Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Aptamer-Based Competitive Binding Assay for One-Step Quantitation of Hepatitis B Surface Antigen

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Figure Legends:

Figure S1 UV absorbance spectra of HBsAg (A), Cy5 (B), and Cy5-labeled HBsAg (C). Both the dye-to-protein ratio of the conjugated protein (i.e. Cy5-labeled HBsAg) and the molar concentrations of the sample were calculated based on the Beer's law, as described in the text. The average dye-to-protein ratio was estimated to be 1.01.

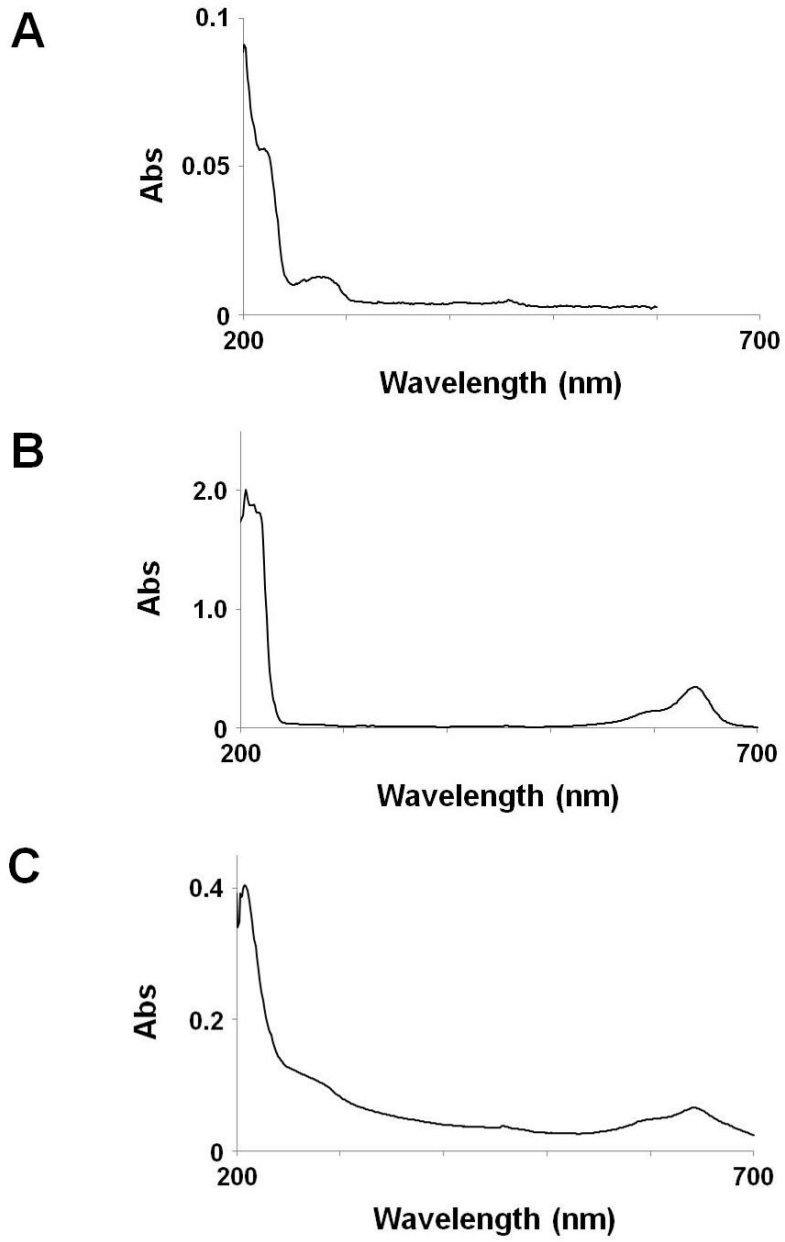
Figure S2 UV absorbance spectra of thiol-functionaled anti-HBsAg aptamer (A), Cy3 (B), and Cy3-labeled aptamer (C). Both the dye-to-RNA ratio of the conjugated material (i.e. Cy3-labeled aptamer) and the molar concentrations of the sample were calculated in the similar manner as above. The average dye-to-RNA ratio was estimated to be 1.04.

Figure S3 Control experiment in the absence of Cy5-labeled HBsAg, demonstrating that the fluorescence intensity of Cy3-labeled aptmaer is not affected by the increased analyte concentration.

