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# Rapid Colorimetric Determination of Procalcitonin Using Magnetic Separation and Enzymatic Catalysis

Yun Zhang[a,b,†], Xiaohui Si[a,b,†], Miaomiao Zhang[a,†], Xue Yang[a,b], Huan Yuan[a,b], Xiangpeng Wang[a,b], Yang Zhang[c], and Hui Wang[a,b]

[a]Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan, China; [b]Henan Key Laboratory of Immunology and Targeted Therapy, Xinxiang Medical University, Xinxiang, Henan, China; [c]School of Science, Harbin Institute of Technology (Shenzhen), Shenzhen, Guangdong, China

**ABSTRACT**

Human procalcitonin is an early diagnostic biomarker for sepsis and bacterial infections and can be used in distinguishing bacterial infections from viral infections. In this study, a colorimetric sensing platform for the rapid determination of procalcitonin was developed. The approach involves the capture of procalcitonin by immunomagnetic beads, and a detection antibody labeled with horseradish peroxidase to perform sandwich format, where it catalyzes the oxidation of 3,3′,5,5′–tetramethylbenzidine to produce the colorimetric signal. Under the optimal conditions, a detection limit of 0.04 ng/mL ($3\sigma$) was obtained within the calibration range 0.1–10 ng/mL. The proposed method was performed in less than 90 min and exhibited good specificity without interferences from other biomarkers including C-reactive protein and human serum albumin. Overall, the proposed method provided a new alternative strategy for procalcitonin detection due to its sensitive, rapid, specific, and simple characteristics. This method is suitable for rapid screening of various biomedical targets.

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**KEYWORDS**
Colorimetric determination; enzymatic catalysis; human procalcitonin; magnetic beads

## Introduction

Bacterial infections and sepsis along with consecutive multiple organ dysfunction or failure are the major reasons for morbidity and mortality in modern intensive care units (Carrigan, Scott, and Tabrizian 2004; Yang et al. 2014). Timeliness in a correct diagnosis is necessary to discriminate viral and bacterial sepsis or in the rapid identification of infection origin (Bone et al. 1989; Karzai et al. 1997; Schuetz et al. 2013). Human procalcitonin is a precursor protein of calcitonin with a 13 kDa molecular mass, and acts as

**CONTACT** Hui Wang huiwang65@yeah.net School of Science, Harbin Institute of Technology (Shenzhen), Shenzhen, Guangdong, 518055, China; Yun Zhang zhangyun0126@126.com Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan, 453003, China; Yang Zhang zhangyang07@hit.edu.cn Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine School of Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan, 453003, China.

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[†]These authors contributed equally to this work.

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a well-known biomarker for bacterial infection and sepsis (Assicot et al. 1993; Bloos and Reinhart 2014). Compared with other biomarkers such as C-reactive protein and tumor necrosis factor alpha, procalcitonin demonstrated the best specificity and the greatest sensitivity (Gogos et al. 2000; Sener et al. 2013). Procalcitonin level can be as low as 0.1 ng/mL in healthy state, while it can reach up to more than 100 ng/mL during bacterial infections and sepsis (Herzum and Renz 2008; Westwood et al. 2015). Therefore, accurate detection and monitoring of procalcitonin can be utilized to reflect the severity of sepsis and provide useful information to differentiate the diagnosis of infectious and noninfectious diseases and guide in antibiotic therapy (Rucker and Schumacher 2013; Schuetz, Raad, and Amin 2013).

Several immunoassays based on specific-recognition of antigen-antibody have been widely applied in bioanalysis and clinical chemistry (Murugaiyan et al. 2014; Valera, McClellan, and Bailey 2015). To date, several detection systems have been successfully developed for the determining procalcitonin, such as immunochromatographic tests (Toriyama et al. 2015), fluorescence immunoassay (Baldini et al. 2009), chemiluminescence immunoassay (Qi et al. 2013; Tian et al. 2014) and electrochemical immunoassay (Liu et al. 2014; Li et al. 2015; Shen et al. 2015). However, rare metal usage, complicated nanomaterial preparations, professional staff, or sophisticated instruments were required in these methods. Thus, more simpler and cost-effective methods are warranted.

Magnetic beads served as an ideal platform candidate due to their high target-capturing efficiency, rapid-binding kinetics, and simple processing procedures (Woolley and Hayes 2015; Zhang et al. 2016; Atanasova, Vasileva, and Godjevargova 2017). Owing to these merits of magnetic beads, an increased sensitivity and short assay time would be achieved. In our previous report, magnetic beads combining with catalytic fluorescence immunoassay have been proposed for HIV-1 p24 antigen detection (Zhang et al. 2016). Although fluorescent detection provides high sensitivity, colorimetric assays have gained considerable attention due to their simplicity and practicality, and can be performed just with ultraviolet-visible spectrometry or even possible with the naked eye without the need for expensive or complicated instruments (Jiang et al. 2010; Li et al. 2011; Ren et al. 2013).

