Smartphone-based rapid and highly sensitive immunoassay for human fetuin A

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Summary

A rapid and highly-sensitive smartphone-based immunoassay (SIA) is reported for the detection of human fetuin A (HFA), an important biomarker that needs to be determined in cases of atherosclerosis, diabetes, inflammatory disorders, cardiovascular diseases and other pathophysiological conditions. The developed SIA employs a highly simplified procedure based on covalently bound capture antibody (Ab) and one-step kinetics-based IA. The dynamic range, limit of detection (LOD) and limit of quantification (LOQ) were 0.1-243 ng mL-1, 0.1 ng mL-1 and 0.51 ng mL-1, respectively.

1   Material and Methods

The carbodiimide-activated anti-human CRP capture antibody (Ab) was admixed with 1% (v/v) 3-aminopropyltriethoxysilane (APTES) in 1:1 (v/v) and subsequently dispensed onto a KOH-pretreated microtiter plate (MTP) [1], which led to the leach-proof covalent binding of Ab to the MTP. The one-step kinetics-based immunoassay (IA) [2] enables the formation of sandwich immune complex in just 15 min employing the least number of process steps (Fig. 1A). The readout of the colorimetric IA was performed by our proprietary smartphone-based colorimetric reader (SBCR) (Fig. 2B) [3, 4] and a novel image analysis algorithm (Fig. 3C).

2   Results

The developed IA detected HFA with a dynamic range, limit of detection (LOD) and limit of quantification (LOQ) of 0.1-243 ng mL-1, 0.1 ng mL-1 and 0.51 ng mL-1, respectively. Therefore, it detects the entire clinically-relevant pathophysiological range of HFA i.e. 0.15-600 µg mL-1 after appropriate sample dilution (Fig. 3D). The procedure enables highly specific HFA detection without any interference from different immunological reagents, as shown by the use of various experimental process controls (Fig. 3E). The HFA levels in the ethylenediaminetetraacetic acid (EDTA) plasma samples of patients correlat-ed well with those obtained by the conventional enzyme-linked immunosorbent assay (ELISA), thereby demonstrating the high analytical precision SIA. Moreover, it also detects HFA in complex sample matrix of human whole blood and serum, as demonstrated by the spiking of HFA in these matrices. The functional stability of the Ab-bound and BSA-blocked MTPs for SIA was also assessed, as they are routinely employed in the existing bioanalytical settings such as clinical diagnostic labs, where greater number of samples need to be analysed in shortened time. There was no significant loss of functional activity for 6 weeks.

3   Conclusions and Outlook

The use of our low-cost SBCR is a major advance, which makes the developed IA highly prospective for the development of cost-effective and reliable in vitro diagnostics (IVD) for the developing nations and decentralized settings. We have developed various proprietary SBCRs [3, 4] that are as highly sensitive as the expensive MTP readers but have critically reduced cost. Thereby, the developed SIA is a highly affordable IA format that can be reliably employed for HFA detection in any bioanalytical lab.
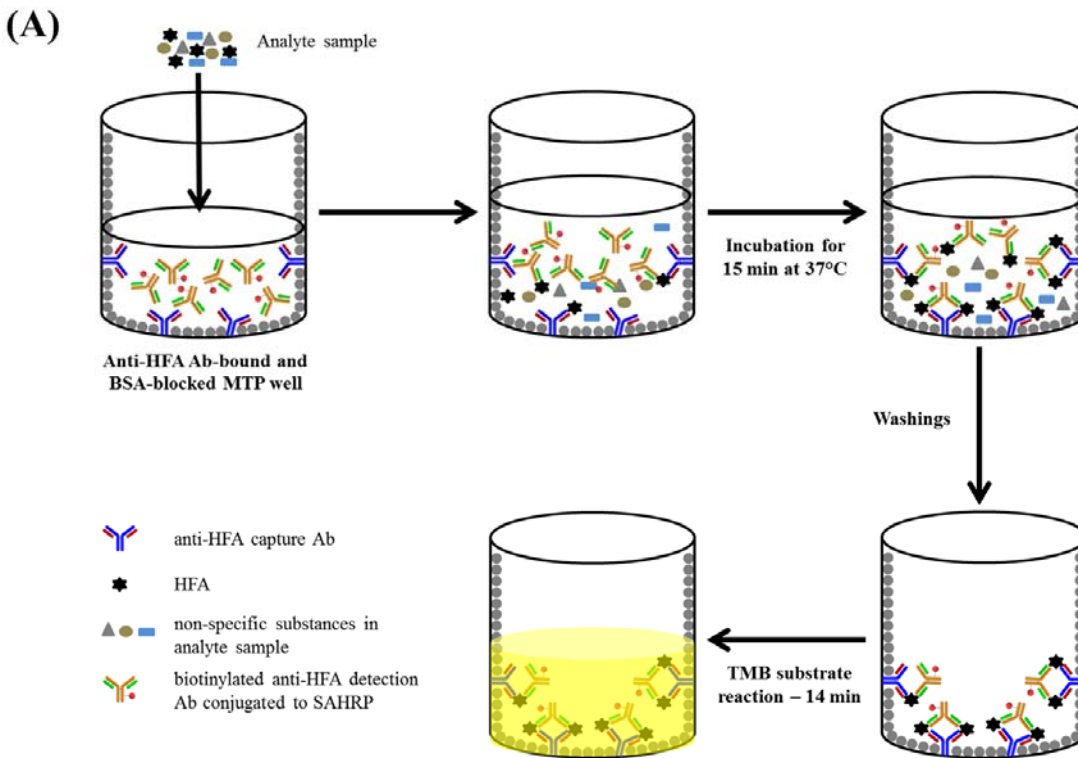
Figure 1: (A) Developed HFA IA.
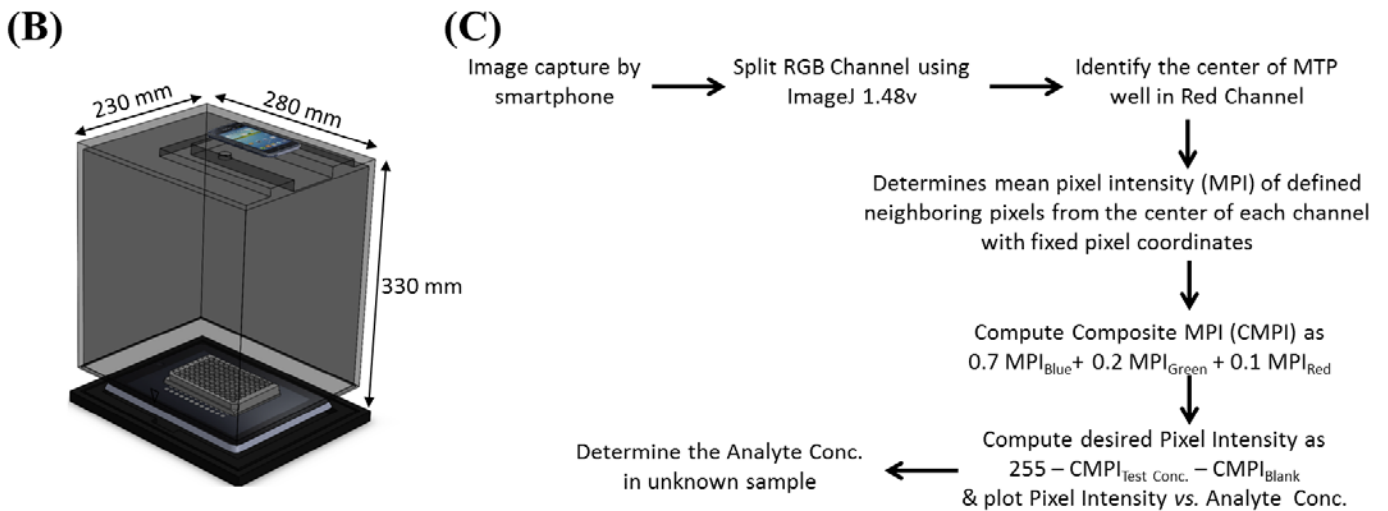
Figure 2: (B) Smartphone readout using iPAD4 screen-saver-based bottom illumination. (C) Image analysis algorithm.

