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PAPER

Paper-based multiplexed vertical flow assay for point-of-care testing

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We developed a multiplexed point-of-care immunodiagnostic assay for antibody detection in human sera made through the vertical stacking of functional paper layers. In this multiplexed vertical flow immunodiagnostic assay (xVFA), a colorimetric signal is generated by gold nanoparticles captured on a spatially-multiplexed sensing membrane containing specific antigens. The assay is completed in 20 minutes, following which the sensing membrane is imaged by a cost-effective mobile-phone reader. The images are sent to a server, where the results are rapidly analyzed and relayed back to the user. The performance of the assay was evaluated by measuring Lyme-specific antibodies in human sera as model target antibodies. The presented platform is rapid, simple, inexpensive, and allows for simultaneous and quantitative measurement of multiple antibodies and/or antigens making it a suitable point-of-care platform for disease diagnostics.

Introduction

Point-of-care testing (POCT) refers to rapid and simple diagnostic tests designed to shorten the sample-to-answer timeline, enabling effective treatment faster.[1-3] Resource-limited regions benefit from the decentralized nature of POCT, leading the World Health Organization (WHO) to outline the "ASSURED" criteria - that is Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable – to aid in the development and selection of POC tests for a given need.[4-5]

Lateral flow assays (LFAs) or immunochromatographic assays (ICAs) form one of the most widely spread examples of POCTs because they utilize low-cost, easy-to-handle paper-based materials, and generate color signals that can simply be read by the naked eye therefore meeting much of the ASSURED criteria.[6-8] As a result, they have penetrated the market in a variety of industries, such as food safety, agriculture, drug testing, and diagnostics.[6-13] However, despite this success, there is a demand to further improve the utility of POCT by enabling the simultaneous and/or quantitative measurement of multiple targets. To this end, many new POCT schemes have been reported in the literature. Multiplexed micro-fluidic platforms, for example, have been demonstrated for applications in cancer diagnosis, cardiovascular disease, and other biomarker quantification.[3, 14-16] However, many of these devices still have limited use for POCT use because they require relatively bulky and/or expensive syringe pumps for fluid handling and benchtop read-out devices for assay analysis.[17] Multiplexed LFA schemes have also been developed.[18-22] For example, single channel designs containing multiple test-lines as well as functionalized dot arrays have both been demonstrated, but these designs are subject to cross-reactivity and signal interference due to their inline geometry.[18] The vertical flow format employs a large 2D sensing membrane with isolated immunoreaction spots, therefore, enables simultaneous measurement of >25 analytes with no cross-reactivity or cross-talk among the spatially isolated channels. Other immunoassay platforms based on a vertical flow format have also been reported.[23-25] These former platforms allow for rapid and simple operation, however they do not demonstrate analysis with field-portable and cost-effective readers. Additionally, the vertical flow designs that are based on syringes, though demonstrating multiplexing capabilities via the micro-array platform, are prone to inter-user variability due to their manual operation, and require larger sample volumes per test (~mL level). Therefore, further development of vertical flow assay technology is still needed for enabling diagnostics at the point-of-care and for personalized medicine related applications that require cost-effective and rapid analysis of a panel of biomarkers.[26, 27]

Herein we demonstrate a multiplexed vertical flow assay (xVFA) which performs parallel detection of a panel of Lyme antibodies in a single test analyzed by a low-cost mobile phone reader. Functional paper layers, optimized through empirical testing are stacked inside a 3-D printed cassette to enable

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repeatable and uniform vertical flow rates, thus minimizing signal deviations among tests as well as across our multiplexed immunoreaction spots. Furthermore, we developed a mobile phone reader and an automated image processing algorithm for the accurate quantification of multiple absorbance-based signals from our assay.

We demonstrate our paper-based sensing platform as a potential POCT tool through the simultaneous measurement of three different Lyme disease (LD)-specific antibodies (anti-OspC, anti-BmpA and anti-P41) as a model target sample for a proof-of-concept demonstration of the quantitative xVFA platform. These target analytes were chosen because Lyme disease is a common illness in the United States and Europe that requires a multiplexed POC diagnostic tool at early stages to prevent long-term complications.[28-32] We therefore show multiplexed signals from human samples confirmed to be LD IgM-positive and IgM-negative, and compare the results to the gold-standard ELISA measurements used for diagnoses. Taken together, this xVFA platform shows potential for multiplexed and quantitative POC immunodiagnostics, enabling rapid assays far from central laboratory settings.

