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A novel strategy for rapid detection of NT-proBNP

Qiyao Cui¹, Honghao Sun¹ and Hui Zhu²

¹School of Bioengineering and Food, Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Hubei University of Technology, Wuhan 430068, China
²Wuhan NCD biotechnology co., LTD

Abstract. In order to establish a simple, rapid, sensitive, and specific quantitative assay to detect the biomarkers of heart failure, in this study, biotin-streptavidin technology was employed with fluorescence immunochromatographic assay to detect the concentration of the biomarkers in serum, and this method was applied to detect NT-proBNP, which is valuable for diagnostic evaluation of heart failure.

1 Introduction

Rapid detection and accurate identification of biomarker is critical in clinical laboratory testing, biological warfare, food industry, and environmental monitoring. Numerous studies have shown that N-terminal pro-brain natriuretic peptide (NT-proBNP) testing was valuable for diagnostic evaluation, NT-proBNP can be used as a strong prognostic marker of the severity of heart failure [1]. Concentrations of NT-proBNP increase in patient with heart failure and other cardiovascular (CV) diseases. The measurement of NT-proBNP are diagnostically useful and of prognostic significance [2]. At present, the detection methods to determine the concentration of NT-proBNP mainly include radiation immunity analysis (RIA), enzyme-linked immunoassay (ELISA), electrochemiluminescence immunoassay (ECLIA) and fluorescence immunochromatographic assay, etc [3-5]. Because of the samples or ¹²⁵ I mark is not stable, RIA method often appear larger error, the disadvantages of low sensitivity and specificity, complex determination method, long-term, and large required sample size, make the RIA not a ideal method despite of its low cost. In recent years, enzyme-linked immunoassay in clinical diagnosis of disease has achieved widespread application [6]. But there are many limitations of this present method, such as miscellaneous steps, the difficulty of reagent preparation and long time is necessary [6]. Besides, its precision and sensitivity are lower than luminescence immune technology. The electrochemical luminescence immunoassay has extensive application in the quantitative determination of NT-proBNP, Roche Elesys electrochemical luminescence immunoassay analysis system always adopted for the rapid detection because of its high sensitivity and accuracy [7]. But this method is not suitable for small and medium-sized hospitals and individuals to the NT-proBNP concentration detection because large size sample is necessary. In addition, expensive apparatus and reagents needed for the test limits the extensive application of chemiluminescence immunoassay [8]. Fluorescence immunoanalysis method is widely adopted with simple operation, low cost, small volume and convenient carrying [9]. Furthermore, the result can be obtained quickly, which is significant in clinical diagnosis. But the sensitivity is not satisfactory. Therefore, in this study, a more sensitive fluorescence immunochromatographic assay for rapid detection of NT-proBNP was developed. Combining biotin-streptavidin technology to fluorescence immunochromatographic assay will remarkably improve the intensity of rapid detection. We take advantage of the strong affinity
between biotin and streptavidin, the multi-stage amplification effect make the BAS immune markers and the tracer analysis is more sensitive, has become the current widely used in trace antigen and antibody qualitative and quantitative detection and location observational study of new technology. Streptavidin have the ability to bind up to four biotin molecules, the high affinity constant between biotin and streptavidin is $10^{15}$ L/mol. The bond formation between biotin and streptavidin is very rapid, and once formed, is unaffected by extremes of pH, temperature, organic solvents and other denaturing agents [10]. These features are useful for purifying or detecting proteins conjugated to either component of the interaction.

Fig.1 The fluorescence immunoassay process combined with Biotin-streptaviding technology

2 Experiment

2.1 Materials
1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC), N-hydroxy-succinimide (NHS) and 2-morph-oioethanesulfonic acid (MES) were purchased from Sinopharm Chemical Reagents Company. Bovine serum albumin (BSA), biotin and streptavidin were obtained from Sigma Aldric. 400nm fluorescent microspheres were purchased from RAMP. Mouse monoclonal anti-NT-proBNP 15C4 and 13G12 antibodies and NT-proBNP were purchased from HyTest Ltd, goat anti-mouse immunoglobulin (IgG) was from Abcam, blood samples were gifted by Xiaogan Central Hospital. Deionized water (18.4 MU cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

2.2 Preparation of fluorescent microspheres labeled by antibody
For the coupling of antibodies to fluorescent microspheres, 100μL of 400nm fluorescent microspheres (1mg/mL) was washed several times with 0.1 M MES (pH 6.0), followed by incubation for 45 min with 1 mL of 10 mM MES containing 5 mmol of EDC and 7.5 mmol of NHS. After that, the mixture was washed three times with 10mM PB (pH 7.4), and incubated with 1.64μL of 9.2 mg/mL anti-NT-proBNP antibody 15C4 in 1 mL of 10 mM PB at 4°C overnight. Then 250μL of 10 mM PB containing 10% (w/v) BSA was added to the solution to incubate for 2h to block the surface of fluorescent microspheres. The conjugates were washed several times with 10 mM PB (pH 7.4). Finally the product was dispersed in 250μL of 10 mM PB, after which the solution was stored at 4°C before use.

