Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Paper-based Multi-well Depletion ELISA

Dohwan Lee[1], Norh Asmare[1], and A. Fatih Sarioglu[1, 2, 3*]

[1]*School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, USA*

[2]*Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, USA*

[3]*Institute for Electronics and Nanotechnology, Georgia Institute of Technology, Atlanta, USA*

*Corresponding author: sarioglu@gatech.edu

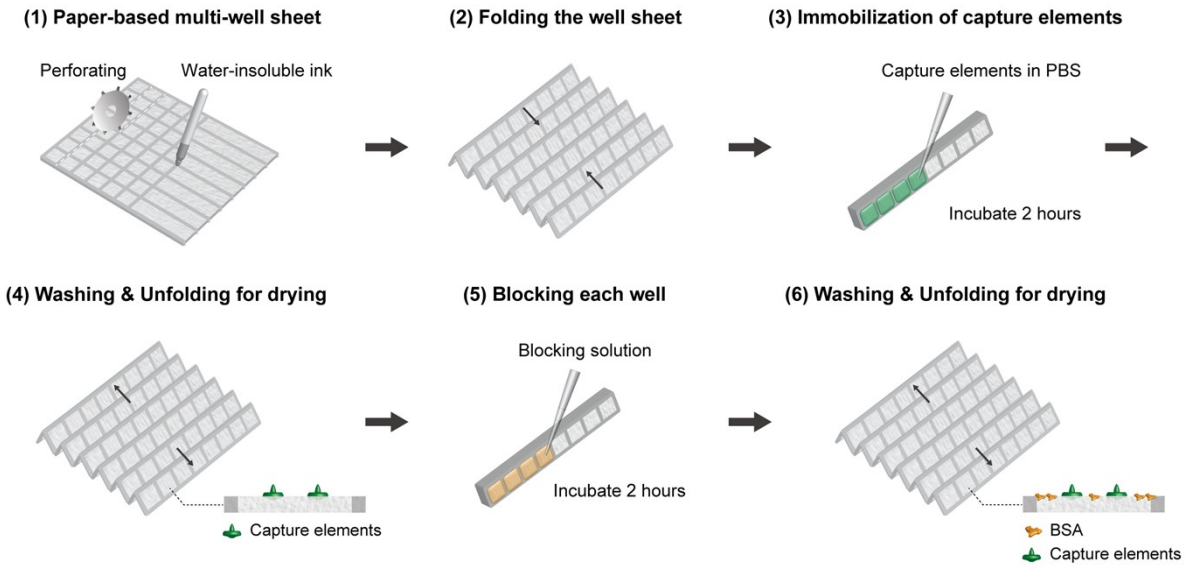

Fig. S1. A schematic showing the developed process to fabricate the paper-based multi-well sheet: (1) Cellulose paper was patterned by drawing lines with a water-insoluble ink. The patterned sheet was perforated along vertical lines for easy alignment when folding. (2) The well sheet was folded to stack wells. (3) Stacked wells were functionalized by loading a controlled amount of capture elements followed by 2 hours of incubation at room temperature. (4) Each well stack was washed 3 times and dried. (5) The well sheet was folded again and a blocking buffer was applied to each well stack followed by 2 hours of incubation. (6) Lastly, each well stack was washed 3 times before being unfolded to dry.