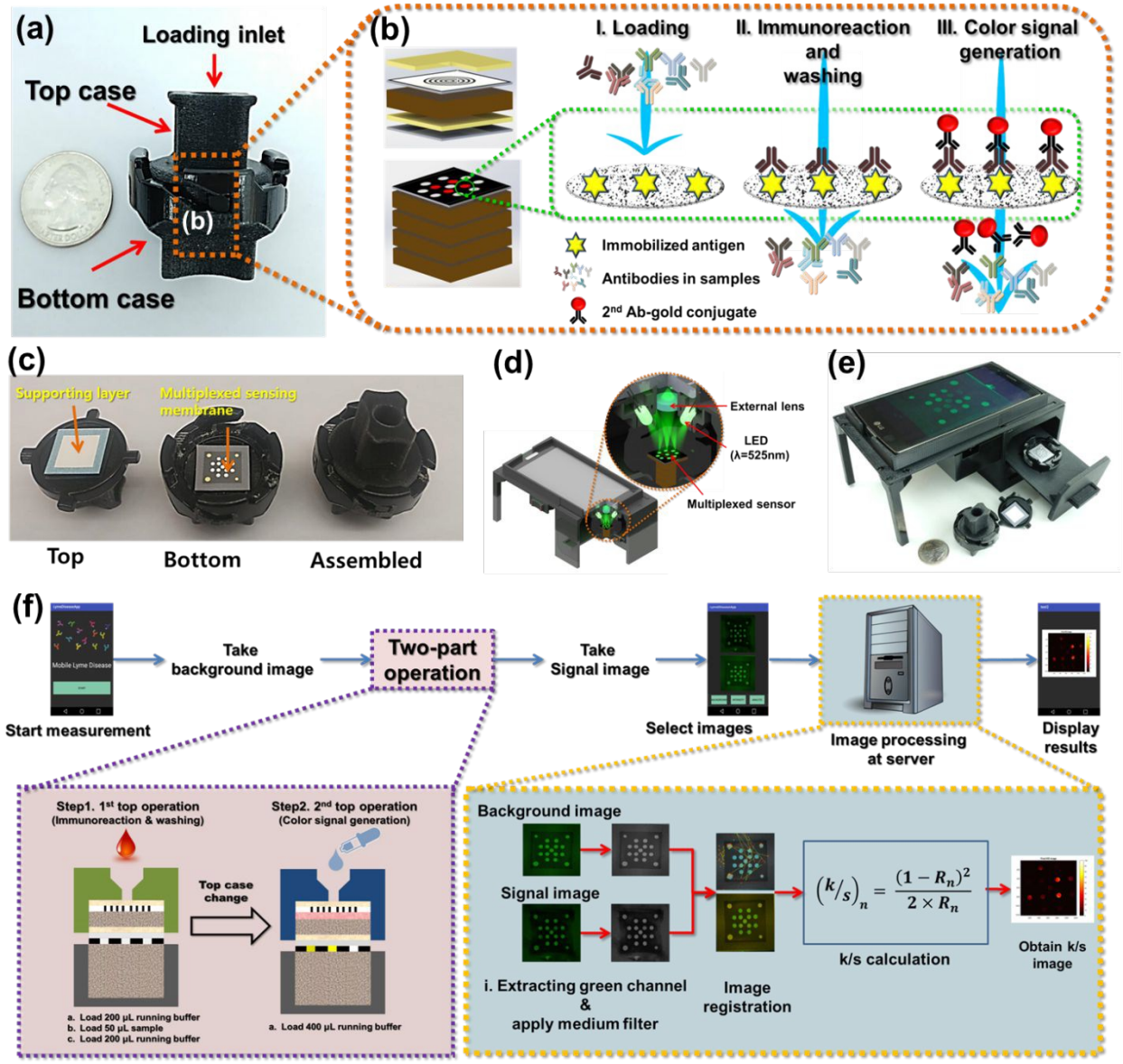

Fig. 1. Paper-based multiplexed vertical flow assay (xVFA) and a custom-designed mobile-phone reader. (a) Image of the assembled sensor cassette and (b) the vertically stacked paper layers inside the cassette (yellow: asymmetric membrane, white: vertical flow diffuser, brown: absorption pad, black: multiplexed sensor). The sandwich immunoassay operation is shown to the right. (c) Picture of an opened sensor cassette (top and bottom) and a closed sensor cassette. (d) Mobile phone reader for reflection-based detection of the colorimetric signals from the multiplexed sensing membrane. (e) Picture of the mobile phone reader and sensor cassettes. (f) Schematic overview of the complete assay operation and image processing. Cost of materials per test: 26.7 ¢ (see Table S1).

