Supporting Information
for
Development of a microfluidic paper-based immunoassay for rapid detection of allergic protein in foods

Marti Z. Hua, Xiaonan Lu*

Department of Food Science and Agricultural Chemistry, McGill University Macdonald Campus. 21111 Lakeshore Road, Sainte-Anne-de-Bellevue, Quebec, H9X 3V9, Canada.
Food, Nutrition and Health Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, V6T 1Z4, Canada.
*Corresponding Author: xiaonan.lu@mcgill.ca

Details of preparation of immunological reagents

Synthesis of gold nanoparticles

In an aqua regia-washed 500-mL round bottom flask, 2 mL of 1% (w/w) $HAuCl_4$ was added into 200 mL of ultrapure water. With magnetic stirring, ca. 150 µL of 1 M NaOH was added to reach a final pH of 4. The solution was heated to 100-105°C via an oil bath with stirring. Then, 1 mL of 500 mM sodium citrate was quickly added and mixed, and the reaction was kept at 100-105°C for 2 h. The resulted colloid was cooled down to room temperature and stored in the dark.

Optimization of the conjugation of AuNPs and antibody

NaCl-induced aggregation test was used to determine the minimum amount of antibody required to coat AuNPs. Briefly, the pH of AuNPs was first adjusted to 7.5 for polyclonal antibody or 8.2 for monoclonal antibody with 0.2 M K_2CO_3. Then, 25 µL of AuNPs and 25 µL of antibody (containing 0-20 µg antibody per 1 mL AuNPs) were mixed and incubated at room temperature for 15 min, followed by the addition of 50 µL of 10% NaCl. The absorbance (524 nm) reading of the resulted mixture was acquired with the plate reader.

Antibody pairing

Antibody pairs were screened using a simplified conventional system to determine the best candidate for recognizing and capturing antibodies. On a sheet of HF135 nitrocellulose membrane (Millipore, US), 0.5 µL of each antibody (*i.e.*, M291, M292, M293, and Poly; 1 µg/µL) was spotted 8 times in a row. The membrane was dried at 37°C for 15 min. The membrane was then blocked with 1% BSA (Goldbio, US) at room temperature for 10 min, followed by a quick rinse with PBST (0.1% Tween 20 in phosphate-buffered saline [PBS]). The excess amount of PBST was removed by gently absorbing with Kimwipes (Kimtech, US), and the membrane was dried in the oven at 37°C for 30 min. The dried membrane was then attached to an H5072 absorbent pad (Jieyi Bio, China) via a DB-7 backing card (Jieyi Bio, China), and then cut into strips as appropriate. In a 96-well plate (Corning, US), 100 µL of PBS or 100 ppm ovalbumin in PBS were mixed with 1 µL of each conjugate (*i.e.*, Au-M291, Au-M292, Au-M293, and Au-Poly). The strips were placed in the wells for 5 min to seek the desired combination of sandwiching antibodies.

