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Detection of NT-pro BNP using fluorescent protein modified by streptavidin as a label in immunochromatographic assay

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ABSTRACT

A novel fluorescent immunochromatographic assay for the detection of NT-proBNP in human serum has been developed. Based on a sandwich-type immunoassay format, analytes in samples were captured by one monoclonal antibody labeled with fluorescent protein and "sandwiched" by another monoclonal antibody immobilized on the nitrocellulose membrane, the fluorescence and concentration of analytes were measured and then calculated by fluoroanalyzer. The fluorescent protein is a fusion protein and was prepared through the application of Streptavidin gene SA, β subunit cpcB of Phycocyanin, lyase alr0617, and phycoerythrobilin synthetase gene ho1, pebA, pebB for covalent binding. It is characterized with higher stability, good solubility in water and it is not easy to quench fluorescence. Take the advantages of fluorescent protein, the immunochromatographic assay exhibited a wide linear range for NT-proBNP from 200 pg ml^{-1} to 26,000 pg ml^{-1}, with a detection limit of 47 pg ml^{-1} under optimal conditions. Compared with chemiluminescence immunoassay (CLIA), 131 human serum samples were analyzed and the correlation coefficient of the developed immunoassay was 0.978. These results demonstrated that fluorescent immunochromatographic assay is a more rapid, sensitive, specific method and could be developed into a platform for more biomarkers determination in clinical practice.

1. Introduction

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is derived from the precursor peptide, namely proBNP. The proBNP is synthesized mainly in heart ventricles [1] and is cleaved into active hormone BNP and inactive N-terminal proBNP (NT-proBNP) [2]. Compared with BNP, the NT-proBNP contains 76 amino acids and has better stability in circulating blood. In light of this, it has been widely applied as a biomarker of cardiovascular disease [3] and in particular a marker of heart failure [4,5], coronary artery disease [6,7], chronic kidney disease [8], diabetes mellitus and systemic hypertension [9], and holds promise as a tool for screening of the general population both for the detection of underlying cardiac structural and functional abnormalities as well as the early detection of potential cardiovascular events [10].

During the past decades, several analytical methods have been developed for the detection of serum levels of NT-proBNP, including radioimmunoassay (RIA) [11,12], immunoradiometric assay (IRMA) [13], the enzyme-linked immunosorbent assay (ELISA) [14] and recently-developed electrochemiluminescence immunoassay (ECLIA) [15]. While ELISA needs more sample volume and a long detection time, RIA and IRMA have more radionuclide pollution problem and less automation, ECLIA characterized by high sensitivity, specificity and easily-operated automation, still needs high cost, laboratory-oriented large analytical instrument, well-trained personnel sample transportation and increased waiting time. Therefore, investigating a novel, simple, rapid, sensitive and specific method to detect NT-proBNP at an early stage and to improve the treatment success has garnered considerable interest.

Recent improvements about methods for detecting the levels of NT-proBNP have focused on developing point-of-care testing (POCT) of NT-proBNP to offer rapid, accuracy testing result for patients, and improve the therapeutic strategies timely. Immunochromatographic assay is a simple, rapid and user friendly immunoassay which has been widely used in clinical diagnosis [16–18], food analysis [19,20], environment monitoring [21]and agriculture [22,23]. The immunosensor of immunochromatographic assay is based on various labels, e.g., colloidal gold [24], fluorescent immunoliposomes [25], nanoparticle [26–28], fluorescent microspheres [29] and quantum dot [30]. Colloidal gold possesses long-term stability, user-friendly format, a short period of results obtainment and inexpensive cost, while it is just qualitatively and shows serious sensitivity limitations. Although other immunosensors based on fluorescence or electrochemical have brought quantitative determination to clinical practice, various sensitivity, specificity, linear

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range and detection limits are still existing. Efforts to develop a novel quantitatively immunochromatographic assay is still imperative in order to improve accuracy of the testing results for patients.

Herein we first report the development of a novel fluorescent immunochromatographic assay for the sensitive and rapid detection of NT-proBNP in human serum, plasma and whole blood using fluorescent-streptavidin fusion protein which has been applied for china national invention patent (ZL200710030603.6) as an immunosensor. The fluorescent-streptavidin fusion protein gene sequences comprise Streptavidin gene SA, β subunit cpcB of Phycocyanin, lyase alr0617, and phycoerythrobilin synthetase gene ho1, pebA, pebB. And the fusion protein prepared by genetic engineering recombinant technologies are used to conjugate to anti-NT-proBNP antibody labeled with biotin for amplifying signal output. The phycocyanin-streptavidin fusion protein can emit strong fluorescence with good light absorption properties and high quantum yield. It has a wide range of excitation and emission in the visible region of the spectrum. It can be applied to the functional material fields of food, health care and medicine, in particular to the fluorescent probe in the biological and medical molecular monitoring field. Therefore, we choose the fusion protein as the immunosensor and it is characterized with higher stability, good water solubility, shorter analysis time and shows a wider linear range. In this study, a total of 131 human serum samples were analyzed by our developed immunoassay. Compared with conventional immunoassay, these data showed good correlation. Thus, this assay has significant promise to offer a new avenue for POCT of more future important biomarkers.