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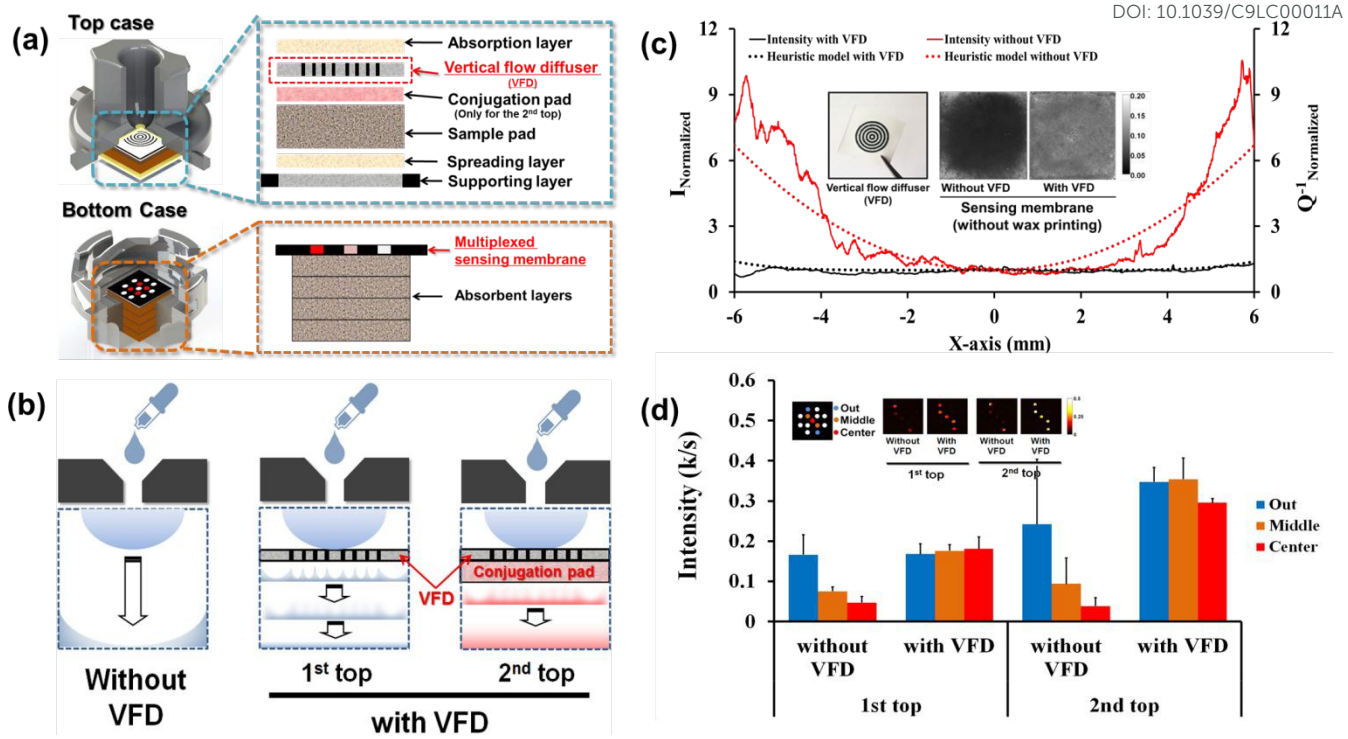

Fig. 2. The effect of the vertical flow diffuser (VFD) on flow uniformity. (a) Cross-sectional view of the vertically stacked paper layers within the top and bottom case. (b) Conceptual diagram demonstrating the improvement of vertical flow uniformity by the VFD. (c) Signal intensity distribution with and without the VFD at the sensing membrane, plotted with a heuristic model of vertical flow rate along the x-axis (lateral dimension) using Darcy's law (see Fig. S4). The insert shows an image of the vertical flow diffuser, next to raw images of the sensing membrane activated with and without the VFD. (d) Comparison of the signal intensity from different immunoreaction spots located in the outer (out), middle, and center of the multiplex sensing membrane with and without the VFD (insert: k/s intensity images).

Experimental Section

Materials

Borrelia burgdorferi antigens (OspC (BOR-004), BmpA (BOR-006), P41(BOR-001)) were purchased from Prospec Inc. An anti-BmpA rabbit IgG (ab106085) and anti-P41 rabbit IgG (ab93713) were purchased from Abcam and anti-OspC rabbit IgG (ABIN964717) was purchased from Antibodies-online, Inc. An anti-rabbit IgG (unlabeled (4030-01) and HRP conjugated (4030-05)) was acquired from Southern Biotech and anti-human IgM (unlabeled (ab99741) and HRP conjugated (ab99744)) was purchased from Abcam. Blocker™ BSA (37525) was purchased from Bio-Rad. Nitrocellulose membranes (0.22µm (11327) and 0.45µm (11036)) were purchased from Sartorius Stedim North America Inc. A vivid plasma separation membrane (grade GX) was purchased from Pall Co., and the sample pad (CF7) and the conjugation pad (Grade Standard 14) were sourced from GE Healthcare Biosciences Corp. The absorbent pad (Whatman Grade 707) was acquired from OpticsPlanet, Inc. The gold colloidal solution (40 nm colloid, 15707-1) was purchased from Ted Pella, Inc. Foam tape (Super-Cushioning Food-Grade Polyethylene Foam Sheets 1/16") was purchased from McMaster. Lyme positive human plasma was purchased from SeraCare Life Sciences and two negative control human sera were obtained from the UCLA Health system.

