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Enhanced sensitivity of lateral flow tests using a two-dimensional paper network format

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Abstract

Point-of-care diagnostic assays that are rapid, easy-to-use, and low-cost are needed for use in low-resource settings; the lateral flow test has become the standard bioassay format in such settings because it meets those criteria. However, for a number of analytes, conventional lateral flow tests lack the sensitivity needed to have clinical utility. To address this limitation, we are developing a paper network platform that extends the conventional lateral flow test to two dimensions. The two-dimensional structures allow incorporation of multi-step processes for improved sensitivity, while still retaining the positive aspects of conventional lateral flow tests. Here we create an easy-to-use, signal-amplified immunoassay based on a modified commercial strip test for human chorionic gonadotropin, the hormone used to detect pregnancy, and demonstrate an improved limit of detection compared to a conventional lateral flow assay. These results highlight the potential of the paper network platform to enhance access to high-quality diagnostic capabilities in low-resource settings in the developed and developing worlds.

Keywords

two-dimensional paper networks; lateral flow assays; chemical signal amplification; gold enhancement of gold nanoparticles; immunoassay

1.1 Introduction

Lateral flow strip tests have been developed for many applications ranging from home testing for pregnancy and drugs of abuse, to disease detection in low-resource settings. The main advantages of these strips over conventional laboratory-based tests, such as ELISA, are that they are rapid, low-cost, readable by eye with visible signals, instrument-free, and use reagents stored in dry form. However, conventional lateral flow strip tests often only measure a single analyte per strip, and for many targets lack the sensitivity required for clinical utility. Much of the recent work in paper-based devices has been to extend the capability of conventional lateral flow strips to detect multiple analytes using two- and three-dimensional patterns in paper to link a single inlet with multiple detection regions [1–7]. The power and applicability of such simple analytical devices would be greatly enhanced if limits of detection (LOD) were improved. For example, recent evaluation of commercially-available point-of-care tests for chlamydia [8, 9] and influenza [10–13] indicate very poor sensitivity and highlight the need for tests with improved limits of detection.

Previous work to improve LOD in lateral flow strips has involved employing particle labels (*e.g.* quantum dots, up-converting phosphors, and paramagnetic particles) that require additional instrumentation and cost [14, 15]. Chemical signal amplification has been used to improve LOD in laboratory-based bioassays, most notably, in ELISA. The utility of paper-based tests could be greatly extended by enabling sophisticated processes, such as chemical signal amplification. There have been several investigations of the utility of colorimetric signal amplification systems for enhancing the sensitivity of conventional lateral flow tests. Horton *et al.* reported a 100-fold reduction of the LOD [16] by immersion of a lateral flow strip into a silver enhancement solution. More recently, several groups have applied signal amplification methods by flowing solutions across the width of the detection region of the lateral flow strip. Reports include improvements in LOD of approximately 10-fold [17, 18] using an enzymatic amplification system, and an approximately 50-fold gain in LOD using a silver enhancement solution [19]. However, in these studies, the user was still required to perform numerous timed steps to apply rinse and signal amplification solutions to the lateral flow strip, thus limiting the appropriateness of the formats for use at the point of care (POC).

We have previously demonstrated the potential of two-dimensional paper networks (2DPNs) for automating multi-step chemical processing [20–23]. A key feature of the 2DPN is the configuration of the network, composed of multiple inlets per detection region, which functions as a program for the timed delivery of multiple reagent volumes within the network. Using a 2DPN, we demonstrated the sequential delivery of multiple reagents, including a signal amplification solution [21]. Here, we show that in a 2DPN format based on a conventional lateral flow strip, LOD can be improved by adding rinse and signal amplification steps. For the demonstration, we chose a model system, detection of human chorionic gonadotropin (hCG) in a commercial lateral flow strip, and used a commercially-available gold enhancement reagent. We first present the effects of rinse and chemical amplification steps on assay LOD in a lateral flow format using manually-performed sequential applications. We then translate the desired processes to a paper network format that can perform signal amplification with an ease of use appropriate for minimally-trained users in low-resource settings. The current study represents the first demonstration of the use of a paper network assay to achieve an improved limit of detection.