2. Materials and methods

2.1. Chemicals and reagents

Biotin, Bovine serum albumin (BSA), dimethylformamide (DMF), polyvinyl alcohol AH-26, polyglucosan20000, D-trehalose and Tween-20 were obtained from Sigma-Aldrich. (St. Louis, MO, USA). The antibody pairs, namely murine anti-NT-pro BNP mAb 4NT1-15F11 and grNT1-02, Goat anti-mouse IgG were purchased from Fitzgerald (North Acton, MA, USA). Nitrocellulose membrane, glass fiber membrane, absorbent pad, sample pad and conjugate pad were purchased from Millipore (Bendford, MA, USA). Fluorescent-streptavidin fusion protein was produced in our laboratory. All solvents and other chemical were of analytical reagent grade and used as received.

2.2. Apparatus

The test strip fabrication system consists of an R5DDA automatic dispenser, a FR-900 sealer, a P2-40 laminator, and the Guillotine cutting system C-6, which were purchased from Shanghai Hangan Electronic Technology Co, Ltd. The Multifunctional fluorescence detector 425-406 was obtained from HIDEX (Finland) to collect the fluorescence signal of the assay on the test strip.

2.3. Labeling of anti-NT-proBNP antibody with biotin

The procedure of anti-NT-proBNP antibody (grNT1-02) coupled with biotin was described as following. Briefly, the anti-NT-proBNP antibody was diluted in 0.8% NaCl PBS buffer to a concentration of 6.5 mg ml^{-1} by using a centrifuge tube before labeling. Biotin was dissolved in DMF solution to a final concentration of 0.01 mol l^{-1}. Then 65 μl of 0.01 mol l^{-1} biotin was added into 1 ml of 6.5 mg ml^{-1} anti-NT-proBNP antibody in a centrifuge tube with shaking 30 s and the reaction mixture was incubated at 30 °C for 30 min. Afterward, 2.6 μl of 1 mol l^{-1} NH_4Cl was added into the mixture for blocking labeling reaction at 30 °C for 10 min. The excess unreacted NH_4Cl and biotin were eliminated from the mixture by dialysis with 0.8% NaCl PBS buffer (pH 7.4) at 4 °C for 2 h. The dialysis step was repeated 3 times and each time the dialysis solution should be changed. Finally, the biotin-labeled anti-NT-proBNP antibody (6.5 mg ml^{-1}) was stored at −20 °C before use.

2.4. Preparation of fluorescent protein modified by streptavidin

The fluorescent protein is a fusion protein and was prepared by genetic engineering recombinant technology. The gene sequences of the fluorescent protein consist of Streptavidin gene SA, β subunit cpcB of Phycocyanin, lyase alr0617, and phycoerythrobilin synthetase gene ho1, pebA, pebB. Specific forward and reverse primers were designed to amplify the target fragment. Then the gene fragment of the fluorescent protein was transferred to plasmid, and the fusion gene that simultaneously containing β subunit cpcB of Phycocyanin, Streptavidin and histag was screened. By expression, identification and purification of the fusion protein, the fluorescent protein modified by streptavidin was obtained.

2.5. Preparation of the test strip

The test strip consists of six components: the sample application pad, fluorescent marker conjugation pad, labeled antibody conjugation pad, nitrocellulose membrane, absorption pad and backing card. The preparation of the test strip was described as follows. The sample application pad (1.5 cm × 30 cm) was made of glass fiber and it was firstly treated with PBS buffer (0.1 M, pH 7.4) containing 2% polyvinyl alcohol AH-26, 2% D-trehalose, 1% BSA, 10% Sodium azide and 2% Tween-20 for

