Supplementary information

Section A

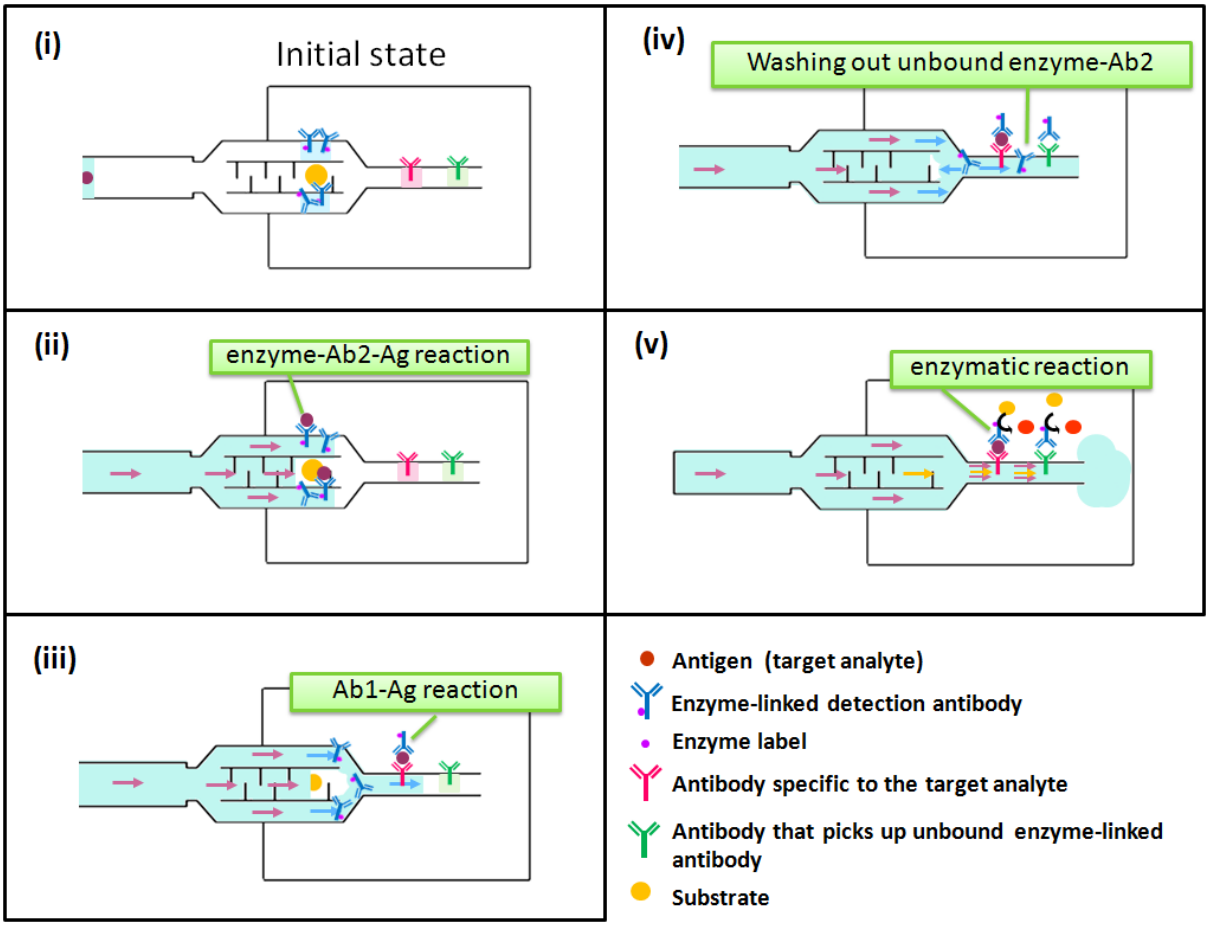
The automated paper-based device made using patterns 1 and 2 for sandwich ELISA

