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

Supporting Information

Converting Colour to Length Based on Coffee-Ring Effect for Quantitative Immunoassays Using a Ruler as Readout

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Chemicals and materials

Glucose, human IgG, 10 mM phosphate-buffered saline (PBS, pH 7.4), Tween-20, bovine serum albumin (BSA) were purchased from Sigma-Aldrich. GOX-conjugated polyclonal rabbit anti-human IgG was purchased from Chang Liang medical Reagent Co.,Ltd (Shenzhen, China). Starch-iodide paper (1 cm × 6 cm), laminating film, glass slides, a sealed chamber and silica gel desiccant were obtained from Sinopharm Chemical Reagent Co.,Ltd (Shanghai, China). Double-sided adhesive tape was purchased from Soken Chemical& Engineering Co., Ltd (Tokyo, Japan). All solutions were prepared with deionised water (18.0 MΩ cm, Milli-Q Gradient System, Millipore). All reagents were used as received without further purification.

Experimental procedures

The starch-iodide paper was first attached to the double-sided adhesive tape and then patterned using a hole puncher into circle reaction reservoirs with a diameter of 6.0 mm. The patterned paper reservoirs were attached to a glass slide which was cleaned using acetone and ethanol. To initiate the human IgG detection, 4.0 µL aliquot of a sample containing human IgG prepared with 10 mM phosphate buffered saline (PBS) was spotted on the paper using a micropipette and allowed to dry for 10 minutes at ambient conditions. Then 4.0 µL aliquot of a blocking buffer (0.1% (v/v) Tween-20 and 1% (w/v) bovine serum albumin (BSA) in PBS) was added to block each test zone and allowed to dry for 10 minutes under

ambient conditions. A 4.0 μL aliquot of a solution containing the GOX-conjugated rabbit anti-human IgG antibody in PBS was added to each zone and allowed to incubate for 5.0 minute. Each test zone was washed for three times by adding 4.0 μL aliquot of wash buffer (0.1% (v/v) Tween-20 in PBS). An absorption pad was used to remove the wash buffer. After the wash step, we placed 4.0 μL aliquot of a solution containing the enzyme substrate (20 mM glucose in PBS) onto our paper-based test wells to react for 5 min. Other than the circle paper reservoir, a paper strip (20×1.4mm) was used for the assay. The paper strip was fabricated by patterning the starch-iodide paper using a paper cutter. We also laminated the paper strip using an office laminator to improve the assay results. A hole in the middle of the lamination pouch was punched for introduction of assay reagents and sample. Both ends of the paper strip were exposed to the air.

The homemade dry chamber is made of polypropylene, which is 25 cm(L), 17 cm(W) and 9.5 cm(H). About 150 g silica gel desiccant was included in the dry chamber. The homemade dry chamber was used as the reaction chamber to maintain a constant humidity for all assays. After about 120 min, the relative humidity of the chamber was kept at about 17%, which was measured using a hygrometer. An office scanner (BenQK802) with a resolution of 1200 DPI was used to obtain optical images of the paper reservoir. The color intensity of the detection reservoir was obtained from histogram

of the selected area of the image imported into the Photoshop software.

Supporting Figures

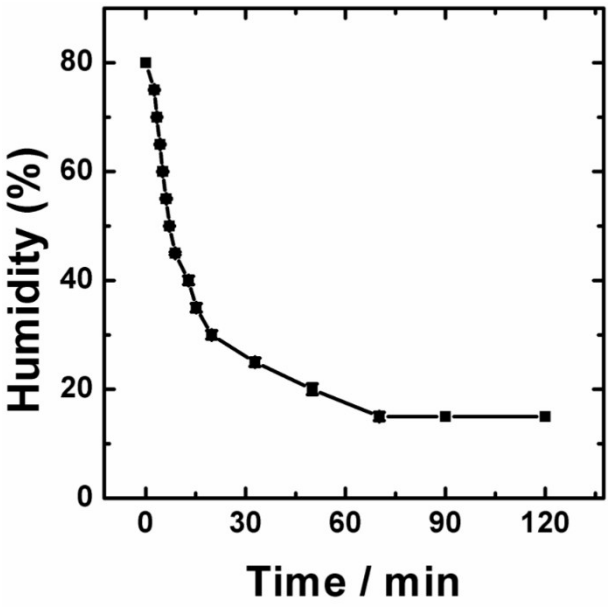

Figure S1. Relative humidity inside the sealed chamber as a function of time.