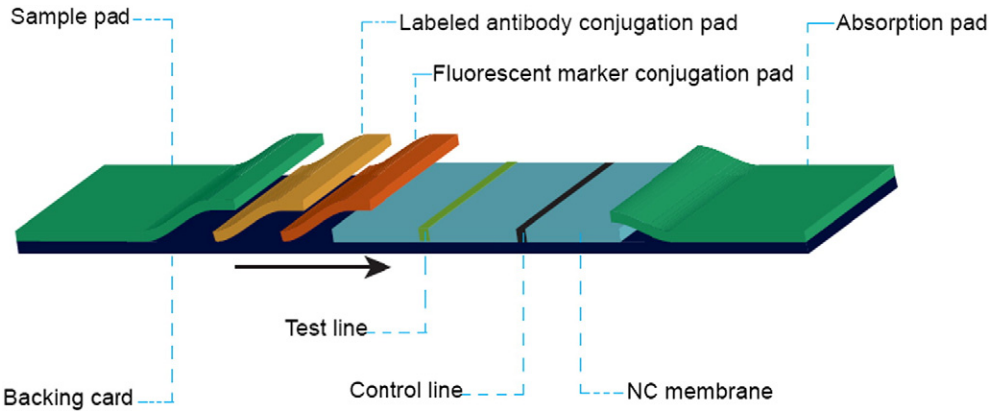

Fig. 1. Schematic diagram of the immunochromatographic test strip.

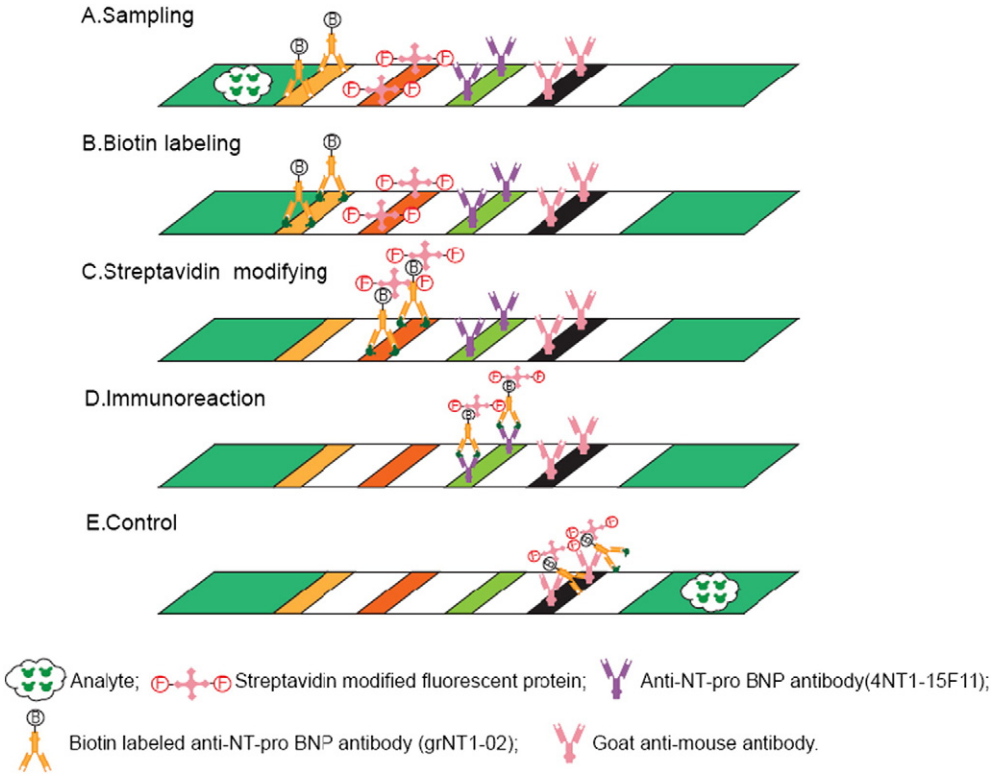

Fig. 2. Schematic illustration of the principle of fluorescent immunochromatographic assay. (A) Liquid sample containing analytes (NT-proBNP) was added to the sample pad. (B) Analyte was bound with anti-NT-proBNP antibody (grNT1-02) labeled with biotin and migrated along the other end of the test strip by capillary action. (C) The complex NT-proBNP-anti-NT-proBNP antibody (grNT1-02)-biotin was conjugated to the fluorescent protein modified by streptavidin through the streptavidin-biotin amplification system. (D) Anti-NT-proBNP antibody (4NT1-15F11) immobilized on the test line capture the complex NT-proBNP-anti-NT-proBNP antibody (grNT1-02)-biotin-fluorescent protein and form sandwiched complexes. (E) Some of the analytes continue to flow toward the control line and forms a fluorescent band regardless of the presence or absence of NT-proBNP. Finally, excess analytes continue to flow into the absorption pad at the end of the strip.