Herein, a simple magnetic beads coupled enzymatic catalysis sensing platform was developed for colorimetric determination of procalcitonin. As shown in Figure 1, a sandwich-type immunoassay format was adopted using horseradish peroxidase labeled antibody as an enzyme tracer and 3,3′,5,5′-tetramethylbenzidine/$H_2O_2$ as the substrate to detect procalcitonin. Compared with the reported plasmonic sensors (de la Rica and Stevens 2012; Liang et al. 2015; Xianyu, Chen, and Jiang 2015), the proposed method did not utilize the noble metal nanoparticles and all of the required reagents are commercially available.

## Experimental

### *Materials and reagents*

Carboxyl-terminated magnetic beads (800 nm in diameter) at 10 mg/mL and magnetic separation racks were obtained from Jiayuan Quantum Dots (Wuhan, China) and Goldmag Biotech (Xi'an, China), respectively. The paired mouse anti-procalcitonin monoclonal antibody (1 mg/mL), horseradish peroxidase-mouse anti-procalcitonin

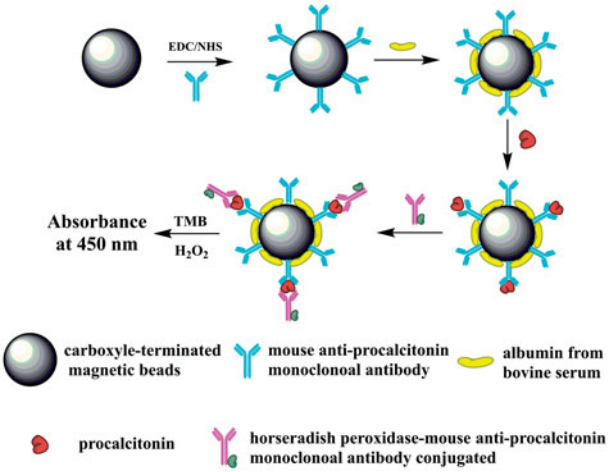


**Figure 1.** Schematic representation of the colorimetric sensing platform for the detection of procalcitonin where EDC is as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, NHS is as *N*-hydroxysuccinimide, and TMB is 3,3′,5,5′-tetramethylbenzidine.

monoclonal antibody conjugated (1 mg/mL), human recombinant procalcitonin (1 mg/mL), C-reactive protein (1 mg/mL), and human serum albumin (1 mg/mL) were obtained from Kitgen Biotech (Hangzhou, China). Human serum was obtained from Fankel Biological Technology (Shanghai, China). *N*-hydroxysuccinimide and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were acquired from Aladdin Industrial (Shanghai, China). Bovine serum albumin and skimmed milk powder were supplied by Roche (Germany) and Oxoid (England), respectively. 3,3′,5,5′-tetramethylbenzidine solution used for colorimetric detection was purchased from Biodragon Immunotechnologies (Beijing, China).

96-well serological microplates were acquired from Jet Bio-Filtration (Guangzhou, China). 0.01 mol/L phosphate-buffered saline (pH 7.4) was prepared by dissolving 8 g sodium chloride, 1.15 g disodium hydrogen phosphate, 0.24 g potassium chloride, and 0.2 g potassium dihydrogen phosphate in 1000 mL double distilled water, and 0.01 mol/L phosphate-buffered saline (pH 6.8) was adjusted by 0.1 mol/L hydrochloric acid. Phosphate-buffered saline with Tween 20 was processed by dissolving 500 µL of Tween-20 with 1000 mL 0.01 mol/L phosphate-buffered saline (pH 7.4). Other reagents and chemicals used were of analytical grade reagents and double distilled water (18.2 Ω).

## *Apparatus*

Ultraviolet-visible spectra were recorded by an ultraviolet-visible spectrophotometer UV-3600 (Shimadzu, Japan). Optical measurements were recorded using a Synergy H1 multimode microplate reader (Biotek, USA). Incubation was carried out by an incubator shaker ZWY-200D (Labwit Scientific, Shanghai, China). The magnetic beads were dispersed using an Oscillator Vortex Genie 2 (USA) to disperse the magnetic beads. A Delta 320 pH meter (Mettler Toledo, Switzerland) was used to adjust and measure the pH of buffer solutions.

### Preparation of the immunomagnetic beads

Carboxyl-terminated magnetic beads were utilized to prepare immunomagnetic beads by using a classic 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/*N*-hydroxysuccinimide amidization reaction. Magnetic beads (1 mg) were washed twice using 100 μL of 0.01 mol/L phosphate-buffered saline (pH 6.8) to balance the salt concentration and then were resuspended in 120 μL of 0.01 mol/L phosphate-buffered saline (pH 6.8). After that, 40 μL of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (50 mg/mL) and 40 μL of *N*-hydroxysuccinimide (25 mg/mL) were added and incubated.