2.3 Preparation of biotin-antibody
For the coupling of antibodies to biotin, 7.15μLof 7.0 mg mL anti-NT-proBNP antibody 13G12 were added into 50μL biotin (1000 mg/ mL) in PB. The solution was incubated for 4h at room temperature, and then dilute the solution to 250μL with 10mM PB, dialysis for 48 h at 4°C.
2.4 Immunochromatography
The method of liquid fluorescent immune chromatography were adopt to prepare strips. Strip is assembled by overlapping sample pad, cellulose nitrate capsule and blotting paper progressive on the polyvinyl chloride (PVC) board. Streptavidin solution (10μg/μL) and the goat anti-mouse IgG secondary antibody(1μg/mL) were sprayed onto the nitrocellulose membrane as the test line and control line by membrane machine, respectively. 0.5 μL fluorescent microspheres labeled by anti-NT-proBNP antibody 15C4 mixed with 2 μL the biotin-antibody prepared above, and then join the sample that under test for 1 min , immune conjugate formed. Droping the conjugate into the well of the immune chromatography test paper, use 500 mM PB (pH7.4) as chromatography buffer ,chromatography 30000 ng/L, 15000 ng/L, 10000 ng/L, 7500 ng/L, 5000 ng/L, 2500 ng/L, 1000 ng/L, 750 ng/L, 500 ng/L, 250 ng/L, 125 ng/L and 20 ng/L standard samples respectively, and use fluorescent quantitative detector to detect the fluorence intensity of the test line(T) and the control line(C), compare the T/C.

3 Result and discussion

![Image](image)

**Fig. 2** The fluorescence spectra of the fluorescence immunoassay result (excitation: 550 nm, emission: 590 nm)

![Image](image)

**Fig. 3** Calibration curve of the fluorescence immunoassay

Fig. 2 shows the fluorescence spectra of the test line and the control line at different NT-proBNP concentrations, which indicated that this method was completely qualified for the detection of NT-proBNP. All the NT-proBNP samples in Fig. 2 were already detected by using a POCT fluorescence immunoassay quantitative analyser (RAMP200).

Fluorescence intensity ratio analysis was adopted to study the linear relation between fluorescence intensity and the NT-proBNP concentration of the sample. The performance of biotin-streptavidin system based fluorescence immunoassay was tested with different concentrations of NT-proBNP.
(20–30000 ng/L). Quantitative detection was performed by recording the fluorescence intensity at 590 nm with a fluorescence spectrophotometer (inset of Fig. 3). It was observed that the fluorescence intensity increased with the concentration of NT-proBNP, the concentration of NT-proBNP were found linear in concentration range of 20-30000 ng/L.

4 Conclusion
In this study, we reported a sensitive and convenient method to detect the concentration of NT-proBNP. Based on biotin-streptavidin technology, a new fluorescence immunoassay for NT-proBNP detection was developed. In the presence of NT-proBNP, fluorescent microspheres bearing 15C4 and biotin linked with 13G12 form a sandwich structure, because of the strong affinity between biotin and streptavidin, the sandwich structure conjugate can be captured by the streptavidin on the test line, the bond formation between biotin and streptavidin is extremely rapid. Fluorescent microspheres offer a stable and amplified fluorescence signal which can quantify NT-proBNP concentration from the standard curve. This new method has several advantages for biomarker (NT-proBNP) detection, such as easy and simple operation, needlessness of trained technicians and centrifugation, and high sensitivity and specificity, as well as a quick detection time (in 5 min), all of which make it suitable for POCT.

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Reference
[1] Hunt P. J. (1997), Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP): a new marker of cardiac impairment. Clin Endocrinol (Oxf) 47:87–96.
[2] Kikula K. (1996), Increases plasma levels of B-type natriuretic peptide in patients with unstable angina. Am Heart J 132: 101-106.
[3] Collinson, P. O. (2004), Analytical performance of the N terminal pro B type natriuretic peptide (NT-proBNP) assay on the Elecsys™ 1010 and 2010 analysers. The European Journal of Heart Failure 6: 365–368.
[4] James L. J. (2006), NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients. European Heart Journal 27: 330–337.
[5] Maisel A. S. (2002), Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 347:161–167.
[6] Li C. Z. (2011), Paper based point-of-care testing disc for multiplex whole cell bacteria analysis. Biosensors and Bioelectronics 26: 4342–4348.
[7] Qi H. (2011), A rapid and highly sensitive protocol for the detection of Escherichia coli O157: H7 based on immunochromatography assay combined with the enrichment technique of immunomagnetic nanoparticles[J]. International Journal of Nanomedicine 6: 3033-3039
[8] Pratikkumar S. (2013), Development of paper-based analytical kit for point-of-care testing. Expert Rev. Mol. Diagn. 13(1): 83–91.
[9] Eshaq (Isaac) S. (2003), Determination of ractopamine in animal tissues by liquid chromatography—fluorescence detection and liquid chromatography / tandem mass spectrometry[J]. Analytica Chimica Acta 483: 137—145.
[10] Zhang J. (2009), Fluorescent avidin-bound silver particle: a novel strategy for single target molecule detection on cell membrane. Anal Chem. 81(3): 883–889.