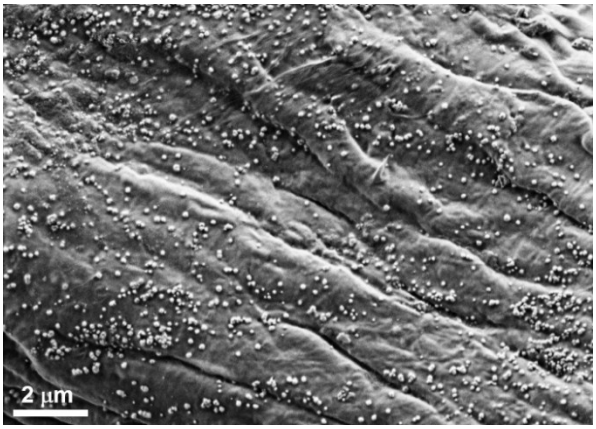

Figure S2. SEM image of the coloured stain on the paper reservoir after completion of the assay.

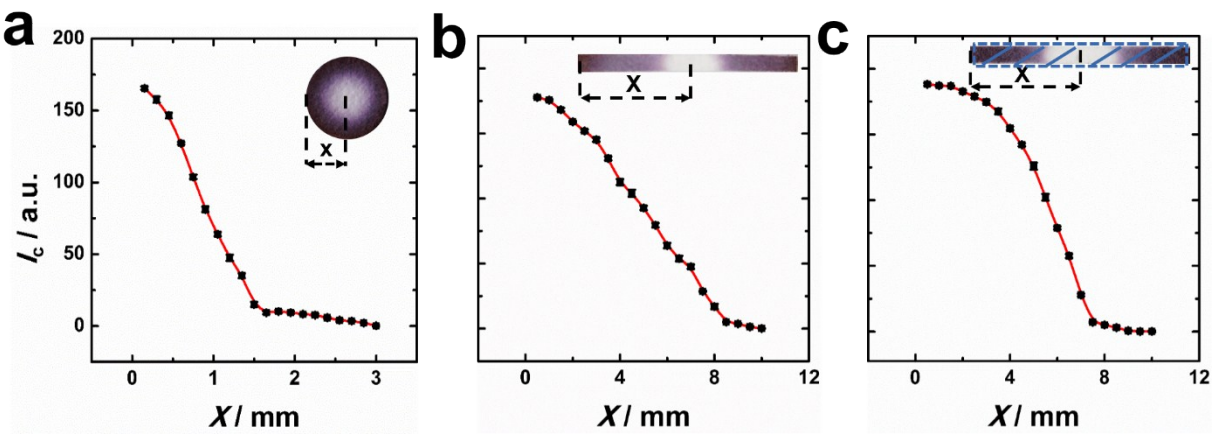

Figure S3. Colour intensity measured as a function of distance from the edge after the completion of test. The human IgG concentration in the sample was 500 ng/mL. (a) circle paper reservoir (b) paper strip without lamination (c) partially laminated paper strip. Blue dashed lines show where the lamination was on the strip.

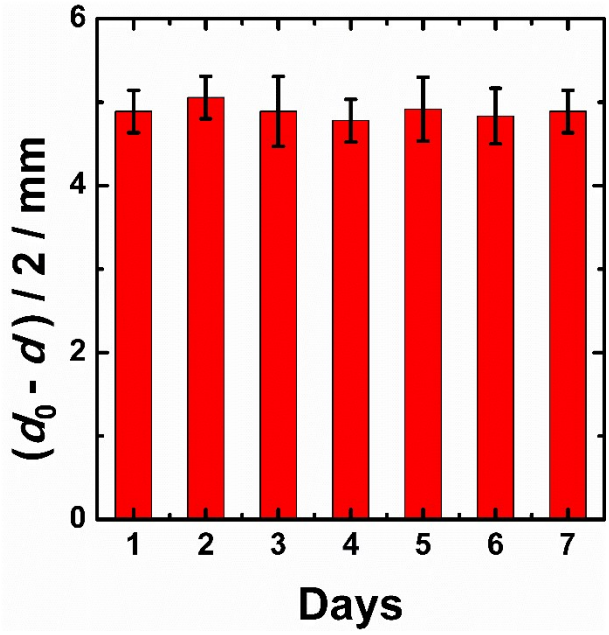

Figure S4. The results of testing of the same sample in a week. The error bars represent standard deviation for 20 replicated tests.