After 30 min of activation, the magnetic beads were washed twice with 200 μL of 0.01 mol/L phosphate-buffered saline (pH 7.4) to remove 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and *N*-hydroxysuccinimide and dispersed in 100 μL of mouse anti-procalcitonin monoclonal antibody (0.5 mg/mL). After 4 h of reaction with gentle rotation at 37 °C and 180 rpm, 200 μL of phosphate-buffered saline with Tween 20 were used to wash the immunomagnetic beads twice to remove unconjugated mouse anti-procalcitonin monoclonal antibodies. The antibody-coated magnetic beads were blocked in 1 mL of 0.01 mol/L phosphate-buffered saline (pH 7.4) containing 5% bovine serum albumin and 15% skimmed milk at 37 °C for 2 h. After magnetic separation, the immunomagnetic beads were washed five times to remove the residual bovine serum albumin and skimmed milk, and then resuspended in 1 mL of 0.01 mol/L phosphate-buffered saline (pH 7.4) containing 0.1% bovine serum albumin and 0.1% $NaN_3$. Finally, the prepared immunomagnetic beads were stored at 4 °C until use.

### Procedure for the colorimetric sensing platform

For procalcitonin detection, colorimetric sensing platform was performed as outlined in Figure 1. Firstly, 15 μg of immunomagnetic beads were incubated (180 rpm) with 100 μL procalcitonin at 37 °C for 30 min. After magnetic separation, the resulted procalcitonin-loading immunomagnetic beads were washed five times with phosphate-buffered saline with Tween 20 and then incubated with 100 μL of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated at 1:2000 dilution ratio (dilution ratio is utilized to indicate the dilution factor) at 37 °C for 30 min. The formed sandwich structure of immunomagnetic beads/procalcitonin/horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated was washed five times by phosphate-buffered saline with Tween 20 and then incubated with 100 μL of chromogenic substrate containing 3,3′,5,5′-tetramethylbenzidine and $H_2O_2$ at 37 °C for 10 min. After that, 100 μL of 2 mol/L $H_2SO_4$ was added to terminate the enzymatic reaction. Following magnetic separation, the supernatant was transferred into the microplate for detecting the absorbance (450 nm).

## Results and discussion

### Optimization of the experimental conditions

Before initiation of the measurements, we ensured that the antibodies were conjugated to carboxyl-terminated magnetic beads. As shown in Figure S1, ultraviolet-visible absorption spectrophotometry was used to reveal the changes in the electronic spectra

before and after incubating mouse anti-procalcitonin monoclonal antibody with magnetic beads, indicating that the mouse anti-procalcitonin monoclonal antibody was successfully immobilized on the magnetic beads. The optimal experimental conditions were then characterized.

Generally, the sensitivity of an immunoassay was greatly influenced by the concentration of immuno-reagents and the conditions of immuno-reaction. In this work, in order to attain proper dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated, the amount of immunomagnetic beads added and the incubation time were optimized to obtain the highest $A/A_0$ ratio, where $A$ is the absorbance signal at 450 nm with the samples containing procalcitonin, while $A_0$ is the absorbance of phosphate-buffered saline under the same conditions.

The dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated was a critical factor that influences the sensitivity. The dilution ratio effects of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated on $A$, $A_0$, and $A/A_0$ were investigated. The dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated was optimized, ranging from 1:1000 to 1:5000. Figure 2(A) shows that too high a dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated may lead to unsaturated reaction, while low dilution ratio would cause non-specific adsorption on immunomagnetic beads and lower the sensitivity. As shown in Figure 2(B), the highest $A/A_0$ ratio was attained when the dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated was 1:2000. Hence, this ratio was selected for the subsequent measurements. The conditions were 15 μg immunomagnetic beads and the incubation times of the first and second binding events were both 30 min.

The effect of the addition of immunomagnetic beads on the sensitivity was evaluated by measuring $A$ and $A_0$, as the mass of immunomagnetic beads was increasing from 5 to 25 μg. As shown in Figure 2(C), when the addition of immunomagnetic beads was lower than 15 μg, $A$ increased rapidly, and if it was more than 15 μg, $A$ went up slowly. At the same time, $A_0$ increased gradually with a continuous increase of the mass of immunomagnetic beads added, which in turn manifests a non-specific adsorption of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated on excessive immunomagnetic beads and leads to poorer sensitivity (Zhang et al. 2017).

