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ORIGINAL RESEARCH

A Rapid Method Based on Colloidal Selenium Nanoparticle to Detect NT-proBNP Antigen in Serum for Monitoring Acute Heart Failure

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Purpose: Rapid and accurate detection of acute heart failure (AHF) enables effective treatment of HF. This study aimed to to establish a timely test using colloidal selenium for initial screening and risk assessment of HF in primary care settings and at home.

Methods: Colloidal selenium was synthesized by reduction of sodium selenite with vitamin C under ambient conditions. In order to improve the stability of colloidal selenium and the coupling efficiency of the antibody, a novel synthetic method to coat polyethylene glycol 20000 (PEG20000) and sodium dodecyl sulfate (SDS) on colloidal selenium was developed. In order to improve the detection performance of colloidal selenium test strips, the labeling conditions and construction processes were optimized by the controlled variable method, and finally the test strips were successfully prepared.

Results: PEG20000 and SDS modified colloidal selenium had a very low detection limit of 250pg/mL, which met the sensitivity criteria for the diagnosis of acute heart failure in the Chinese Guidelines for the Diagnosis and Treatment of Heart failure (2024) that NT-proBNP ≤300 pg/mL can exclude acute heart failure, and ≤125 pg/mL can exclude chronic heart failure.

Conclusion: The developed single-step immunochromatographic method utilizing PEG20000 and SDS-modified colloidal selenium demonstrates meaningful potential for the rapid and reliable detection of NT-proBNP in serum samples from clinical patients. The colloidal selenium immunochromatographic technique developed in this study was assessed from the aspect of material synthesis, and the conditions for the application of this test paper were screened to verify the high specificity of the test paper, which met the criteria of the Chinese Heart Failure Diagnosis and Treatment Guidelines 2024 for acute heart failure.

Keywords: colloidal selenium, NT-proBNP, test strip, heart failure

Introduction

Heart failure (HF) is a syndrome characterized by the heart's inability to pump enough blood and oxygen to support the metabolic needs of other organs.[1] There are approximately 64 million people with HF worldwide. The prevalence of HF is increasing due to an aging population, increased comorbidities and risk factors for HF, and longer survival times after myocardial infarction.[1,2] The most prevalent population includes those over 70 years of age, with a prevalence of more than 10% and a 5-year mortality rate of up to 50%.[3–5] AHF is the leading cause of hospitalization in patients >65 years of age and has a very poor prognosis, with a hospitalization mortality rate of 12%, a 1-year rehospitalization rate of about 45%, and a 1-year mortality rate of about 22%. The initial diagnosis (suspicion) of AHF mostly begins with dyspnea as the prominent clinical manifestation. Early detection and diagnosis is therefore critical to avoid advanced stages of AHF and improve overall prognosis.[6] Electrocardiogram (ECG) and cardiac magnetic resonance (CMR) are usually the criterion standard for the diagnosis of definite AHF, but the application of these tools requires high costs and high requirement of expertise, and they are not applicable for use as screening tools in the general population,[6,7] which poses a

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number of difficulties in the early diagnosis of cardiovascular diseases and aggravates the health risks of patients. In order to break the current deadlock, we have tried to adopt the relatively mature emerging technologies such as immunochromatography and microfluidics,[8] which have the advantages of low cost, simple instrumentation and easy integration.[9,10] However, Microfluidic chips have limited sample handling capacity and risk of clogging and contamination.[11,12] In contrast, immunochromatographic technology is not only easy to operate, rapid detection, high specificity, good stability, and easy to carry, has been widely used in clinical diagnosis, food safety, drug testing, environmental pollution and other fields. N-terminal B-type natriuretic peptide precursor (NT-proBNP) is a biomarker of cardiac function, and it is the preferred biomarker for the diagnosis and differential diagnosis of AHF,[13,14] as well as for the assessment of severity and prognosis, and it has been recommended by several clinical practice guidelines as a grade I.[15] The Chinese Guidelines for the Diagnosis and Treatment of Heart failure (2024) mention that NT-proBNP ≤300 pg/mL can exclude acute heart failure, and ≤125 pg/mL can exclude chronic heart failure.[13,16] Regarding the current methods of detecting NT-proBNP, the main ones include the following, as shown in Figure 1.

