Electronic Supplementary Information (ESI)

Smartphone-based Microplate Reader for High-throughput Quantitation of Disease Markers in Serum

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Additional experimental details and the validation of smart-ELISA system with the detection of human serum albumin (HSA) (5 pages).

1. Additional Experimental Details

1.1. Details of the components to build the optical attachment. The LED lights were purchased from Anhui Aihaidi Lighting Technology Co., LTD (Huangshan, China). Their specifications are: 12 V/20mA, 3920 lux, with emission from 400 to 730 nm. As shown in Figure S1, we have determined that there are two main peaks (450 nm and 550 nm) in the emission spectrum. The polystyrene (PS) diffuser (130 × 90 × 1.0 mm; diffuse index: ≥0.75; haze: mix 99%; light transmittance: standard value ± 3 %) and polymethyl methacrylate (PMMA) light guide plate (130 × 90 × 2.0 mm; impact strength: 15 J/m; haze: ≥92.5%; flexural strength: 3300 MPa) were purchased from Fengsheng Opto-electronic Co., Ltd (Changzhou, China).

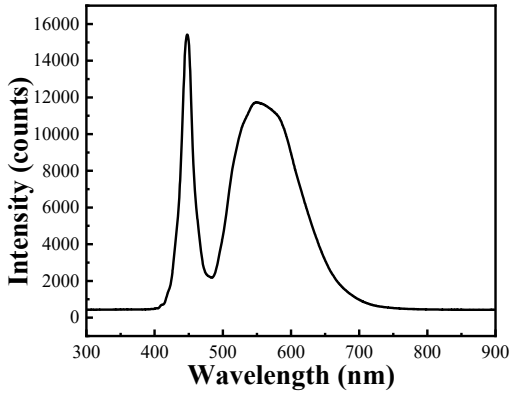

Figure S1. The emission spectrum of the LED lights used to build the optical attachment for performing smart-ELISA measurements.

1.2 Coding 96 wells for imaging and software analysis. As shown in Figure S2, for the convenience of imaging and data analysis, the standard 96-well microplate was coded by numbering the two dimensions with alphabets and numbers, respectively.

	H	G	F	E	D	C	B	A
1	H1	G1	F1	E1	D1	C1	B1	A1
2	H2	G2	F2	E2	D2	C2	B2	A2
3	H3	G3	F3	E3	D3	C3	B3	A3
4	H4	G4	F4	E4	D4	C4	B4	A4
5	H5	G5	F5	E5	D5	C5	B5	A5
6	H6	G6	F6	E6	D6	C6	B6	A6
7	H7	G7	F7	E7	D7	C7	B7	A7
8	H8	G8	F8	E8	D8	C8	B8	A8
9	H9	G9	F9	E9	D9	C9	B9	A9
10	H10	G10	F10	E10	D10	C10	B10	A10
11	H11	G11	F11	E11	D11	C11	B11	A11
12	H12	G12	F12	E12	D12	C12	B12	A12

Figure S2. Coding of a standard 96-well microplate for imaging and data analysis.

1.3. Conversion formula of RGB to CMYK values. After obtaining the image of a 96-well microplate using the smartphone App, the RGB values of each well was analyzed and converted to CMYK values according to the following equations. The value range of R, G or B is from 0 to 255, while C, M, Y, K is from 0 to 100.

$$R' = R/255$$

$$G' = G/255$$

$$B' = B/255$$

$$K = 1 - \max(R', G', B')$$

$$C = (1 - R' - K) \times 100 / (1 - K)$$

$$M = (1 - G' - K) \times 100 / (1 - K)$$

$$Y = (1 - B' - K) \times 100 / (1 - K)$$

1.4. Analysis of blank microwells for normalization. To further explore the background interference during the process of image analysis, the Y values of blank microwells (denoted as Y_0) and yellow ink filled ones (Y) were both determined with the smart-ELISA. Afterwards, the ratios of Y/Y_0 were calculated for comparison (Figure S3).

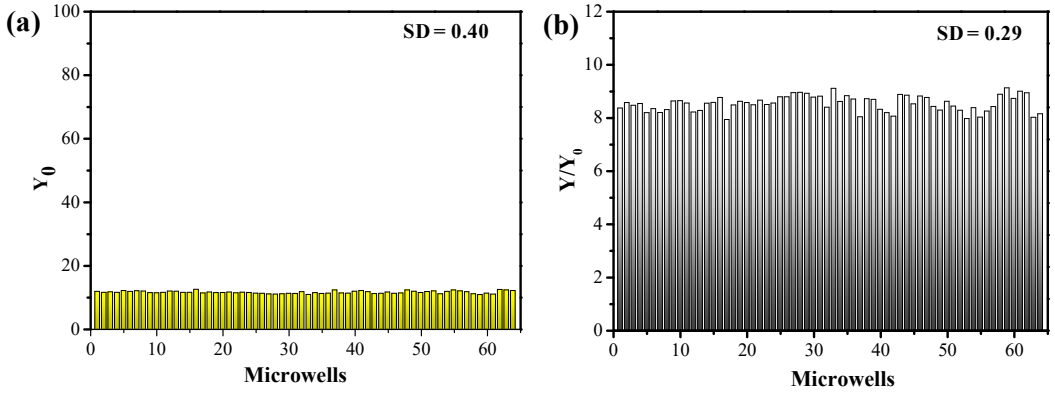

Figure S3. (a) Y values of 64 blank wells obtained using the smart-ELISA system (denoted as Y_0); (b) ratio of Y/Y_0 in each well, for which the Y values were obtained by filling these wells with the same yellow ink solution as used in Figure 3 of the main text.