10 min. Then it was dried for 15 h at 37 °C and stored in a desiccator at room temperature. The conjugation pad (1 cm × 30 cm) was also made up of glass fiber and it was firstly pretreated with PBS buffer (0.1 M, pH 7.4) containing 2% polyvinyl alcohol AH-26, 0.5% Glucan 20000, 2% D-trehalose, 2% BSA, 10% Sodium azide and 2% Tween-20 for 10 min. Then it was dried for 15 h at 37 °C and stored in a desiccator at room temperature. For preparation of fluorescent marker conjugation pad, the fluorescent marker was diluted to concentration of 1.0 mg ml^{-1} with PBS buffer (0.1 M, pH 7.4) and dispensed at 3.9 μl cm^{-1} onto the pretreated glass fiber pad using an automatic dispenser, followed by being dried for 6 h at 37 °C and then stored at room temperature in a sealed foil bag. For preparation of labeled antibody conjugation pad, the biotin labeled antibody grNT1-02 was diluted to concentration of 0.5 mg ml^{-1} with PBS buffer (0.1 M, pH 7.4) and dispensed at 3.9 μl cm^{-1} onto the pretreated glass fiber pad using an automatic dispenser, after drying for 6 h at 37 °C, it was stored at room temperature in a sealed foil bag. The detection zone of the nitrocellulose membrane involves immobilized goat anti-mouse antibodies as control line and anti-NT-proBNP antibodies as test line (0.3 and 2 mg ml^{-1} in 0.1 M PBS buffer, pH 7.4, respectively) dispensed at 1 μl cm^{-1} using an automatic dispenser. Then the nitrocellulose membrane was dried for 6 h at 50 °C and stored in a desiccator at room temperature for using. The absorption pad was stored at room temperature without any treatment. All of the above parts were assembled on a plastic adhesive backing card in proper order as follows. Firstly, fixed the nitrocellulose membrane to the bottom of the backing card, and then fixed the absorption pad, fluorescent marker conjugation pad, labeled antibody conjugation pad and the sample application pad to the top. Finally, the test strip (Fig. 1) with a 4.1 mm width was cut using the Guillotine cutting system C-6 and assembled into strip cassettes for the following assay.

2.6. Sample preparation

131 serum samples, 50 plasma and 50 whole blood samples were collected from Nanfang Hospital (Guangdong, China) and stored at −20 °C until use. The Ethical Committee of Science and Technology Department of the Southern Medical University approved collection of these samples.

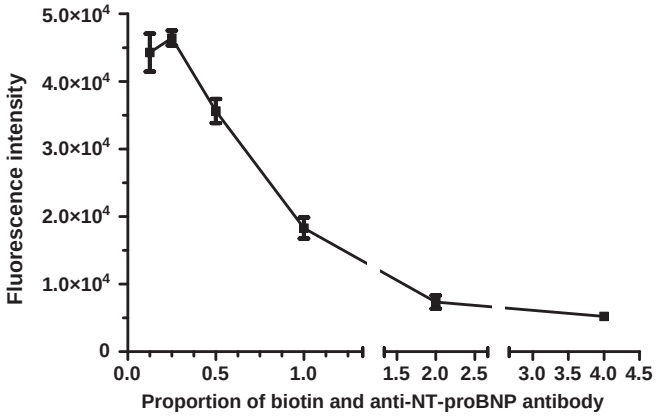

Fig. 3. Optimization of the marking proportion of biotin and anti-NT-proBNP antibody grNT1-02 (0.125:1, 0.25:1, 0.5:1, 1:1, 2:1, 4:1).

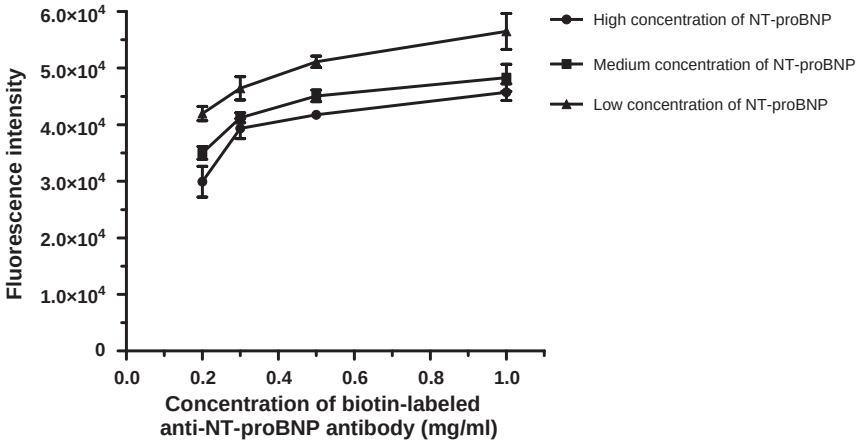

Fig. 4. Optimization of the concentration of biotin-labeled anti-NT-proBNP antibody (grNT1-02) applied (varied from 0.2 to 1.0 mg ml^{-1}). The NT-proBNP standard samples with three different concentrations (50, 1500 and 15,000 pg ml^{-1}) were detected in this assay by using 1.0 mg ml^{-1} of fluorescent protein, 2.0 mg ml^{-1} of anti-NT-proBNP antibody (4NT1-15F11) and 0.3 mg ml^{-1} of goat anti-mouse antibody.