Figure S4 Case report forms of 25 HBsAg-positive patients (A) and 19 HBsAg-negative patients (B). Quantitation of HBsAg was obtained from Abbott Architech HBsAg assay, and DNA quantitation from Abbott RealTime PCR HBV assay. The assays were performed according to the manufacturer’s protocols, and the amount of HBsAg in the sample was calculated based on the relative light units (RLUs) detected by the Architect Immunoassay System optics.

Figure S5 Experimental results of aptamer- and FRET-based quantitative assay for 25 HBsAg-positive (A) and 19 HBsAg-negative samples (B). The label of y-axis is the fluorescence intensities at 570 nm obtained from the aptamer- and FRET-based competitive binding assay, which can be converted to the amount of HBsAg in the sample based on the results shown in Fig. 3A.

Figure S1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0
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Wavelength (nm)
B
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0
Abs
200
700
Wavelength (nm)
C
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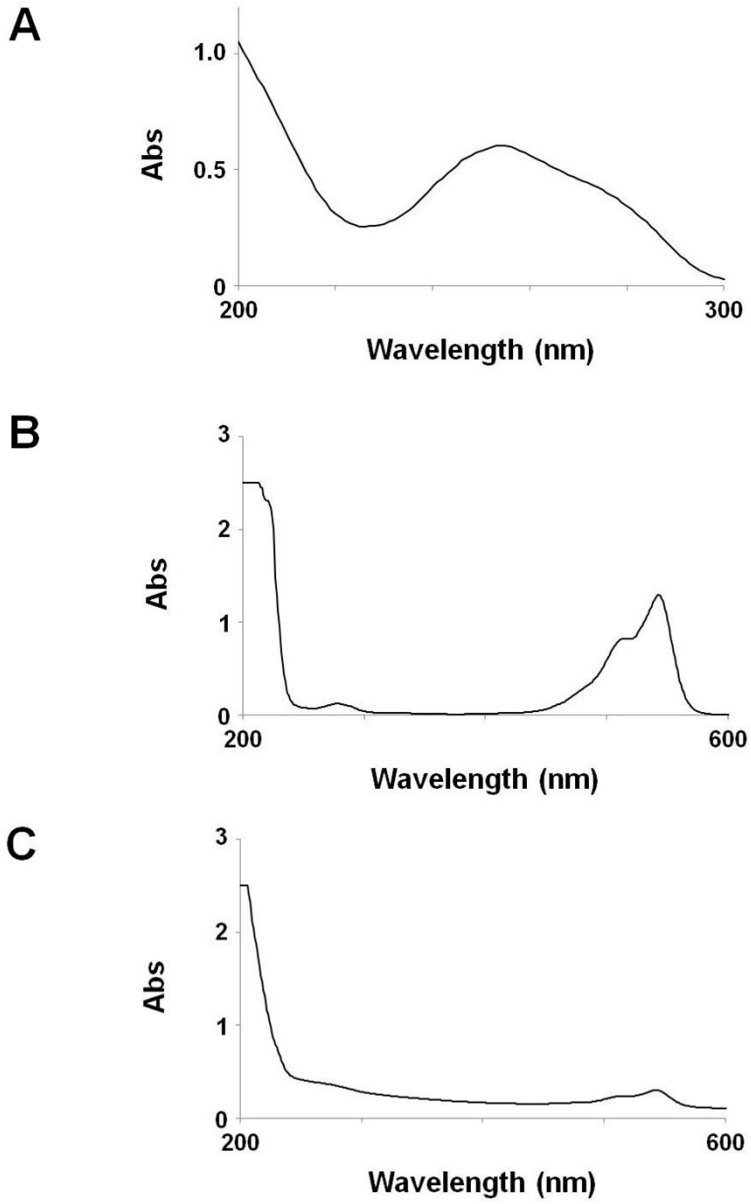
0.2
0
Abs
200
700
Wavelength (nm)

Figure S2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0
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300
Wavelength (nm)
B
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2
1
0
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200
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Wavelength (nm)
C
3
2
1
0
Abs
200
600
Wavelength (nm)

