Paper-based multiplexed vertical flow assay for point-of-care testing

Supplementary Information

H.-A. Joung[1], Z. S. Ballard[1,2], A. Ma[3], D. K. Tseng[1], H. Teshome[4], S. Burakowski[5], O. B. Garner[6], D. D. Carlo[5,7], A. Ozcan*[1,2,7]

[1]Electrical & Computer Engineering Department, [2]California NanoSystems Institute (CNSI), [3]Biochemistry Department, [4]Neuroscience Department, [5]Mechanical Engineering Department, [6]Department of Pathology and Laboratory Medicine, [7]Bioengineering Department, University of California, Los Angeles, California 90095, United States. * Corresponding Author. Email: ozcan@ucla.edu

Supplementary Figures

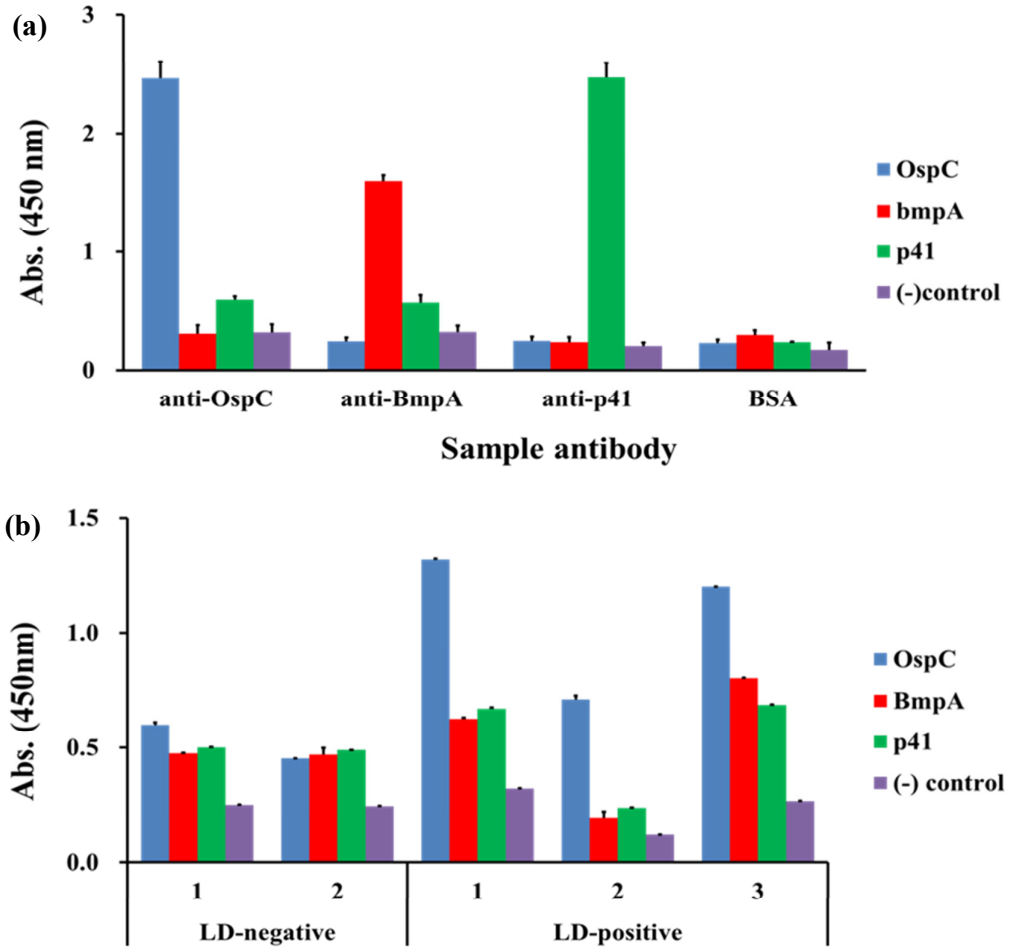

Fig. S1 (a) ELISA results of the three LD-specific rabbit antibodies (anti-OspC, anti-BmpA and anti-P41) tested on the xVFA (results shown in Figure 4 of the main text) **(b)** and ELISA results of anti-OspC, anti-BmpA and anti-P41 measurements made in the real human samples tested by the xVFA (results shown in Figure 5 of the main text).