2.7. Detection of NT-proBNP

The immunochromatographic assay on the test strip was performed as follows: 100 μl of sample was added to the sample application pad, after 10 min reaction at room temperature, the test strip was inserted into the multifunctional fluorescence detector, then the fluorescence signal was measured at the wavelength of 555 nm.

3. Results and discussion

3.1. Principle of the immunochromatographic assay

In this study, the basic principle of this method is based on a sandwich-type immunoassay format. Analytes in samples were captured by one monoclonal antibody labeled with fluorescent protein and "sandwiched" by another monoclonal antibody immobilized on the nitrocellulose membrane, then fluorescence was measured by fluoroanalyzer and the concentration of analytes could be calculated. The detailed principle of the proposed immunochromatographic assay is illustrated in Fig. 2. During the assay, a certain amount of sample containing NT-proBNP was applied onto the sample application pad as shown in Fig. 2A. Subsequently, NT-proBNP migrated along the porous membrane by capillary action and then bound to anti-NT-proBNP antibody (grNT1-02) labeled with biotin (Fig. 2B). Then, the ternary complex could be conjugated to the fluorescent protein modified by streptavidin through the streptavidin-biotin amplification system (Fig. 2C). The formed complexes continued to migrate along the membrane to the test zone and were selectively captured by the anti-NT-proBNP antibody (4NT1-15F11) which were immobilized on the test zone of the nitrocellulose membrane to form sandwiched complexes as shown in Fig. 2D. Some of the analytes will continue to flow toward the control line and form a fluorescent band regardless of the presence or absence of NT-proBNP (Fig. 2E). Finally, the fluid fraction containing excess analytes continue to flow into the absorption pad at the end of the strip. After a complete assay, quantitative analysis was obtained by reading the fluorescence intensities of the test line with fluoroanalyzer. The more NT-proBNP in the sample, the more fluorescence conjugates would bind to the capturing antibodies in the test line, which leads to the increase of the fluorescence intensity. Therefore, the concentration of the NT-proBNP could be calculated due to the fluorescence signal is proportional to the analyte concentrations in the sample. Using this strategy, a simple, rapid, sensitive and specific assay has been developed for the detection of NT-proBNP in the human sample.

3.2. Optimization of the marking proportion of biotin and anti-NT-proBNP antibody grNT1-02

Optimization of the marking proportion of biotin and anti-NT-proBNP antibody grNT1-02 (0.125:1, 0.25:1, 0.5:1, 1:1, 2:1, 4:1) was directly tested using a nitrocellulose membrane coated with excess goat anti-mouse antibodies. The fluorescence intensity increased with

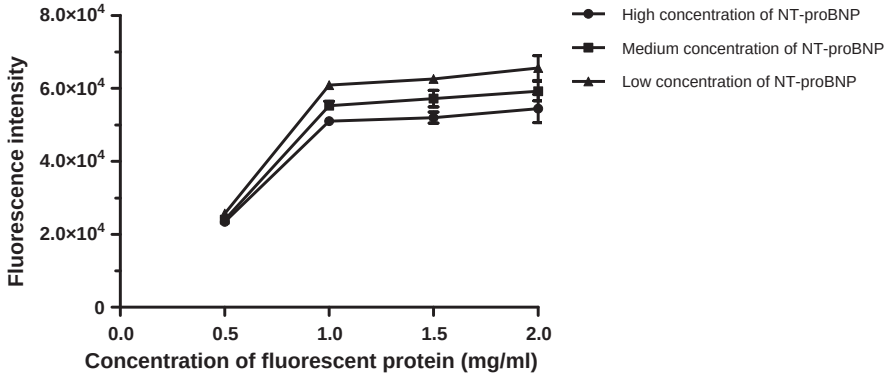

Fig. 5. Optimization of the concentration of fluorescent marking protein applied (varied from 0.5 to 2.0 mg ml^{-1}). The NT-proBNP standard samples with three different concentrations (50, 1500 and 15,000 pg ml^{-1}) were detected in this assay by using the concentrations of biotin-labeled anti-NT-proBNP antibody (grNT1-02) 0.5 mg ml^{-1}, immobilized anti-NT-proBNP antibody (4NT1-15F11) 2.0 mg ml^{-1}, and immobilized goat anti-mouse antibody 0.3 mg ml^{-1}, respectively.