As illustrated in Figure 2(D), $A/A_0$ had the maximum value using 15 μg of immunomagnetic beads. Therefore, 15 μg of immunomagnetic beads provided adequate binding sites and possessed an efficient capturing capacity for procalcitonin. Hence, 15 μg was selected for further measurements. The conditions were 1:2000 dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated and the other parameters the same as shown in Figure 2(A).

The formation of the sandwich biocomplex involved two binding events including the binding event between immunomagnetic beads and procalcitonin and the binding event between procalcitonin and horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated. The incubation time of these two binding events affected the formation of the biocomplex. Therefore, the influence of incubation time on $A$, $A_0$, and $A/A_0$ was characterized from 10 min to 60 min. During the first binding event, as shown in Figure 2(E), $A$ reached a plateau after 30 min, which suggested a binding saturation of procalcitonin to immunomagnetic beads, and $A_0$ remained at a relatively low level in

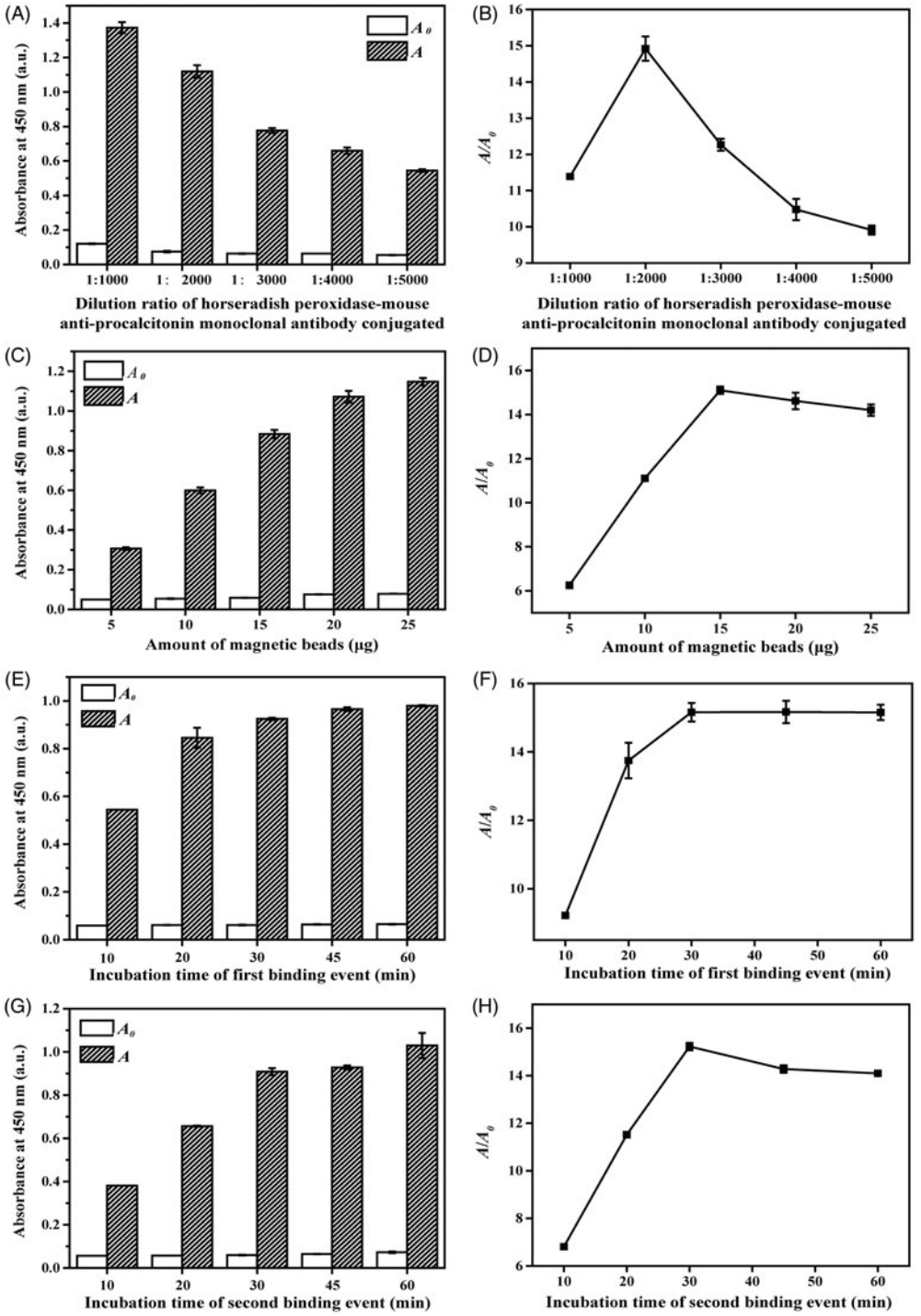


**Figure 2.** Optimization of experimental conditions for the colorimetric sensing platform, where $A$ is the absorbance at 450 nm with the samples containing 10 ng/mL procalcitonin and $A_0$ is the absorbance at 450 nm for phosphate-buffered saline using the same conditions as for $A$. Three independent measurements were obtained from three individual preparations for each measurement. The error bars represent the standard deviations.

spite of the increasing time. As displayed in Figure 2(F), $A/A_0$ was parallel to $A$ (Figure 2(E)). The incubation time of the first binding event was selected to be 30 min due to saturated binding. The conditions were 1:2000 dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated; 15 µg immunomagnetic beads; and the other parameters the same as those for Figure 2(A).

