

Washing-free Chemiluminescence Immunoassay for Rapid Detection of cTnI in Whole Blood Samples

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Abstract

Background: Chemiluminescence immunoassay (CLIA) has always been a great challenge in detecting whole blood samples without centrifugation because of the interference of red blood cells and low sensitivity.

Results: In this scheme, the antigens and erythrocytes in the blood were captured by the antibodies immobilized on the magnetic particles, recognized by another biotin-conjugated cTnI antibody and detected by streptavidin/acridine ester-conjugated PCMS. After magnetic separation, the supernatant was transferred and measured. No significant difference was noted between the cTnI concentrations of the serum samples, plasma samples and whole blood. The prepared PCMS provided more functional areas to conjugate streptavidin and acridinium ester, so the immunoassay has highly sensitive, the limits of blank at 0.012 ng/mL, and functional sensitivity at 0.019 ng/mL with a CV of 20%, and 0.058 ng/mL with a CV of 10%. Total precision of any sample type ranged from 2.62~5.67%. The assay was linear over the studied range of 0.01–50.00 ng/mL, and no hook effect was found when cTnI concentrations reached 1900 ng/mL. No significant interference was noted with the potential endogenous interfering substances. Compared with the commercial kit (Abbott assay kit), the correlation coefficient was 0.9859.

Conclusions: A washing-free chemiluminescence immunoassay (CLIA) was established for the rapid detection of cardiac troponin I (cTnI) in human whole blood, using erythrocyte capture antibodies-conjugated magnetic nanoparticles for eliminating the influence of erythrocytes and polychloromethylstyrene microspheres (PCMS) for signal amplification, which showed great potential in clinical application.

Background

In recent years, the main cause of death and attack is cardiovascular disease worldwide [1]. Biomarkers are the most commonly used indicators in screening patients with acute myocardial infarction (AMI). When stress or myocardial injury leads to cardiomyocyte necrosis, cardiac troponins expressed exclusively in cardiomyocytes are released into the circulation [2]. Therefore, the accurate determination of cardiac troponin I is the preferred biomarker for the diagnosis of AMI or emergency patients with suspected symptoms and signs of AMI presenting to the emergency department [3, 4]. To detect a low concentration of cTnI in patients with early myocardial necrosis, the experts recommended that the assays with high analytical sensitivity are considered as high-sensitivity cardiac troponin I (hs-cTnI) [5]. According to the recommendations, Low concentration precision and limits of the assay are the two most critical performances of hs-cTnI [3–7]. Various analysis techniques for cTnI detection have been developed to improve precision and sensitivity, including enzyme-linked immunosorbent assays (ELISA) [8], chemiluminescence immunoassays (CLIA) [9], and lateral flow immunoassays (LFIA) [10, 11]. With the rapid development of signal amplification strategy and magnetic nanoparticles technology, chemiluminescence immunoassay has become the most ideal method for the detection of hs-cTnI [9, 12–14].

For the sample types used for cTnI assay, the whole blood samples without any pre-treatment are more preferred than serum or plasma because of a short reporting time. In this study, a cTnI assay with whole blood samples was established, and the assay duration between sampling and the result reporting was 15.6 minutes, which met the needs of the central laboratory to initiate effective, evidence-based medical management and revascularization [15, 16]. Shortened sampling and outcome times will lead to rapid medical decisions when treating patients with symptoms of AMI and have a significant positive impact on their lives.

To shorten the detection time and maintain high sensitivity in this study, a sandwich washing-free CLIA applying cTnI/erythrocyte antibodies-conjugated magnetic nanoparticles in combination with streptavidin/acridinium ester-conjugated polychloromethylstyrene microspheres (SA/AE-PCMS) for further signal amplification was performed, and it is schematically shown in Fig. 1. To eliminate the need for washing steps, the change of the chemiluminescence intensity of the supernatant of the reaction solution was measured to indicate the concentration of the analyte in the sample. However, the sensitivity of the change rate of the supernatant was insufficient compared with that of the magnetic bead precipitation. Employing polychloromethylstyrene microspheres to provide a larger specific surface area, the labeling efficiency was greatly improved, so that the change rate of the supernatant was enough to indicate the concentration of the analyte. Secondly, the erythrocytes in the whole blood samples have a great influence on the detection intensity of chemiluminescence. The application of erythrocytes antibody-coated magnetic nanoparticles makes the erythrocytes in the supernatant almost invisible to the naked eye. Finally, the low sensitivity of the supernatant and the interference of red blood cells was improved, and the washing-free CLIA method could be easily realized. The analytical performance of cardiac troponin I (cTnI) was evaluated according to Clinical Laboratory Standards Institute (CLSI) documents on the Getein MAGICL6000 immunoassay analyzer (Getein Biotechnology, China).

Results And Discussion

Characterization

The SEM image of the prepared PCMS is shown in Fig. 2A, and the enlarged image is shown in Fig. 2B, both images show a discernible structure with an average size of ~ 100 nm. The Z-average particle sizes of the PCMS were 116.0 nm, and the polydispersity was 0.005. Therefore, the particle size distribution confirmed the SEM characterization. To compare the labeled ratio of acridine ester to streptavidin between direct labeling and indirect labeling amplified with PCMS, the RLUs of acridinium ester labeled streptavidin were measured at the same protein concentration, and the RLU curves were shown in Fig. 2C. The amount of proteins (streptavidin) coated to the microspheres was measured by the BCA protein assay kit. The concentration of acridinium ester in the PCMS was calculated by the acridinium ester standard solution-RLU standard curve. Considering the molecular weight of streptavidin (MW: 66KD) and acridine ester (MW: 740.85), the acridinium ester labeling efficiency (the molar ratio of acridinium ester to SA) of the PCMS amplified method was about 12.35, and that of the direct labeling method as the control

was 2.55. The proposed labeling method improves the efficiency of acridinium ester - labeling by about 5 times, which is essential to improve the analytical sensitivity.