2.1 Materials and Methods

Manual format

An hCG dipstick test (Meditests, Bensalem, PA) was run by laying the strip test on a flat surface and sequentially pipetting sample and reagents onto the strip test at timed intervals. Modifications to the strip test included trimming a well-defined length off the bottom of the strip to access the sample pad and adding a cellulose pad (Millipore, Billerica, MA) to the existing absorbent wick to allow for the processing of an increased total volume for rinse and signal amplification reagents. Mock samples were created by adding known amounts of human chorionic gonadotropin (hCG) (Sigma-Aldrich, St. Louis, MO) to fetal bovine serum (Invitrogen, Carlsbad, CA). The hCG concentrations in the mock samples were determined using the Beckman Coulter Access Immunoassay System as performed by University of Washington Research Testing Services. Rinse solutions consisted of either phosphate buffered saline (PBS) at pH 7.4 or a phosphate buffer at pH 2 (low-pH PB). Gold enhancement (GE) solution (Nanoprobe, Yaphank, NY) was used as the signal amplification reagent. Equal volumes of each of the four components were mixed together within 30 minutes of application to the device. Reagents were applied to the strip test as described in the following. First, 35 μ l of a mock sample consisting of a known concentration of hCG spiked into fetal bovine serum was added at the start. In the case of a rinse step, 20 μ l of rinse buffer was applied at approximately 10 minutes. In the case of an amplification step, 25 μ l of the gold enhancement chemistry was applied at approximately

20 minutes. Device detection regions were imaged for end-point quantitation using a high-resolution scanner (Epson Perfection V700, Nagano, Japan). Scans were performed at approximately 30 minutes for sample only and sample plus rinse cases. For the PBS rinse plus amplification case, scans were performed at 40 minutes, while for the low pH PB rinse plus amplification case, the scans were performed at a slightly longer time, 45 minutes. Strips were run in batches of 5 with at least 12 replicate measurements for the zero-concentration sample (FBS only) and 4 measurements each for non-zero concentrations of analyte.

Automated 2DPN card

The modified hCG dipstick test (Meditests, Bensalem, PA) constituted a main component of the 2DPN card. Glass fiber pads (Ahlstrom, Helsinki, Finland) were used to construct the rinse and amplification pieces of the paper network, and as source pads for applying wet buffer. Cellulose pads (Millipore, Billerica, MA) were used as the absorbent wick. The housing of the card was composed of Mylar™ and adhesive layers (Fraylock, San Carlos, CA). Materials were cut to appropriate shapes on a CO₂ laser system (Universal Laser Systems, Scottsdale, AZ). The rinse solution consisted of phosphate buffered saline (PBS) at pH 7.4. As above, the GE solution was used for signal amplification. A web camera (Logitech, Fremont, CA) and image acquisition software (AZcendant, Tempe, AZ) were used to record images of flow. For end-point quantification, the scanner described above was used to acquire images of the detection region.

The procedure for running 2DPN folding cards follows. For each run, 90 µl of PBS buffer and 140 µl of GE solution were added to appropriate pads, and then 50 µl of a mock sample consisting of a known concentration of hCG spiked into fetal bovine serum was added to the sample pad. The card was then closed at 20 min, which activated the 2DPN network and initiated subsequent rinse and amplification steps. Scans were performed at 35 min. For each concentration, 3 to 5 replicate measurements were performed.

Strip test comparison

The LOD of the modified commercial strip test without rinse and amplification was measured for comparison. In that case, 50 µl of mock sample was added to the sample pad and the scan performed at 20 min.