During the second binding event, $A$ exhibited similar tendency as the positive signal shown in Figure 2(E), while $A_0$ indicates a slight augmentation with prolonged incubation time (as illustrated in Figure 2(G)), suggesting that horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated non-specific adsorption on immunomagnetic beads. Figure 2(H) shows that the highest $A/A_0$ value was attained when the incubation time of the second binding event was 30 min. The conditions were 1:2000 dilution ratio of horseradish peroxidase-mouse anti-procalcitonin monoclonal antibody conjugated; 15 µg immunomagnetic beads; the incubation time of the first binding event was 30 min; and the other parameters are the same as those in Figure 2(A).

### *Determination of procalcitonin*

Under optimal conditions, a series of procalcitonin concentrations were prepared and examined. Figure 3(A) showed that as the concentration of procalcitonin increased, the absorbance at 450 nm was enhanced. The calibration curve between the concentration of procalcitonin and the absorbance intensity ranging from 0.1 ng/mL to 10 ng/mL exhibited a good linear relationship suitable for trace levels of human procalcitonin. The regression equation was $Y = 0.087X + 0.078$, where $X$ is the concentration of procalcitonin (ng/mL) and $Y$ is the absorbance of the reaction solution at 450 nm. The determination coefficient ($R^2$) was calculated to be 0.997. The limit of detection ($3\sigma$, where $\sigma$ is the standard deviation of a blank solution processed similarly as the samples containing procalcitonin, $n = 9$, data were shown in Supplemental File, Table S1) was determined to be 0.04 ng/mL. The measurements were completed in less than 90 min. Additionally, the reproducibility of the proposed method was evaluated from 1 ng/mL procalcitonin variability data

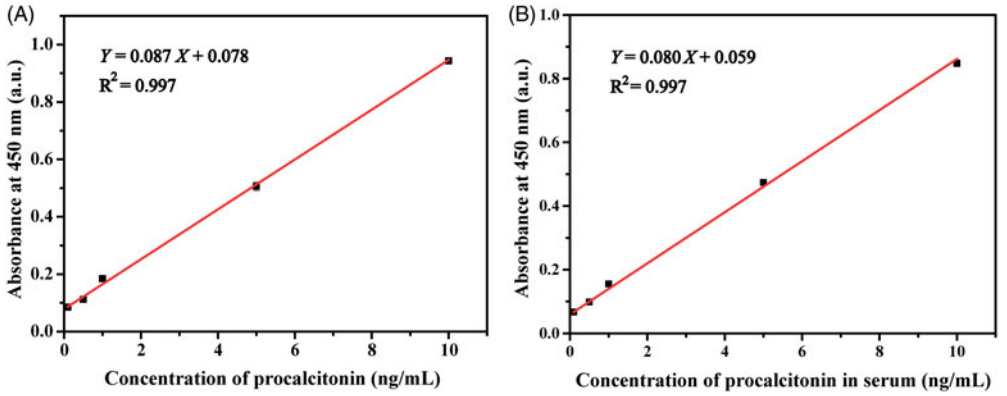


**Figure 3.** Calibration curve of absorption intensity as a function of procalcitonin concentration under the optimal conditions in (A) buffer and (B) serum. Three independent measurements were obtained from three individual preparations for each condition. The error bars represent the standard deviations.

of 11 replicates, wherein the relative standard deviation was 4.02% (the results are shown in Supplemental File, Table S2). These measurements show that the method was reliable with and suitable reproducibility for the determination of procalcitonin.

Commercial human serum samples, spiked with different amounts of procalcitonin, were applied to validate the analytical application of the proposed method. Similar concentration gradient as in the buffer was set for determining procalcitonin in the human serum. Figure 3(B) shows the linearity of the calibration curve with a determination coefficient of 0.997. As shown in Table 1, the recovery of procalcitonin for the proposed method ranged from 97.9% to 104.0%, and the relative standard deviation values were acceptable. These measurements demonstrate that the sensing platform had considerable potential to determine procalcitonin in the clinical serum samples.

The specificity of the proposed method was investigated by testing the absorbance intensity to the interfering proteins. Possible interferents such as C-reactive protein and human serum albumin were utilized in this study. As shown in Figure 4, the absorbance with procalcitonin was significantly higher than with the interferents, and the mixture produced a signal that was similar to procalcitonin. These results demonstrated that the sensing platform possessed excellent specificity for procalcitonin detection.