FIGURES

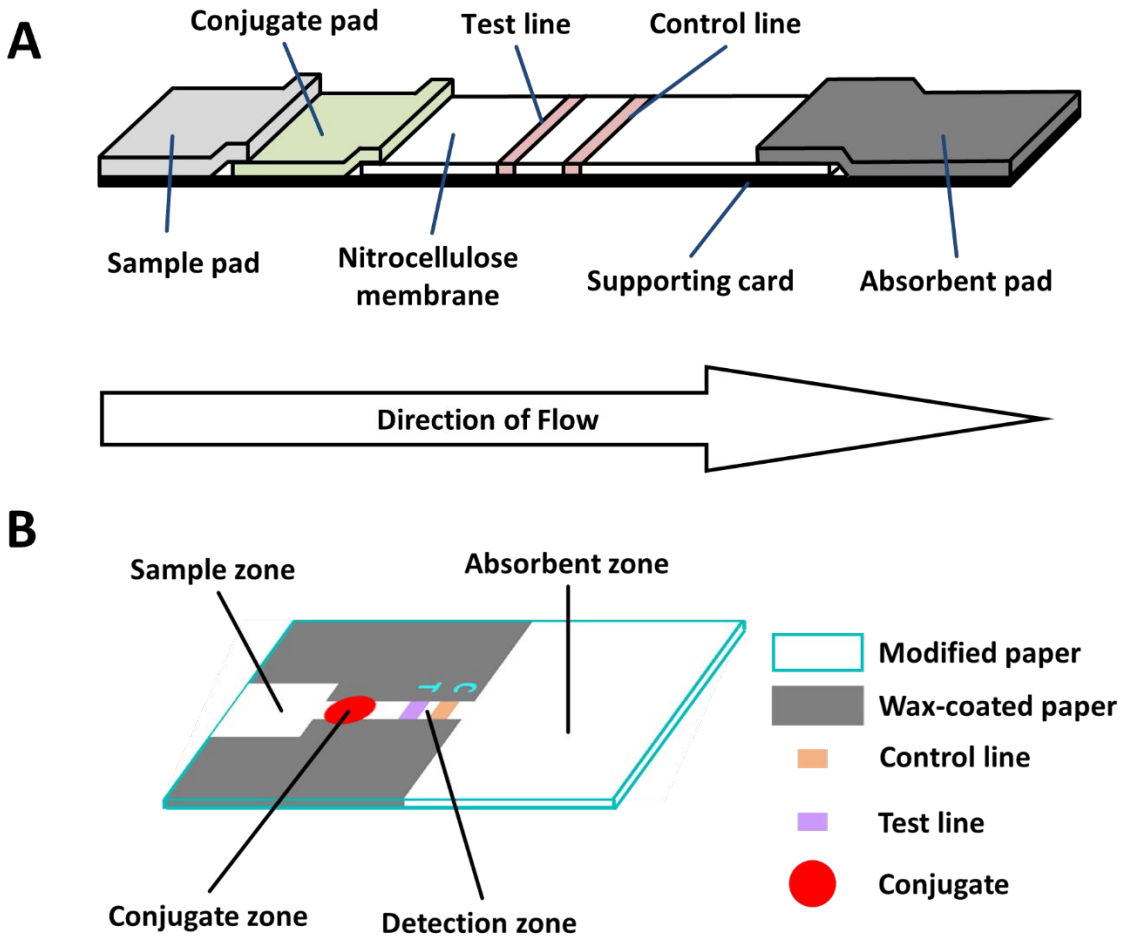

Figure S1. Scheme of (A) a conventional lateral flow immunoassay and (B) a single-piece paper-based device.

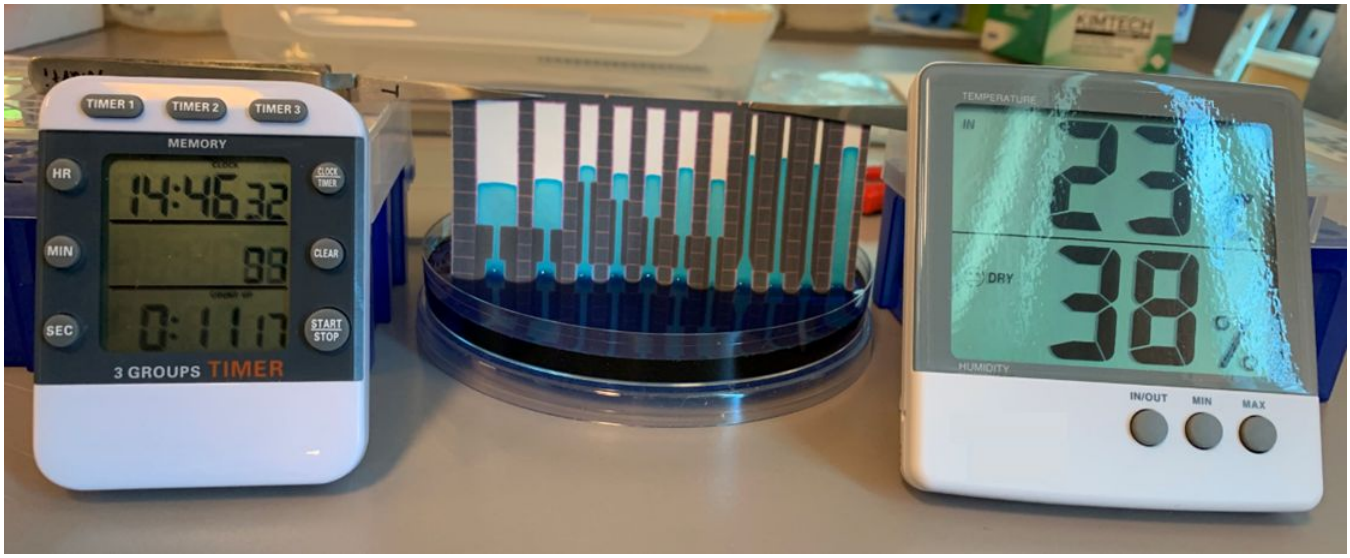

Figure S2. Settings to determine the effect of patterning geometry on flow rate.

