A rapid one-step kinetics-based immunoassay procedure for the highly-sensitive detection of C-reactive protein

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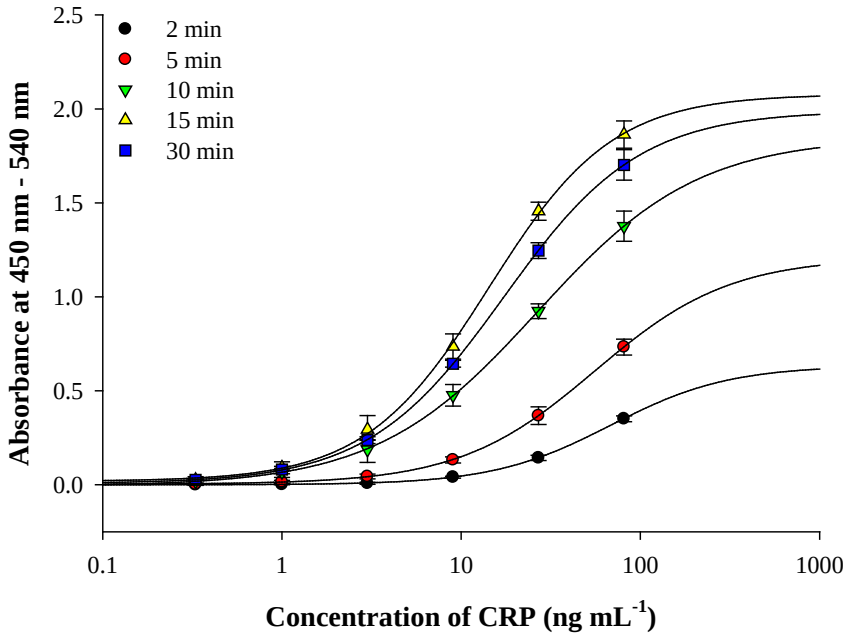
(A)



(B)

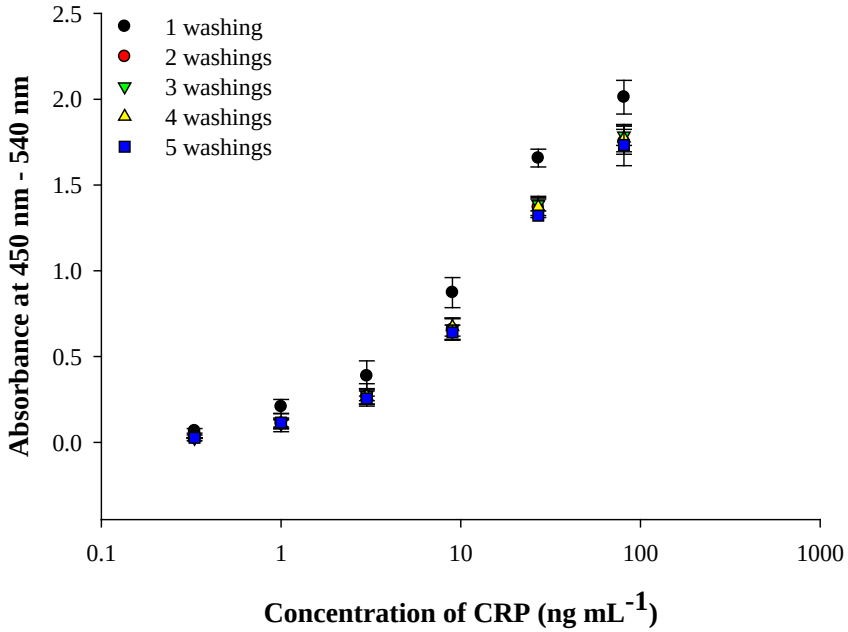
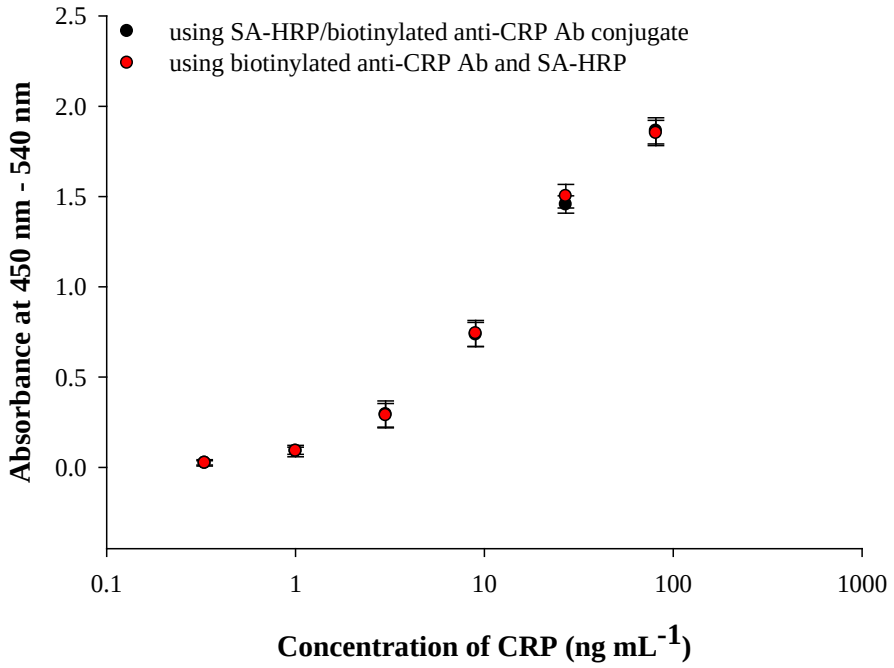


Figure S1. Optimization of (A) incubation time and (B) number of washings for one-step kinetic-based sandwich ELISA[1]. All experiments were performed in triplicate and the error bars represent the standard deviation. Reproduced with permission from Elsevier Inc.