Erythrocyte antibodies coated magnetic nanoparticles were applied to capture erythrocytes in the whole blood samples to eliminate the influence of erythrocytes on immunoassay. As shown in Fig. 2D, the relatively large amounts of whole blood and erythrocyte antibodies coated magnetic nanoparticles are used to distinguish the change of turbidity in solution with the naked eye.

Sample types

A matrix comparison study was performed using serum, plasma, and whole blood samples. Serum reference tubes, EDTA plasma, and EDTA whole blood tubes were used. 40 blood donors were tested using each sampling tube was spiked with cTnI range from 0.026–48.51 ng/mL. No significant difference was noted between the cTnI concentrations of the serum samples and plasma samples or EDTA whole blood samples as shown in Fig. 3.

Regression analysis (Deming) analysis:

Serum vs. K2-EDTA plasma: slopes = 0.9997 (± 0.0027); intercepts = -0.0259 (± 0.049) ng/mL. Serum vs. K2-EDTA whole blood: slopes = 0.9939 (± 0.0053); intercepts = -0.0125 (± 0.095) ng/mL;

Bland and Altman analysis:

Serum vs. K2-EDTA plasma: Mean absolute bias = -0.08 ng/mL (95% CI, from -0.21 to 0.03 ng/mL); Mean percentage bias = 0.58% (95% CI, from -0.27–1.44%). Serum vs. K2-EDTA whole blood: Mean absolute bias = -0.05 ng/mL (95% CI, from -0.21 to 0.11 ng/mL); Mean percentage bias = -0.002% (95% CI, from -0.89–0.89%).

Limit of blank (LoB) and functional sensitivity

The cTnI assay needs to be highly sensitive to become a preferred biomarker for the diagnosis of heart attacks. Following CLSI EP17-A2, LoB and functional sensitivity of the proposed method were studied. A representative standard curve (RLU values against cTnI concentrations of 0.01–50 ng/mL) was successfully obtained as shown in Fig. 4A. It showed negative slopes with increasing cTnI concentrations, and the square of the correlation coefficient was 0.9997 indicating good linearity. LoB was determined to be 0.012 ng/mL (M-2SD, n = 20), and the functional sensitivity was determined to be 0.058 ng/mL with a CV of 10%, and 0.019 ng/mL with a CV of 20% as shown in Fig. 4B, which showed high accuracy in low concentration detection of cTnI.

Precision

Six samples (including two serums, two plasma, and two whole blood) were tested in duplicate on 20 different days (2 runs per day) with the established method using MAGICL6000 chemiluminescence analyzer. The intra and inter-day precision were calculated based on recommendations from the CLSI EP 5-A3 document as shown in Table 1. Total precision of serum samples for various cTnI concentrations

ranged from 2.62 to 4.74%, plasma samples ranged from 2.67 to 4.94%, and whole blood ranged from 3.10 to 5.76%.

The determination of an analyte concentration at very low concentrations in a blood sample requires both precision and accuracy. Sensitivity is closely related to precision since sensitivity is the concentration of the analyte below which imprecise is unacceptable. The imprecision of the measurement method is caused by the combined influence of several sources of variation, mainly determined by manipulation errors, separation, washing, detection, and antibody characteristics. In conventional magnetic immunoassay, the washing process of immune complexes on magnetic nanoparticles is the main factor leading to imprecision. In the improved washing-free CLIA, the supernatant is directly tested after magnetic separation, which avoids the loss and error in the process of magnetic bead washing. The results showed that the improved CLIA without washing process in the presence of PCMS and erythrocyte antibody-coated magnetic nanoparticles can be applied to quantify cTnI in a variety of human sample types with excellent precision.

Table 1
The CV% of various cTnI sample types of at 95% confidence limits

Levels	CV%	Serum		Plasma		Whole blood	
		Lower Limit	Upper Limit	Lower Limit	Upper Limit	Lower Limit	Upper Limit
Level 1	Repeatability	1.86%	3.06%	1.79%	3.04%	2.01%	3.32%
	Precision	3.38%	4.74%	3.22%	4.94%	3.69%	5.76%
	Between-Run	0.51%	0.93%	0.50%	0.90%	0.57%	1.02%
	Between-Day	0.11%	0.21%	0.11%	0.22%	0.12%	0.25%
Level 2	Repeatability	1.73%	2.52%	1.77%	2.49%	1.95%	2.83%
	Precision	2.62%	3.69%	2.67%	3.83%	3.10%	4.49%
	Between-Run	0.83%	1.53%	0.86%	1.48%	1.04%	1.66%
	Between-Day	0.18%	0.37%	0.17%	0.37%	0.20%	0.41%

Linearity and high-dose hook effect

A linearity check was performed with a first linear regression, and then with higher-order models of quadratic and cubic. The linearity hypothesis over the whole concentration range of 0.01–50.00 ng/mL was accepted and a linear response was obtained according to the linearity data ($y = 1.01x - 0.1863$, $R^2 = 0.9984$). Hook effect can occur in the presence of high concentrations of the analyte, resulting in false-negative or inaccurately low results. Therefore, we need to find hook effect points by testing increasing concentration samples to improve the detection performance. The study demonstrates that assay is free of hook effects up to 1900 ng/mL of cTnI.

