Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary information

Vertical flow immunoassay (VFA) biosensor for a rapid one –step immunoassay

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Experimental Section

Reagents and materials. CRP-free serum (90R-100), surfactant 10G (95R-103), and bovine serum albumin (BSA) (30-AB74) were purchased from Fitzgerald (USA). CRP (309-51191) was ordered from Wako (Japan). CRP polyclonal antibody and monoclonal antibody were ordered from Abcam Inc. (UK). The nitrocellulose membrane was purchased from Millipore (HFB02404, USA). Sample pads (P/N BSP-133-20, USA) and asymmetric membranes (Vivid Plasma Separation-GX, USA) were ordered from Pall Co. Conjugate pads (fusion5 8151-6621, USA) and sealing tape (UnisealTM 7704-0009, USA) were obtained from Whatman. Gold colloid solutions were ordered from BBInternational (EM.GC10, 15, 20, 40, UK). Micropunches were purchased from Ted Pella Inc. (Harris Uni-coreTM hole; .5–20 mm; USA). Double-sided polyvinylchloride tape was purchased from ACT (Korea). Polyvinylpyrrolidone (PVP10), sucrose (S7903), sodium azide (S8032), and other chemicals were obtained from Sigma-Aldrich (USA). All the buffers and reagent solutions were prepared with water that had been purified using a Milli-Q water-purification system. To prepare the LFA sensor, Inter pads were ordered from Whatman (MF1 8122–1250, UK), hosing case strips were obtained from Infopia Co. Ltd, and laminated cards were purchased from Millipore (HF000MC100, USA).

Instruments. The NC membrane was processed using a laser device (LD2030; Femtoscience, Korea). A drying oven was obtained Korea Auto.Control & Scientific Instrument Co. (KO-100, Korea). Centrifuge was purchased from HANIL (micro 17TR, Korea). A cutter device for cutting each pad was obtained from Taewoocutex (TBC-50TS, Korea). A Scanner to obtain image was ordered Epson (V700, Japan). A dispenser was purchased by Zeta Corporation (DCI100, Korea).

Preparation of CRP antibody-conjugated AuNPs and the conjugate pad. To prepare the antibody-AuNPs, anti-CRP antibody in PBS (10 µL of 1 mg mL^{-1}) was added to several mixtures of 1 mL AuNP colloid (10, 15, 20, or 40 nm in diameter) and 0.1 mL of borate buffer (0.1 M, pH 8.5). After incubation for 30 min at room temperature, 0.1 mL of 10 mg mL^{-1} BSA in PBS was added to the solution to block the AuNP surface. After incubation for 15 min at room temperature, the mixture was centrifuged at 14,000 rpm and 4 °C for 30 min. The supernatant was discarded, and 10 mM Tris-HCl (pH 7.4) was added to the antibody-AuNPs to be resuspended. The centrifugation and suspension processes were repeated twice, and the final suspension solution was retained in a storage buffer (pH 7.4) containing 0.1 % BSA, 0.05 % NaN3, 1 % sucrose and 10 mM Tris-HCl. The final concentrations were determined based on absorbance at 520 nm. All the synthesis processes were confirmed by dynamic laser scattering (DLS) and the absorbance scan. Mixed solutions containing 1 % PVP, 0.5 % surfactant 10G, and 10 µL of conjugate mixture were pipetted onto each conjugate pad. The pads were dried at 60 °C for 10 min and then stored in a desiccator (humidity below 20 %).

Preparation of the VFA sensor. The VFA sensor consists of several components. sealing tape, sample pad, double-sided tape, conjugate pad, asymmetric membrane, mesh, adhesive film with holes, and the NC membrane. All the components were vertically arranged in sequence. To inject the sample solution, the sealing tape (1.2 cm × 1.2 cm) used to fix the sample pad was perforated in the middle to create a 10-mm-diameter hole. The sample pad (7.5 mm × 3.5 mm) was prepared from cellulose fiber. The conjugate pad (7 mm × 3.5 mm)

was prepared by dispensing the desired volume of antibody-AuNP solution onto a glass fiber pad and then drying it at 60°C for 10 min. The pad was stored in a desiccator at RT. An "I"-shaped nitrocellulose membrane (1.6 cm × 1.6 cm) was processed by a laser device and was used to immobilize the capture and control antibodies at different locations to form control and test zones for independent reactions.
Hundreds of micro-sized holes (500, 750, 1000, and 1200 μm) were drilled into an adhesive film (0.9 cm × 0.9 cm) was made in 2 holes at an interval of 0.5 cm between holes. After attaching the film above the NC membrane, 0.5 μL of 0.2 mg mL^{-1} anti CRP and anti-mouse antibody were injected through each hole before drying in a convection oven for 10 min at 60 °C.