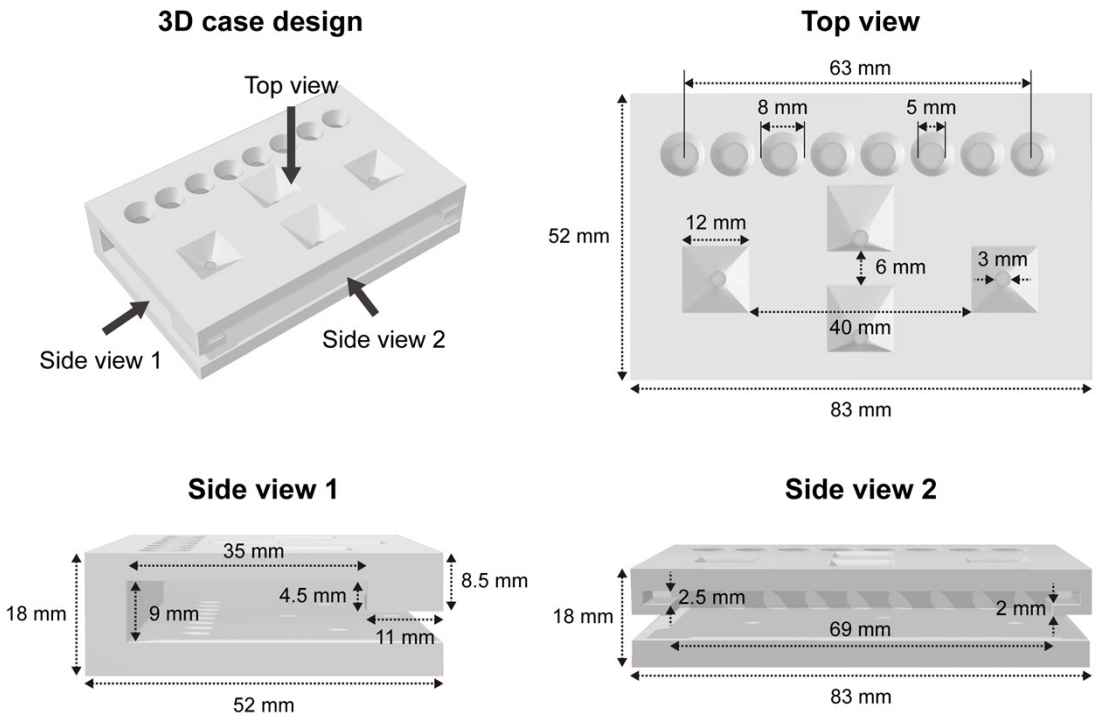

Fig. S2. A schematic showing the detailed design of the 3D printed case from different views. The dimensions of various features are marked on the drawings.