Figure S3

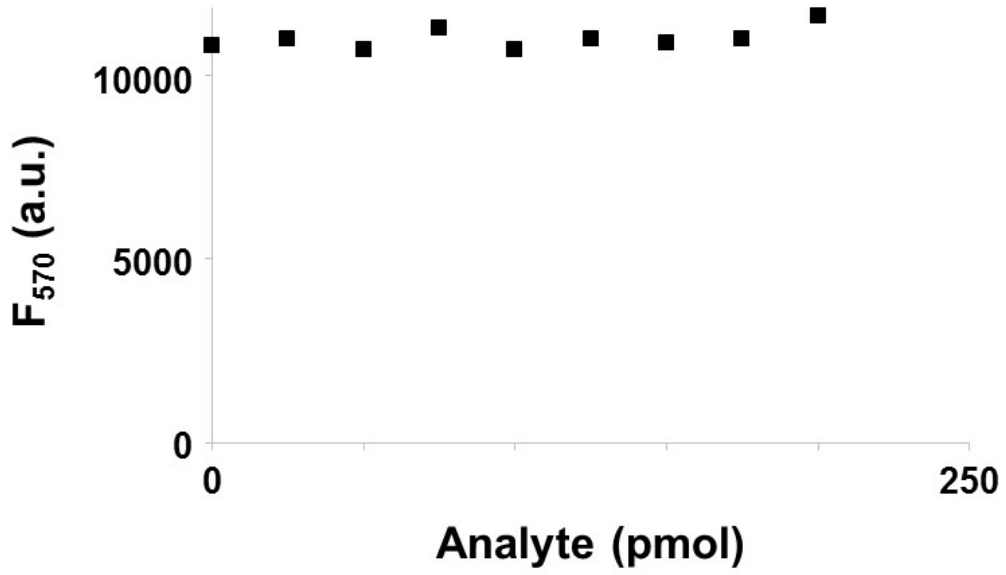


Figure S4

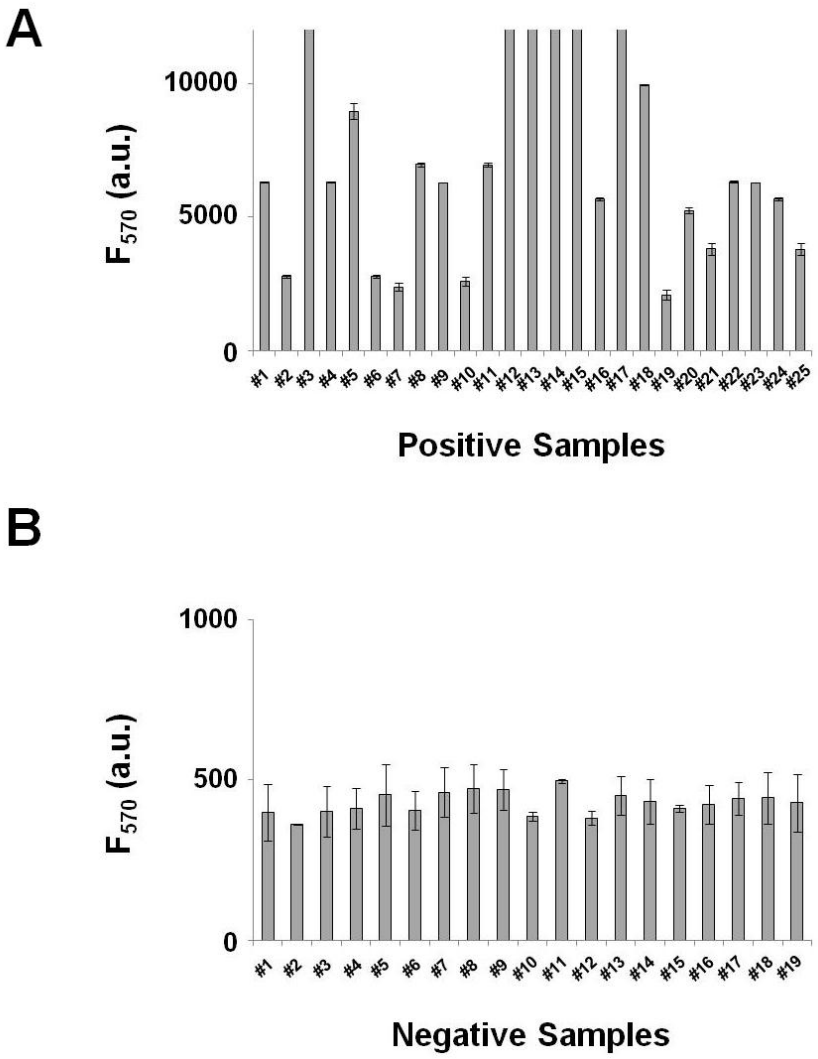
A

Serial number	Sex	Age	Test Date	HBsAg Positive	HBV DNA
1	M	45	2013-11-29	Positive(Pt's S/CO: 3677.82, Cut-off S/CO:≥1.00)	10,682 IU/mL
2	M	51	2013-12-02	Positive(Pt's S/CO: 801.51, Cut-off S/CO:≥1.00)	167 IU/mL
3	M	34	2013-12-03	Positive(Pt's S/CO: 4287.52, Cut-off S/CO:≥1.00)	12,103 IU/mL
4	F	57	2013-12-04	Positive(Pt's S/CO: 3673.50, Cut-off S/CO:≥1.00)	171 IU/mL
5	M	58	2013-12-05	Positive(Pt's S/CO: 5009.77, Cut-off S/CO:≥1.00)	167 IU/mL
6	F	52	2013-12-06	Positive(Pt's S/CO: 786.20, Cut-off S/CO:≥1.00)	791,774,064 IU/mL
7	M	72	2013-12-06	Positive(Pt's S/CO: 172.54, Cut-off S/CO:≥1.00)	30 IU/mL
8	M	54	2013-12-09	Positive(Pt's S/CO: 4750.84, Cut-off S/CO:≥1.00)	829 IU/mL
9	M	56	2013-12-10	Positive(Pt's S/CO: 4935.18, Cut-off S/CO:≥1.00)	544 IU/mL
10	M	48	2013-12-10	Positive(Pt's S/CO: 455.73, Cut-off S/CO:≥1.00)	427 IU/mL
11	M	51	2013-12-10	Positive(Pt's S/CO: 4042.94, Cut-off S/CO:≥1.00)	39 IU/mL
12	M	46	2013-12-10	Positive(Pt's S/CO: 3782.10, Cut-off S/CO:≥1.00)	2,886,916 IU/mL
13	M	42	2013-12-11	Positive(Pt's S/CO: 4212.30, Cut-off S/CO:≥1.00)	65,600 IU/mL
14	F	59	2013-12-11	Positive(Pt's S/CO: 3935.72, Cut-off S/CO:≥1.00)	827,771 IU/mL
15	M	58	2013-12-12	Positive(Pt's S/CO: 6470.34, Cut-off S/CO:≥1.00)	1,002,038 IU/mL
16	M	54	2013-12-12	Positive(Pt's S/CO: 2798.36, Cut-off S/CO:≥1.00)	2,030 IU/mL
17	M	41	2013-12-13	Positive(Pt's S/CO: 4662.87, Cut-off S/CO:≥1.00)	3,520,462 IU/mL