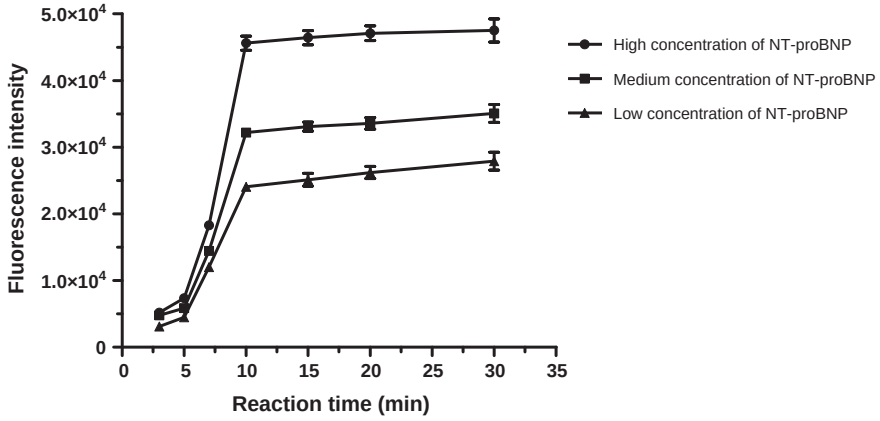

Fig. 6. Optimization of the reaction time (varied from 3 to 30 min). The NT-proBNP standard samples with three different concentrations (50, 1500 and 15,000 pg ml^{-1}) were applied in this assay.

decreasing proportion of biotin and anti-NT-proBNP antibody grNT1-02 (Fig. 3). However, if the marking proportion were less than 0.25:1, the fluorescence intensity tended to reach a maximum. Therefore, the marking proportion of 0.25:1 was selected as the optimum because satisfactory signal intensity was achieved.

3.3. Optimization of the concentration of biotin-labeled anti-NT-proBNP antibody grNT1-02

Biotin-labeled anti-NT-proBNP antibody grNT1-02 loaded on the glass fiber by physical absorption were used to capture NT-proBNP in this sandwich-type immunoassay. Hence, the linear range of this method was determined mostly by the concentration of biotin-labeled anti-NT-proBNP antibody grNT1-02. In order to save the cost of materials and heighten the sensitivity of the assay, the concentration of biotin-labeled anti-NT-proBNP antibody grNT1-02 was optimized. To ensure the sensitivity of the proposed method, a nitrocellulose membrane test strip was prepared with 1.0 mg ml^{-1} of fluorescent protein concentration, 2.0 mg ml^{-1} of anti-NT-proBNP antibody 4NT1-15F11 concentration and 0.3 mg ml^{-1} of goat anti-mouse antibody concentration. The sensitivity was investigated with concentrations of biotin-labeled anti-NT-proBNP antibody grNT1-02 (0.2, 0.3, 0.5 and 1.0 mg ml^{-1}) and three different concentrations (50, 1500 and 15,000 pg ml^{-1}) of NT-proBNP were added to the test strip to detect the fluorescence signals. The results were shown in Fig. 4. Under the conditions of high, medium and low concentrations of NT-proBNP, fluorescence intensity increased as the increasing concentration of biotin-labeled anti-NT-proBNP antibody grNT1-02, and 0.5 mg ml^{-1} of biotin-labeled anti-NT-proBNP antibody grNT1-02 concentration has fulfilled the reaction demands. Therefore, 0.5 mg ml^{-1} of biotin-labeled anti-NT-proBNP antibody grNT1-02 concentration was chosen as the optimal working concentration for subsequent assays.

3.4. Optimization of the concentration of fluorescent marking protein

The fluorescent marking protein loaded on the glass fiber by physical absorption was used to conjugate with biotin-labeled anti-NT-proBNP antibody grNT1-02 (the conjugation principle has been illustrated at Section 3.1) in this assay. The sensitivity of this method for NT-proBNP was closely related to the concentration of fluorescent marking protein. Optimization of the concentrations of fluorescent marking protein (0.5, 1.0, 1.5 and 2.0 mg ml^{-1}) was also done using NT-proBNP standard samples of concentrations of high, medium and low. The assay was carried out on a nitrocellulose membrane test strip which was prepared with the concentrations of biotin-labeled anti-NT-proBNP antibody grNT1-02 0.5 mg ml^{-1}, immobilized anti-NT-proBNP antibody 4NT1-15F11 2.0 mg ml^{-1}, and immobilized goat anti-mouse antibody 0.3 mg ml^{-1}, respectively. As shown in Fig. 5, a higher fluorescence intensity was achieved with the increasing concentration of fluorescent marking protein and this then remained unchanged with an increase in the fluorescent marking protein concentration. And 1.0 mg ml^{-1} of fluorescent marking protein concentration has met the reaction demands, increasing the concentration of fluorescent marking protein would enhance the background fluorescence interference, which might interfere the judgement of results. Therefore, 1.0 mg ml^{-1} of fluorescent marking protein concentration was selected as the optimal concentration for the following experiments.

