side of the device, which prevents sample leakage during tests and thereby reducing the cost of laminating both sides of the device. We also report a new design and method for preparing the test zone of paper based microfluidic device with hassle-free spotting of reagents and treatment of the test zone, which requires washing and regeneration. Functionality of the device was successfully demonstrated for colorimetric assays using fabricated test zones with different reagents (chemicals, proteins, etc). The fabricated device presents the possibility of controlled loading of varied biomolecules (antibody, protein, aptamers, DNA, etc.) as well as chemical reagents on the test zones without any reagent crossover to surrounding sites of the device. The prepared device has the potential for leak proof detection of analyte, requires low sample volume, involves reduced cost of production (\$0.03, excluding reagent and lamination cost), and enables the integration of customized test zones. Furthermore, the prefabrication test zone approach might reduce the overall costs associated with low temperature storage of the device by minimizing the storage volume for the product as the storage of only prefabricated test zones with embedded heat labile reagents would suffice instead of storing the whole assembly with base device. Limitations of the constructed device though present can be easily overcome. First, the coffee staining effect during nonspecific detection mainly occurs in the case of non-covalent immobilization of reagents on the test zone. However, this limitation can be overcome by either controlling the sample flow rate or covalently immobilizing the reagents on the test zone. Second, the design strategy though validated with only photolithography may be integrated with different fabrication processes like wax printing, AKD printing, inkjet printing, etc. Third, for effective commercialization, batch to batch reproducibility and integration with supporting imaging and analysis components need to be studied. However, considering the easy and simple fabrication method, reproducibility can be easily achieved. Similarly, the platforms for image capture and analysis can be integrated into a single format like cellphones equipped

with suitable algorithms to negate the effect of lighting variance, imaging distance, and inter-phone difference.

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