Recently, some new immunoassays for procalcitonin detection have been reported (Sener et al. 2013; Yang et al. 2017; Zhou et al. 2018). For example, the detection of

**Table 1.** Recovery of procalcitonin in the human serum samples.

| Sample | Added concentration (ng/mL) | Found concentration (ng/mL) | Recovery (%) |
|---|---|---|---|
| 1 | 0.5 | 0.49 ± 0.01 | 97.9 |
| 2 | 2 | 2.07 ± 0.04 | 103.4 |
| 3 | 5 | 5.20 ± 0.11 | 104.0 |

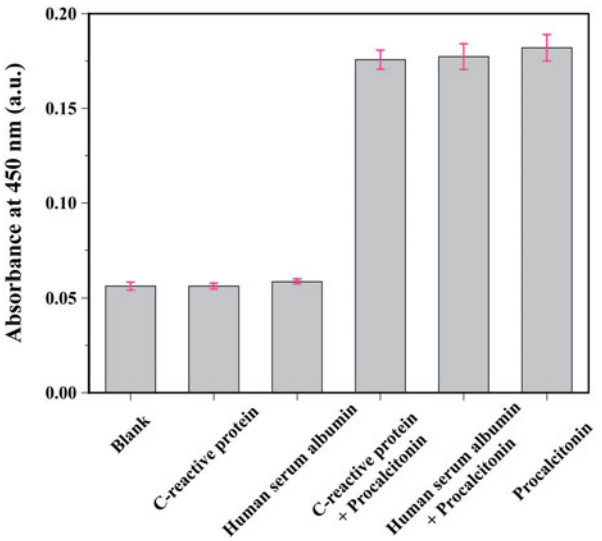


**Figure 4.** Specificity study for the immunomagnetic bead based assay for the determination of procalcitonin. The concentrations of procalcitonin, C-reactive protein, and human serum albumin were 1, 100, and 100 mg/mL, respectively. Three independent measurements were obtained from three individual preparations for each condition. The error bars represent the standard deviations.

procalcitonin using microcontact imprinted surface plasmon resonance biosensor was completed in approximately 1 h, making this biosensor a promising candidate for real-time procalcitonin monitoring (Sener et al. 2013). However, the detection limit was 9.9 ng/mL, which did not allow the determination of trace levels of human procalcitonin.

Cu/Mn double-doped $CeO_2$ nanocomposites were utilized to assemble the electrochemical sensor, which detected a minimum concentration of 0.03 pg/mL procalcitonin (Yang et al. 2017). However, this protocol required a long fabrication time, which is unsuitable for future point-of-care testing requirements. An immunosensor based on fluorescence resonant energy transfer between $NaYF_4$ upconversion nanoparticles and CdTe quantum dots determined a low concentration of 0.25 ng/mL procalcitonin (Zhou et al. 2018), but this approach required complicated material preparations. Compared with other immunoassays, the detection limit of the proposed method was not that low. However, this protocol achieved an acceptable detection limit with less demanding experimental conditions and simpler techniques, meeting the needs of clinical examination.

## Conclusions

In summary, a rapid and sensitive colorimetric approach has been developed to determine procalcitonin by coupling magnetic separation with enzymatic catalysis. The proposed method linearly responded to the procalcitonin concentrations with a detection limit of 0.04 ng/mL in less than 90 min. The proposed method exhibited acceptable sensitivity, good reproducibility, and ideal selectivity. Moreover, this method was efficient for the detection of procalcitonin in human serum. In conclusion, the fabrication process of this sensing platform was simple, and possessed great potential for the detection of the severity of sepsis and microbial infections through procalcitonin detection in clinical samples.

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## ORCID

Yun Zhang http://orcid.org/0000-0002-3274-2792

## References

Assicot, M., D. Gendrel, H. Carsin, J. Raymond, J. Guilbaud, and C. Bohuon. 1993. High serum procalcitonin concentrations in patients with sepsis and infection. *Lancet* 341 (8844):515–518. doi:10.1016/0140-6736(93)90277-N.

Atanasova, M., N. Vasileva, and T. Godjevargova. 2017. Determination of aflatoxin M1 in milk by a magnetic nanoparticle-based fluorescent immunoassay. *Analytical Letters* 50 (3):452–469. doi:10.1080/00032719.2016.1187626.

Baldini, F., L. Bolzoni, A. Giannetti, M. Kess, P. M. Kramer, E. Kremmer, G. Porro, F. Senesi, and C. Trono. 2009. A new procalcitonin optical immunosensor for POCT applications. *Analytical and Bioanalytical Chemistry* 393 (4):1183–1190. doi:10.1007/s00216-008-2547-1.