2DPN card with dry reagents

For the final demonstration, the GE reagent was stored dry in pads on the 2DPN card. Equal volumes of each of the components were added to glass fiber pads, dried at 37 °C overnight, and stored in a desiccator. The pads were layered and incorporated into the 2DPN card. The dry GE reagent was rehydrated using deionized water.

Image analysis and calculation of limit of detection

Images were processed using a custom analysis program (Matlab, Natick, MA). The algorithm for defining the test line region of interest (ROI) was as follows. First, in a graphical display of the test region image, the user marked the upstream edge of the control line. A starting location for the center of the test line ROI was set at 190 pixels upstream of the mark and the ROI swept over a 20 pixel range (along the direction of sample flow) about the starting position. For each location of the test line ROI, the average grayscale intensity was calculated and the test line ROI was set at the location of the minimum average grayscale intensity. Once this test line ROI was defined, a reference ROI of the same size was then defined 50 pixels upstream of the test line ROI and the average grayscale intensity of the reference ROI measured. Each ROI was either 130 by 38 pixels (manual format) or 50

by 38 pixels (2DPN card). The assay signal was defined as the average grayscale intensity in the test line ROI subtracted from the average grayscale intensity in the reference ROI. The average and standard deviation for each concentration was calculated and reported. A percentage of the cards failed (20%) due to high nonspecific adsorption in the background region and were not processed further. The average intensity within the reference region was compiled for each card, and across cards, the distribution of this quantity was bimodal, as evidenced by the fact that a two-gaussian mixture model better described the distribution than a single-gaussian (as determined by AIC, BIC, and CAIC). Because the reference region serves as a negative control for the assay, observation of high background (low intensity) in the reference region is a basis for the user to reject the card. The lower-intensity component of the reference region average intensity distribution represented cards with anomalously high background. The best-fit mixture model indicated that the intensity of best separation between the two gaussian components was 110,000 and we therefore uniformly applied a minimum cutoff for the intensity in the background region. The slope of the linear portion of the signal vs. concentration curve near zero (using the three lowest concentration measurements) was calculated for each case. Inspection of the largest zero-concentration dataset ($N = 22$ for PBS rinse with amplification) indicated that the replicate zero-concentration signal data were not normally distributed ($P < 0.01$, Shapiro-Wilk test), but were instead better described by a log-normal distribution. Accordingly, the mean μ and standard deviation σ of the log-transformed signal data were then computed. Per standard practice, the LOD was defined as the signal value at which the probability of a type I error was 1%. This was calculated as the 99th percentile of the lognormal signal distribution based on the parameters μ and σ , calculated in Microsoft Excel using the NORMINV function, $e^{[\text{NORMINV}(0.99, \mu, \sigma)]}$, divided by the slope. LOD uncertainty was estimated using a Monte Carlo procedure. For each assay i , with N_i zero-concentration signal measurements, $N_i \times 10^6$ log-signal values were sampled from the normal distribution with parameters μ and σ ; an LOD was computed for each of the 10^6 sets of N_i values as described above; finally, the LOD uncertainty was computed as the median absolute deviation of the resulting 10^6 values.

Using the Monte Carlo method ($N = 10^6$), we calculated the distributions of LOD for each assay type, based on the replicate experimental data for that assay type. To compare the LOD measurements for the two assay types, we calculated Herrnstein's ρ statistic. For pairs of samples from the LOD distributions for two assays A and B, ρ is the fraction of pairs where the LOD for assay A is greater than the LOD for assay B. This statistic is related to the area of overlap in the two LOD distributions and is a measure of the statistical significance that the LODs arise from different distributions.