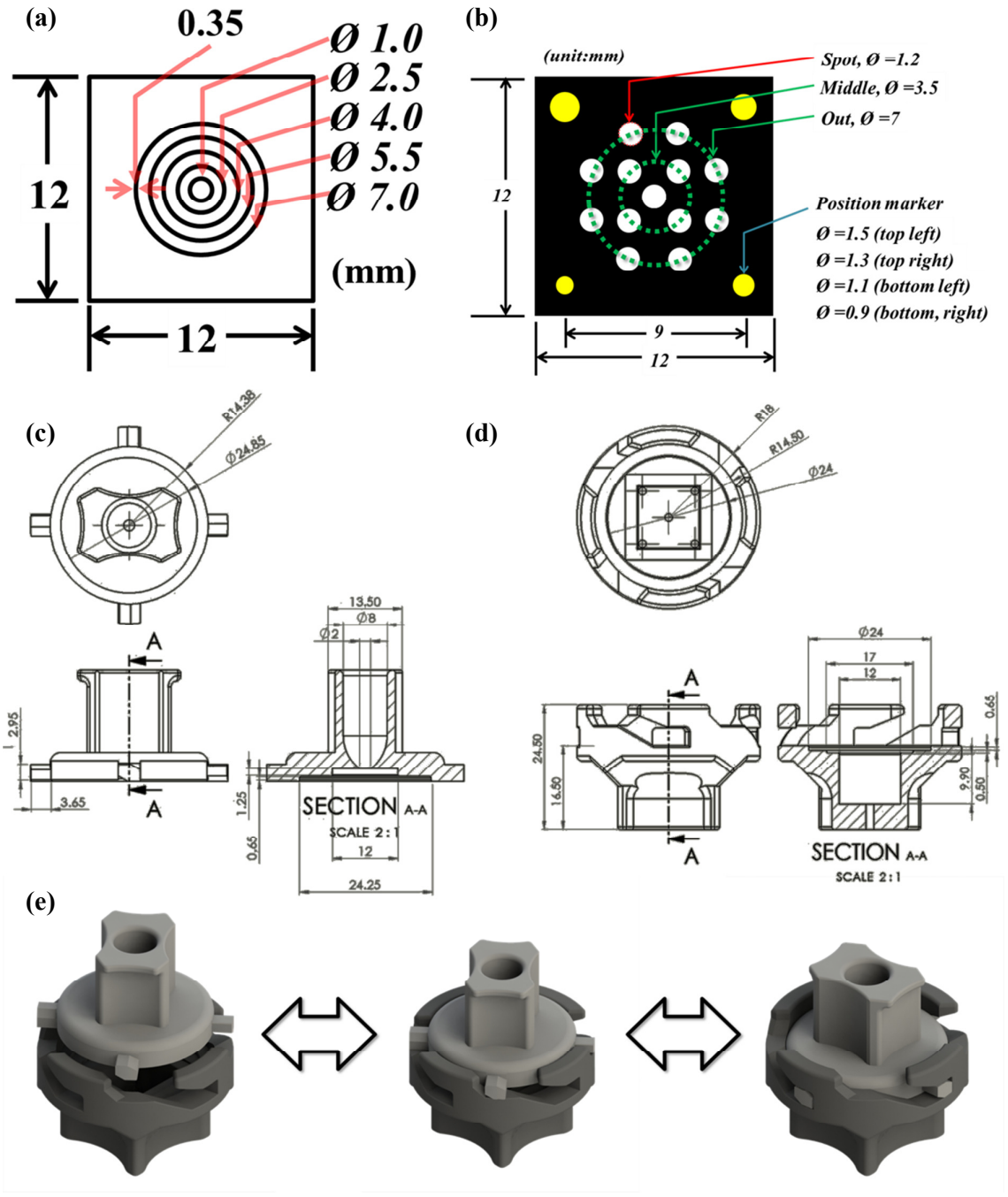

Fig. S2 (a) Drawings of the vertical flow diffusor (VFD), **(b)** multiplexed sensing membrane, **(c)** top **(d)** and bottom sensor cases (unit: mm) and **(e)** schematic of the cassette's 'twist on-off' mechanism for easily separating the top and bottom case.

