Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Lateral Flow Assay Ruler for Quantitative and Rapid Point-of-care Testing

Zhao Li,[‡a] Hui Chen,[‡a] and Ping Wang[*a]

[a] Department of Pathology and Laboratory Medicine, University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104, United States

‡ These authors contributed equally to this work.

* Corresponding author:

Ping Wang

Department of Pathology and Laboratory Medicine,

University of Pennsylvania

3400 Spruce Street, Founders 7.103

Philadelphia, Pennsylvania 19104

Office: 215-614-6062

Email: Ping.Wang2@uphs.upenn.edu

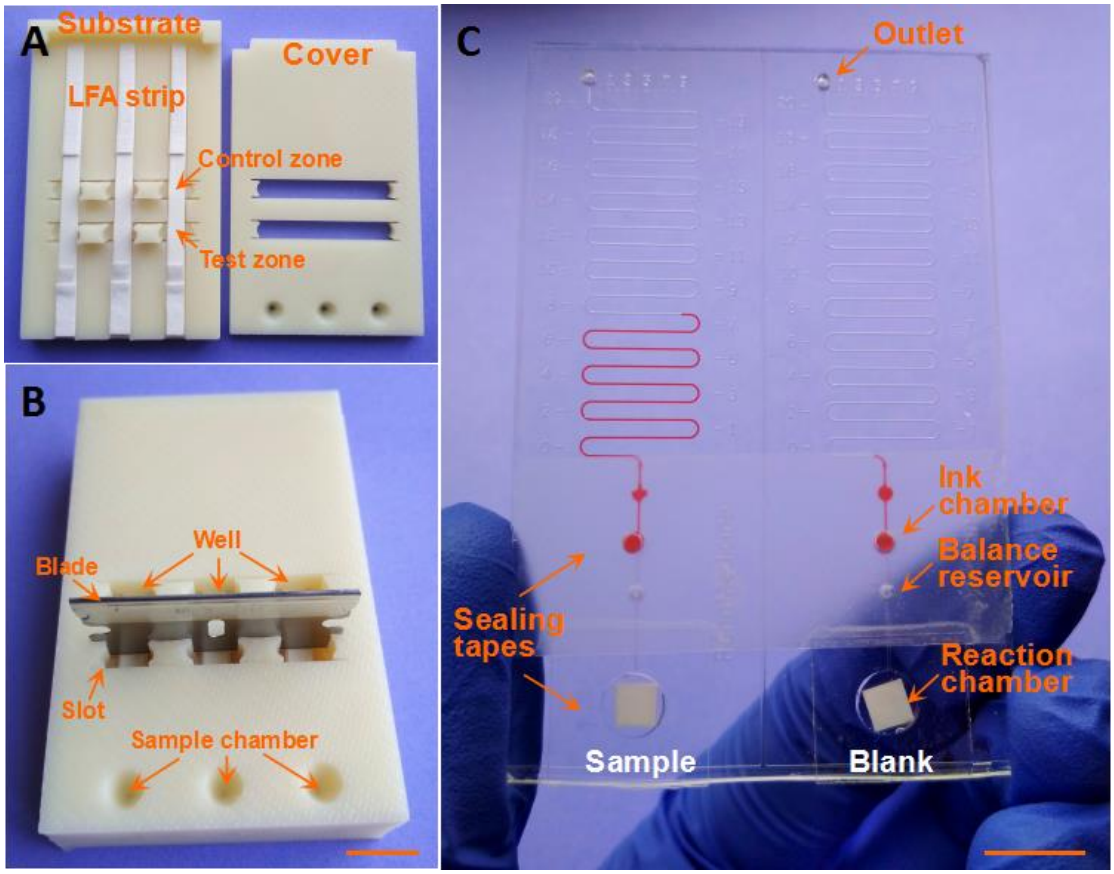


Figure S1. Photographs of the 3D printed strip cassette (A and B) and LFA ruler (C). After assembling the substrate and cover of strip cassette, the operation of LFA strip tests can start from loading sample solution into the sample chamber. After LFA completion, a blade is inserted into the slots to cut the test zone of LFA strips. Then the pads are transferred from the well of strip cassette to the reaction chamber in the LFA ruler. The LFA ruler contains microchannel, distance markers, ink chamber, balance reservoir, reaction chamber, outlet and sealing tapes. The ink advancement distance is proportional to the amount of target molecules in the sample, which achieves instrument-free quantitative detection of biomarkers by the naked eye. Scale bar, 1 cm.