Effect of potentially interfering substances

The five endogenous interfering substances evaluated by the washing-free cTnI assay had no effect on the performance at concentrations reasonably and consistently found in clinical situations. Samples with cTnI concentrations of 0.045 ng/mL and 0.450 ng/mL showed interference within $\pm 10\%$ when hemoglobin up to 160 mg/dL, biotin up to 0.03, intralipid up to 1000 mg/dL, bilirubin up to 10 mg/dL, and rheumatoid factor up to 1500 IU/mL. Considering that imprecision of the assay may cause a deviation of $\pm 10\%$, therefore, interference within $\pm 10\%$ can be considered clinically irrelevant.

Comparison study

165 samples collected from the Second Hospital of Nanjing were analyzed to further assess the feasibility of the established method for clinical application. A comparison study was performed between our method and the clinical method (Abbott kit assay) in Fig. 5. The linear regression equation and the square of the correlation coefficient were given according to the comparison data: $y = 1.0067x - 0.0569$, $R^2 = 0.9859$. The Bland-Altman analysis showed that the mean absolute bias was -0.0124 ng/L (95% CI, -0.19 to 0.17), and the mean percentage bias was -8.2% (95% CI, -13.1% to -3.1%), suggesting a good consistency with the control reagent. Therefore, our washing-free CLIA could be used as a clinical examination assay to detect cTnI in real samples, human blood, with excellent precision and high sensitivity, showing a good application prospect for early diagnosis of patients with AMIs.

Conclusions

A washing-free assay for the detection of cTnI in human whole blood based on cTnI/erythrocyte antibodies-conjugated magnetic nanoparticles and streptavidin/acridine ester-conjugated PCMS was presented. To reduce the analysis time a washing-free assay was employed to test whole blood samples, including sample addition, incubation, magnetic separation, and measurement of supernatant. In terms of whole blood analysis, it was found that erythrocyte antibodies coated magnetic nanoparticles could be used without effect on test results and PCMS amplified label method could significantly improve the sensitivity. This strategy presents an excellent platform for CLIA that allows direct analysis of whole blood samples without washing steps, improving the detection efficiency by reducing operation steps for the non-professionals.

Materials And Methods

Adipic dihydrazide acridinium ester (NSP-DMAE-ADH) was provided by Materwin (China). Styrene, sodium styrene sulfonate, divinylbenzene, chloromethyl styrene, potassium persulfate, and chloromethyl styrene monomer were purchased from Aladdin Reagent (China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Bovine serum albumin (BSA), N-hydroxysuccinimide (NHS), and NHS-Biotin were purchased from Sigma-Aldrich (USA). Carboxyl magnetic nanoparticles, Streptavidin and erythrocyte capture antibodies were provided by Getein Biotechnology (China). 4 monoclonal antibodies that

recognize different aminoacid sequences of the cTnI molecule were provided by HyTest Ltd (Finland). Two antibodies recognize the sequences 190–196 (clone: MF4) and 82–93 (clone: 560) as biotin-labeled antibodies. Another two antibodies recognizing the aminoacid sequences 18–35 (clone: 810) and 41–49 (clone: 19C7) were coated on the magnetic nanoparticles.

Synthesis of the polychloromethylstyrene microspheres (PCMS)

PCMS were prepared per the published procedure and slightly modified [17, 18]. First, a 500mL three-neck round bottom flask with a condenser was used as the reaction vessel. 200 mL of deionized water was added to the three-neck flask, and a layer of nitrogen was kept over the reaction solution during polymerization after nitrogen was bubbled into the water for 20 minutes to remove oxygen. 50 mg of sodium styrene sulfonate was added to the flask and mechanically stirred at 35°C for 10 minutes. Then 8 mL of styrene, 0.5 mL of divinylbenzene (DVB, 50% in ethyl vinylbenzene), and 1 mL of chloromethyl styrene were placed in the reaction flask, and the mixture was continuously stirred for at 75°C for 20 minutes. Next, 300 mg of potassium persulfate as the initiator was added to the reaction solution stirred for 50 minutes. Finally, 1 mL of chloromethyl styrene monomer was added slowly and stirred for 18 hours. The prepared microspheres were washed with ethanol for 4 times by centrifuged at 3000g for 20 minutes, and then stored in water for a short time or freeze dried for a long time.

Preparation of streptavidin/acridinium ester (SA/AE) - conjugated polychloromethylstyrene (PCMS)

10 mg prepared microspheres were added into the buffer solution containing 1 mg streptavidin and 0.1 mg acridine ester with amino ligands to complete the coupling reaction. After the mixture was stirred at 37°C for 24 hours, the product was washed four times by centrifugation with PBST (0.1 M PBS containing 0.9% NaCl, 0.1% Tween-20 and 0.1% ProClin 300, pH 7.0), and the coupled microspheres were analyzed for protein concentration and relative light units (RLU). The amount of streptavidin and acridine ester conjugated to the microspheres depended on the protein concentration and RLU in the product. Labeling efficiency was defined as the ratio of the molar concentration of streptavidin to the molar concentration of acridine ester labeled on the PCMS. The molar concentration of acridinium ester on the PCMS was determined by a standard curve, where the X-axis was the gradient concentrations of acridinium ester standard solution and the Y-axis was the RLU values.

