

Multicolored Silver Nanoparticles for Multiplexed Disease Diagnostics: Distinguishing Dengue, Yellow Fever, and Ebola Viruses

Chun-Wan Yen,^{a,b} Helena de Puig,^c Justina Tam,^{a,b} José Gómez-Márquez,^d Irene Bosch,^{a,b} Kimberly Hamad-Schifferli,^{c,e*} and Lee Gehrke^{a,f*}

^aInstitute for Medical Engineering and Science, Massachusetts Institute of Technology Cambridge, MA USA 02139

^bWinchester Engineering Analytical Center, Food and Drug Administration. Winchester MA USA 01890

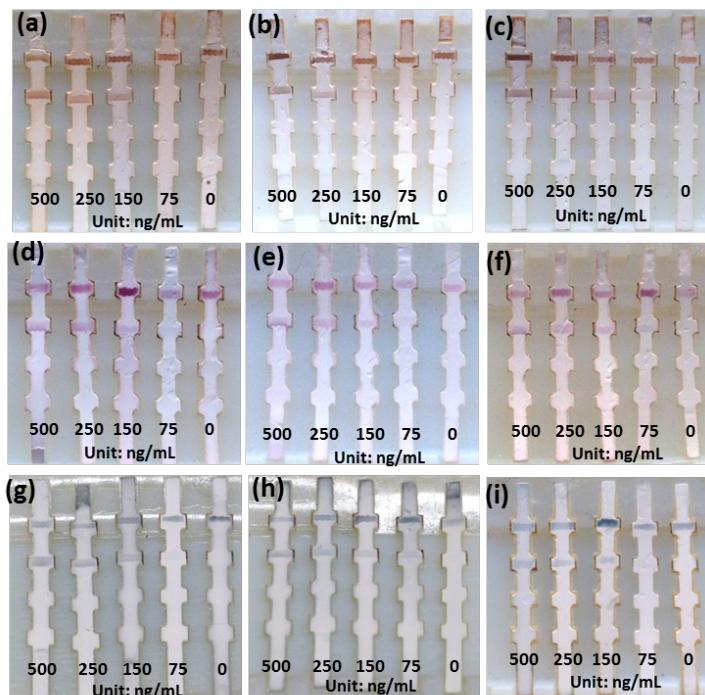
^cDept. of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