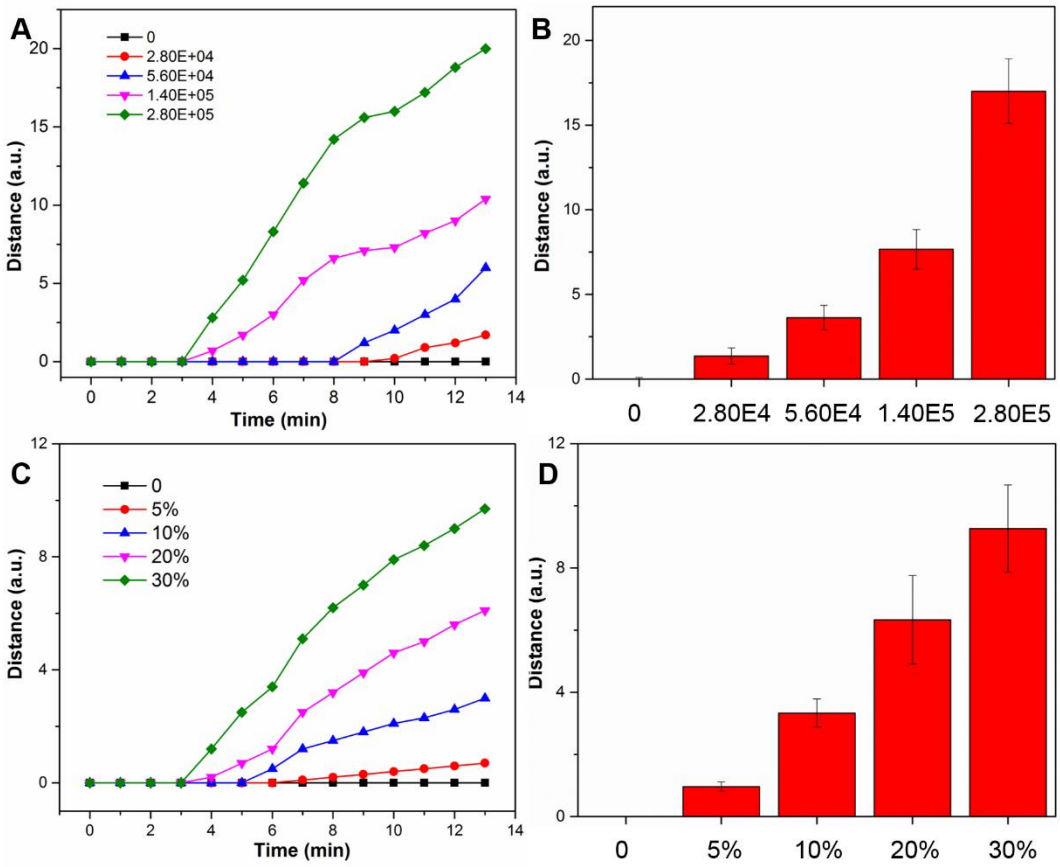


Figure S2. (A) Time-dependent ink advancement with the application of H_2O_2 (30%) and different concentrations of PtNPs. The number of PtNPs is 0, 2.8×10^4, 5.6×10^4, 1.4×10^5, and 2.8×10^5, respectively. (B) Ink advancement after 12 min plotted against the number of PtNPs (H_2O_2, 30%). (C) Time-dependent ink advancement with the application of PtNPs (1.4×10^5) and different concentrations of H_2O_2. The number of H_2O_2 is 0, 5%, 10%, 20%, and 30%, respectively. (D) Ink advancement after 12 min plotted against the concentrations of H_2O_2 (PtNPs, 1.4×10^5).