18	M	58	2013-12-16	Positive(Pt's S/CO: 5227.40, Cut-off S/CO:≥1.00)	265 IU/mL
19	F	45	2013-12-16	Positive(Pt's S/CO: 26.01, Cut-off S/CO:≥1.00)	20 IU/mL
20	M	64	2013-12-17	Positive(Pt's S/CO: 2237.65, Cut-off S/CO:≥1.00)	959 IU/mL
21	M	54	2013-12-17	Positive(Pt's S/CO: 1814.23, Cut-off S/CO:≥1.00)	18 IU/mL
22	F	54	2013-12-18	Positive(Pt's S/CO: 3681.98, Cut-off S/CO:≥1.00)	1,479,190 IU/mL
23	F	54	2013-12-18	Positive(Pt's S/CO: 3592.93, Cut-off S/CO:≥1.00)	15 IU/mL
24	F	68	2013-12-19	Positive(Pt's S/CO: 2774.99, Cut-off S/CO:≥1.00)	781 IU/mL
25	M	59	2013-12-19	Positive(Pt's S/CO: 1148.95, Cut-off S/CO:≥1.00)	612,216,641 IU/mL

B

Serial number	Sex	Age	Test Date	HBsAg Negative	HBV DNA	HBV Ab(IU/L)
1	M	58	2013-12-11	Negative		<10
2	M	73	2013-12-11	Negative		<10
3	F	71	2013-12-12	Negative		<10
4	F	48	2013-12-12	Negative		<10
5	F	58	2013-12-13	Negative		<10
6	M	43	2013-12-13	Negative		<10
7	F	49	2013-12-13	Negative		<10
8	F	47	2013-12-16	Negative		<10
9	F	58	2013-12-16	Negative		<10
10	F	59	2013-12-16	Negative		<10
11	F	43	2013-12-17	Negative		<10
12	M	72	2013-12-17	Negative		<10
13	F	50	2013-12-18	Negative		<10
14	M	55	2013-12-19	Negative		<10
15	F	67	2013-12-19	Negative		<10
16	M	54	2013-12-19	Negative		<10
17	F	54	2013-12-20	Negative		<10
18	M	51	2013-12-20	Negative		<10
19	M	61	2013-12-20	Negative		<10

Figure S5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Positive Samples
B
1000
500
0
F_{570} (a.u.)
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18 #19
Negative Samples