Preparation of cTnI/erythrocyte capture antibodies-conjugated magnetic nanoparticles

0.2mg NHS solution and 1mg EDC solution were added to 10 mg magnetic nanoparticles and the reaction mixture was shaken at 37°C for 30 minutes to activate carboxyl magnetic nanoparticles. After the nanoparticles were washed by magnetic separation three times, 0.2mg monoclonal cTnI antibodies (19C7/810) and 0.2mg erythrocyte antibodies were added to carboxyl activated magnetic nanoparticles

and the mixture was shaken at 37°C for another 3 hours. 2% BSA was added to the reaction tube to block the unreacted NHS- activated group. After blocking, the magnetic nanoparticles solution was placed on the magnet for 1 minute to remove the supernatant. Next, cTnI/erythrocyte antibodies-conjugated magnetic nanoparticles were washed by magnetic separation three times with PBST, and the storage concentration of conjugated magnetic nanoparticles was 2.5 mg/mL after resuspension with 4 mL PBS containing 2% BSA.

Preparation of biotin-labeled antibodies

0.15 mg anti-cTnI antibody solution (MF4/560) and 0.01 mg NHS-biotin (dissolved in DMF) were placed to the bottom of a reaction tube and mixed by a vortex with 150 μ L PBS. After 30 minutes of water bath reaction at 37.0°C, the tube was taken out. 20 μ L lysine solution was added to block the unreacted NHS-Biotin and then incubated at 37.0°C for 30 minutes. Zeba Spin Desalting Columns (ThermoFisher) were used to remove the unconjugated NHS-Biotin. The reaction mixture was added to the desalting column after the column was washed 3 times by PBS, and the eluent was collected by centrifugation at 1000 \times g for 2 minutes. Finally, the eluent containing biotin-labeled antibody was added with 50 μ L PBS containing 2% BSA and 200 μ L 50% glycerol and stored at -20°C.

Immunoassay

The following operations are all performed by the MAGICL6000 chemiluminescence analyzer. The obtained SA/AE - PCMS were applied to detect proteins of interest by one step without washing. In the cTnI assay, 100 μ L of cTnI sample, 10 μ L of cTnI/erythrocyte antibodies-conjugated magnetic nanoparticles (2.5mg/mL), 20 μ L of biotin-labeled cTnI antibodies (1.94 μ g/mL), and 20 μ L of SA/AE - PCMS (0.387 μ g/mL) were added to a reaction cup. The mixed solution was incubated at 37°C for 10 minutes and then separated by a magnet. 20 μ L supernatant was transferred to a special detection tube and triggered by pre-trigger and trigger solutions. The chemiluminescence intensity of the supernatant was detected immediately, and the cTnI concentration was calculated automatically through the standard curve. The standard curve was obtained by measuring various concentrations of troponin calibrators. The generated standard curve was fitted by the four-parameter logistic curve, and the concentration of cTnI in the unknown sample was estimated by the equation. The total analysis time is 15.6 minutes, including instrument sampling, magnetic separation, and measurement process.

Sample types

Serum (with thrombin-based clot activator and without separator), plasma (K_2 EDTA as the anticoagulant), and whole blood (K_2 EDTA as the anticoagulant) samples were collected from each patient to evaluate the potential impact of anticoagulants and the blood cells on the measured cTnI concentration. Forty patients (3 different sample types were collected from each patient) with various cTnI concentrations were collected from AMI patients admitted to Nanjing Second Hospital. Accurate cTnI concentrations in whole blood could be corrected by software calculation of hematocrit values (Hct), following the formula: the whole blood cTnI = measured cTnI/[(1-Hct/100)].

Analysis performance evaluation

The limit of blank and functional sensitivity

To evaluate the sensitivity of the proposed method, the limits of blank (LoB) and functional sensitivity were studied. The mean value (M) and standard deviation (SD) were calculated by measuring the RLU of the negative sample 20 times so that the M-2SD value was obtained. The LoB of this method was calculated by substituting the RLU of M-2SD to the standard curve fitting equation. The cTnI samples with low concentrations of 0.00 ng/mL, 0.020 ng/mL, 0.04 ng/mL, 0.06 ng/mL, 0.08 ng/mL and 0.10 ng/mL were measured for 10 times, and the coefficient of variation of the measured values was obtained. The lowest concentrations with a CV less than 10% or 20% represented the functional sensitivity of this method.

Precision

The precision study of the cTnI assay was conducted on the MAGICL6000 chemiluminescence analyzer by using the proposed method over 20 days. A panel of serum, plasma, and whole blood samples from different patients at two cTnI concentrations was tested in duplicate in 2 runs per day (Level 1: 0.12 ng/mL, Level 2: 1.25 ng/mL). The protocol was performed using a nested components-of-variance design based on the CLSI EP5-A3 guideline for each sample. (20 days × 2 runs × 2 replicate, the total number of replicates: 80 per site).

Linearity

Linearity was assessed following the CLSI EP 6-A guideline. An EDTA blood pool (pool A; cTnI concentration: 50.000 ng/mL) was diluted with an EDTA blood pool at low concentration of cTnI (pool I; cTnI concentration: 0.010 ng/mL), at a ratio of 7/1, 6/2, 5/3, 4/4, 3/5, 2/6, and 1/7. By using this protocol, seven various samples (pool B, C, D, E, F, G, and H) with reference concentrations of 43.751, 37.503, 31.254, 25.005, 18.756, 12.508, 6.259 ng/mL were prepared, and each of the nine pools was measured three replicas in a single run. Linearity was evaluated by comparing the first, second, and third-order polynomial regression analysis for all test samples.