Preparation of the LFA sensor. The LFA sensor consists of 5 components: sample pad, conjugate pad, inter pad, nitrocellulose membrane, and absorbent pad. For most components, the materials were the same as those used in the VFA sensor, and these were affixed to a general backing card (typically an inert plastic, e.g., polyester). The conjugate pad (7 mm × 3.5 mm) was prepared using the same method as for the VFA sensor pad. The NC membrane (25 mm × 30 cm) was used to immobilize the capture and control antibodies at different zones by using a dispenser capable of delivering 0.8 μL cm^{-1}. The distance between the test zone and control zone was approximately 3 mm. Each zone was blocked by 1 μL cm-1 of 1 % BSA solution by using a dispenser, to prevent adsorption of other materials. The loaded membrane was then dried at 37 °C for 1 hr. After the NC membrane was dried, it was cut into 3.8-mm-wide strips. An absorbance pad (1.5 cm × 3.8 mm) was attached to the top of the strip, followed by an inter pad (1 cm × 3.8 mm), conjugate pad (7 mm × 3.5 mm), and sample pad (1.5 cm × 3.8 mm), consecutively assembled on a plastic adhesive backing (60 mm × 3.8 mm) containing NC membrane-immobilized antibodies and the absorbent pad. Each part overlapped the previous layer by 1.5 mm to facilitate migration of the solution during the assay. Finally, the manufactured strip was inserted in a housing case (1.8 cm × 6.6 cm).

Analysis of hsCRP. The hsCRP solutions of various concentrations were prepared in CRP-free human serum solution. The prepared sample solutions were incubated on the VFA and LFA sensor. The images were obtained using an Epson scanner (V700; Suwa, Nagano, Japan) and then, the color intensity was measured using Multi gauge version 3.0 software (Fujifilm, Tokyo, Japan).