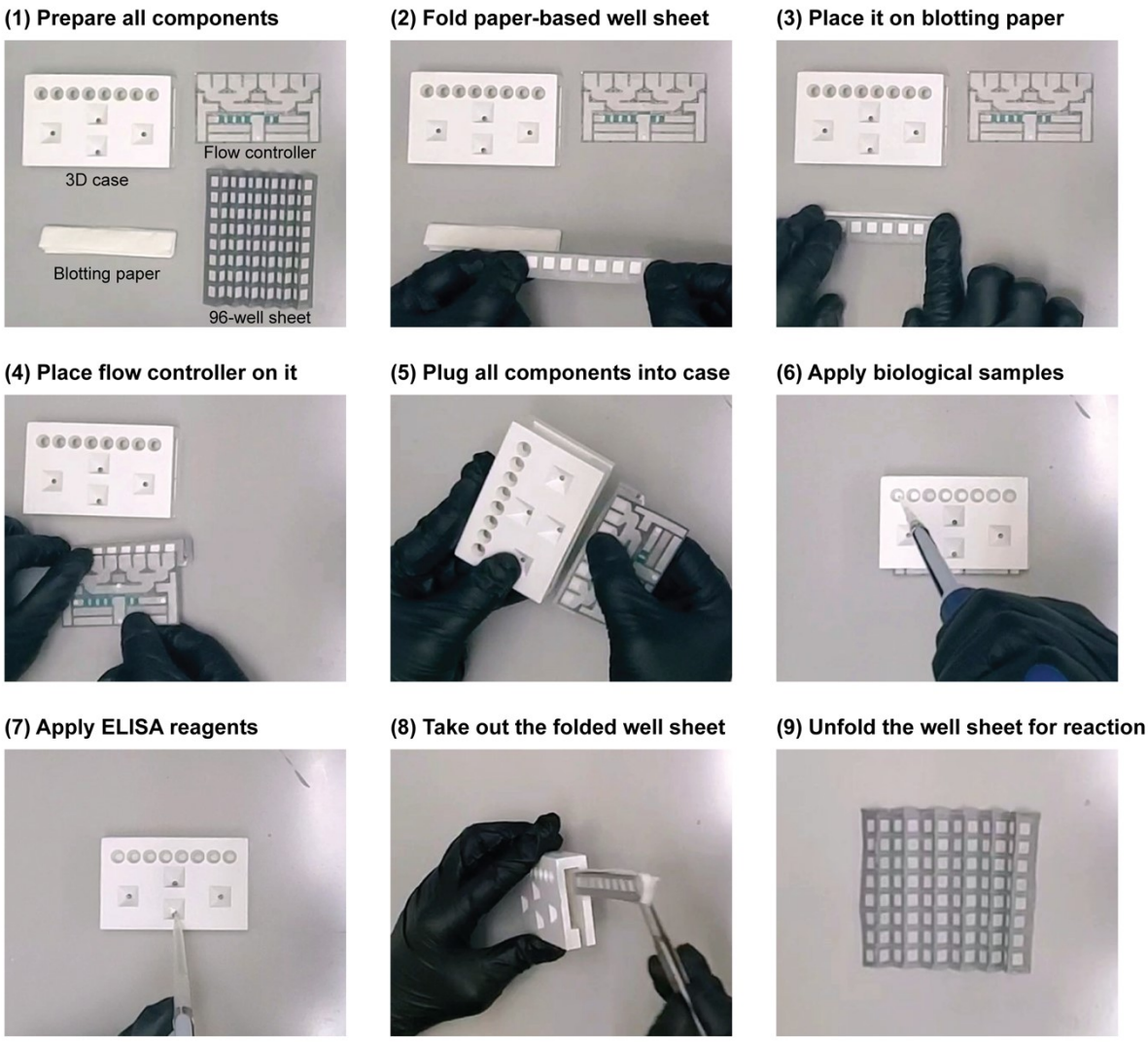

Fig. S3. A series of photos showing the device assembly process and the device operation. (1) Gathering of prepared components: a 3D case, a flow controller, a blotting paper, and a paper-based 96-well sheet. (2) Folding the 96-well sheet. (3) Aligning and placing the folded 96-well sheet on the blotting paper. (4) Placement of the flow controller on top of the folded well sheet after alignment. (5) Insertion of the grouped components into the 3D case. (6) Deposition of the samples on designated inlets. (7) Deposition of the ELISA reagents to the designated inlets. (8) Extraction of the folded well sheet from the device case after the completion of the assay after ~ 35 mins. (9) Unfolding of the 96-well well sheet for reading the assay results.

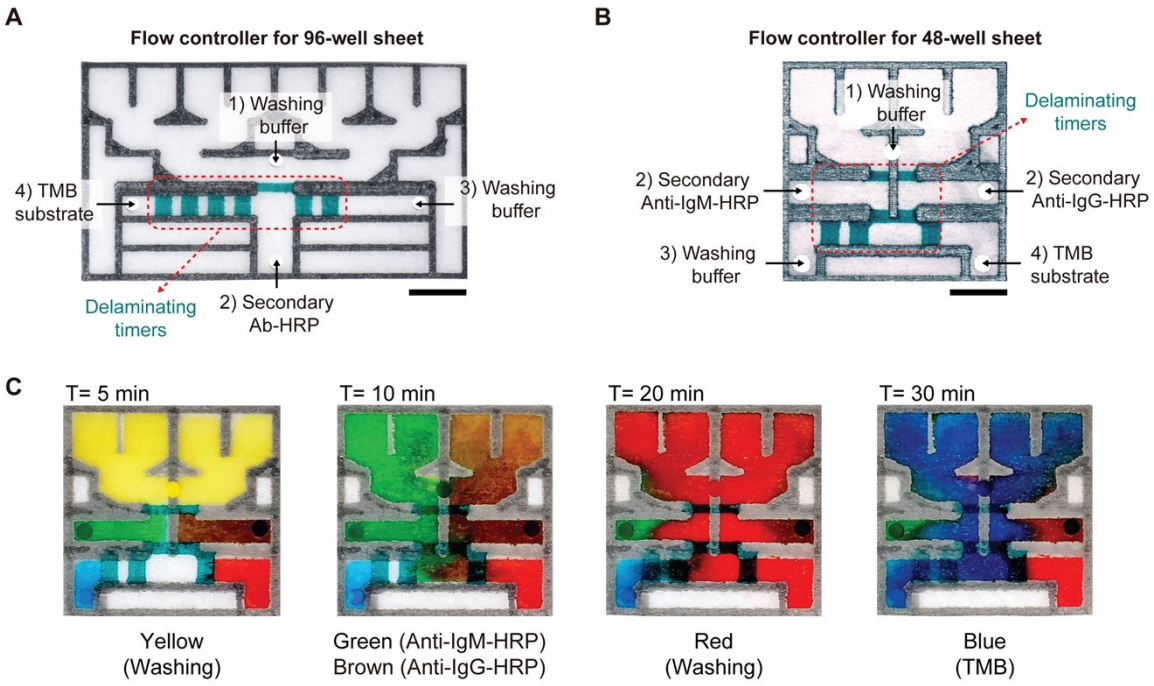

Fig. S4. Design of the capillary flow controllers employed in this work. Photos of the paper-based flow controllers built to deliver multiple reagents to the reaction to perform the ELISA assay for (A) a 96-well sheet and (B) a 48-well sheet. The scale bar is 1 cm. Both photos show the imprinted features for capillary flow manipulation (C) Time-lapse images showing the sequential delivery of different reagents by the flow controller designed to perform ELISA for the 48-well sheet. The silver lines guided the capillary flow and the green lines were used as timers to stall the flow for the desired duration. The volume of the washing buffer and TMB substrate was all 300 µl and each volume of secondary anti-IgM-HRP and anti-IgG-HRP was 150 µl.

Calculated dilution factors in each row								Average	Standard deviation
Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8		
~ 3.19 fold	~ 3.13 fold	~ 3.17 fold	~ 3.30 fold	~ 2.81 fold	~ 3.14 fold	~ 2.72 fold	NC	~ 3.07 fold	0.21 fold

Table S1. Calculated well-to-well dilution factors observed for the depletion ELISA assay.