High-dose hook effect

To test for High Dose “Hook Effect” associated with immunoassays, cTnI samples with extremely high concentrations were diluted among 29.7–1900 ng/mL (29.7, 59.4, 119, 238, 475, 950, and 1900 ng/mL) in EDTA blood by double dilution method. Each of the seven samples was tested in duplicate on a reagent lot on the MAGICL6000 chemiluminescence analyzer.

Effect of potentially interfering substances

cTnI negative samples and five potentially interfering substances at high concentrations (hemoglobin, 800.0 mg/dL; biotin, 0.1 mg/dL; intralipid, 2000 mg/dL; bilirubin, 50 mg/dL; rheumatoid factor, 1500 IU/mL) were added in fixed ratios (10/0, 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, 1/9, 0/10) to generate the samples containing gradient concentrations of interfering substance. Each concentration of potential interferent was spiked into the blood at two cTnI concentrations (S1: 0.045 ng/mL; S2: 0.450 ng/mL). All

testing was performed in duplicate, and the interference was evaluated by calculating the deviation of cTnI concentrations between potential interferent-spiked blood and unspiked (no potential interferent) blood. No interference was defined as $\leq 10\%$ difference for the average of all concentrations tested.

Comparison study

The washing-free cTnI method was compared with the clinical cTnI method (Abbott ARCHITECT i2000 analyzer) based upon the CLSI-EP 9 guideline. 165 cTnI samples ranging from 0.02 to 50.00 ng/mL were tested over five days both on the proposed system and the Abbott system. The correlation coefficient and regression equation were performed to evaluate the correlation between the two methods, and the 95% confidence interval and the mean relative error were estimated by the Bland-Altman method to determine the consistency.

Characterization

Scanning electron microscopy (SEM, JSM-5600LV, Japan) was used to characterize the morphologies of the microspheres, and Zeta Potential Analyzer (Zeta Plus, USA) was used to determine the particle size distribution and ζ -potential of PCMS. More details of the experiment were given in the supplementary article.

Statistical analysis

With SPSS 17.0 software, statistical analysis on a completely randomized design was conducted using the one-way analysis of variance (ANOVA) procedure. MedCalc Software (MedCalc, Mariakerke, Belgium) was performed to analyze the Bland-Altman plot and Passing-Bablok regression. All data analyses were performed as mean \pm SD, and when $p < 0.05$ the differences were accepted as significant.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data and materials in this manuscript are all available.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Huan Zhao: Writing-original draft. **Enben Su:** Experiment design. **Li Huang:** Part Experiment. **Yunfeng Zai:** Data analysis. **Yuan Liu:** Formal analysis. **Zhu Chen:** Conceptualization. **Song Li:** Writing - review & editing. **Yan Deng:** Funding acquisition. **Lian Jin:** Funding acquisition. **Nongyue He:** Supervision.

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References

1. Virani Salim S, Alonso A, Benjamin Emelia J, Bittencourt Marcio S, Callaway Clifton W, Carson April P, Chamberlain Alanna M, Chang Alexander R, Cheng S, Delling Francesca N, et al: **Heart Disease and Stroke Statistics—2020 Update: A Report From the American Heart Association.***Circulation* 2020, **141**:139-151.
2. Jaffe AS, Vasile VC, Milone M, Saenger AK, Olson KN, Apple FS: **Diseased Skeletal Muscle: A Noncardiac Source of Increased Circulating Concentrations of Cardiac Troponin T.***J Am Coll Cardiol* 2011, **58**:1819-1824.
3. Thygesen K, Alpert Joseph S, Jaffe Allan S, Chaitman Bernard R, Bax Jeroen J, Morrow David A, White Harvey D, et al: **Fourth Universal Definition of Myocardial Infarction (2018).***J Am Coll Cardiol* 2018, **72**:2231-2264.
4. Wu A, Christenson R, Greene D, Jaffe A, Kavsak P, Ordóñez-Llanos J, Apple F: **Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine.***Clin Chem* 2018, **64**:645-655.
5. Apple FS, Jaffe AS, Collinson P, Mockel M, Ordonez-Llanos J, Lindahl B, Hollander J, Plebani M, Than M, Chan MHM: **IFCC educational materials on selected analytical and clinical applications of high**