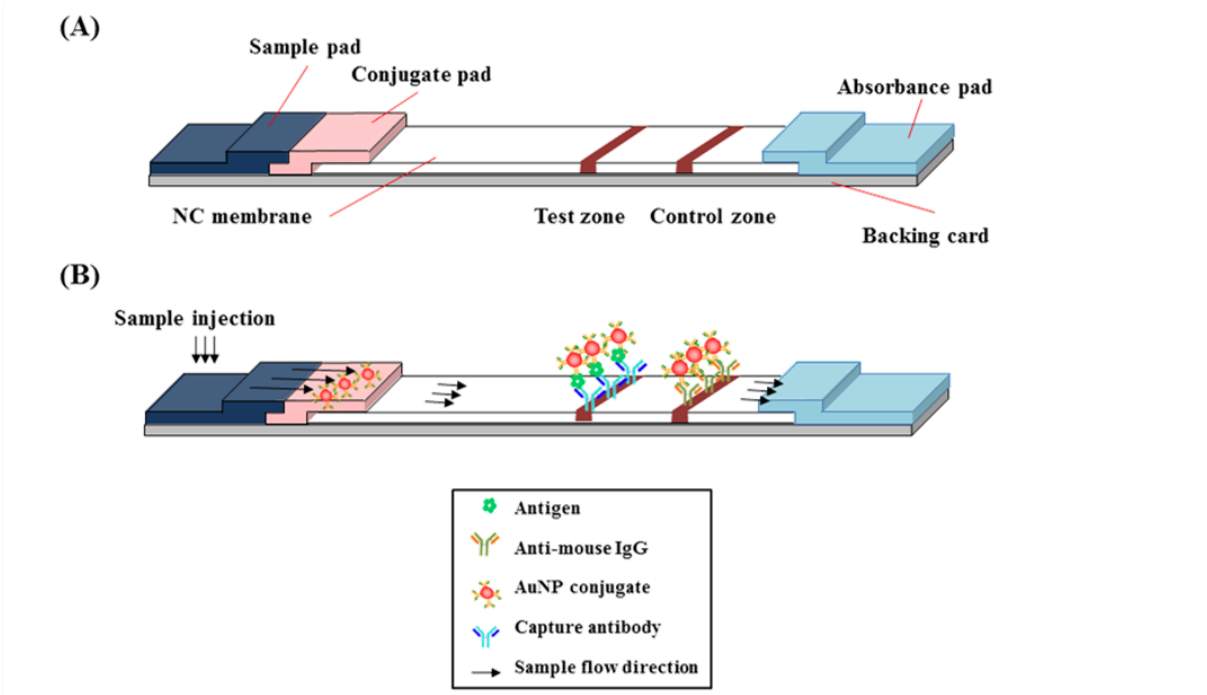


Figure S1. Schematic illustration of lateral flow assay methods. (A) is structureof LFA sensor and (B) is sample flow direction and immunoassay reaction in each zones

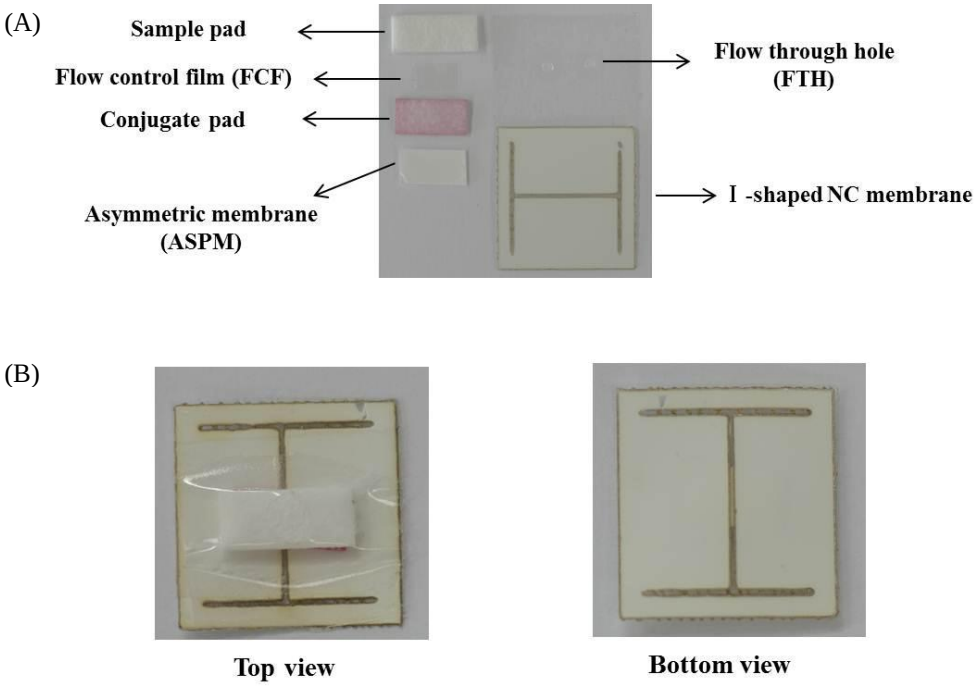
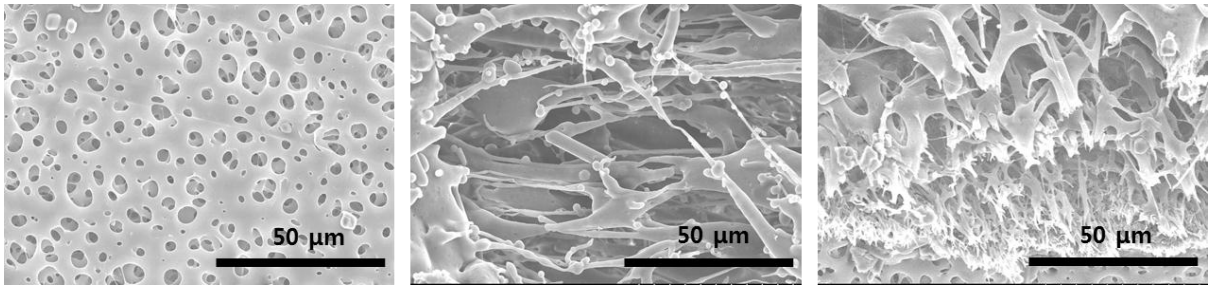


Figure S2. Photographsof each component of VFA sensor (A) and top, bottom view(B) of the sensor.