Bloos, F., and K. Reinhart. 2014. Rapid diagnosis of sepsis. *Virulence* 5 (1):154–160. doi:10.4161/viru.27393.

Bone, R. C., C. J. Fisher Jr., T. P. Clemmer, G. J. Slotman, C. A. Metz, and R. A. Balk. 1989. Sepsis syndrome: a valid clinical entity. Methylprednisolone severe sepsis study group. *Critical Care Medicine* 17 (5):389–393. doi:10.1097/00003246-198905000-00002.

Carrigan, S. D., G. Scott, and M. Tabrizian. 2004. Toward resolving the challenges of sepsis diagnosis. *Clinical Chemistry* 50 (8):1301–1314. doi:10.1373/clinchem.2004.032144.

de la Rica, R., and M. M. Stevens. 2012. Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. *Nature Nanotechnology* 7 (12):821–824. doi:10.1038/nnano.2012.186.

Gogos, C. A., E. Drosou, H. P. Bassaris, and A. Skoutelis. 2000. Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: A marker for prognosis and future therapeutic options. *The Journal of Infectious Diseases* 181 (1):176–180. doi:10.1086/315214.

Herzum, I., and H. Renz. 2008. Inflammatory markers in SIRS, sepsis and septic shock. *Current Medicinal Chemistry* 15 (6):581–587. doi:10.2174/092986708783769704.

Jiang, Y., H. Zhao, Y. Lin, N. Zhu, Y. Ma, and L. Mao. 2010. Colorimetric detection of glucose in rat brain using gold nanoparticles. *Angewandte Chemie International Edition* 49 (28):4800–4804. Engl. doi:10.1002/anie.201001057.

Karzai, W., M. Oberhoffer, A. Meier-Hellmann, and K. Reinhart. 1997. Procalcitonin – A new indicator of the systemic response to severe infections. *Infection* 25 (6):329–334. doi:10.1007/BF01740811.

Li, P., W. Zhang, X. Zhou, and L. Zhang. 2015. C60 carboxyfullerene-based functionalised nanohybrids as signal-amplifying tags for the ultrasensitive electrochemical detection of procalcitonin. *Clinical Chemistry* 48 (3):156–161. doi:10.1016/j.clinbiochem.2014.09.017.

Li, T., K. Zhu, S. He, X. Xia, S. Liu, Z. Wang, and X. Jiang. 2011. Sensitive detection of glucose based on gold nanoparticles assisted silver mirror reaction. *The Analyst* 136 (14):2893. doi:10.1039/c1an15256d.

Liang, J., C. Yao, X. Li, Z. Wu, C. Huang, Q. Fu, C. Lan, D. Cao, and Y. Tang. 2015. Silver nanoprism etching-based plasmonic ELISA for the high sensitive detection of prostate-specific antigen. *Biosensors and Bioelectronics* 69:128–134. doi:10.1016/j.bios.2015.02.026.

Liu, F., G. Xiang, R. Yuan, X. Chen, F. Luo, D. Jiang, S. Huang, Y. Li, and X. Pu. 2014. Procalcitonin sensitive detection based on graphene-gold nanocomposite film sensor platform and single-walled carbon nanohorns/hollow pt chains complex as signal tags. *Biosensors and Bioelectronics* 60:210–217. doi:10.1016/j.bios.2014.03.071.

Murugaiyan, S. B., R. Ramasamy, N. Gopal, and V. Kuzhandaivelu. 2014. Biosensors in clinical chemistry: An overview. *Advanced Biomedical Research* 3 (1):67. doi:10.4103/2277-9175.125848.

Qi, S., Q. Li, W. Rao, X. Liu, L. Yin, and H. Zhang. 2013. Determining the concentration of procalcitonin using a magnetic particles-based chemiluminescence assay for the clinical diagnosis of sepsis. *Analytical Sciences* 29 (8):805–810. doi:10.2116/analsci.29.805.

Ren, T. L., C. J. Yang, Y. X. Hang, H. Shi, H. X. Zhou, D. Y. Fang, G. X. Shi, and X. J. Ji. 2013. G-quadruplex-based DNAzyme as a sensing platform for ultrasensitive colorimetric adenosine deaminase detection. *Analytical Letters* 46 (15):2444–2453. doi:10.1080/00032719.2013.800543.

Rucker, G., and M. Schumacher. 2013. Procalcitonin as a diagnostic marker for sepsis. *The Lancet Infectious Diseases* 13 (12):1012–1013. doi:10.1016/S1473-3099(13)70303-7.

Schuetz, P., B. Muller, M. Christ-Crain, D. Stolz, M. Tamm, L. Bouadma, C. E. Luyt, M. Wolff, J. Chastre, F. Tubach, and M. Briel. 2013. Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections. *Evidence-Based Child Health: A Cochrane Review Journal* 8 (4):1297–1371. doi:10.1002/ebch.1927.