- sensitivity cardiac troponin assays.*Clin Biochem* 2015, **48**:201-203.**
6. Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Ganiats TG, Holmes DR, Jaffe AS, Jneid H, Kelly RF, Kontos MC, et al: **2014 AHA/ACC Guideline for the Management of Patients With Non–ST-Elevation Acute Coronary Syndromes: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines.***J Am Coll Cardiol* 2014, **64**:2645-2687.
 7. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, Huber K, Plebani M, Biasucci LM, Tubaro M, et al: **How to use high-sensitivity cardiac troponins in acute cardiac care†.***Eur Heart J* 2012, **33**:2252-2257.
 8. Jiao L, Zhang L, Du W, Li H, Yang D, Zhu C: **Au@Pt nanodendrites enhanced multimodal enzyme-linked immunosorbent assay.***Nanoscale* 2019, **11**:8798-8802.
 9. Zhao H, Lin Q, Huang L, Zhai Y, Liu Y, Deng Y, Su E, He N: **Ultrasensitive chemiluminescence immunoassay with enhanced precision for the detection of cTnI amplified by acridinium ester-loaded microspheres and internally calibrated by magnetic fluorescent nanoparticles.***Nanoscale* 2021, **13**:3275-3284.
 10. Huang L, Zhang Y, Su E, Liu Y, Deng Y, Jin L, Chen Z, Li S, Zhao Y, He N: **Eight biomarkers on a novel strip for early diagnosis of acute myocardial infarction.***Nanoscale Adv* 2020, **2**:1138-1143.
 11. Huang L, Su E, Liu Y, He N, Deng Y, Jin L, Chen Z, Li S: **A microfluidic device for accurate detection of hs-cTnI.***Chin Chem Lett* 2020.
 12. Tang C, He Z, Liu H, Xu Y, Huang H, Yang G, Xiao Z, Li S, Liu H, Deng Y, et al: **Application of magnetic nanoparticles in nucleic acid detection.***J Nanobiotechnol* 2020, **18**:1-19.
 13. Dong H, Tang C, He Z, Liu H, Xu Y, Huang H, Yang G, Xiao Z, Li S, Deng Y, et al: **Rapid identification of diarrheagenic Escherichia coli based on barcoded magnetic bead hybridization.***Chin Chem Lett* 2020, **31**:1812-1816.
 14. Hussain M, Zhu S, Yang P, An Y, Li Z, Ali I, Liu B, Shen H, He N: **Rapid Detection System for Hepatitis B Surface Antigen (HBsAg) Based on Immunomagnetic Separation, Multi-Angle Dynamic Light Scattering and Support Vector Machine.***IEEE Access* 2020, **8**:107373-107386.
 15. Mehta SR, Granger CB, Boden WE, Steg PG, Bassand J-P, Faxon DP, Afzal R, Chrolavicius S, Jolly SS, Widimsky P, et al: **Early versus Delayed Invasive Intervention in Acute Coronary Syndromes.***N Engl J Med* 2009, **360**:2165-2175.
 16. Antman EM, Cohen M, Bernink PJLM, McCabe CH, Horacek T, Papuchis G, Mautner B, Corbalan R, Radley D, Braunwald E: **The TIMI Risk Score for Unstable Angina/Non–ST Elevation MIA Method for Prognostication and Therapeutic Decision Making.***JAMA* 2000, **284**:835-842.
 17. Margel S, Nov E, Fisher I: **Polychloromethylstyrene microspheres: Synthesis and characterization.***J Polym Sci, Part A: Polym Chem* 1991, **29**:347-355.
 18. Kedem M, Margel S: **Synthesis and characterization of micrometer-sized particles of narrow size distribution with chloromethyl functionality on the basis of single-step swelling of uniform polystyrene template microspheres.***J Polym Sci, Part A: Polym Chem* 2002, **40**:1342-1352.