2. Validation of the Smart-ELISA System with the Detection of Human Serum Albumin (HSA)

2.1 Sample preparation. HSA is the most abundant protein in blood plasma with a concentration of approximately 35–50 g/L (3.5–5.0 g/dL).[S1] As a transporter for small hydrophobic molecules, it plays a critical role in maintaining oncotic pressure and regulating the fluid distribution in human body. To verify the feasibility of the smartphone-based ELISA reading system, HSA was selected as the model analyte. The original concentration of HSA standard solution from the testing kit is 42.9 g/L, to get a wider concentration gradient, pure HSA was added, then diluted it with citrate buffer to obtain a series of HSA standard samples (1 – 200 g/L).

2.2 Principle of human serum albumin (HSA) detection. The detection principle of HSA was based on its immediate chromogenic reaction with bromocresol (an indicator).[S2,S3] The binding of bromocresol green to HSA changes the solution color from blue-green to yellow-green. Figure S4a is the UV/Vis absorption spectra of the solution with different concentrations of HSA. The two characteristic peaks at 420 nm and 630 nm changes substantially; Figure S4b shows the linear

correlation (R^2 of 0.9994) between the absorbance at 630 nm and the concentration of HSA in the standard solutions.

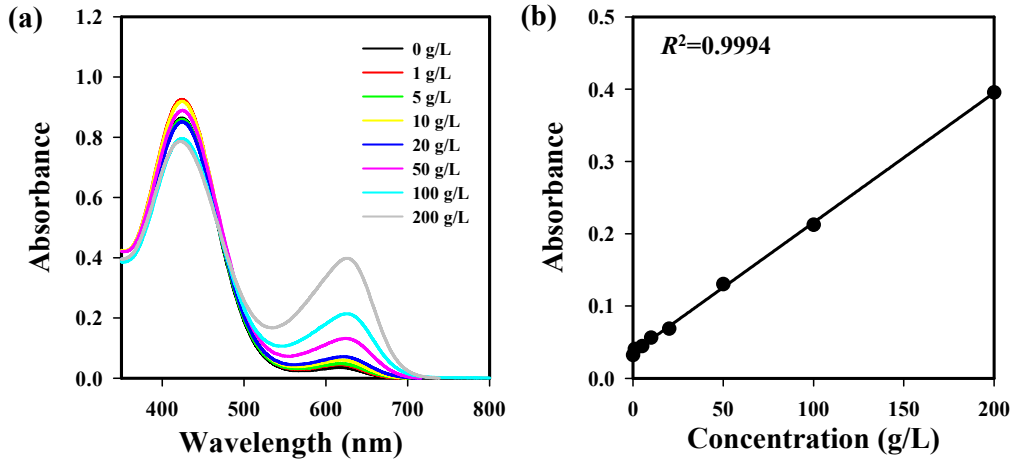

Figure S4. (a) UV-vis absorption spectra of the chromogenic solutions in the presence of different concentrations of HSA; (b) the absorbance at 630 nm as function of the HSA concentration in the standard solutions. The solid line is the best linear fit to the experimental data with the R^2 value listed.

2.3 Performance evaluation of the smart-ELISA system. As the chromogenic reaction mentioned above could be used for the quantitative detection of HSA, we further performed the quantitation of HSA with the smart-ELISA system. As showed in Figure S5a, the G values of the HSA standard samples decrease with increasing the HSA concentrations, and there is an excellent correlation between them (R^2 = 0.9935). This result proved the feasibility of this system for the quantitative detection of HSA. To further assess the accuracy and reliability of the detected results, the same set of HSA standard samples were also analyzed using a commercial plate reader. As shown in Figure S5b, the results of smart-ELISA are consistent with the conventional ELISA tests (R^2 = 0.9994 for the liner fit between the two sets of data), which built our confidence to proceed with the detection of multiple biomarkers in serum as reported in the main text.

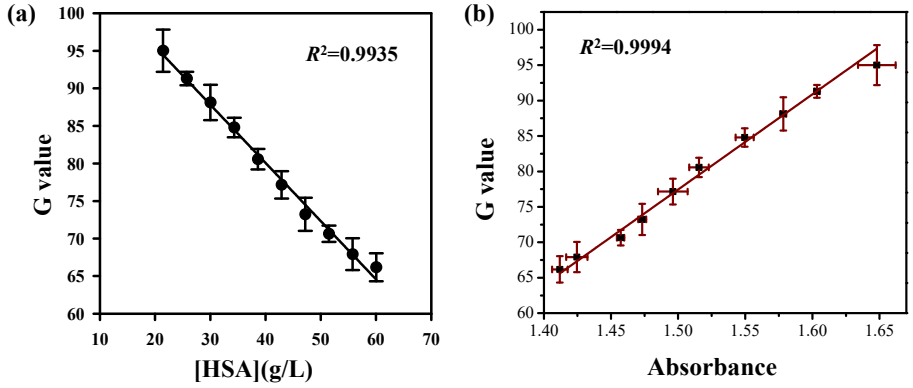

Figure S5. (a) The dependence of the color intensity (G value) on the concentration of HSA; (b) the correlation plot of the color intensity and the absorbance for the same set of HSA standard samples. The solid line is the best linear fit to the experimental data.

References

S1. "Harmonisation of Reference Intervals" (PDF). https://www.acb.org.uk/asset/EEA82309-7E93-417D-9B8991B0C65C52FB/, accessed Dec. 15, 2022.

S2. B. T. Doumas, W. A. Watson, H. G. Biggs, Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta* 1971, **31**, 87- 96.

S3. J. E. Gustafsson, Improved specificity of serum albumin determination and estimation of acute phase reactants by use of the bromocresol green reaction. *Clin. Chem.* 1976, **22**, 616-622.