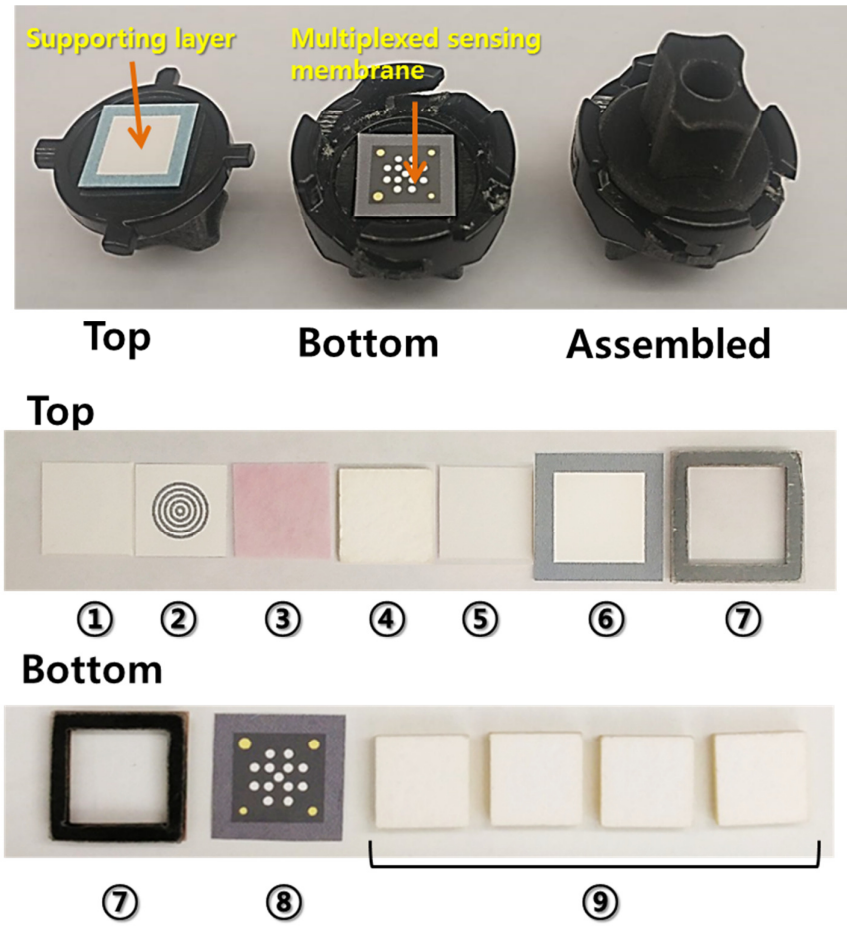

Fig. S3 Images of the assembled top and bottom case, fully assembled cassette, and optimized paper layers: ①Absorption layer (plasma separation membrane (asymmetric), large pore side up), ②Vertical flow diffuser (nitrocellulose membrane, thickness: 0.45 µm) ③Conjugate pad (for 2nd top case only), ④Sample pad (thickness: 1.2 mm), ⑤Spreading layer (plasma separation membrane (asymmetric), large pore side down), ⑥Supporting layer (nitrocellulose membrane, pore size: 0.22 µm), ⑦Double-sided foam tape, ⑧Multiplexed sensing membrane (nitrocellulose membrane, pore size: 0.22 µm) , ⑨Absorbent layer (Absorption pad stack, thickness: 1.8 mm each absorption pad).