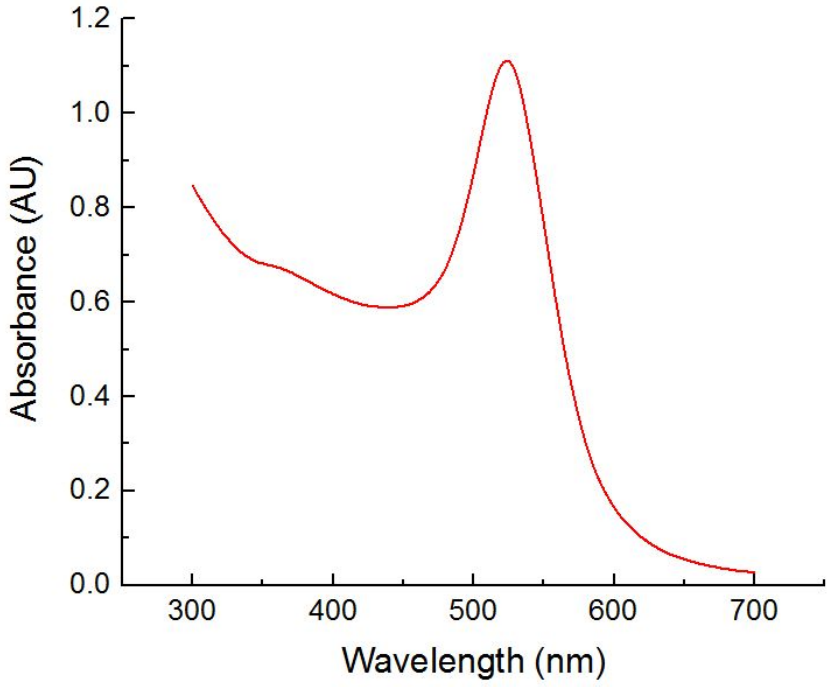

Figure S3. UV-Vis spectrum of the synthesized gold nanoparticles (surface plasma resonance peak at 523.7 nm).