Preparation of conjugation pad

Gold nanoparticle (AuNP)-antibody conjugates were synthesized by adding 900µL of gold nanoparticle solution (1 OD), 100µL 0.1M borate buffer (pH 8.5) and 10µL of the antibody (1mg/mL) to a sterile Eppendorf. The materials were incubated for 1 hour at room temperature, then 100µL BSA (1 % in PBS) was added and incubated for 30 minutes at room temperature, acting as a blocking protein to prevent non-specific binding. The AuNP conjugates were centrifuged at 4 °C for 15 min at 8000 rpm, and then washed with 1 mL 10 mM Tris buffer (pH 7.4) three times. After the final wash, 100 µL of storage buffer (0.1 M pH 8.5 borate buffers with 0.1% BSA and 1% sucrose) was added to the supernatant, and the final concentration of AuNP antibody conjugate was confirmed by optical density measurement at 525 nm. Lastly, 70 µL of the 2 OD conjugate solution was pipetted onto each conjugation pad (1.2 × 1.2 cm), and the pads were dried at 37 °C for 1 hour.

Preparation of sensing membrane and paper layers

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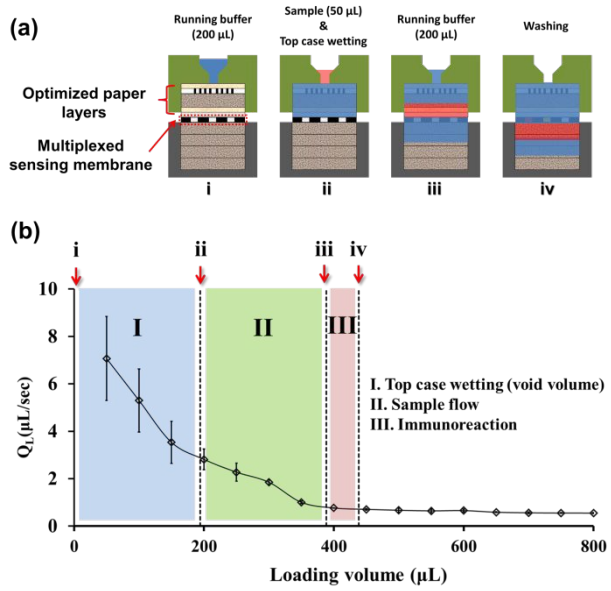

Fig. 3. Characterization by volumetric flow rate analysis. (a) Schematic of the loading flow rate measurement. (b) Diagram of the vertical flow timing during the assay operation. (c) Volumetric flow rate change at the loading inlet versus total loading volume. Three regimes of the paper based vertical sample flow are defined as I: top case wetting, II: sample flow, and III: immunoreaction. The assay loading protocol is designed so that the immunoreaction occurs when the paper layers are fully wetted and the vertical flow rate is limited by the multiplexed sensing membrane, thus converging to a uniform sample flow rate, as shown in regime III and beyond.

The multiplexed sensing membrane was produced using a NC membrane (0.22 µm pore-size) and wax printer (Xerox ColorQube 8580). The membrane was placed on an A4 copy paper and fixed using a tape (paper: transparency, print quality: photo). Thirteen spatially isolated immunoreaction spots were defined by wax printed barriers, allowing for different capture antigens to be spotted on the nitrocellulose membrane. After printing, the sensing membranes were incubated for 30 seconds at 120 ℃ in an oven to allow the printed wax to melt and diffuse downward into the nitro-cellulose. Each of the sensing spots was then loaded with 1 µL of capture-antigen solution, and allowed to dry for 30 minutes at room temperature. Then the membrane was submerged in 1% BSA in PBS solution for 30 minutes to block non-specific binding, and the sensing membranes were again dried for 10 min at 37°C in a convection dry oven. The supporting layer (0.22 µm pore-size NC membrane) and vertical flow diffuser (0.45µm pore-size) were similarly produced with a wax printer, and the BSA blocking procedure was performed for these paper layers following the same procedure as for the multiplexed sensing membrane. The absorbent pad (1.2 × 1.2 cm), foam tape (1.7 × 1.7 cm for outside, 1.2 × 1.2 cm for inside), and asymmetric membrane (1.2 × 1.2 cm, i.e. spreading layer) were all laser-cut (60W Speedy 100 CO_2 laser from Trotec) to achieve precise dimensions and high-throughput production. The other paper layers were hand-cut to avoid possible damage from smoke to their immobilized capture chemistries.