Schuetz, P., I. Raad, and D. N. Amin. 2013. Using procalcitonin-guided algorithms to improve antimicrobial therapy in ICU patients with respiratory infections and sepsis. *Current Opinion in Critical Care* 19 (5):453–460. doi:10.1097/MCC.0b013e328363bd38.

Sener, G., E. Ozgur, A. Y. Rad, L. Uzun, R. Say, and A. Denizli. 2013. Rapid real-time detection of procalcitonin using a microcontact imprinted surface plasmon resonance biosensor. *The Analyst* 138 (21):6422. doi:10.1039/c3an00958k.

Shen, W. J., Y. Zhuo, Y. Q. Chai, Z. H. Yang, J. Han, and R. Yuan. 2015. Enzyme-free electrochemical immunosensor based on host-guest nanonets catalyzing amplification for procalcitonin detection. *ACS Applied Materials & Interfaces* 7 (7):4127–4134. doi:10.1021/am508137t.

Tian, Y., J. Lv, J. Wang, Y. Qin, J. Liu, and W. Zhao. 2014. A light initiated chemiluminescent immunoassay for procalcitonin. *Acta Biochimica et Biophysica Sinica* 46 (9):817. doi:10.1093/abbs/gmu061.

Toriyama, K., T. Suzuki, T. Inoue, H. Eguchi, S. Hoshi, Y. Inoue, H. Aizawa, K. Miyoshi, M. Ohkubo, E. Hiwatashi, and Y. Ohashi. 2015. Development of an immunochromatographic assay kit using fluorescent silica nanoparticles for rapid diagnosis of acanthamoeba keratitis. *Journal of Clinical Microbiology* 53 (1):273–277. doi:10.1128/JCM.02595-14.

Valera, E., M. S. McClellan, and R. C. Bailey. 2015. Magnetically-actuated, bead-enhanced silicon photonic immunosensor. *Analytical Methods* 7 (20):8539–8544. doi:10.1039/C5AY01477H.

Westwood, M., B. Ramaekers, P. Whiting, F. Tomini, M. Joore, N. Armstrong, S. Ryder, L. Stirk, J. Severens, and J. Kleijnen. 2015. Procalcitonin testing to guide antibiotic therapy for the treatment of sepsis in intensive care settings and for suspected bacterial infection in emergency department settings: A systematic review and cost-effectiveness analysis. *Health Technology Assessment* 19 (96):1–236. doi:10.3310/hta19960.

Woolley, C. F., and M. A. Hayes. 2015. Sensitive detection of cardiac biomarkers using a magnetic microbead immunoassay. *Analytical Methods* 7 (20):8632–8639. doi:10.1039/C5AY01071C.

Xianyu, Y., Y. Chen, and X. Jiang. 2015. Horseradish peroxidase-mediated, iodide-catalyzed Cascade reaction for plasmonic immunoassays. *Analytical Chemistry* 87 (21):10688–10692. doi:10.1021/acs.analchem.5b03522.

Yang, S. K., L. Xiao, H. Zhang, X. X. Xu, P. A. Song, F. Y. Liu, and S. Liu. 2014. Significance of serum procalcitonin as biomarker for detection of bacterial peritonitis: A systematic review and meta-analysis. *BMC Infectious Diseases* 14 (1):452. doi:10.1186/1471-2334-14-452.

Yang, Z., S. Ren, Y. Zhuo, R. Yuan, and Y. Chai. 2017. Cu/Mn double-doped $CeO_2$ nanocomposites as signal tags and signal amplifiers for sensitive electrochemical detection of procalcitonin. *Analytical Chemistry* 89 (24):13349–13356. doi:10.1021/acs.analchem.7b03502.

Zhang, Y., C. Yan, H. Yang, J. Yu, and H. Wei. 2017. Rapid and selective detection of *E. coli* O157:H7 combining phagomagnetic separation with enzymatic colorimetry. *Food Chemistry* 234:332–338. doi:10.1016/j.foodchem.2017.05.013.

Zhang, Y., H. Yang, J. Yu, and H. Wei. 2016. Rapid and sensitive detection of HIV-1 p24 antigen by immunomagnetic separation coupled with catalytic fluorescent immunoassay. *Analytical and Bioanalytical Chemistry* 408 (22):6115–6121. doi:10.1007/s00216-016-9722-6.

Zhou, Y., X. Shao, Y. Han, and H. Zhang. 2018. Detection of procalcitonin (PCT) using the double antibody sandwich method based on fluorescence resonant energy transfer between upconversion nanoparticles and quantum dots. *Analytical Methods* 10 (9):1015–1022. doi:10.1039/c8ay00039e.