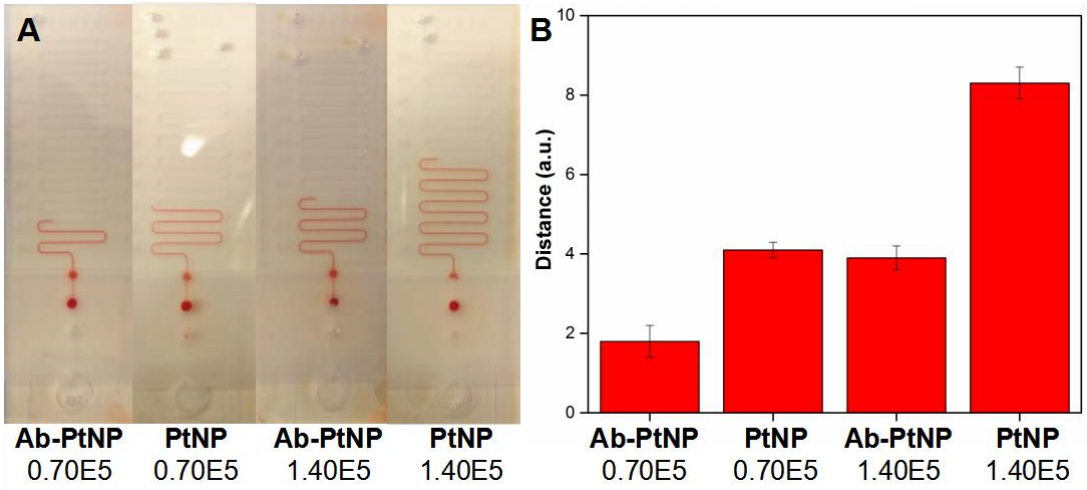


Figure S3. Comparison of catalytic activity of PtNP and Ab-PtNP. The number of tested nanoparticles is 0.7×10^5 and 1.4×10^5. (A) The ink advancement in LFA rulers. (B) The distance value histogram (mean ± standard error), tested in triplicates. At the same concentration, the catalytic activity of Ab-PtNP is approximately half that of PtNP.

