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Smartphone-based immunoassay for the highly-sensitive point-of-care detection of human C-reactive protein in whole blood and serum

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We have developed a highly sensitive and cost-effective smartphone-based immunoassay (IA) for the point-of-care (POC) detection of human C-reactive protein (hCRP) in human whole blood and serum in ~20 min. The precise determination of CRP is essential for the diagnosis and management of neonatal sepsis, cardiovascular diseases, infectious and inflammatory conditions, meningitis and diabetes. It obviates the need of expensive instruments and special analytical skills, and employs the one-step kinetics-based sandwich IA procedure (Fig. 1A) that is superior to the commercial kit and our IA procedures[1-4] as it employs a highly simplified procedure with least number of steps and significantly reduced IA duration. The procedure involves the dispensing of capture anti-CRP antibody (Ab)-bound dynabeads (2.8 µm dia) and biotinylated anti-CRP detection Ab prebound to horseradish peroxidase (HRP)-labeled streptavidin (SA) to the bovine serum albumin-preblocked 96-well microtiter plate wells[5]. Subsequently, CRP spiked in 1:100 diluted human whole blood or serum is provided and incubated for 15 min at room temperature that leads to the formation of immune complex, which is then captured by the magnet and washed twice with washing buffer. The hCRP concentration is determined by smartphone-based image capture of the colorimetric product formed after 3,3□,5,5□-tetramethylbenzidine (TMB) substrate reaction, and subsequent image processing that provides nearly precise predictive absorbance readings from the pixel intensity of the captured image (Fig. 1C). The developed hCRP IA in human whole blood has dynamic range, limit of detection, analytical sensitivity, correlation coefficient (R^2), percentage coefficient of variance and half-maximal effective concentration (EC_{50}) of 0.33-81 ng/mL, 0.27 ng/mL, 0.5 ng/mL, 0.999, 0.5-4.6, and 16.8 ng/mL, respectively (Fig. 1). It has same precision as CRP ELISA (Pearson correlation coefficient of 1) (Fig. 1E). The developed technology has immense potential to develop low-cost POC *in vitro* diagnostic kits/devices to detect analytes in various bioanalytical settings.

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(A)

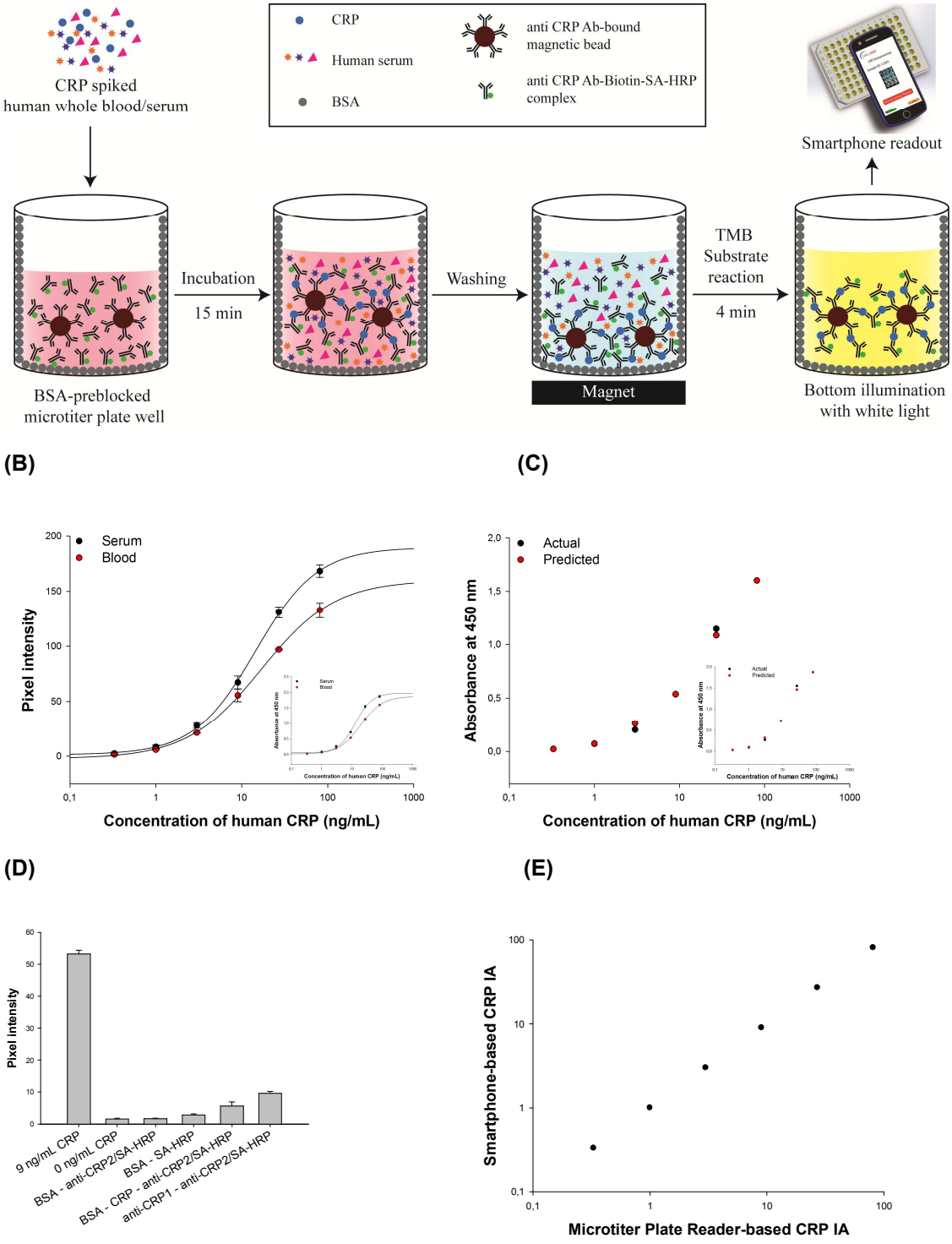

Figure 1. Smartphone-based human C-reactive protein (hCRP) immunoassay in 20 min. (A) Schematic, (B) Smartphone-based detection of CRP in human serum and whole blood (inset shows the results obtained by the microtiter plate reader), (C) Overlay of smartphone-based predictive absorbance readings and the microtiter plate reader-based actual absorbance readings in human whole blood (inset shows the overlay of same curves for serum), (D) Experimental process controls (anti-CRP1 and anti-CRP2 are the capture and detection antibodies, respectively), and (E) Correlation of developed smartphone-based CRP immunoassay in whole blood with the microtiter plate reader-based immunoassay.

Keywords: Smartphone, Immunoassay, Point-of-Care, Colorimetric readout