## **Supplementary Information**

## Capillary Driven Microfluidic Sequential Flow Device for Point-of-Need ELISA: COVID-19 Serology Testing

Cody Carrella<sup>‡</sup>, Ilhoon Jang<sup>a,b<sup>‡</sup></sup>, Jeremy Link<sup>a</sup>, James S. Terry<sup>d</sup>, Zachary Call<sup>a</sup>, Yosita Panraksa<sup>e</sup>,

Orawon Chailapakul<sup>e</sup>, David S. Dandy<sup>c,f</sup>, Brian J. Geiss<sup>d,f</sup>, and

Charles S. Henry<sup>a,c,f\*</sup>

<sup>a</sup>Department of Chemistry, Colorado State University, CO, USA, 80523

<sup>b</sup>Institute of Nano Science and Technology, Hanyang University, Seoul, Korea, 04763

<sup>e</sup>Department of Chemical and Biological Engineering, Colorado State University, CO, USA, 80523

<sup>d</sup>Department of Microbiology, Immunology and Pathology, Colorado State University, CO, USA, 80523

<sup>e</sup>Program in Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, 10330

<sup>f</sup>School of Biomedical Engineering, Colorado State University, CO USA 80523

\*Corresponding author: <u>Chuck.Henry@colostate.edu</u>

<sup>‡</sup> C.C. and I.J. contributed equally to this work.



**Figure S1** Design of each layer of the dELISA. Layers are currently assembled by hand with the aid of a cold-press laminator. During assembly, the marked section on layer 4 of DSA is cut out to complete the connection in the channel. The DSA material that is cut is included initially to hold all layers together.



Figure S2 The signal and noise (blank) values used to generate Fig. 3 in the main text.

4-Parameter Logistic model used to fit the data from the dose-response curve. Y is the signal, x is the target concentration, a is the expected response (Y) at x=0, b is the slope of the curve at point c, which is the target concentration that corresponding to Y=(a+d)/2, and d is the expected response when the target concentration is infinitely high (Assuming no hook effect).

$$Y = d + \frac{a - d}{1 + \left(\frac{x}{c}\right)^b}$$
(Eq S1)

The equation used to calculate the detection limit from the 4-parameter logistic fit. Parameters a, b, c, and d are defined in Equation S1 above.  $SD_b$  is the standard deviation of the blank and  $R_b$  is the response or signal of the blank.

$$LOD = c \times \left( \left( \frac{(a-d)}{(3 \cdot SD_b + R_b) - d} \right) - 1 \right)^{\frac{1}{b}}$$
(Eq S2)