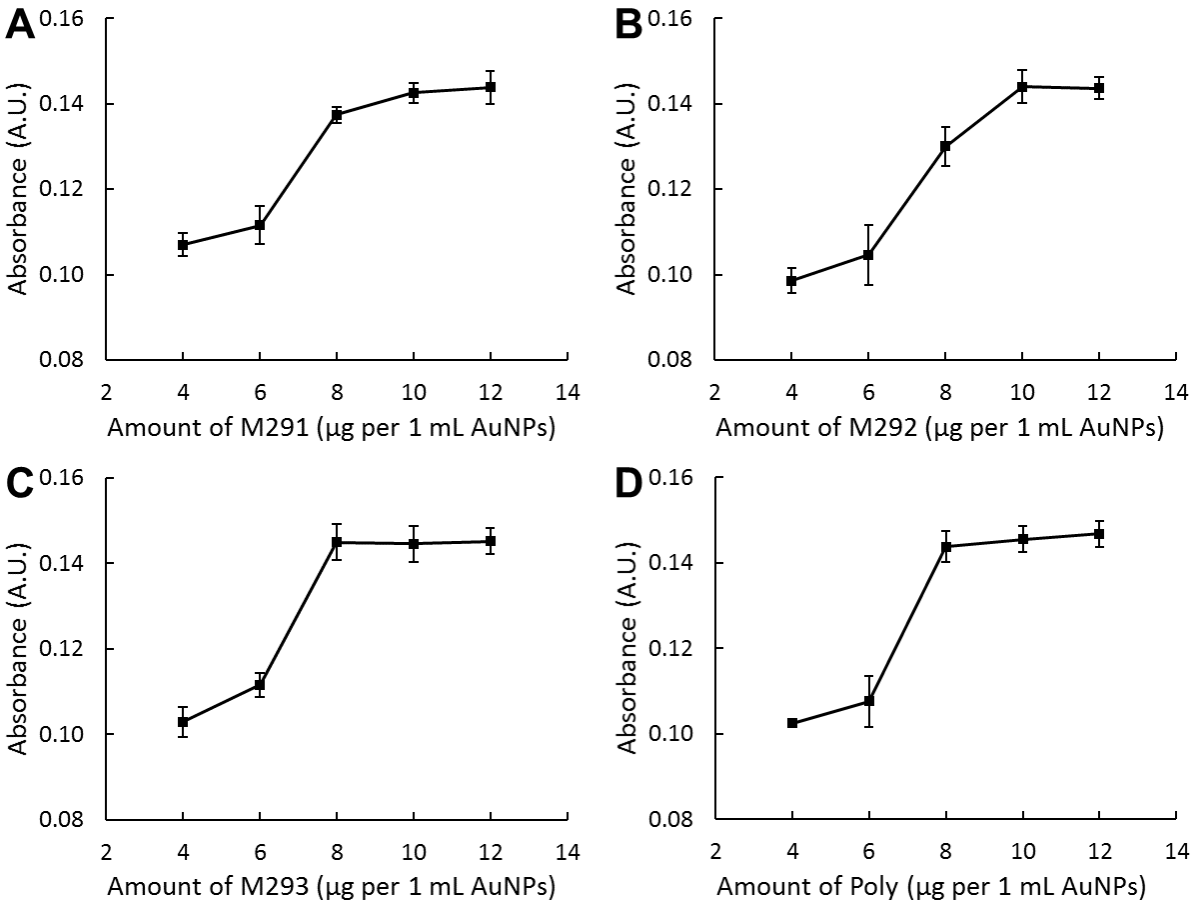

Figure S4. Absorbance (524 nm) of the gold-conjugated (A) M291, (B) M292, (C) M293, and (D) Poly at different ratios (*i.e.*, 4, 6, 8, 10, and 12 μg antibody per 1 mL AuNPs) after mixing with NaCl.

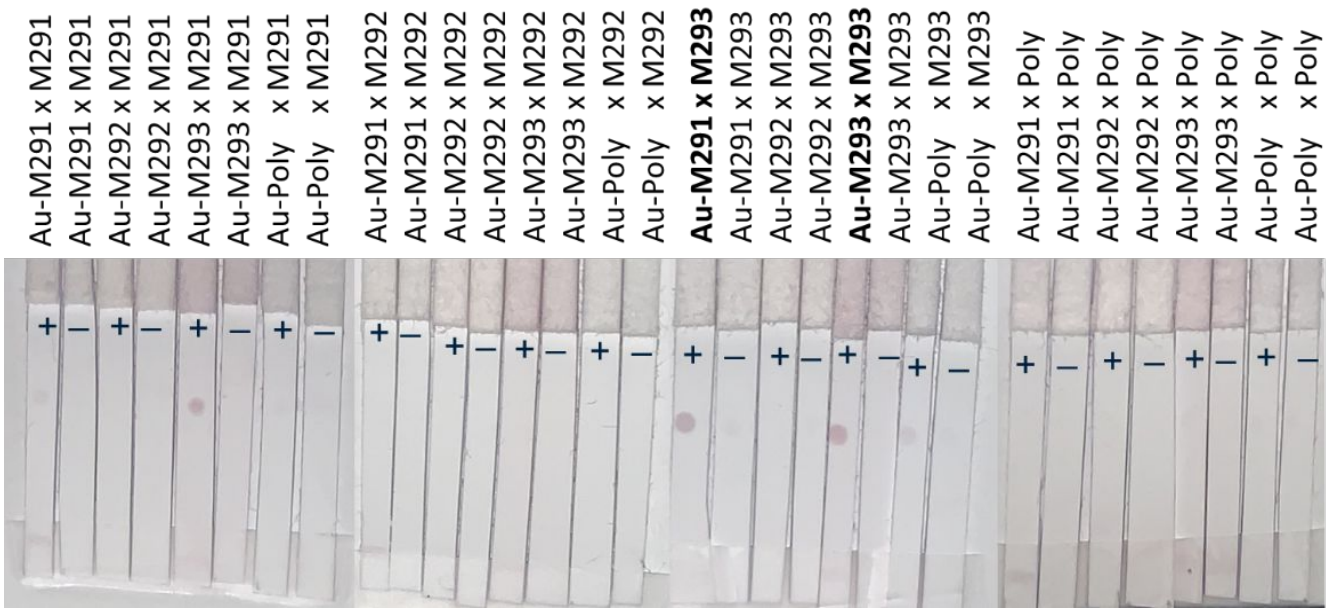

Figure S5. Result of antibody pair screening. Au-M291 x M293 was selected due to high signal intensity (darker spot) and a lower background than Au-M293 x M293.