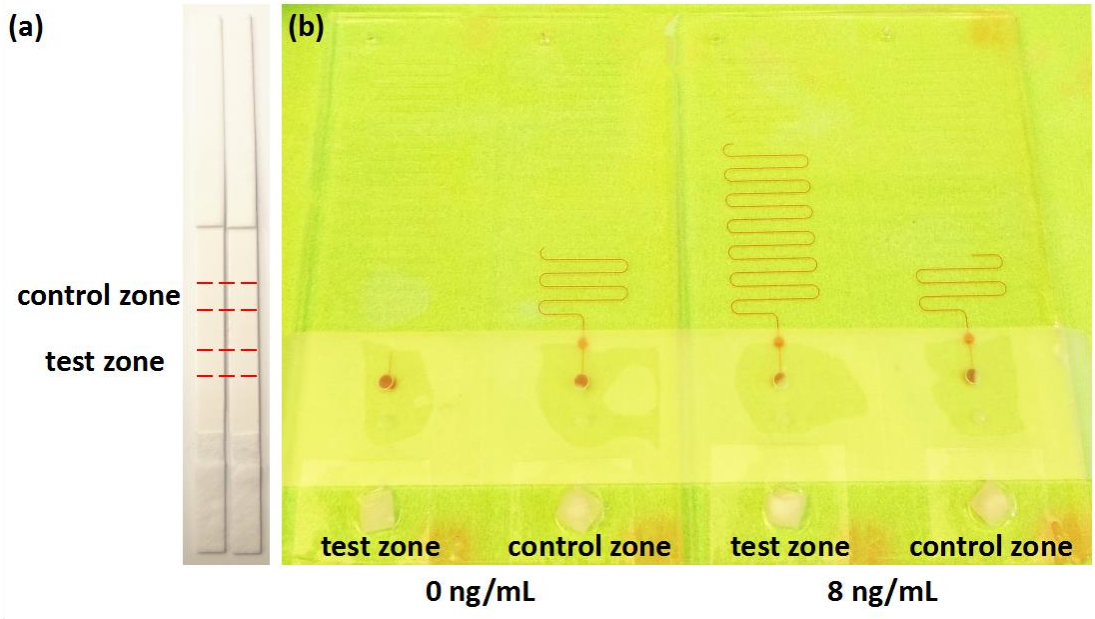


Figure S4. (A) The images of LFA strips. There is no difference in color between the blank strip (0 ng/mL PSA) and the positive strip (8 ng/mL PSA) with naked eyes. (B) The ink advancement distances of the test/control zone from the blank strip (0 ng/mL PSA) and the positive strip (8 ng/mL PSA) is significantly different in the LFA rulers.

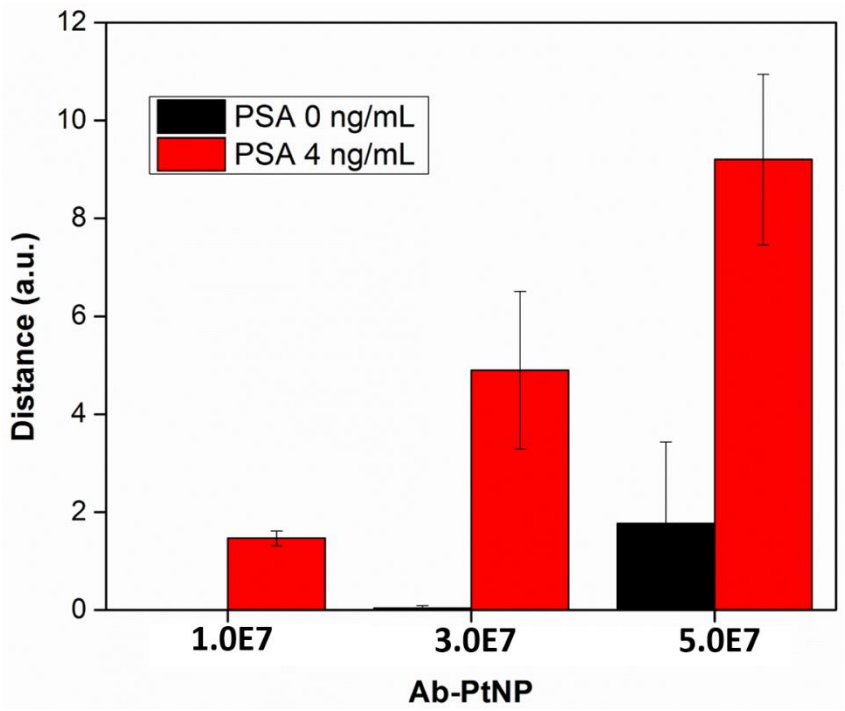
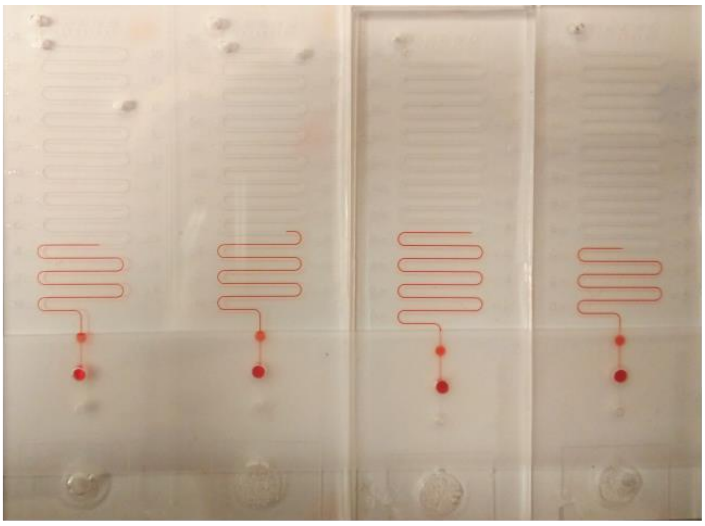


Figure S5. Optimization experiment of Ab-PtNP conjugates for PSA LFA strips. The number of Ab-PtNP conjugates added to the LFA strips was 1.0×10^7, 3.0×10^7 and 5.0×10^7, respectively. The concentration of PSA was 0 ng/mL (blank strip, black column) and 4 ng/mL (positive strip, red column). The ink advancement distances of positive strips increased significantly when adding more Ab-PtNP conjugates. However, the ink advancement distances of blank strip far exceeded "0" when adding 5.0×10^7 Ab-PtNP conjugates. Thus, we chose to add 3.0×10^7 Ab-PtNP conjugates to the LFA strips in the subsequent PSA testing experiments.