Figures

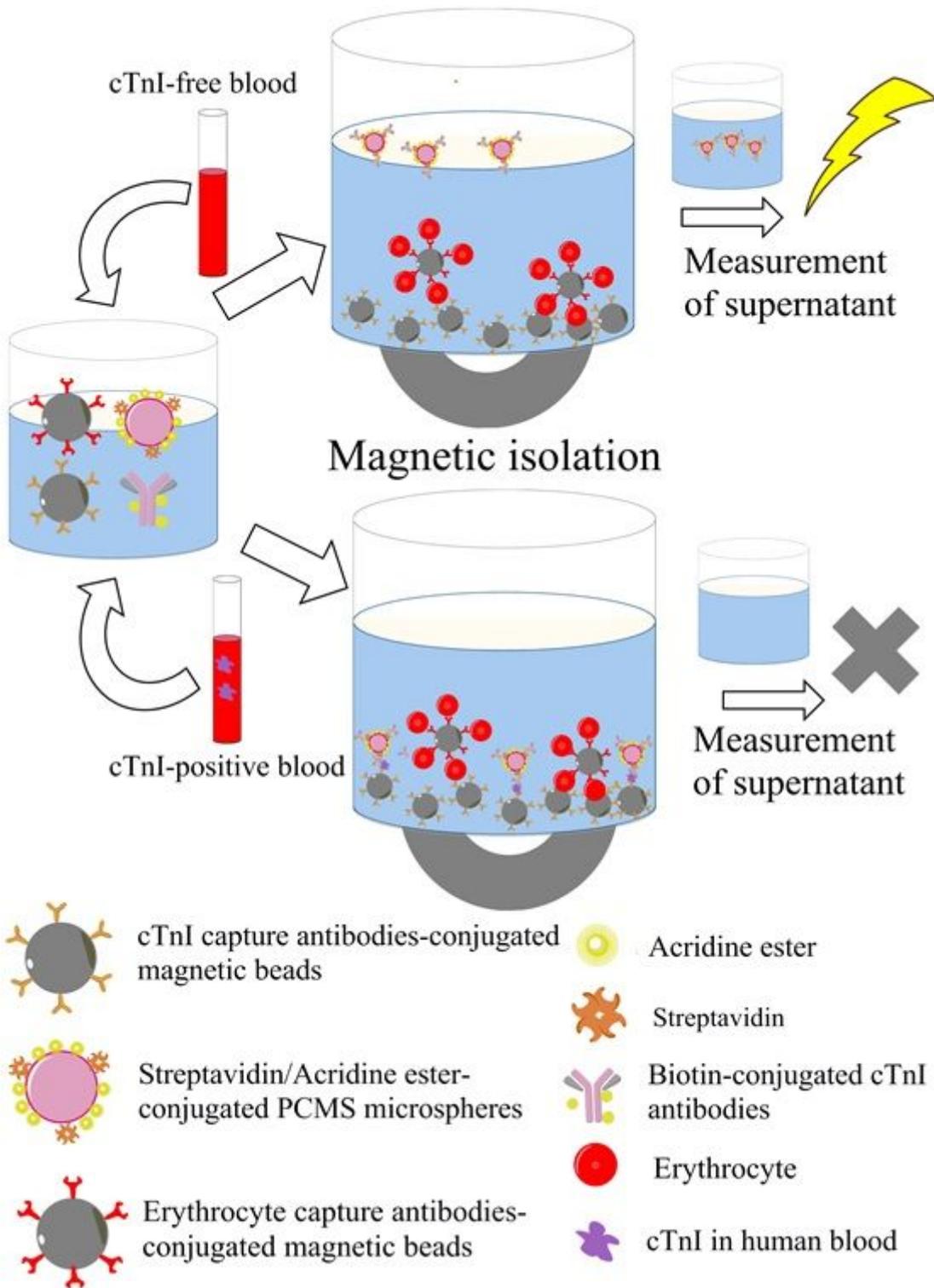


Figure 1

Schematic illustration of a washing-free CLIA for rapid detection of cTnI in whole blood samples. The sample solution is added to the solution containing cTnI/erythrocyte antibodies-conjugated magnetic nanoparticles, Biotin-conjugated cTnI antibodies, and streptavidin/acridine ester-conjugated PCMS. The

target protein is bound in between the magnetic nanoparticles and PCMS. Magnetic nanoparticles are magnetically isolated together with the target protein-bound PCMS, then the supernatant is transferred, and the luminous intensity of the supernatant is measured.

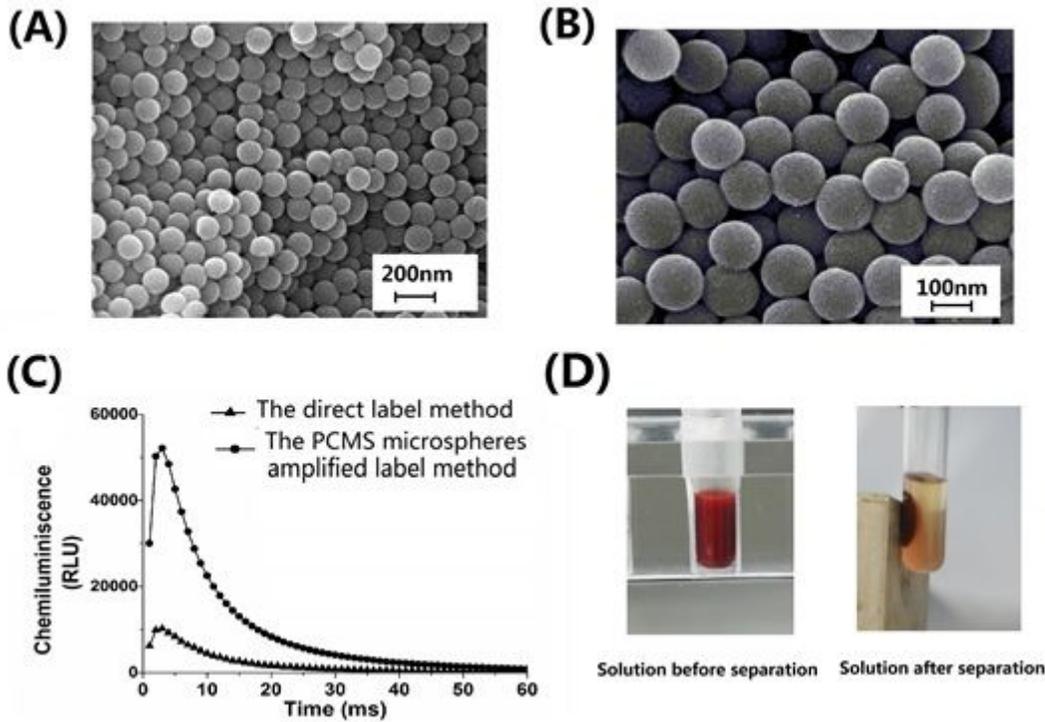


Figure 2

Characterization of PCMS and erythrocyte antibodies-conjugated magnetic nanoparticles. (A) and (B) SEM photomicrographs of PCMS. (C) The chemiluminescence intensity (RLU) of acridinium ester by direct label method and PCMS amplified label method was measured by MAGICL6000 immunoassay analyzer. (D) Demonstration of the washing-free immunoassay based on erythrocyte antibodies-conjugated magnetic nanoparticles. Before magnetic isolation, the reaction solution was affected by the whole blood sample and was very turbid. After magnetic isolation, erythrocytes were separated by magnets and the supernatant became very clear.