Figure 1: Schematic illustration of the automated paper-based device with 2 different designs for the sandwich ELISA. Pattern 1 was our first concept for controlling the reagent flow time on 1 piece of the device. Pattern 2 was created to improve the sensitivity of the device over pattern 1. The automated paper-based device consisted of 3 parts. The first was an input “leg” at which the solution was applied to the device. The second was the reaction area; this was where the delaying channel patterns were created and where all the substances were located. The substrate and enzyme-linked detection Abs (enzyme-Ab2) were prepared in separate channels to prevent them from mixing together, which was accomplished by beginning the detection reaction after the Ab-Ag complex had been washed to remove the nonspecific bound Ag and free enzyme-Ab2 (background). The third was the absorbent area created above the end of the channel (square area part in dotted line) to improve the wicking properties of the device. Four substances were applied on the device at different locations as follows: 1) the immobilized Ab that binds to free (Ag-unbound) enzyme-Ab2; 2) the immobilized Abs (Ab1) specific to the target Ag; 3) enzyme-Ab2; and 4) substrate.

Section B

The sandwich ELISA procedure on our novel automated paper-based device

A



B

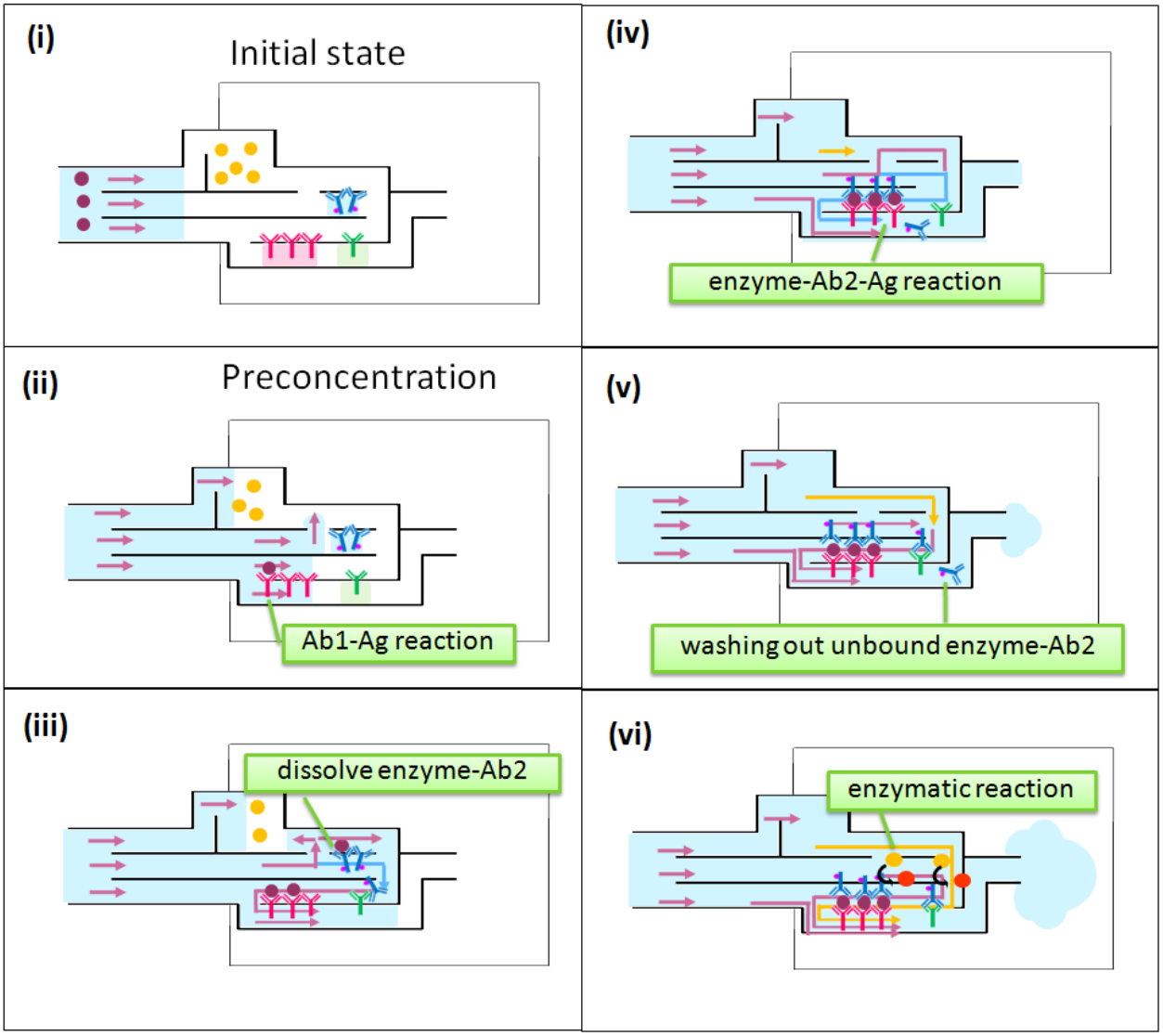


Figure S1: Schematic representation (based on the flow of real fluid in the pattern) of the procedure for the sandwich ELISA in planar views of the automated paper-based device for pattern 1 (A) and pattern 2 (B). The purple arrow represents the sample flow direction; the blue arrow represents the flow direction of the sample containing enzyme-linked detection antibody; and the yellow arrow represents the flow direction of the sample containing substrate.

The sandwich ELISA procedure is schematically shown in Fig. S2 and is briefly summarized as follows. First, for the automated paper-based device with pattern 1 (A), after the sample solution was applied to the device, (i) the sandwich ELISA automatically ran through the sequential reaction; (ii) the analyte solution migrated to the prespotted region of enzyme-Ab2 in the nondelaying channel, and the appropriate Ag(s) bound to the solubilized enzyme-Ab2 