Assembly of vertical flow sensor

A 3-D printed cassette, which opens, closes, and locks through a simple twisting mechanism (see Fig. S2), was developed for housing the vertical stack of paper layers, and allowed for ease-of-handling during sensor operations. Prepared paper materials were stacked inside of the cassette as follows (ordered top to bottom); for the top case; asymmetric membrane (absorption layer), vertical flow diffuser, sample pad, asymmetric membrane (spreading layer) and supporting layer, for the bottom case; multiplexed sensing membrane and four absorbent pads (see Fig. S3). The outer edges of the supporting layer in the top case and the multiplexed sensor in the bottom case were secured with foam tape.

Operation protocol

Initial background images of the prepared sensing membrane are taken using the mobile-phone reader prior to running the assay. Then, the *first* top case and bottom case are assembled together, and 200 µL of running buffer (1.6% BSA and 3% tween 20 in PBS)[33] is dispensed into the top case. After 1 minute, 50 µL of the sample is dispensed into the top case, followed by another 200 µL of running buffer one minute later. Then the fluids are allowed to flow through the paper-based layers for 6 minutes, before the *first* top case is exchanged with the *second* top case containing the conjugation pad layer between the vertical flow diffuser and sample pad. An additional 400 µL of running buffer is dispensed into the *second* top case and allowed to flow for 8 minutes, marking the end of the sandwich immunoassay. The second top case is then removed and an image of the sensing membrane is again taken with the mobile-phone reader.

Mobile phone reader and data processing

The 3D-printed (Dimension Elite, Stratasys) mobile phone based-reader contains four 525 nm wavelength LEDs paired with diffusers to evenly illuminate the sensing membrane (Fig. 1d and e). An external lens is used along with the phone lens and camera to image the sensing membrane in reflection mode. The images are taken with raw .dng format using the standard Android camera app of the smartphone (LG G4 H810). The images are then sent to a server where a custom MATLAB script converts them to tiff format and selects the green channel. The background and signal images are then registered, and the signal image is divided by the background image, and used to calculate the k/s value per pixel as defined by a special case of Kubelka-Munk theory where the reflection substrate is considered opaque (i.e. infinite thickness).[34]

$$\left(k/s\right)_n = \frac{(1-R_n)^2}{2 \times R_n}$$

where k is the absorption coefficient, s is the scattering coefficient of the layer, R_n is per pixel intensity ratio between the registered signal and background image, and n is the pixel index. The k/s image, along with the average k/s value per sensing spot is calculated and then sent back to the custom smartphone application. The results are then displayed in the

graphical user interface (GUI) of the application revealing to the user the relative Lyme-specific antibody concentrations in the sample (Fig. 1F).

ELISA well-plate assay procedure

Lyme antigens (50 µL, 200 ng/mL) in pH 9.0 0.1M carbonate buffer were incubated for two hours in a 96 well-plate at room temperature. Wells were washed with PBST three times then incubated with 100 µL of 1% bovine serum albumin (BSA) in PBS for 2 hours at room temperature to prevent non-specific binding. After washing with PBST three times, 50 µL of the spiked anti-Lyme antibodies (anti-OspC, anti-BmpA and anti-P41) 1% BSA in PBS (1 µg/mL) and the plates were incubated for 2 hours at room temperature. Plates were washed with 100 µL PBST and 50 µL goat anti-rabbit IgG HRP conjugate (4000 times dilution in 1% BSA in PBST) was added to each well and incubated at room temperature for 30 minutes. The plate was washed with PBST (three times), D.I. water (three times), and tetramethylbenzidine (TMB) was added to each well to react for 15 minutes. The reaction was stopped with 50 µL of 0.2M H_2SO_4 and absorbance was measured at 450 nm using a well plate reader (Synergy, BioTek®, Winooski, VT, USA). For the control human sample assay, the same assay was performed with the samples diluted 20 times in 1% BSA.

Results and discussion

Paper-based multiplexed vertical flow immunodiagnostic assay (xVFA)

Fig. 1 outlines the xVFA platform. A sandwich immunoassay is performed at the multiplexed sensing membrane, which contains wax-printed hydrophobic barriers to form 13 isolated immunoreaction spots (Fig. 1a). Above the sensing membrane are the functional paper layers which are responsible for i) generating the assay color signal, ii) achieving uniform vertical flow, and iii) reducing test-to-test variations. The functional layers include a conjugate pad, custom vertical flow diffusers, spreading layers, and absorption layers. A 3D printed cassette encapsulates these stacked paper layers and provides the compression necessary for repeatable flow between layers. The user-friendly cassette includes a simple, sample loading inlet and 'twist-on/off' mechanism for opening and closing (see Fig. S2). The overall assay completes within 20 minutes based on a simple two-part protocol performed by the user (described in the Methods section). In the first part, the sample is loaded and flows through the sensing membrane where the target analyte is specifically captured. In the second part, conjugated gold-nanoparticles (AuNPs) are released from a pre-made conjugate pad with the addition of buffer to generate a color signal (Fig. 1b). Lastly, after the completion of the assay, a mobile phone reader along with automated image processing allows for rapid measurement of the multiplexed signals (Fig. 1c and e).