3.5. Optimization of the reaction time

It is well known that the reaction time can dramatically influence the immunoassay sensitivity and the immunoreaction between antigen and antibody. In this assay, the effect of reaction times was investigated at 3, 5, 7, 10, 15, 20 and 30 min by detecting high, medium and low concentrations of NT-proBNP samples. The results would be judged from the fluorescence intensity of each concentration, the reaction repeatability and integrity. As shown in Fig. 6, the fluorescence intensity of the

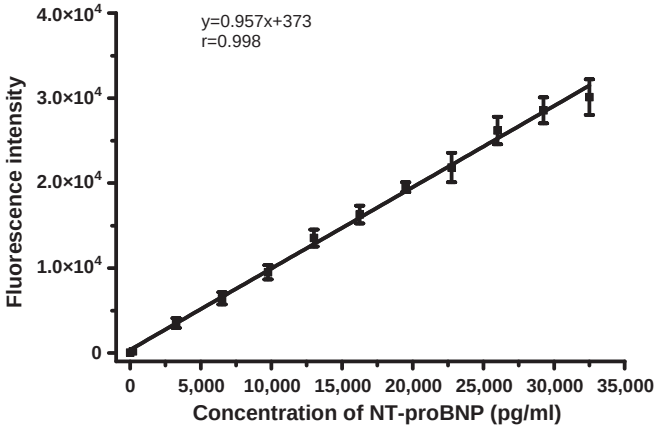

Fig. 7. The standard curve for the fluorescent immunochromatographic assay for the detection of NT-proBNP under optimized experimental conditions. Fluorescence intensity and standard deviations were calculated from a set of four measurements. *X* axis, represents the concentration of NT-proBNP. *Y* axis, represents the fluorescence intensity.

Table 1
Inter-assay and intra-assay reproducibility (precision of the assay).

Spiked (pg ml^{-1})	Inter-assay precision (n = 20)		Intra-assay precision (n = 20)	
	Mean ± SD (pg ml^{-1})	CV (%)	Mean ± SD (pg ml^{-1})	CV (%)
500	503.2 ± 45.58	9.1	503.2 ± 47.93	9.5
5000	5031.7 ± 483.93	9.6	5071.3 ± 483.90	9.6

Table 3
Comparison of the detection of serum samples to the whole blood and plasma samples.

Pair	Sample	N	Mean ± SD (pg ml^{-1})	t	p
Pair1	Serum	50	5719.37 ± 7183.61	1.411	0.164
	Whole blood	50	5662.54 ± 7123.81		
Pair2	Serum	50	5719.37 ± 7183.61	−0.611	0.544
	Plasma	50	5741.19 ± 7215.29		

$p < 0.05$, statistically significant difference.

three samples increased along with reaction time and did not achieve equilibrium before 10 min of reaction, and longer reaction times did not obviously change the fluorescence intensity, the reaction repeatability and integrity. Considering the time consumed, 10 min was selected as the most suitable reaction time for the following experiments.

3.6. Linear range and the analytical sensitivity

Under optimized experimental conditions, a series of NT-proBNP standards with different concentrations (0, 200, 3250, 6500, 9750, 13,000, 16,250, 19,500, 22,750, 26,000, 29,250 and 32,500 pg ml^{-1}) were continuously measured four times for each concentration, and the calibration curve for NT-proBNP was illustrated in Fig. 7. The results were fit by SPSS13.0 to a regression curve. Under optimal conditions, the calibration curve for NT-proBNP was fitted by the equation: $y = 0.957x + 373$ with a correlation coefficient of 0.998. It showed a linear range from 200 to 26,000 pg ml^{-1}. The blank sample was repeatedly measured 20 times and calculated the mean and standard deviation. The resulting mean plus two standard deviations were defined as the limit of detection, which was 47 pg ml^{-1}. These results indicated that the sensitivity reached permitted determination of very low levels of NT-proBNP. And most important of all, the entire analysis can be completed within 15 min. Thus, the results suggested that the fluorescent immunochromatographic assay possessed high sensitivity and wider detection range and could be used as a potential clinical application for the detection of NT-proBNP.