to form enzyme-Ab2-Ag complexes; (iii) the complexes were captured further along with immobilized Ab1 at the test zone; (iv) the free enzyme-Ab2 (not Ag-bound) was captured by the immobilized Ab at the control zone and washed away by sample solution; however, since some of the enzyme-Ab2-containing fluid may migrate to the delaying channel, contacting substrate containing sample fluid, color may be generated upstream of the test zone; and (v) the slower substrates migrating through the delaying channel were gradually dissolved and pass through the test zone and the control zone, allowing the enzyme reaction to occur and the color to develop (change) at the test and control zones.

Second, for the automated paper-based device with pattern 2 (B), after the sample solution was applied on the device, (i) the solution migrated along the device, entering all channels. The assays were run sequentially as follows: (ii) the analyte solution migrated upward to the testing channel where the Ag was captured with the pre-immobilized specific Ab1 at the test zone to form Ab1-Ag complexes (the Ag was preconcentrated in this step at the test zone); (iii) the analyte solution migrated to the prespot region with an excess of the enzyme-Ab2. The solubilized enzyme-Ab2 was rehydrated. The unbound enzyme-Ab2 and enzyme-Ab2 bound with Ag(s) then migrated upward to the test zone, and the affected analyte solution (in channel 1 above the gap) migrated back to the testing channel, increasing the amount of antigen captured at the test zone. Additionally, the sample fluid that had passed through the gap between channels 2

and 3 reached the enzyme-Ab2-containing fluid, first at the top of channel 2, followed by the substrate; this minimized the background from the enzyme-Ab2 that interacted with the substrate before the test zone. (iv) Unbound enzyme-Ab2 captured Ab1-Ag at the test zone. (v) Excess unbound enzyme-Ab2 was then captured by immobilized Ab at the control zone. Free enzyme-Ab2 and low avidity Ags nonspecifically bound to the enzyme-Ab2 were removed and washed away from the test zone. (v) Finally, migrating substrate passed through the test zone and control zone, allowing the enzyme reaction to occur and the color to develop (change) at the test and control zones.