3.1 Results and Discussion

Conventional lateral flow tests are limited to the single-step delivery of chemistries, while the 2DPN card demonstrated here enables automating the implementation of additional processing steps for improved LOD. Table 1 lists the operations performed in a conventional lateral flow test compared to the current 2DPN card. Conventional lateral flow tests can lack two critical components of high-sensitivity laboratory assays such as ELISA. First, a rinse step is performed to remove nonspecific adsorption. And second, a reagent is applied to produce an amplified signal from an original labeling event. Each of these operations can potentially lead to an improvement in the LOD of the assay. Note that programming of additional steps, such as a final rinse, can be implemented through straightforward extension of the paper network. Figure 1A shows schematics of the molecular stack produced in (1) a conventional lateral flow sandwich assay and (2) the amplified 2DPN assay. Figure 1B shows example results from a conventional lateral flow test, and for a lateral flow test with added, manually performed, steps for rinse and chemical signal amplification. Note the significant darkening in the test line after application of the signal amplification reagent. We

chose the model system of human chorionic gonadotropin (hCG) and a signal amplification method of gold enhancement of gold nanoparticle labels [24, 25]. The gold enhancement reagent has been reported to produce LOD improvement in other formats [26, 27].

Figure 2 shows the effects on the LOD of manually adding rinse and chemical amplification steps to the conventional lateral flow format. We first investigated two rinse solutions (Figure 2A), a near-neutral phosphate buffered saline (PBS), and a low pH phosphate buffer (low pH PB). These two were chosen to highlight their different effects on the two quantities that affect the LOD, the 99th percentile of the zero-concentration signal distribution (corresponding to a type I error rate of 1%) and the slope of the signal-versus-concentration curve near zero. Both rinse buffers reduced the slope of the signal-versus-concentration near zero; the PBS rinse resulted in a modest reduction in the slope, while the low pH PB rinse appeared to be higher-stringency and resulted in a larger reduction, almost 2-fold, in the slope. Both rinse buffers resulted in a significant reduction in the 99th percentile of the zero-concentration signal distribution; almost 3-fold for the PBS rinse and almost 2-fold for the low pH PB rinse. The net result on LOD for the low pH PB rinse was little change relative to the LOD for conventional lateral flow. For the PBS rinse, the net result was a decrease in LOD relative to the conventional lateral flow case.

Each rinse step was then coupled to the application of the gold enhancement chemistry (Figure 2B). In the case of the PBS rinse plus amplification, the slope of the signal-versus-concentration curve increased by almost 8-fold compared to conventional lateral flow. The 99th percentile of the zero-concentration signal distribution also increased significantly--by over 2-fold. The net result was a significant reduction in the LOD compared to conventional lateral flow--approximately 4-fold. In the case of the low pH PB rinse plus amplification, the slope increased modestly, by 50%, and the 99th percentile of the zero-concentration signal distribution decreased by almost 3-fold compared to conventional lateral flow, resulting in an almost 4-fold reduction in the LOD. Thus, the rinses in combination with amplification resulted in similarly improved LODs. The more commonly used PBS rinse was chosen for use in the 2DPN card.

The 2DPN card was designed to perform two additional processing steps for improved LOD in an easy to use format. Figure 3A shows an image of a 2DPN card for the automated delivery of rinse and signal amplification reagents with the main components of the card labeled. Figure 3B shows a schematic of the different stages of flow in the 2DPN card. Figure 3C contains an image series depicting 2DPN reagent delivery using colored dyes. The user first loads GE solution, rinse buffer, and sample onto appropriate pads of the card. Sample (yellow fluid) flows into the primary leg of the card that contains the detection region (Figure 3C top) and sample and conjugate bind. Then at 20 minutes, the user folds the card closed to connect a circuit for flow to occur from the rinse and GE reagent pads to the detection region of the main strip. The result is automated delivery of the rinse (red fluid, Figure 3C middle) and amplification (blue fluid, Figure 3C bottom) reagents.