Achieving sample flow uniformity

Flow uniformity laterally across the sensing membrane is critical for our xVFA platform as it relies on spatial multiplexing. Darcy's law states that in fully-wetted porous media, volumetric flow-rate is inversely proportional to flow distance, meaning that the larger the cross-sectional area of the multiplexed VFA the more non-uniform the vertical flow will be as the fluid spreads both laterally and vertically to the edges of the device. This poses clear drawbacks in terms of assay performance and illustrates a trade-off between functional multiplexing area and the signal uniformity. Therefore, we developed and optimized a Vertical Flow Diffuser (VFD) layer, fabricated by wax printing multiple concentric barriers of varying sizes on a nitrocellulose (NC) membrane. When the loaded samples and solution contact the barriers, multiple

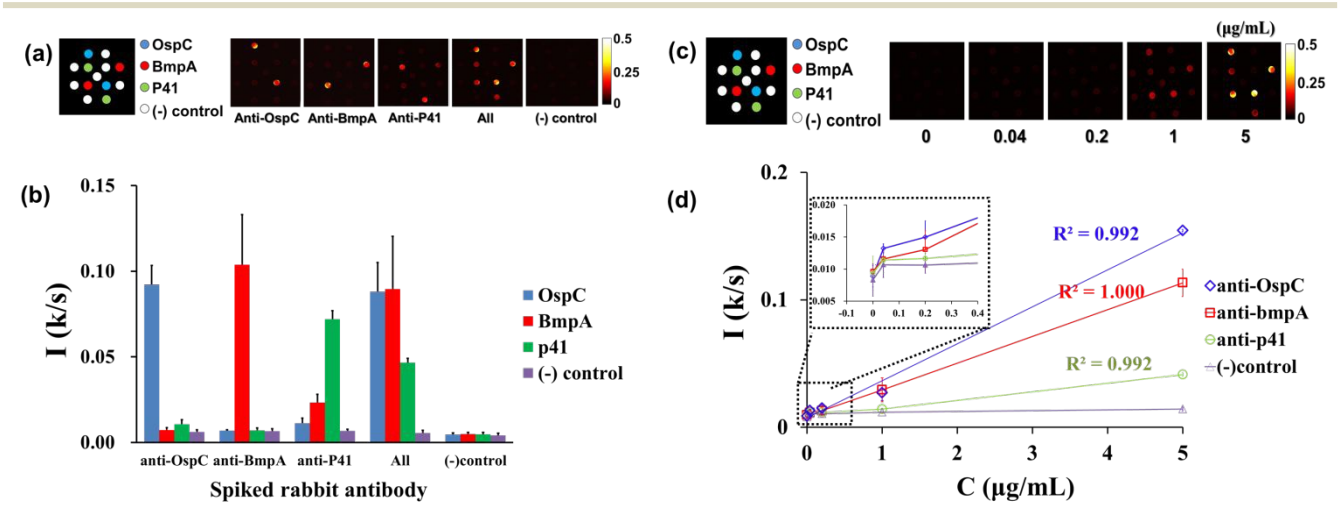

Fig. 4. Evaluation of xVFA with spiked LD-specific rabbit antibodies. (a) Antigen spot legend and k/s images of sensing membranes activated with different sample antibodies (anti-OspC, anti-BmpA and anti-P41). (b) Intensity plot from analyzed k/s images in (a) showing selective signal of the immunoreaction spots (n=2). (c) Antigen spot legend and k/s images of sensing membranes activated with increasing concentrations of three LD-specific rabbit antibodies (anti-OspC, anti-BmpA and anti-P41). (d) Corresponding concentration vs. signal intensity plot from analyzed k/s images in (c) (n=2). The limit of detection (LOD, blank+3SD) was calculated as 209.6 ng/mL for anti-OspC, 162.2 ng/mL for anti-BmpA and 1046 ng/mL for anti-P41.

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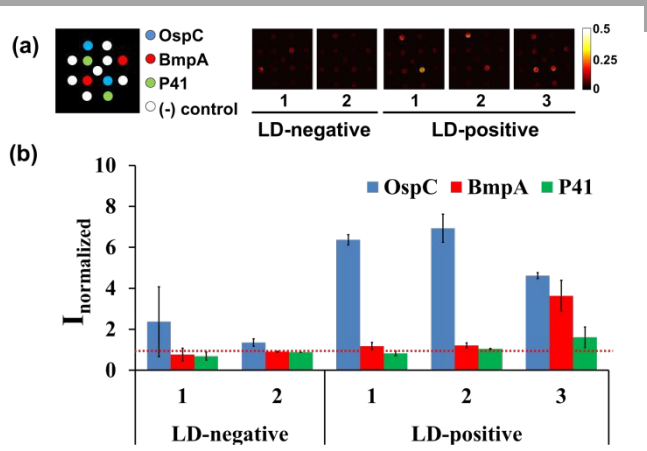