(D)

(E)
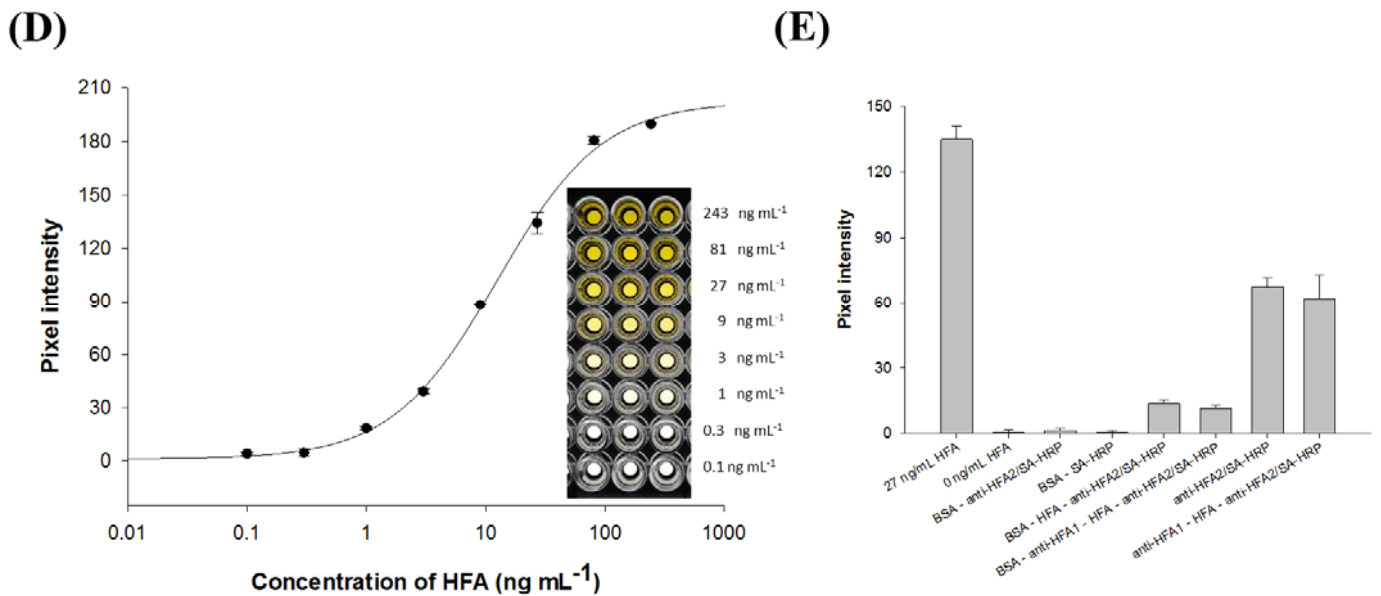
Figure 3: (D) Detection of HFA. (E) Experimental controls.

4 References

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