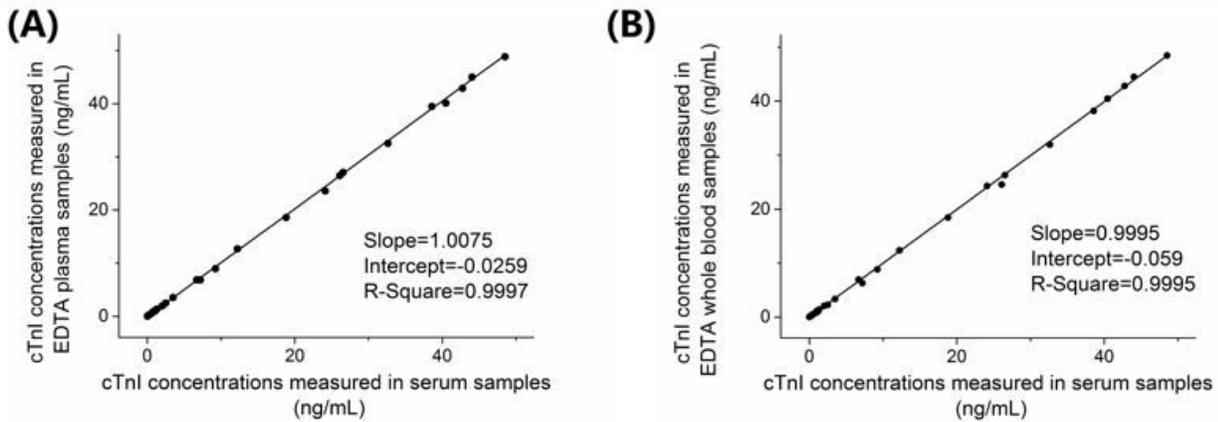


Figure 3

Matrix comparison study of cTnI concentrations measured in different sample types. (A) 40 serum samples vs. matched plasma samples. (B) 40 serum samples vs. matched whole blood samples.

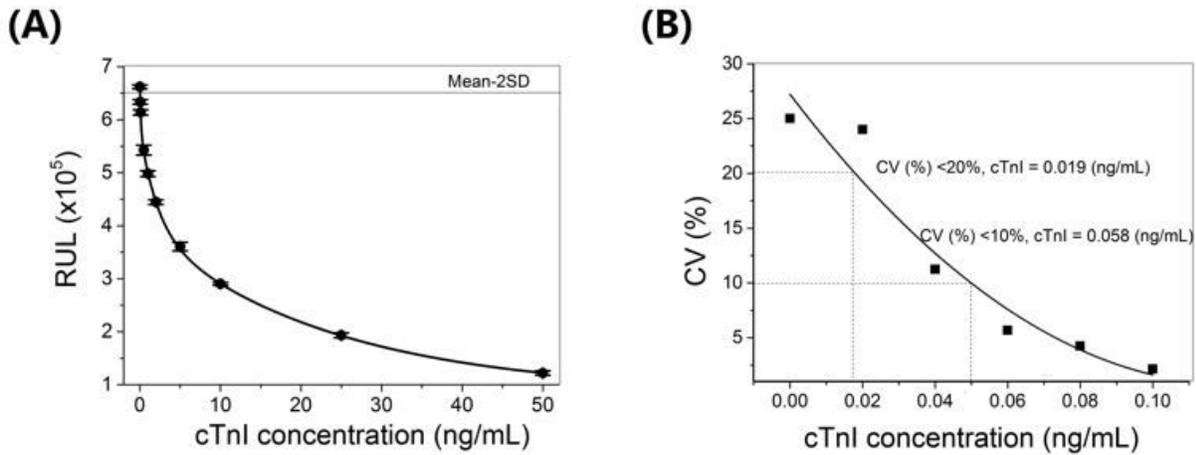


Figure 4

The sensitivity measurements of the proposed method. (A) Standard curve of chemiluminescence intensity (RLU) vs. the concentration of cTnI, with the fitted curve showing the LoB of the immunoassay. (B) The functional sensitivity of cTnI assay at 10% CV and 20% CV.

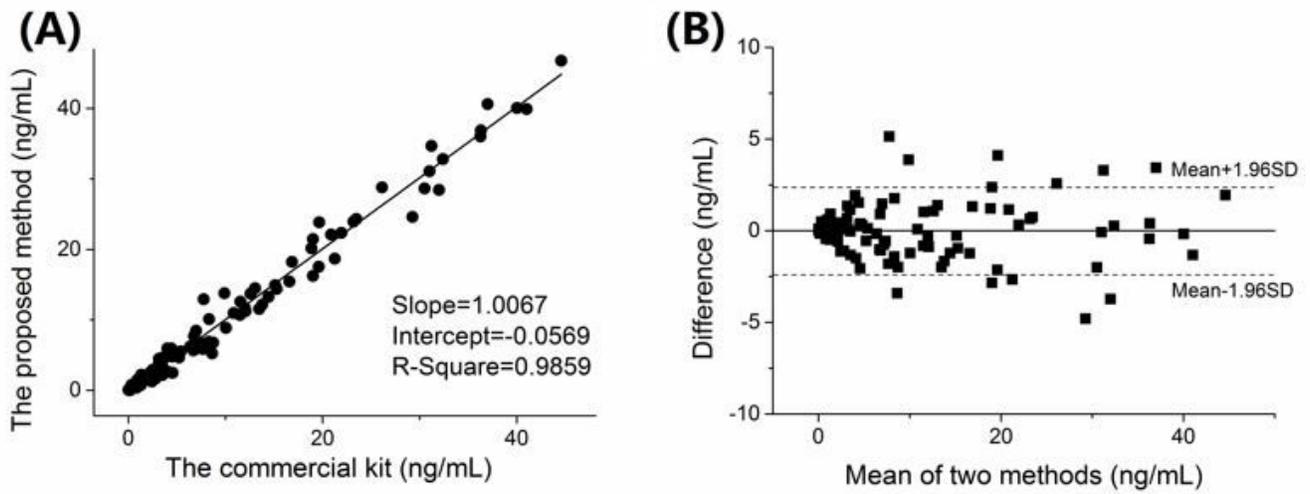


Figure 5

Comparison study between the washing-free CLIA and the clinical method (Abbott assay). (A) Correlation analysis; (B) Bland-Altman analysis.

Supplementary Files

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