An image series of the test and control lines in the 2DPN card for hCG detection is shown in Figure 4A. The signal-versus-concentration curve from a concentration series run on the 2DPN cards is shown in Figure 4B. The LOD, shown in the plot of Figure 4C, was 0.3 ± 0.2 mIU/mL. The LOD at 20 minutes, without rinse and signal amplification steps, was 1.2 ± 0.5 mIU/mL. Note that the implementation of the 2DPN format (Figure 3) is not a direct mapping of the sample/reagent volumes and times from the manual format demonstration (Figure 2). It is therefore not appropriate to directly compare the LOD results from the two sets of data. However, the LOD improvement observed in each of the two data sets with the addition of the rinse and amplification steps were similar, almost 4-fold.

Finally, a focused investigation into the incorporation of dry signal amplification chemistry was performed. The chemical amplification reagents were dried in pads incorporated into the 2DPN card for rehydration at the time of use, such that the user need only handle wet buffers and sample. Specifically for the case of the gold enhancement reagent used here, the use of dry storage on card eliminates the need for the user to mix equal volumes of four different solutions immediately before running the assay, thus greatly improving ease of use. Using the dry signal amplification chemistry, we were able to achieve signal for the 2DPN cards that was comparable to that achieved with wet reagents. Specifically, for 5 mIU/mL, the average signals ($N=3$), agreed to within 5%.

The commercially-available gold enhancement system used here was chosen for ease of use, and has shown promise in other microfluidic formats [26, 27]. However, it is important to note that the 2DPN card format demonstrated here can also be used to implement other signal amplification methods, including silver enhancement of gold nanoparticles [28, 29] and the enzymatic system of horseradish peroxidase/tetramethylbenzidine [30], that have been reported to be useful in lateral flow and other formats, and have great potential for improvement in LOD. Further, for added utility in POC applications, the paper network devices could be straightforwardly modified to accept whole blood samples by the addition of a commercially-available Vivid™ filter (Pall, Port Washington, NY) to remove red cells, white cells, and platelets.

4.1 Conclusion

We have demonstrated a substantially-improved limit of detection using 2DPNs that implement the multi-sequence processes required for rinse and signal amplification steps; the 2DPN format is easy to use in limited-resource settings. The methods demonstrated here could be used to implement a number of other multi-step sample processes in 2DPNs for increased paper-based assay capabilities and performance.

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References

1. Wang W, et al. Tree-shaped paper strip for semiquantitative colorimetric detection of protein with self-calibration. *Journal of Chromatography A*. 2010; 1217(24):3896–3899. [PubMed: 20444459]
2. Martinez AW, et al. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angewandte Chemie-International Edition*. 2007; 46(8):1318–1320.
3. Martinez AW, Phillips ST, Whitesides GM. Three-dimensional microfluidic devices fabricated in layered paper and tape. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(50):19606–19611. [PubMed: 19064929]
4. Zhao WA, et al. Paper-Based Bioassays Using Gold Nanoparticle Colorimetric Probes. *Analytical Chemistry*. 2008; 80(22):8431–8437. [PubMed: 18847216]
5. Dungchai W, Chailapakul O, Henry CS. Use of multiple colorimetric indicators for paper-based microfluidic devices. *Analytica Chimica Acta*. 2010; 674(2):227–233. [PubMed: 20678634]
6. Li X, Tian JF, Shen W. Quantitative biomarker assay with microfluidic paper-based analytical devices. *Analytical and Bioanalytical Chemistry*. 2010; 396(1):495–501. [PubMed: 19838826]
7. Fenton E, et al. Multiplex lateral-flow test strips fabricated by two-dimensional shaping. *Applied Materials and Interfaces*. 2009; 1(1):124–129.
8. van Dommelen L, et al. Alarming poor performance in Chlamydia trachomatis point-of-care testing. *Sexually Transmitted Infections*. 2010; 86(5):355–359. [PubMed: 20876754]