Fig. 5. Detection of LD-specific antibodies in human samples. (a) Antigen spot legend and k/s images of sensing membrane activated by human samples. (b) Normalized intensity plot from analyzed k/s images (n=2). The raw intensity of each antigen was obtained by average of two spots value and normalized by the negative control plus three times the standard deviation. The error bar indicates the standard deviation of duplicate measurement of each sample. The dot line indicates average intensity of negative control spots plus three times the standard deviation. The results of each antigen spot reflect the ELISA results (*see* Fig. S1). Raw signal images are shown n Fig. S8 (a).

concentric flows emanate from the VFD, culminating in an even distribution of flow rates across the sensing membrane. Without the VFD, center-to-edge flow emanating from the loading inlet results in non-uniform volumetric flow-rates across the sensing membrane. This is illustrated in Fig. 2b and c, where we compare a heuristic model of volumetric flow rates with and without the VFD (*see* Fig. S4) to experimental results from the xVFA system. To test the effects of flow uniformity we used rabbit IgG as a capture antibody in the sensing membrane, and anti-rabbit IgG-conjugated gold nanoparticles as model samples in the conjugation pad. When the VFD is absent, a large vertical flow intensity variation (nearly 1000%) between the center and the outside spots is observed, as the colorimetric signal generated during the assay is inversely proportional to the volumetric vertical flow rate of the sample due to the relative immunoreaction time. Therefore, in the presence of VFD, the coefficient of variation (CV, the ratio of the standard deviation to the mean of signal intensity) of signal intensity decreases from 94% to 12.9% across all the active spots (Fig. 2d).

Non-repeatable flow between samples is caused by uncontrolled-factors such as the misalignment of paper-layers and/or an un-even compression ratio by the external plastic case. In order to minimize this random noise and further improve signal variation, we used a hydrophilic asymmetric membrane (i.e. different pore size on top vs. bottom) as a spreading layer. When the small pore side contacts the upper paper layer, the sample flows laterally before it can flow out of the large pore size due to reverse capillary action. This spreading flow reduces the vertical flow non-uniformity and non-repeatability as well as increases the overall signal intensity by slowing the vertical flow rate. The structure of the asymmetric membrane, flow characterization and combined effect with the VFD on signal uniformity and intensity are described in Fig. S6, exhibiting the best results in terms of CV and the overall signal when compared to: i) no functional layers, ii) the VFD only, and iii) the spreading layer only.

Characterization and optimization of the two-part assay procedure

Generally, around 10 mg/mL of IgG and 1-2 mg/mL of IgM are contained in normal adult serum.[35, 36] These levels are 0.1 ~1 million times higher than the detection sensitivity of our presented assay (about 0.1~1 μg/mL), therefore serological tests such as our xVFA are prone to a high degree of nonspecific signal (*see* Fig. S8). We therefore designed a two-part operation, using two separate top pieces of the cassette for the immunoreaction and signal generation steps, respectively. The first part of the assay includes the sequential introduction of 200 μL running buffer for wetting, 50 μL of sample, and 200 μL of running buffer for washing to the sample inlet. Then, after changing to the second top case, 400 μL of running buffer is added, releasing the Au NP solution embedded in the conjugate pad and subsequently washing non-specifically bound particles. In order to determine the optimal loading volumes, incubation times, and sequence of the assay protocol, we performed volumetric flow-rate analysis on our xVFA platform. The volumetric flow rate (Q_L) at the loading inlet was obtained by timing the absorption of 50 μL increments of the running buffer into the vertically stacked paper layers below. In order to ensure that the immunoreaction on the multiplexed sensing membrane proceeds at a set flow rate between different samples, the sample incubation step must occur after the flow rate has converged to this limit. Thus, we divided the flow operation into three phases: I) wetting of the top case, II) sample flow from inlet to sensing membrane, and III) flow of sample through the sensing membrane (i.e. the immunoreaction). The combined void volume of the top paper layers was calculated as 194.3 μL which therefore defines the bounds on phase I. After the void volume of the top case is filled, the 50 μL sample is loaded, and begins to flow to the sensing membrane, followed by another injection of 194.3 μL of the washing buffer. Therefore, due to the limited void volume of the top paper layers, when the second addition of buffer is completely absorbed from the inlet, the sample will have completed its flow through the sensing membrane, ending phase III. The washing of the sensing membrane then occurs before the entire void volume of the vertical stack (top and bottom case) is filled with solution. The volumetric flow rate analysis indicates that with these injection volumes and sequence, the flow-mediated immunoreaction occurs at a constant flow-rate (0.74 ± 0.039 μL/sec). Therefore it was determined for the sensor operation that 200 μL of running buffer be injected before the 50 μL of sample is loaded, and that the sample loading be followed by another 200 μL of running buffer to wash the non-specifically bound proteins or AuNPs. The total time of this loading operation is 5.5 minutes per test, as calculated through a time-average of the volumetric flow rate.