Colloidal gold-labeled qualitative test strips are the most commonly used lateral immunochromatographic test strips in the market,[17,18] but since the preparation of colloidal gold requires high temperature boiling and expensive raw materials,[19] it is still necessary to find a nano-labeling material that is simpler and more economical to prepare. Regarding raw material costs, the core ingredient for colloidal gold is chloroauric acid, which consistently commands a high price. In contrast, the primary component for colloidal selenium is sodium selenite, whose price is significantly lower than that of chloroauric acid. In terms of synthesis processes, colloidal gold requires high-precision control, increasing time and equipment costs. Colloidal selenium preparation is relatively straightforward, typically achieved by reducing selenite at room temperature, with far lower demands on equipment and environmental conditions than colloidal gold. Therefore, colloidal selenium holds significant advantages in both raw material costs and synthesis processes. Colloidal selenium has been gradually used to prepare lateral immunochromatographic test strips.[20–22] Based on its unique properties, colloidal selenium has been widely used in the study of lateral flow immunoassay (LFIA) detection probes in medical diagnostics, drug detection, food safety,[23] etc. PEG20000 was used as a stabilizer coated onto the surface of colloidal selenium particles for stabilizing the colloidal particles independent of the ionic strength, pH, and solvent polarity, because it can produce spatial repulsion when compressing adjacent polymer chains.[24] PEG20000 encapsulated colloidal selenium have better stability, coupling to the antibody without affecting its activity, and also

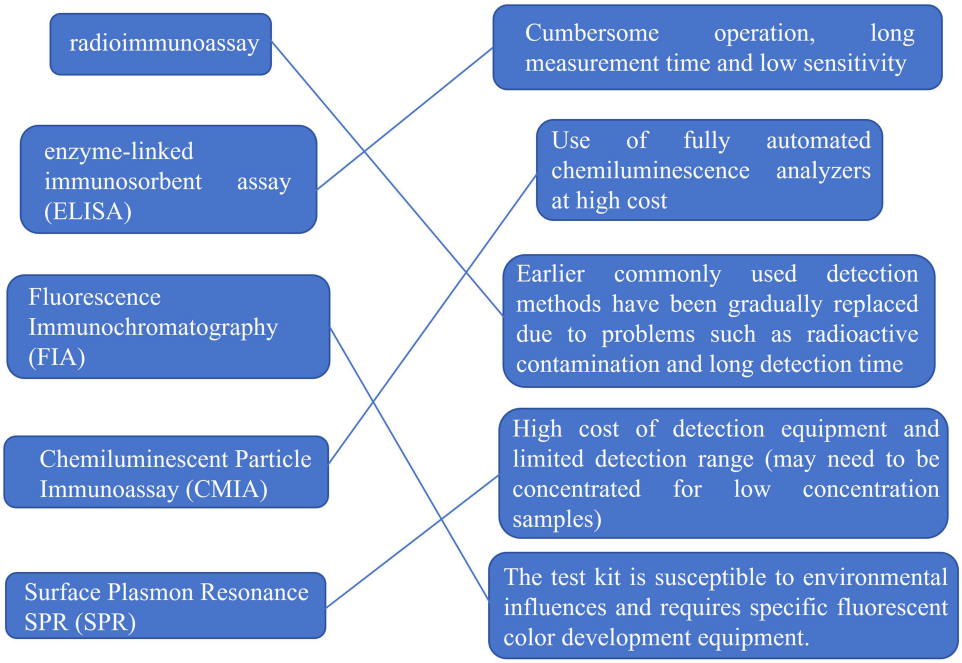

Figure 1 Schematic Diagram of Different Detection Methods.

