One-step kinetics-based immunoassay for the detection of human fetuin A in 25 min

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A novel 1-step kinetics-based immunoassay (IA) was developed for the rapid detection of human fetuin A (HFA) in just 25 min. It involves the preliminary preparation of anti-HFA antibody (Ab)-bound and bovine serum albumin (BSA)-blocked 96-well microtiter plate (MTP) that can be stored at 4ºC in 0.1M PBS, pH 7.4 for 2 months without any decrease in functional activity. The IA (Fig. 1A) involves the sequential dispensing of biotinylated anti-HFA detection Ab pre-conjugated to streptavidin-labeled horse radish peroxidase and analyte sample. The MTP was incubated for 15 min at room temperature, which leads to the formation of sandwich immune complexes, and then washed twice to take out the excess unbound reagents. Finally, the enzyme-substrate reaction was performed by providing the TMB substrate, stopping the reaction after 4 min, and measuring absorbance at 450 nm with reference at 540 nm. The leach-proof Ab-bound MTP was prepared by proprietary 1-step Ab-immobilization strategy[1], where anti-HFA Ab, mixed with 1% (v/v) 3-aminopropyltriethoxysilane (APTES) in the ratio of 1:1 (v/v), was dispensed into MTP wells and incubated for 30 min. The developed IA is the most rapid HFA IA, which has 12- and 7-fold reduced IA duration than the conventional and our previously-developed procedures[2-4], respectively, when Ab-bound and BSA-blocked MTPs were used in all formats. It detects 0.1-283 ng mL^{-1} of HFA with limit of detection, analytical sensitivity and EC_{50} of 0.3 ng mL^{-1}, 1 ng mL^{-1} and 24.2 ng mL^{-1}, respectively. The intra- and inter-day variability were 1.8-7.3 and 2.4-12.1, respectively. The developed IA detects HFA-spiked in diluted human serum and whole blood (Fig. 1B). It has no interference with IA components (Fig. 1C); optimized for incubation time (Fig. 1D) and number of washings (Fig. 1E); and, correlates well with the commercial IA with percentage recoveries between 91-108.

References:

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(A)

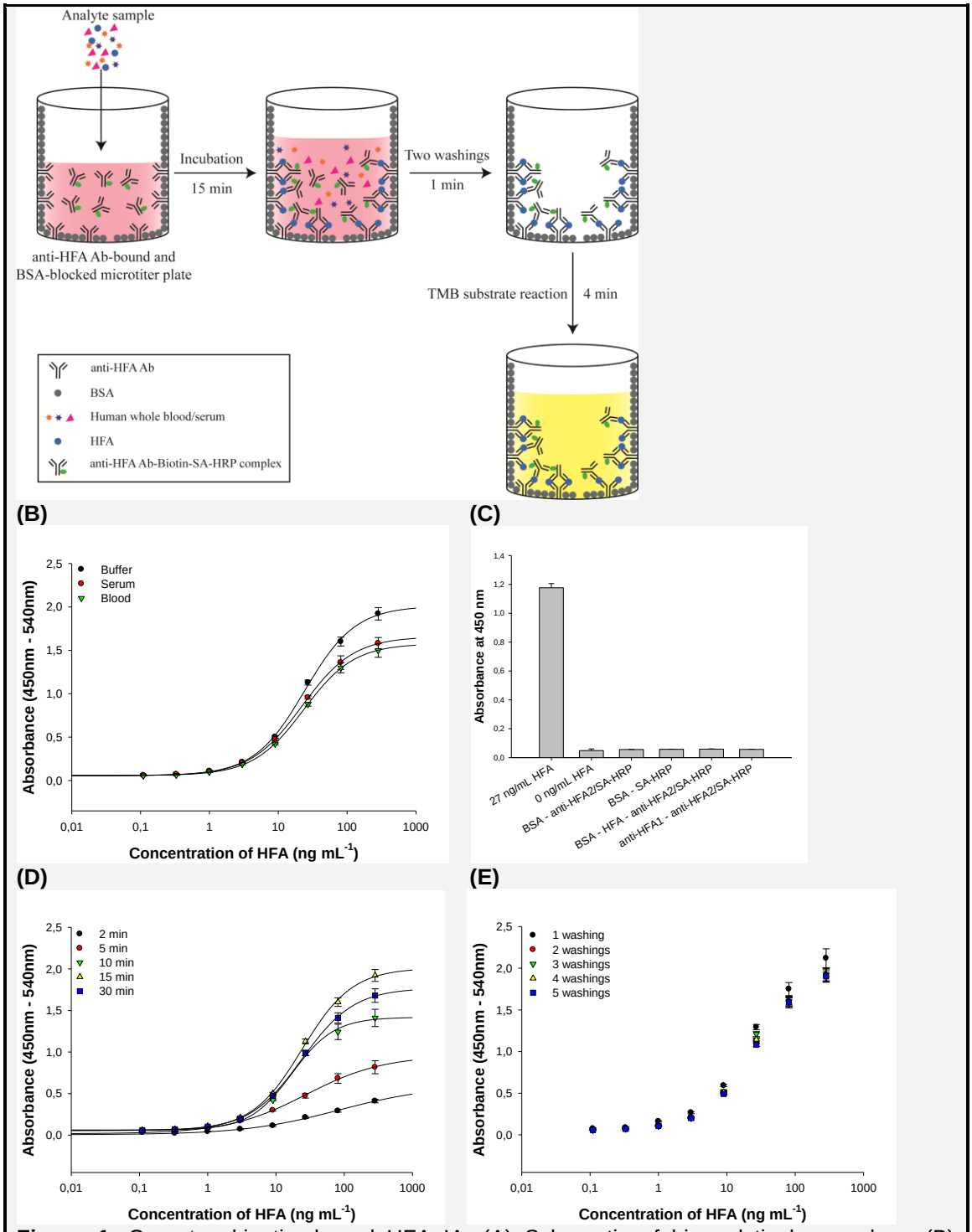

Figure 1. One-step kinetics-based HFA IA. (A) Schematic of bioanalytical procedure, (B) Detection of HFA in buffer and HFA spiked in diluted human serum and whole blood, and (C) Specific detection of HFA with respect to various experimental controls. Optimization of (D) incubation time and (E) number of washings for the developed IA.