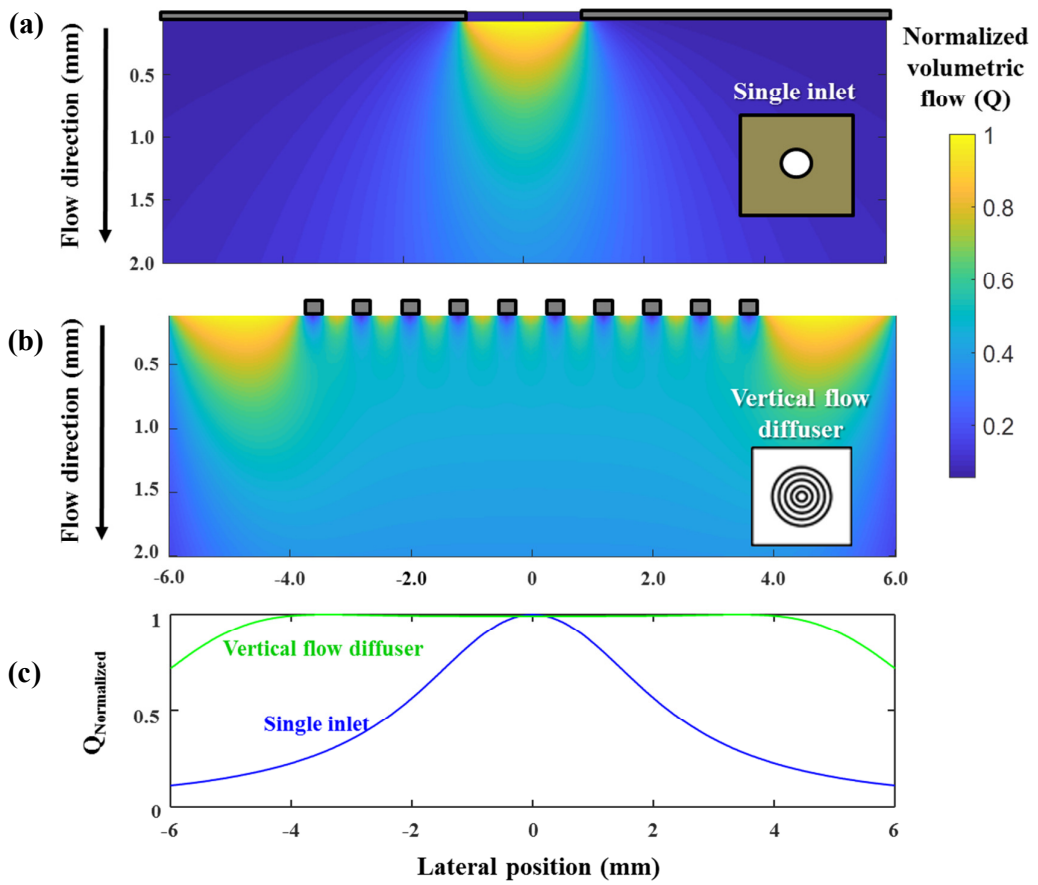

Fig. S4 Heuristic model of the vertical volumetric flow rate, Q, (shown in the color bar) as a function of lateral and vertical position, defined by the flux representation of Darcy's law, where it is inversely proportional to displacement. **(a)** Vertical volumetric flow-rate after loading sample into single inlet and **(b)** vertical volumetric flow-rate after flow through vertical flow diffuser (VFD). **(c).** Normalized volumetric flow rate ($Q_{Normalized}$) over lateral position at the sensing membrane (2.4 mm from inlet).

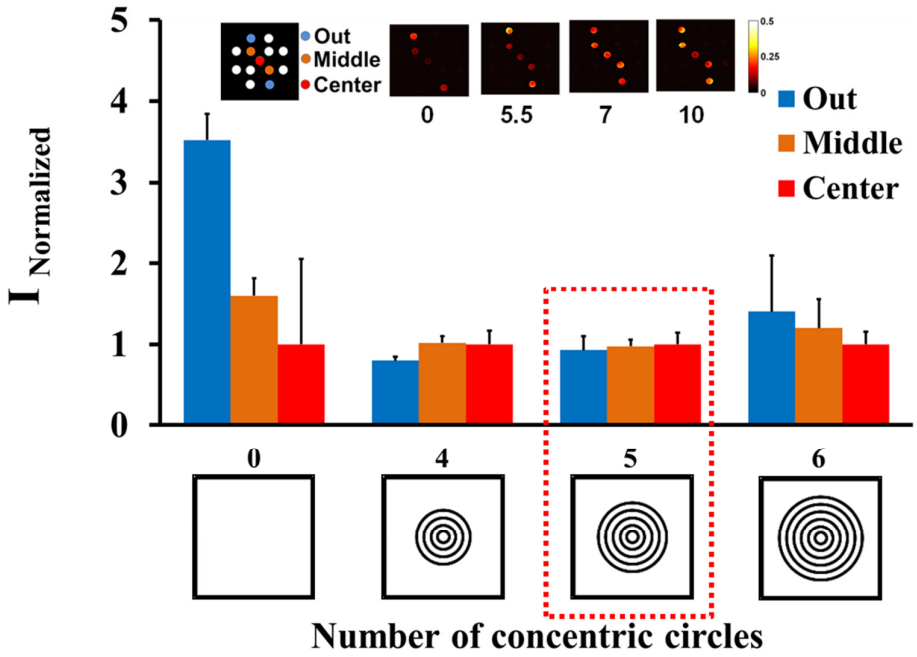

Fig. S5 Empirical characterization of vertical flow diffuser (VFD) according to the number of concentric circles.