3.7. Precision and accuracy of the assay

Precision of the assay was evaluated by intra-assay and inter-assay coefficients of variation (CV) according to the CLSI EP5-A2 guideline [31]. Briefly, we analyzed two separate runs with two test samples of high and low concentration of NT-proBNP daily, and the samples were tested in duplicate per run, two separate runs per day with an interval of at least 2 h. The experiment lasted for 20 days. The CVs at the concentrations of 500 pg ml^{-1} and 5000 pg ml^{-1} were 9.1% and 9.6% for intra-assay, 9.5% and 9.6% for inter-assay. Both the intra-assay and the inter-assay CVs were lower than 10%, and the results are within the criteria recommended by the CLSI guideline (Table 1). The accuracy of the assay was estimated by adding the samples of NT-proBNP at the concentrations of 5000 pg ml^{-1} and 10,000 pg ml^{-1} into the given amount of NT-proBNP standard (1000 pg ml^{-1}) at various analyte levels. All of the samples were measured three times and then the mean recoveries were calculated. As shown in Table 2, the recoveries of the analyte were 97.5% and 99.1%. Therefore, the precision and accuracy of the assay established in our study were acceptable.

Table 2
Recovery (accuracy of the assay).

Original (pg ml^{-1})	Spiked (pg ml^{-1})	Observed Mean ± SD (pg ml^{-1})	Recovery (%)
1000	5000	5984.13 ± 553.35	97.5
1000	10,000	11,023.83 ± 773.13	99.1

3.8. Comparison of the detection of serum samples to the whole blood and plasma samples

To further evaluate the performance of the new immunochromatographic assay, 50 cases of serum, plasma and whole blood samples from patients were detected respectively. And the results were analyzed by paired-samples *t*-test. As shown in Table 3, for pair 1 and pair 2, the comparative analysis of serum samples to whole blood and plasma samples showed no significant difference ($p = 0.164$ or $p = 0.544$). This finding suggested that the immunochromatographic assay could serve for clinical determination of NT-proBNP in human serum, plasma and whole blood.

3.9. Clinical application of the assay

The feasibility of the immunochromatographic assay for clinical application was further investigated by analyzing 131 clinical serum samples from patients. And the results were compared with those detected by a commercial CLIA kit. Linear regression analysis was shown in Fig. 8. The data revealed a good correlation between the immunochromatographic method and CLIA with a correlation coefficient of 0.978. The equation of the regression curve was $y = 0.973x + 121.2$, where x represents the concentration of NT-proBNP calculated by our immunochromatographic method and y represents the concentration of NT-proBNP calculated by the CLIA method. The results indicate that the immunochromatographic assay has good potential clinical application for the detection of NT-proBNP.

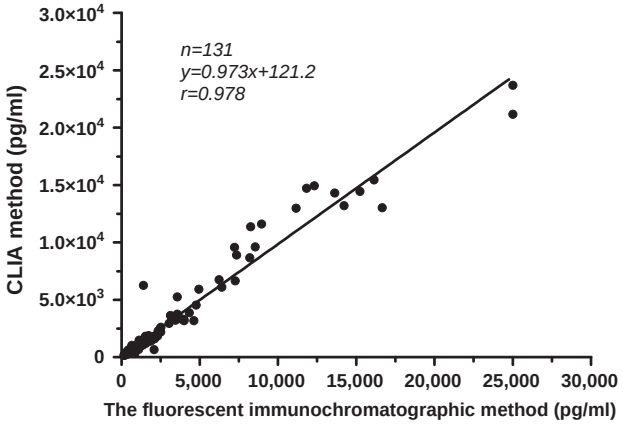

Fig. 8. Comparison between the fluorescent immunochromatographic method and CLIA. The comparison was made by using 131 clinical serum samples. The data revealed a good correlation between the proposed method and CLIA with a correlation coefficient of 0.978.

4. Conclusion

In conclusion, a novel fluorescent immunochromatographic method for detecting NT-proBNP in human serum was successfully developed. The method applied a fluorescent protein modified by streptavidin as a tracer to conjugate the biotin-labeled anti-NT-proBNP antibody on the conjugation pad to facilitate the detection of NT-proBNP in human serum samples. This assay platform revealed several advantages compared with conventional methods, such as higher sensitivity, higher efficiency, broader dynamic assay ranges and less consumption of reagents. Furthermore, the assay showed good properties for detection of NT-proBNP with acceptable precision and accuracy. And it also displayed good correlation with CLIA kit. This immunosensor could be developed into commercially available fluorescent immunochromatographic kits for the clinical detection of NT-proBNP or other biomarkers, and its celerity provides highly promising for in-field and point-of-care diagnosis especially during emergency events.

Conflicts of interest

The authors have declared that no competing interests exist.

Acknowledgements

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