Section C

Effects of printing parameters

Figure 2S shows an image of the printed lines on the NC membrane obtained by applying different printing parameters. The inkjet printing parameters and the gap between the inkjet tip and surface of the NC membrane affected the width of the printed line pattern. These parameters included the following: 1) voltage supply, which must be 1000 V; 2) bias frequency [Fig. 2S (I)], where the printed line width decreased with increasing bias value; 3) frequency [Fig. 2S (II)], which behaved similarly to the bias frequency, where the printed line width decreased with increasing frequency value; and 4) the gap between the inkjet tip and surface of the NC membrane [Fig. 2S (III)], where the barrier width tended to decrease as the gap between the inkjet tip and surface of the NC membrane increased. To obtain a thin barrier width with no leakage, the parameters were adjusted as follows: voltage supply = 1000 V, bias = 200 V, frequency = 300 Hz, and gap between the inkjet tip and surface paper = 250 μm.

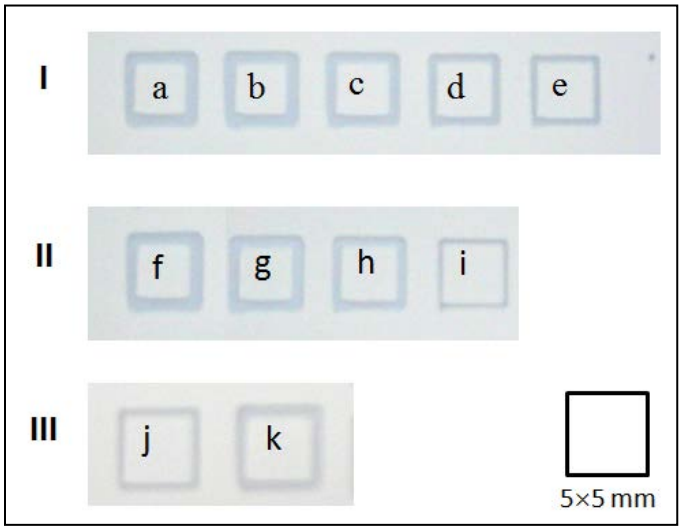


Figure S2: Image of the printed line on the NC membrane. (I) Fixed frequency of 0 V, fixed distance between the nozzle and paper of 250 μm, and varying bias frequencies: a, 0; b, 50; c, 100; d, 500; and e, 1000 Hz. (II) Fixed bias voltage of 0 V, fixed distance between nozzle and paper of 250 μm, and varying frequencies: f, 0; g, 50; h, 100; and i; 500 Hz. (III) Fixed frequency of 300 V and fixed bias of 200 V, with varying distances between the nozzle and surface of the NC membrane: j, 250; and k, 500 μm.

Section D

Analytical Application

Results of the determination of hCG spiked in urine samples are shown in Fig. S3.

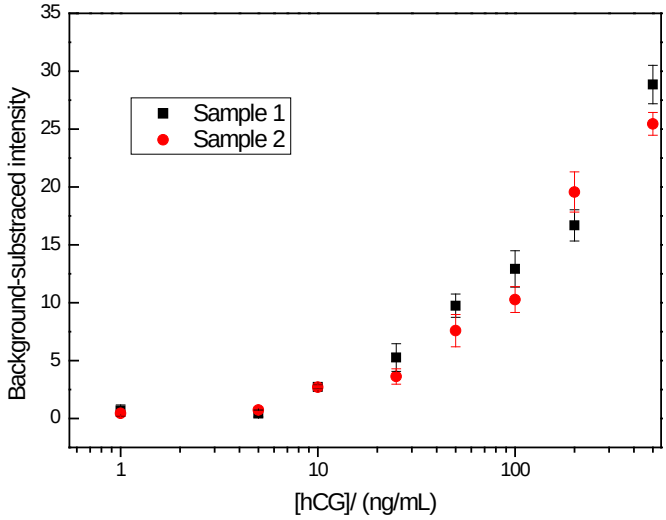


Figure S3: Calibration curve of hCG in urine samples (sample 1 was a urine sample from a man, and sample 2 was a urine sample from a woman). hCG ranged from 1 to 500 ng/mL in substrate buffer solution. The data are derived from 3 replicates.