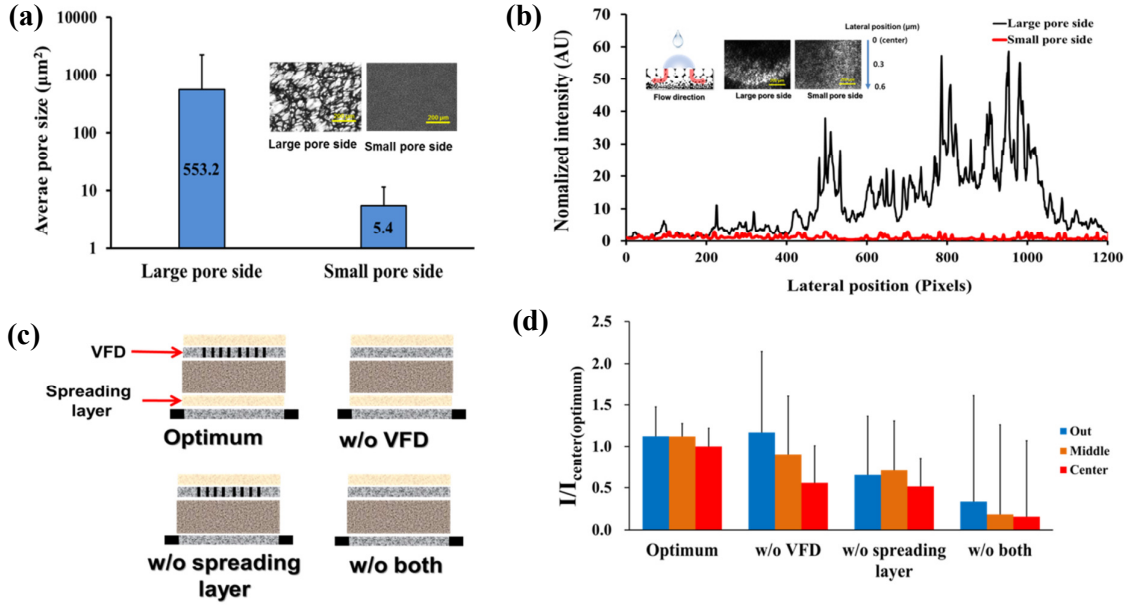

Fig. S6 Characterization of the spreading layer (asymmetric membrane). **(a)** Average pore size of both sides of the asymmetric membrane (Insert: 10X microscope image of both side). **(b)** Normalized signal distribution over lateral position from both sides of the asymmetric membrane. The images and signal were obtained by loading a fluorescent bead droplet onto the center of larger pore side. The fluorescent intensity distribution was then imaged with a benchtop microscope**. (c)** Schematics of vertical paper layer combinations based-on vertical flow diffuser (VFD) and spreading layer. **(d)** Comparison of signal distribution and intensity according to the various combinations of the VFD and spreading layer.

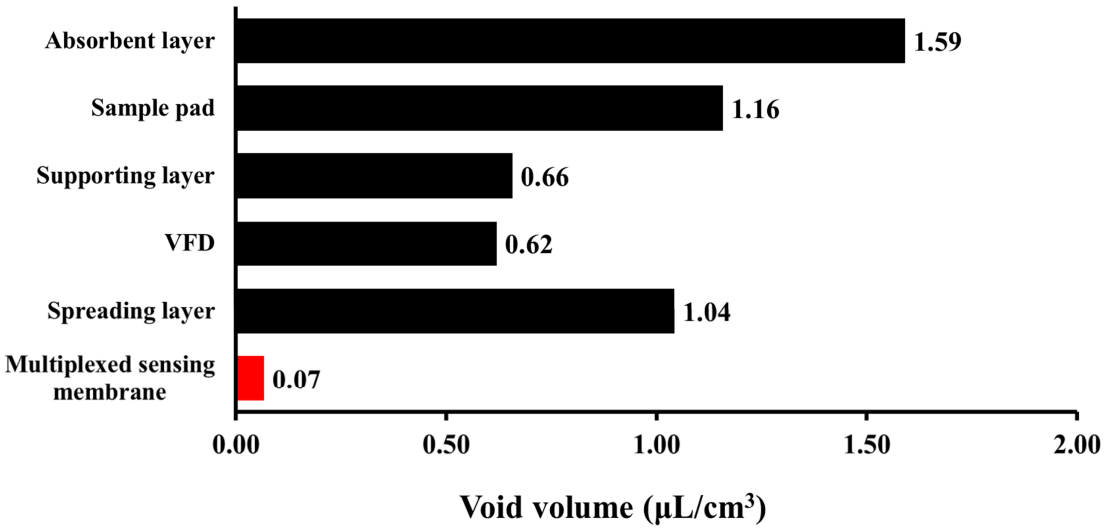

Fig. S7 Void volume per unit volume of each paper layer. The vertical flow rate is limited by multiplexed sensing membrane, due to it having the smallest void volume.