(A) (B) (C)

Figure S3. Scanning electron microscope (SEM) imagesof small (A), large (B) pore side and cross section (C) of asymmetric membrane.

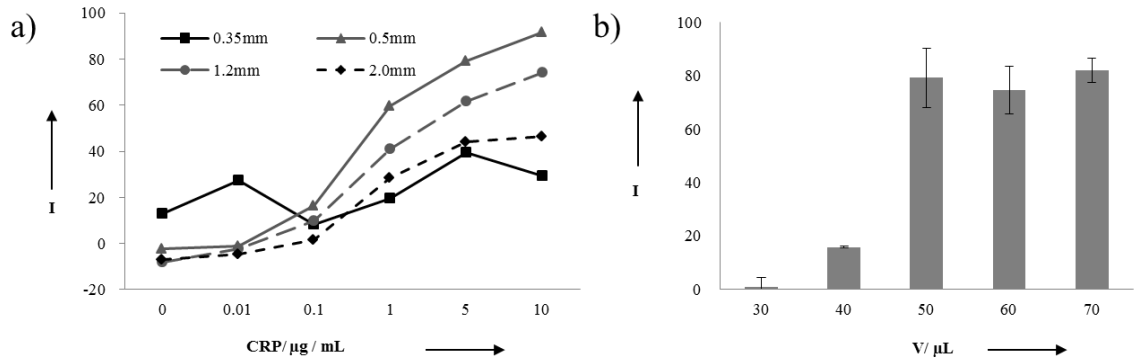


Figure S4. (A) Effect of VFA hole size on various concentrations of CRP. (B) Effect of sample volume on the CRP assay (1 µg mL^{-1})

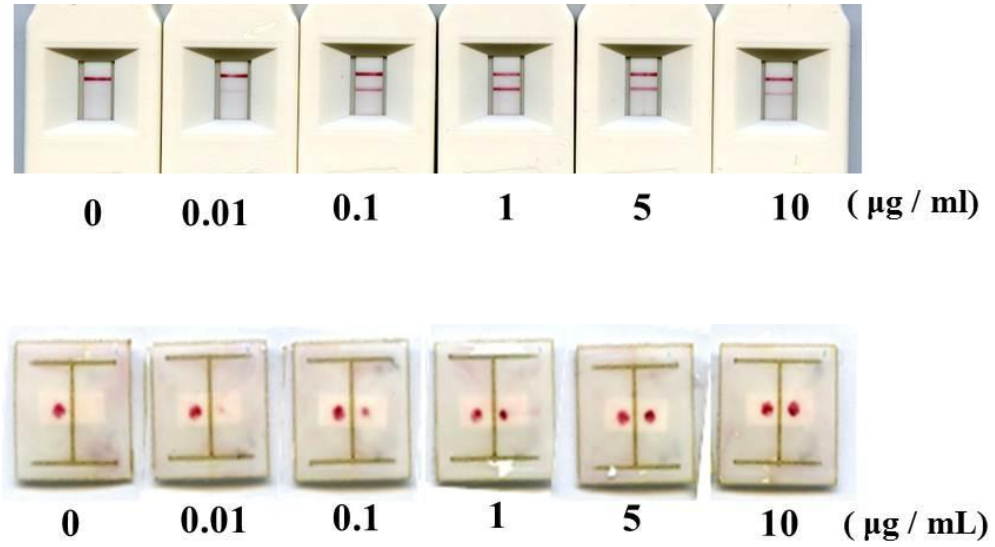


Figure S5. Photographic images of the VFA (control: left spot, test: right spot) sensor at 4 min of 12 reaction a) and LFA (control: upper line, test: below line) sensor b) at 10 min of reaction for various 13 concentrations of CRP in the serum.