AA	0 mM	25 mM	50 mM	100 mM
PtNP	5.60E4	5.60E4	5.60E4	5.60E4

Figure S6. The ink advancement in LFA rulers with the application of PtNP (5.6×10^4), H_2O_2 (30%), and different concentration of ascorbic acid (AA). The concentration of AA is 0, 25, 50, and 100 mM, respectively. The reaction between PtNP and H_2O_2 is not affected by AA, even at the concentrations that are much higher than the blood concentration of people who are supplemented with vitamin C.

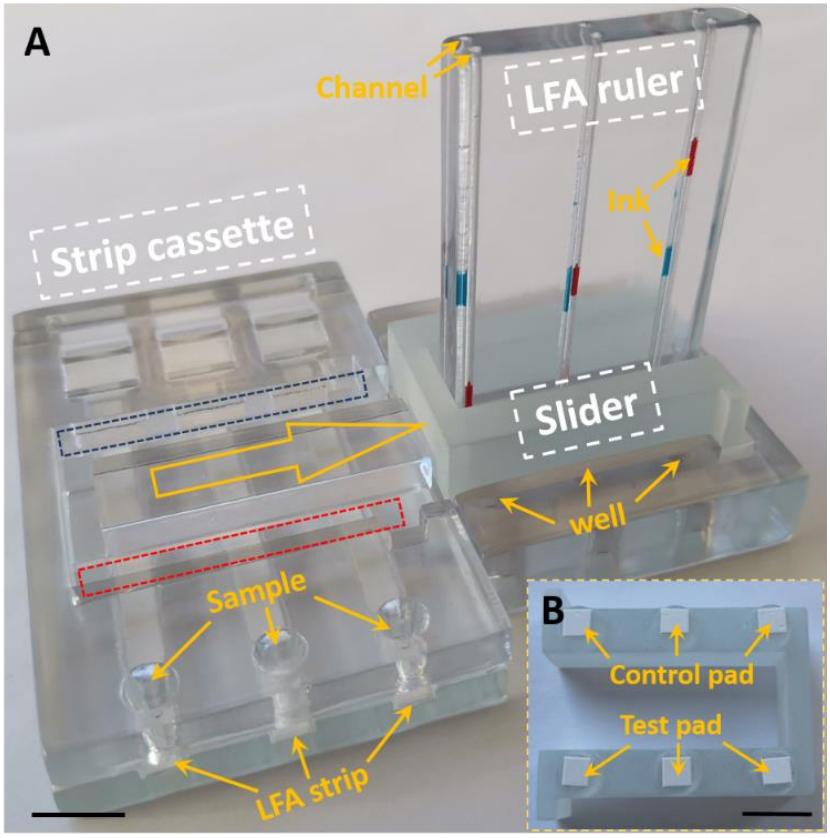


Figure S7. The prototype of fully 3D-printed LFA ruler with the advantages of multiplexed quantification and ease of use. (A) There are three components of 3D-printed LFA ruler, including strip cassette, slider, and LFA ruler. After LFA completion, slider slides into the slot (the sliding direction shown as the yellow hollow arrow), bringing the test/control pads affixed at the bottom of the slider's pillars into the reaction wells in the LFA ruler for the subsequent quantitative tests. The PtNPs on the pads react with H_2O_2 pre-loaded in the reaction wells to generate oxygen that pushes the ink to advance in the channels. The red and blue ink advancement distances correspond to the quantitative results of test and control pads, respectively. (B) Bottom view photo of the slider with control/test pads affixed at the bottom of pillars. Scale bar, 1 cm.