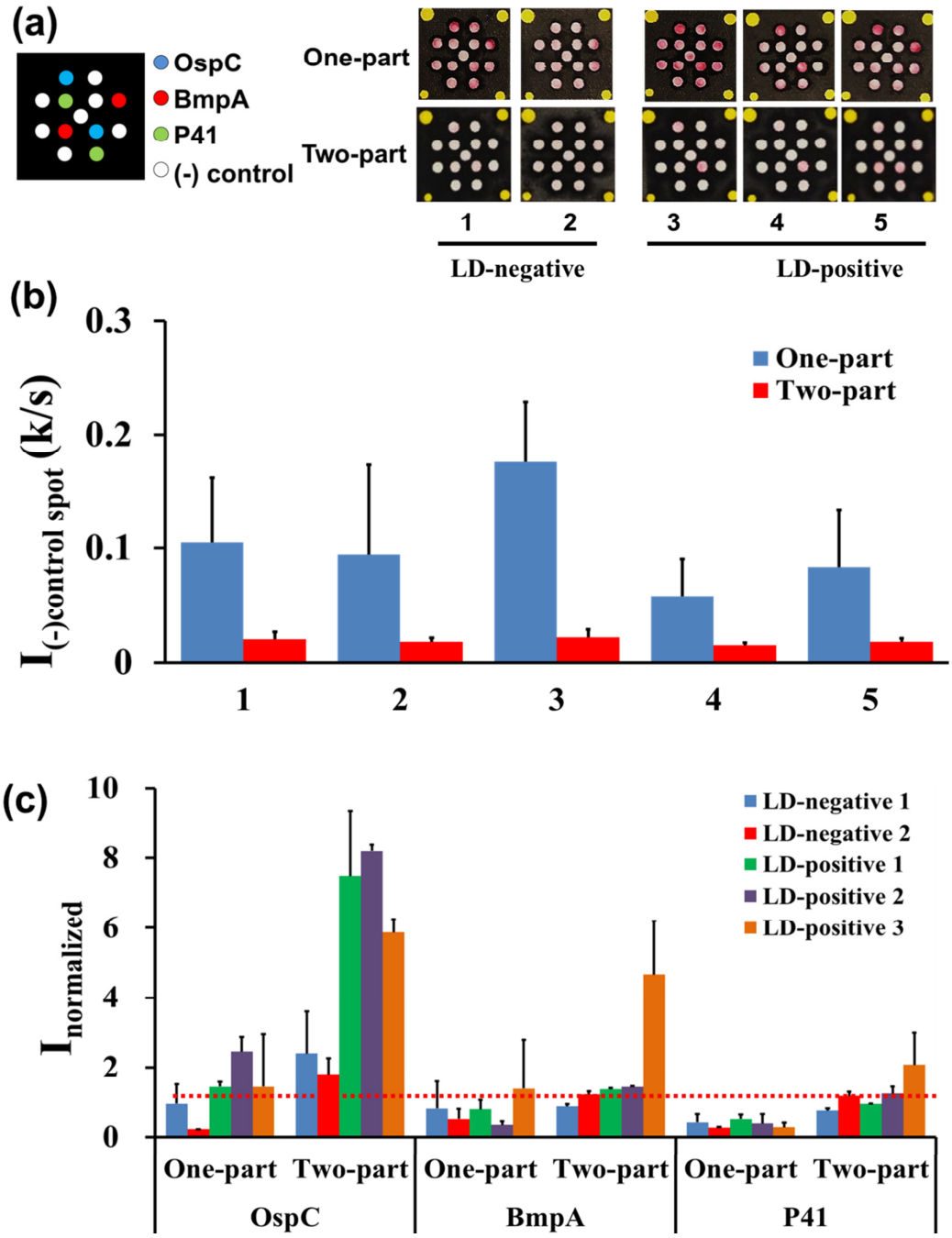

Fig. S8. Comparison of nonspecific signal generated for one- and two-part assays with human serum. **(a)** Antigen spot legend and raw signal images of human IgM control serum measurements. **(b)** k/s value plot of negative control spot. **(c)** Lyme-specific antigen spot signals normalized to the average negative control signal plus three times the standard deviation.