^dMIT Little Devices Lab and the MIT-SUTD International Design Centre

^eMIT Lincoln Laboratory, Lexington MA 02420

^fDept. of Microbiology and Immunobiology, Harvard Medical School, Boston 02115

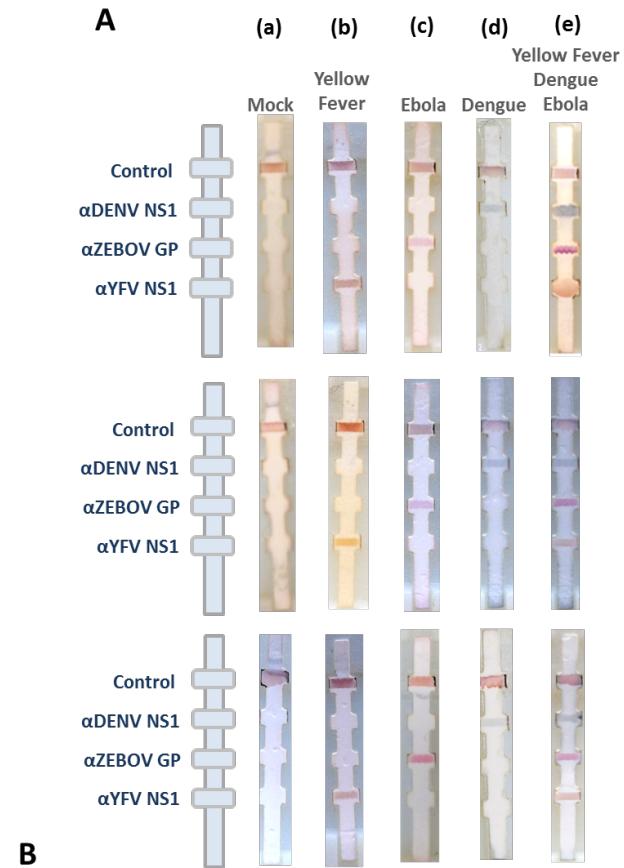
A



B

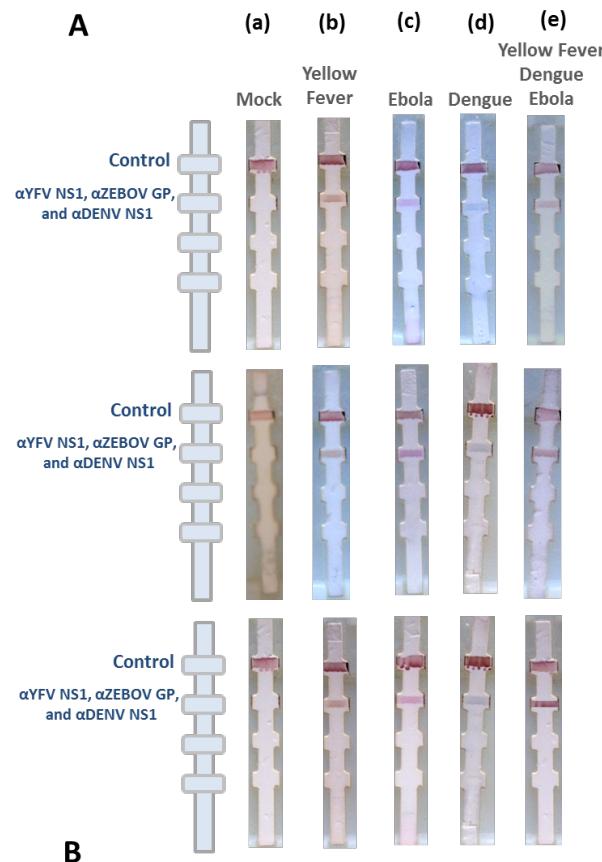
	Yellow Fever test line	Ebola test line	Dengue test line
Sample Conc: 500 ng/mL	148 ± 7	160 ± 8	152 ± 5
Sample Conc: 250 ng/mL	167 ± 5	177 ± 5	169 ± 6
Sample Conc: 150 ng/mL	180 ± 6	191 ± 6	178 ± 7
Sample Conc: 75 ng/mL	212 ± 7	205 ± 5	200 ± 9
Sample Conc: 0 ng/mL	229 ± 5	220 ± 4	210 ± 5

Figure S1: (A) Three independent limits of detection testing of (LOD) of YFV NS1 (a-c), ZEBOV GP (d-f), and DENV NS1 (g-i) proteins using the different AgNP-Abs. a,d, and g appear in the manuscript in Figure 2. (B) Signal intensities were quantified using ImageJ to define the LODs. The images were converted to gray scale to acquire gray level intensities. The intensities are averages of 5 line scans that span the 1 mm test line area for 3 separate flow tests. LOD is defined as the minimum concentration that gives a difference in intensity between the control line and the test line five times larger than the standard deviation of the control intensity.¹



	Yellow Fever test line (R/G/B)	Ebola test line (R/G/B)	Dengue test line (R/G/B)	Control line (R/G/B)
Sample A (Mock)	218 ± 3 200 ± 9 210 ± 12	215 ± 7 218 ± 11 220 ± 8	228 ± 8 217 ± 10 210 ± 11	149 ± 13 92 ± 9 92 ± 14
Sample B (Yellow Fever Virus)	154 ± 16 85 ± 8 70 ± 6	195 ± 5 200 ± 8 231 ± 12	195 ± 8 196 ± 9 239 ± 13	161 ± 8 113 ± 10 102 ± 8
Sample C (Ebola Virus)	239 ± 8 221 ± 9 205 ± 5	211 ± 10 124 ± 11 164 ± 13	238 ± 7 206 ± 10 200 ± 9	168 ± 9 107 ± 11 110 ± 10
Sample D (Dengue Fever Virus)	229 ± 10 209 ± 8 203 ± 7	219 ± 11 215 ± 10 210 ± 9	115 ± 9 112 ± 9 100 ± 8	160 ± 7 118 ± 9 119 ± 9
Sample E (Yellow Fever + Ebola + Dengue)	172 ± 11 98 ± 7 75 ± 10	216 ± 11 144 ± 10 168 ± 8	106 ± 9 106 ± 7 114 ± 10	178 ± 15 135 ± 13 138 ± 10

Figure S2: (A) Three independent multiplexed detection was repeated by printing each biomarker into individual detection area. The bottom panel appears in the manuscript as Figure 3a. (B) RGB analysis, using ImageJ, of repeat detection experiments. The results are averages of 5 line scans that span the 1mm test line area of 3 separate lateral flow tests. Red/Green/Blue value ranges are 0 to 255.² The R/G/B values of white color is 255/255/255.



	Test line (R/G/B)	Control line (R/G/B)
Sample A (Mock)	220 ± 10 227 ± 9 203 ± 8	152 ± 8 95 ± 7 98 ± 10
Sample B (Yellow Fever Virus)	172 ± 10 107 ± 6 162 ± 8	161 ± 9 110 ± 10 100 ± 9
Sample C (Ebola Virus)	208 ± 10 119 ± 7 172 ± 5	158 ± 11 115 ± 9 110 ± 8
Sample D (Dengue Fever Virus)	122 ± 10 109 ± 9 103 ± 11	156 ± 9 106 ± 10 107 ± 7
Sample E (Yellow Fever + Ebola + Dengue)	188 ± 11 122 ± 9 115 ± 9	181 ± 10 141 ± 11 144 ± 12

Figure S3: (A) Three independent experiments of multiplexed detection were repeated by printing the mixed biomarkers into a single detection area. The bottom panel appears as Figure 3b in the manuscript. (B) The repeated multiplexed detection of YFV, DENV, and EV. RGB analysis of the test and control lines using Image J. Red/Green/Blue value ranges are 0 to 255. The R/G/B value of white color is 255/255/255.

Reference :¹ A. K. Badu-Tawiah, S. Lathwal, K. Kaastrup, M. Al-Sayah, D. C. Christodouleas, B. S. Smith, G. M. Whitesides, and H. D. Sikes, *Lab Chip*, 2015, 15, 655-659.

² M. D. Abramoff, P. J. Magelhaes, and S. J. Ram, *Biophotonics International*, 2004, 11, 36-42