9. Skidmore S. Poorly performing point-of-care tests for chlamydia: what can be done? Sexually Transmitted Infections. 2010; 86(5):330. [PubMed: 20876749]
10. Hurt AC, et al. Performance of six influenza rapid tests in detecting human influenza in clinical specimens. Journal of Clinical Virology. 2007; 39(2):132–135. [PubMed: 17452000]
11. Vasoo S, Stevens J, Singh K. Rapid Antigen Tests for Diagnosis of Pandemic (Swine) Influenza A/H1N1. Clinical Infectious Diseases. 2009; 49(7):1090–1093. [PubMed: 19725784]
12. Uyeki TM, et al. Low Sensitivity of Rapid Diagnostic Test for Influenza. Clinical Infectious Diseases. 2009; 48(9):E89–E92. [PubMed: 19323628]
13. Drexler JF, et al. Poor Clinical Sensitivity of Rapid Antigen Test for Influenza A Pandemic (H1N1) 2009 Virus. Emerging Infectious Diseases. 2009; 15(10):1662–1664. [PubMed: 19861069]
14. Chun, P. Colloidal gold and other labels for lateral flow immunoassays. In: Wong, R.; Tse, H., editors. Lateral Flow Immunoassay. Humana Press; New York: 2009. p. 75-94.
15. Faulstich, K., et al. Handheld and portable reader devices for lateral flow immunoassays. In: Wong, R.; Tse, H., editors. Lateral Flow Immunoassay. Humana Press; New York: 2009. p. 75-94.
16. Horton JK, Swinburne S, O'Sullivan MJ. A novel, rapid, single-step immunochromatographic procedure for the detection of mouse immunoglobulin. J Immunol Methods. 1991; 140(1):131–4. [PubMed: 2061609]
17. Zhang C, Zhang Y, Wang S. Development of multianalyte flow-through and lateral-flow assays using gold particles and horseradish peroxidase as tracers for the rapid determination of carbaryl and endosulfan in agricultural products. Journal of Agricultural and Food Chemistry. 2006; 54(7): 2502–2507. [PubMed: 16569035]
18. Cho JH, et al. Plastic ELISA-on-a-chip based on sequential cross-flow chromatography. Analytical Chemistry. 2006; 78(3):793–800. [PubMed: 16448053]
19. Cho I-H, et al. Immunogold-silver staining-on-a-chip biosensor based on cross-flow chromatography. Journal of Chromatography B. 2010; 878(2):271–277. Sp. Iss. SI.
20. Fu E, et al. Controlled reagent transport in disposable 2D paper networks. Lab on a Chip. 2010; 10(7):918–920. [PubMed: 20300678]
21. Fu E, et al. Chemical signal amplification in two-dimensional paper networks. Sensors and Actuators B-Chemical. 2010; 149(1):325–328.
22. Fu E, et al. Transport in two-dimensional paper networks. Microfluidics and Nanofluidics. 2011; 10(1):29–35.
23. Osborn J, et al. Microfluidics without pumps: reinventing the T-sensor and H-filter in paper networks. Lab on a Chip. 2010; 20(10):2659–2665. 10.1039/c004821f [PubMed: 20680208]
24. Brown KR, Natan MJ. Hydroxylamine seeding of colloidal Au nanoparticles in solution and on surfaces. Langmuir. 1998; 14(4):726–728.
25. Hainfeld, JF., et al. Proceedings of the fifty-seventh Annual Meeting, Microscopy Society of America. Springer-Verlag; 1999. Gold-based autometallography.
26. Lei KF, Butt YKC. Colorimetric immunoassay chip based on gold nanoparticles and gold enhancement. Microfluidics and Nanofluidics. 2010; 8(1):131–137.
27. Lei KF, Wong KS. Automated Colorimetric Immunoassay Microsystem for Clinical Diagnostics. Instrumentation Science & Technology. 2010; 38(4):295–304.
28. Yan J, et al. A Gold Nanoparticle-Based Microfluidic Protein Chip for Tumor Markers. Journal of Nanoscience and Nanotechnology. 2009; 9(2):1194–1197. [PubMed: 19441486]
29. Yeh CH, et al. An immunoassay using antibody-gold nanoparticle conjugate, silver enhancement and flatbed scanner. Microfluidics and Nanofluidics. 2009; 6(1):85–91.
30. Kolosova AY, et al. Investigation of several parameters influencing signal generation in flow-through membrane-based enzyme immunoassay. Analytical and Bioanalytical Chemistry. 2007; 387(3):1095–1104. [PubMed: 17146620]

Figure 1.

