Supporting Information

Paper-Based ELISA

Chao-Min Cheng,[1#] Andres W. Martinez,[1#] Jinlong Gong,[1] Charles R. Mace,[1] Scott T. Phillips,[1] Emanuel Carrilho,[1,2] Katherine A. Mirica,[1] and George M. Whitesides[1,*]

[1]Department of Chemistry & Chemical Biology, Harvard University, Cambridge, MA 02138

[2]Instituto de Química de São Carlos, Universidade de São Paulo 13566-590 São Carlos – SP, Brazil

[*]Corresponding author E-mail: gwhitesides@gmwgroup.harvard.edu

[#]These authors contributed equally to this work.

EXPERIMENTAL DETAILS

Fabrication of a Paper 96-Microzone Plate. We fabricated the 96-microzone paper plate using photolithography. Paper was impregnated with SU-8 photoresist (MicroChem), baked on a hot plate (10 minutes, 110 °C), cooled to room temperature and exposed to UV light (13 seconds, IntelliRay 600, UVitron International, Inc.) through a transparency mask. The paper was then baked a second time (5 minutes, 110 °C), cooled, and the patterns were developed in an acetone bath (1 minute) followed by a rinse in acetone and a rinse in 70% isopropyl alcohol. The paper was blotted between two paper towels, rinsed a second time with 70% isopropyl alcohol, blotted, and allowed to dry under ambient conditions for at least 1 hour before use.

Immobilizing Proteins on Paper. Solutions of 50-ng/μL, 25-ng/μL, 12.5-ng/μL, and 0-ng/μLTRITC-labeled rabbit IgG in PBS (3 μL) were spotted in paper test zones and allowed to dry under ambient conditions. Half of the test zones were washed with PBS (2 × 10 μL) by adding PBS to the test zone, and then bringing the bottom of the test zone in contact with a piece of blotting paper. Once dry, the test zones were scanned using a fluorescent scanner (Typhoon Trio, General Electric Co. set to 526-nm excitation, 532-nm emission). The fluorescence intensity of each test zone was quantified by analyzing the image using ImageQuant.

Blocking the Paper. Test zones were blocked with a blocking buffer (1 μL of 0.05% (w/v) Tween-20 and 1% (w/v) BSA in PBS) and allowed to dry for 10 minutes. Solutions of 50-ng/μL, 25-ng/μL, 12.5-ng/μL, and 0-ng/μLTRITC-labeled rabbit IgG in

PBS (3 μL) were spotted in test zones that were either blocked or not blocked. After incubating for one minute, the test zones were washed with PBS (2 × 10 μL) as described above. Once dry the test zones were scanned and the fluorescence intensity of each zone was measured.

Removing Unbound Proteins from Paper by Washing. Solutions of 50-ng/μL and 0-ng/μLTRITC-labeled rabbit IgG in PBS (3 μL) were spotted in blocked test zones, incubated for one minute and then washed using nine different washing protocols. The washing protocols tested the number of washes (from 1 to 3), and the volume of PBS used for each was (5, 10 and 15 μL). Once dry, the test zones were scanned and the fluorescence intensity of each zone was measured.

Indirect P-ELISA for Rabbit IgG Detection. Rabbit IgG (3 μL) in ten fold dilutions (67 mM to 670 nM, corresponding to 67 pmoles to 670 amoles of IgG per zone spotted on the paper) was immobilized in test zones; PBS was used as a negative control. The test zones were blocked, and a solution of ALP-conjugated anti-rabbit IgG produced in goat (3 μL of a 1:1000 dilution the of stock antibody solution in 0.05% Tween-20 in PBS) was added to the test zones and allowed to incubate for one minute. The test zones were then washed with PBS (2×10 μL), and the ALP substrate solution (3 μL of 2.68 mM BCIP, 1.8 mM NBT, 5 mM $MgCl_2$, 100 mM NaCl, 0.05% Tween in 100mM Tris buffer, pH 9.5) was added to each well. After 30 minutes, the test zones were scanned (Perfection 1640SU scanner, EPSON Corp., set to color photo scanning, 600 dpi

resolution), and the image was analyzed in grayscale using ImageJ. All the antibodies and reagents were purchased from Sigma Aldrich.

P-ELISA for HIV-1 Envelope Antigens Detection. HIV-1 envelope antigen (gp41, 1 mg/mL) was diluted 1/10 in PBS and immobilized in test zones. The test zones were blocked using 1% (w/v) BSAand serum from HIV-1 infected patients and healthy control patients was added to the test zones, incubated and washed. A solution of ALP-conjugated anti-human HIV-1 produced in goat (3 μL of a 1:1000 dilution the of stock antibody solution in 0.05% Tween-20 in PBS) was then incubated in the test zones and washed. The procedure is the same as that we developed for rabbit IgG. Five microliters of the ALP substrate solution was added to each well; after 30 minutes, the results were scanned and analyzed in ImageJ. The HIV-1 envelope (gp41) antigen was purchased from ViroGen (Cambridge, MA). The serum samples were purchased from Golden West Biologicals (Temecula, CA).