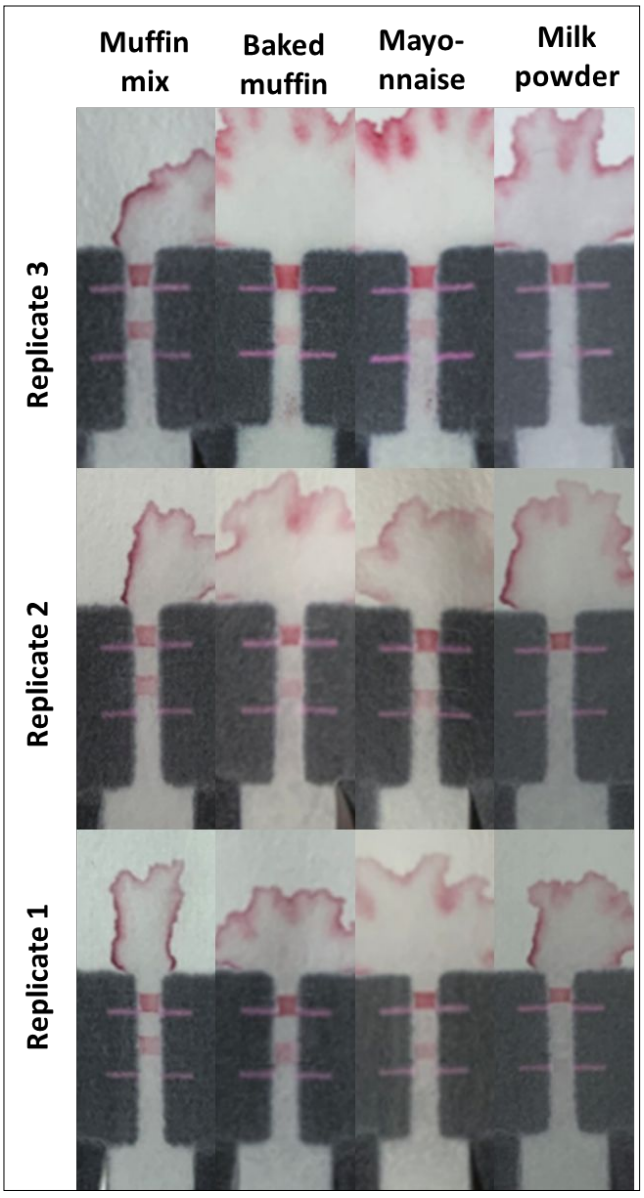

Figure S6. Detection of egg white in muffin mix, baked muffin, mayonnaise, and skim milk powder.

TABLES

Table S1. Summary of claimed performance of commercial LFI products and the developed µPAD.

Brand	Product	Claimed LOD
Romer Labs	AgraStrip Egg	2 ppm dried whole egg 2 µg/25 cm^2 dried whole egg
Neogen	Reveal® 3-D for Egg	5 ppm egg 10 µg/100 cm^2 egg
R-Biopharm	Egg Lateral Flow Allergen Test Kit	sprayed egg powder down to 1 ppm
Morinaga	Rapid Test Pro II for Egg	5 ppm (µg protein /g food) 1 µg protein /swab
Current study	µPAD	1 ppm ovalbumin (<2 ppm egg white) 0.01% egg white in cake mix

* Data from Reference 43-46 of the main article.

(43) Romer Labs. AgraStrip Egg. Available online: https://www.romerlabs.com/shop/agrastrip-r-egg (accessed on 25 October 2019).
(44) Neogen. Reveal 3D for Egg. Available online: https://foodsafety.neogen.com/en/reveal-3-d-egg (accessed on 25 October 2019).
(45) Weber Scientific. Egg Lateral Flow Allergen Test Kit. Available online: https://www.weberscientific.com/egg-lateral-flow-allergen-test-kit-bioavid-diagnostics/r-biopharm (accessed on 25 October 2019).
(46) Morinaga Institute of Biological Science. Rapid Test Pro II for food allergens. Available online: http://www.miobs-e.com/product/food_allergen_lateral_flow/dl/Rapid_Test_Pro%E2%85%A1_Leaflet_rev2.pdf (accessed on 25 October 2019).