(A) Schematic of the molecular system used in the conventional lateral flow sandwich format assay and with chemical signal amplification for improved limit of detection (LOD). In the signal amplification method used here, gold enhancement of gold nanoparticles, catalytic deposition of gold onto the surface of the original particle labels increases their size and results in a change in color of the labels from pink to dark purple. (B) Example images of the results in the unamplified and amplified cases for a concentration series of hCG spiked into fetal bovine serum. The LOD in the amplified case is improved to well below 1 mIU/mL.

Figure 2. Effects of additional processing steps on the limit of detection (LOD) of a conventional lateral flow test in a manual format. (A) Bar plot shows the two quantities that determine the limit of detection, the 99th percentile of the zero-concentration signal distribution and the slope of the signal-versus-concentration curve near zero, for each of the five sets of data acquired in the manual format. For each data set, $N \geq 12$ for the zero concentration and $N = 4$ for each of the non-zero concentrations. The error bars for the slope are the standard error based on the linear regression. The error bars for the 99th percentile of the zero-concentration signal distribution and the LOD were calculated using a Monte Carlo procedure as described within the Materials and Methods section. Note the different effects of the PBS and PB rinse on each quantity for the rinse only cases. B) Bar plot shows the LOD for the same five sets of data shown in (A). Additional processing steps improve the limit of detection. The combination of rinse and amplification steps results in an almost 4-fold improvement in LOD. The Herrinstein's ρ statistic, a measure of the overlap in the distributions for two given LODs was less than 0.05 for the following three pair wise comparisons; PBS+amp and LFT, PB+amp and LFT, PB+amp and PB.

Figure 3.

2DPN card allows for the automation of additional processing steps. (A) Image of a 2DPN card that performs rinse and signal amplification steps. (B) Schematic of the flow sequence in the 2DPN card. The user first loads GE solution, rinse buffer, and sample onto appropriate pads of the card. Sample flows in the primary leg of the card to the detection region (see i). The user folds the card at 20 minutes, such that fluids can flow through the secondary leg to the detection region of the card; buffer (see ii) and then GE solution (see iii). Additional steps could be implemented in a straightforward manner through extension of the network. (C) Image series showing the sequential delivery of fluids in the 2DPN card. (top) Yellow fluid represents the sample. (middle) Red fluid represents the rinse buffer. (bottom) Blue fluid represents the gold enhancement solution.

Figure 4.

2DPN card results in improved limit of detection. (A) Images of the detection region from an hCG concentration series. (B) Plot of signal-versus-concentration for the 2DPN card with rinse and amplification (at 35 minutes, solid circles) and without any rinse or amplification steps (at 20 min., hollow squares). (C) The additional steps of rinse and amplification resulted in an almost 4-fold improvement in the LOD. Note that this 2DPN format can be used with other signal amplification reagents (for enzyme-based methods and silver enhancement) that have shown promise in alternate formats. The Herrinstein's ρ statistic, a measure of the overlap in the distributions for two given LODs, was 0.06 for the comparison of 2DPN card and strip without rinse and amplification.

Table 1

Table of chemical steps in a conventional lateral flow test and additional steps performed in the 2DPN folding card demonstrated here.

Conventional lateral flow test	2DPN folding card
sample-conjugate delivery	sample-conjugate delivery
rinse fluid delivery (in some tests)	rinse fluid delivery
NA	signal amplification chemistry delivery