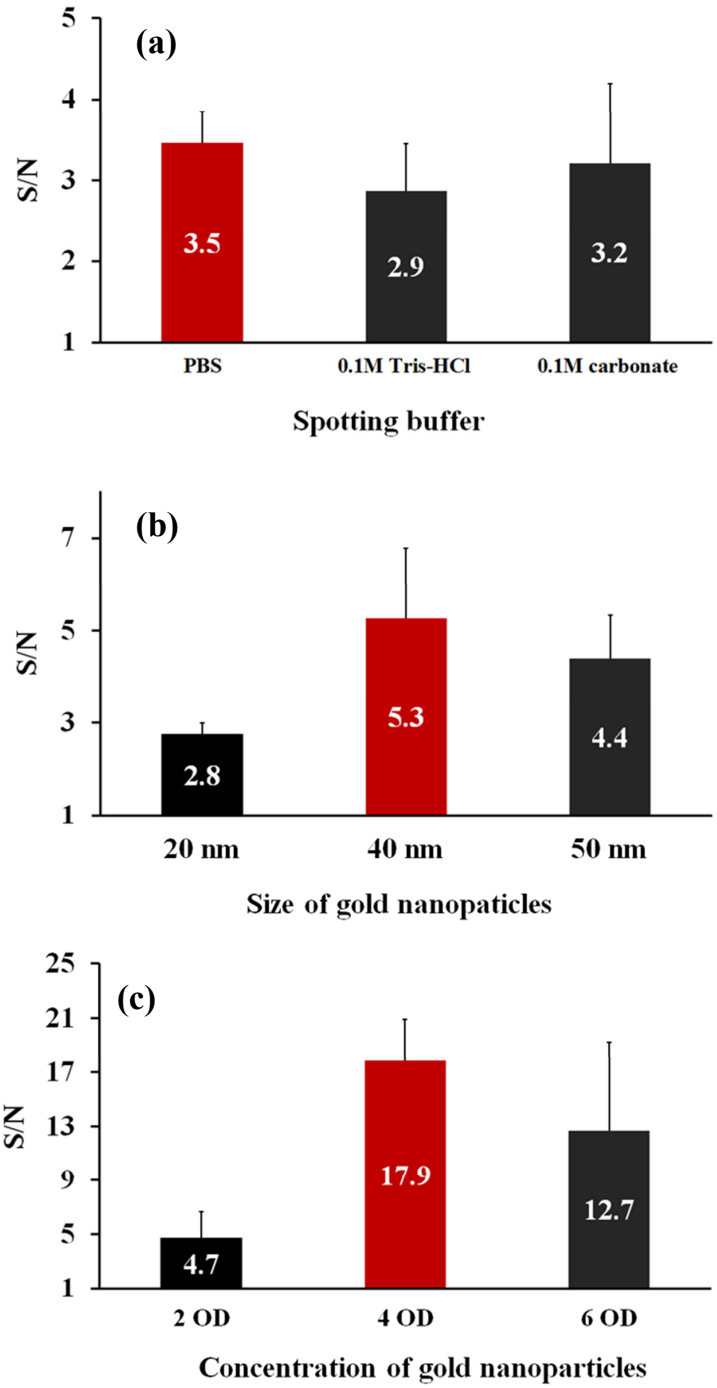

Fig. S9 Further optimization of the xVFA assay. Signal to background/noise (S/N) versus various conditions including **(a)** different buffer conditions **(b)** different gold nanoparticles size **(c)** and different gold nanoparticle concentrations.

Table S1. Total cost of used paper material for single test (unit: ¢)

	Unit price	Number	Sub Total
Absorption layer	0.5	2	1.0
Vertical flow diffuser (VFD)	2.9	2	5.8
Sample pad	1.7	2	3.4
Spreading layer	0.5	2	1.0
Supporting layer	2.9	2	5.8
Multiplexed sensing membrane	2.9	1	2.9
Absorbent pad	1.4	5	7.0
Foam tape	0.22	3	0.7
Total			**27.6 ¢**