The two-part assay procedure is well suited for multiplexed antibody measurement in human sera which can create a high nonspecific signal (Fig. S8), however it is also important to

note that the assay can be simplified to a one-step procedure for target samples with lower concentrations of nonspecific proteins (e.g. urine).

Evaluation of xVFA in a buffer system

The quantitative performance of the assay was evaluated by measuring anti-OspC, anti-BmpA and anti-P41 spiked samples in a clean buffer. For evaluation of specific binding, we immobilized antigens and BSA on the multiplex sensor as positive and negative spots, respectively. We confirmed selective signal depending on the antigen and antibody pair with no-antibody and all-antibody samples along with single-antibody samples (one with anti-OscpC only, one with anti-BmpA only and one with anti-P41 only all at 5 µg/mL concentration) (Fig. 4a). In addition, we obtained a limit of detection (LOD, mean + 3SD) as 209.6 ng/mL for anti-OspC, 162.2 ng/mL for anti-BmpA and 1.05 µg/mL for anti-P41 (Fig. 4b). These results, confirmed by ELISA testing (Fig. S1a), indicate that the proposed sensor is able to simultaneously detect multiple antibodies and provide quantitative results.

Human Lyme IgM control plasma measurements

xVFA platform was also evaluated with Lyme-specific IgM-positive and negative human samples in order to verify its applicability to POCT diagnostics. As shown in Fig. 5, we confirmed a clear difference in signal intensity between the negative and positive samples, and the results match closely with the ELISA results. Interestingly, a large negative control signal in the ELISA test did not appear in the xVFA. In the case of the Lyme positive-1 sample, a negative control level 24.3% of OspC signal can be seen in the ELISA test, compared to the 9.2 % in the xVFA. This result demonstrates that the optimized two-part operation of the xVFA works effectively for human samples, showing potential as a multiplexed POCT tool for diagnosing LD.

Conclusions

This study demonstrated a multiplexed and quantitative POCT platform. Our paper-based test meets the ASSURED criteria and was confirmed to successfully operate using human samples. To enable multiplexed detection, we designed and characterized a vertically stacked structure of functionalized paper layers. The uniformity of vertical sample flow was optimized through the implementation of a vertical flow diffuser in combination with an asymmetric membrane, and the optimal assay operation (i.e. loading volumes, loading timing) was determined by volumetric flow rate analysis at the loading inlet. We employed 13 independent immunoreaction spots in this study but confirmed the operation of our xVFA for up 35 independent reaction spots (data not shown). In addition, all of the paper materials cost less than 30 cents per test (see Table S1), and read-out is performed with a low-cost mobile-phone reader[37] and a mobile application. The entire two-part assay operation reports results within 20 minutes from sample collection, and uses 50 µL of serum sample volume, comparable to lateral flow assays. Further studies are needed to develop an LD decision algorithm, and evaluate the long-term stability of the conjugate pad and used buffers. Additional research and development could also incorporate whole-blood processing steps within the functional layers of the xVFA through e.g., the implementation of a plasma separation membrane. Other automation features can also be explored such as the storage of the necessary buffers within the xVFA cassette. In conclusion, the xVFA is a promising POCT diagnostic tool. It can provide quantitative and multiplexed measurements of target analytes. Without the need for highly trained technicians, the xVFA could potentially be implemented in low-resource areas, for applications such as Lyme-disease diagnosis, enabling more effective prevention of chronic symptoms.

Conflicts of interest

There are no conflicts to declare.

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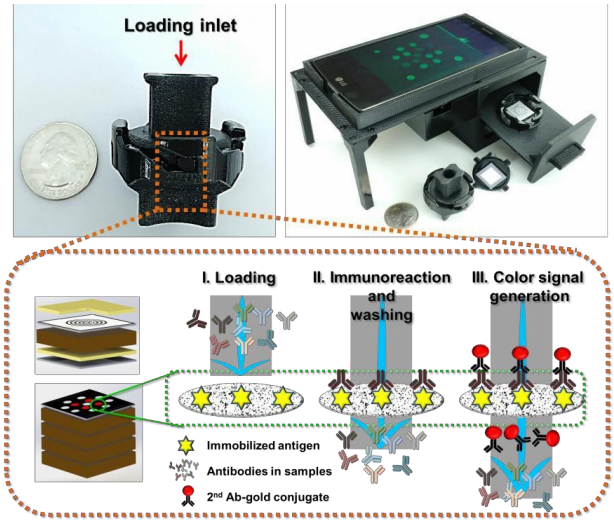
Graphical abstract



The developed platform could serve as a diagnostic tool for point-of-care Lyme diagnosis and can be applied to other diagnoses which require a multiplexed measurement in resource-limited settings.