(A)



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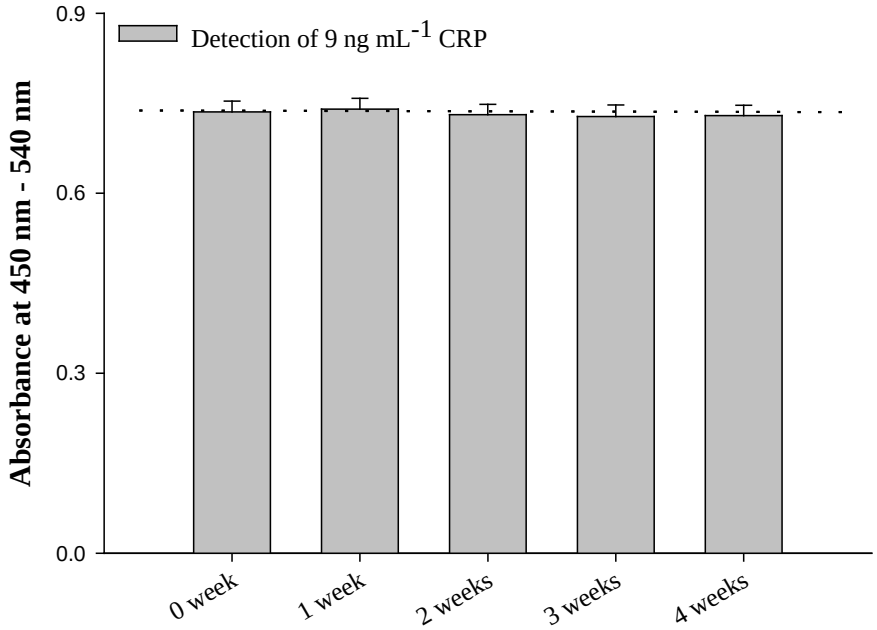

Figure S2. (A) Comparison of one-step kinetics-based sandwich ELISA using the developed SA-HRP/biotinylated anti-CRP Ab conjugate and the two-step binding of biotinylated anti-CRP

Ab and SA-HRP (as used in conventional sandwich ELISA)[1]. (B) Stability of the one-step kinetics-based sandwich ELISA solution, comprising anti-CRP capture Ab-bound Dynabeads® and biotinylated anti-CRP detection Ab preconjugated to SA-HRP, when stored at 4 ºC in BSA-preblocked MTPs[1]. All experiments were performed in triplicate and the error bars represent the standard deviation. Reproduced with permission from Elsevier Inc.

Table S1. Process steps involved in one-step kinetics-based and conventional sandwich ELISA procedures[1]. Reproduced with permission from Elsevier Inc.

One-step kinetics-based sandwich ELISA	**Conventional sandwich ELISA**
Mix all immunoassay components with the analyte sample (1 min)	Add capture antibody & incubate (overnight)
	Wash 6 times
Incubate for 15 min	Add blocking solution & incubate for 1 h
Wash 2 times using a magnetic holder (~2 min)	Wash 6 times
Enzyme-substrate reaction – 4 min	Add sample & incubate for 2 h
	Wash 6 times
Read	Add biotinylated detection antibody & incubate for 2 h
	Wash 6 times
	Add streptavidin-HRP & incubate for 20 min
	Wash 6 times
	Enzyme-substrate reaction for 20 min
	Read

Table S2. Analytical parameters of one-step kinetic-based sandwich ELISA[1]. Reproduced with permission from Elsevier Inc.

Analytical parameters	**One-step kinetics-based sandwich ELISA**
Immunoassay duration	30 min (~12-fold less than conventional procedure (excluding antibody immobilization in both procedures))
Requirement for Reagents	2.5-fold reduced requirement for reagents than that of the conventional procedure
Number of process steps	Employs least process steps
Number of washings required	Only 2 (in comparison to 24 in the conventional procedure)
Detects the clinically-relevant hsCRP range (3-80 µg mL^{-1})	Yes
Detection range	0.3-81 ng mL^{-1}
Linear range	1-81 ng mL^{-1}
LOD (ng mL^{-1})	0.4
Analytical sensitivity (ng mL^{-1})	0.7
% CV	
Intra-day (n=5)	0.7-10.8
Inter-day (n=5)	1.6-11.2

REFERENCES

1. Vashist, S. K. *et al.* One-step kinetics-based immunoassay for the highly-sensitive detection of C-reactive protein in less than 30 minutes. *Anal. Biochem.* **456**, 32-37 (2014).