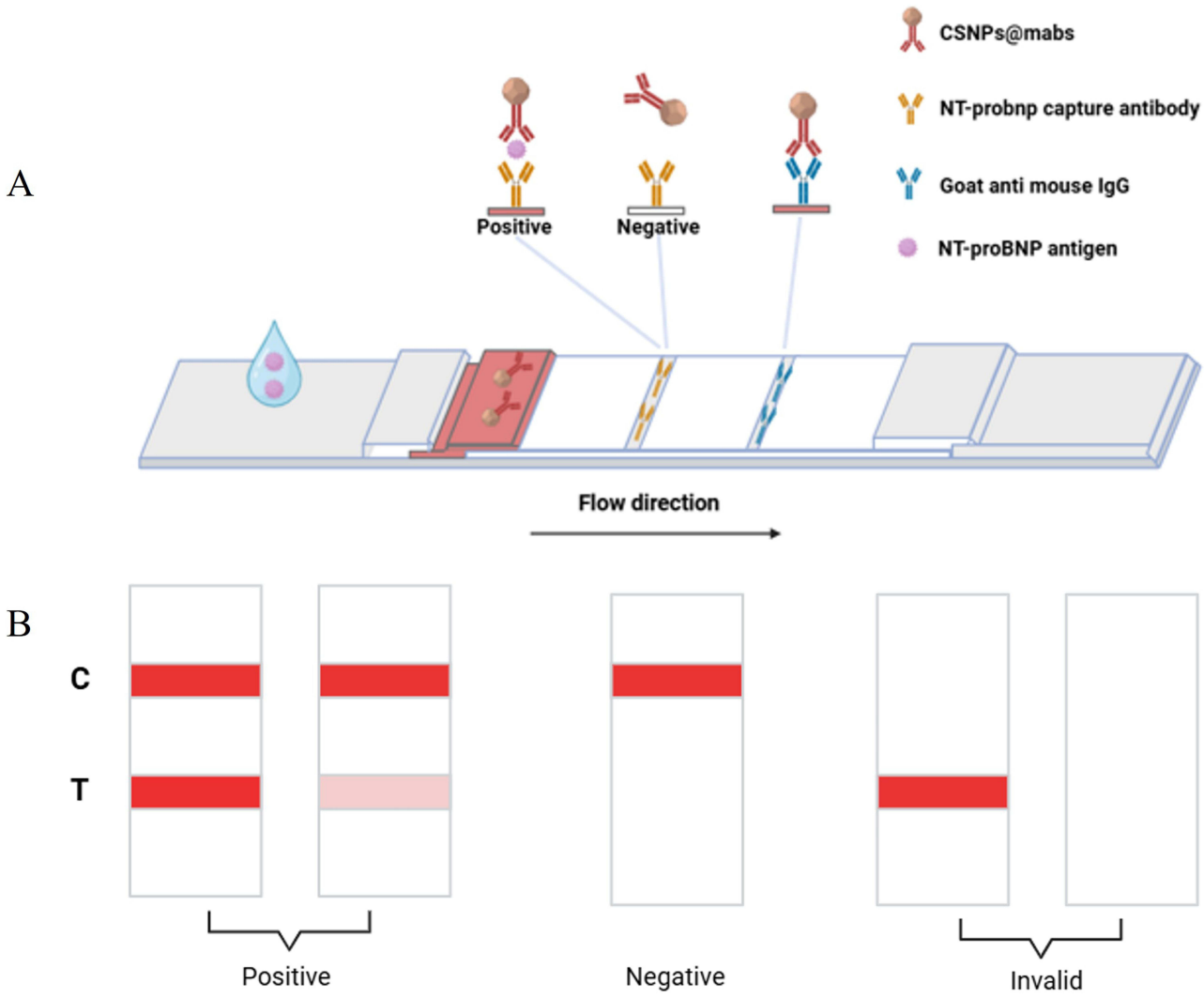

Figure 2 Graphical representation of colloidal selenium test strip. **(A)** The preparation and assembly of the immunoassay NT-proBNP test strip. **(B)** Result judgment of the test strip. C, control line; T, test line.

maintain protein adsorption efficiency, which suggests their advantage in LFIA immunochromatography.[25] Since NT-proBNP is prone to false positives due to its low limit of detection, Sodium dodecyl sulfate (SDS) is an anionic surfactant that minimizes false positives by reducing interference from background impurities.[26]

In this study, the developed rapid immunochromatographic method utilizing PEG20000 and SDS-modified colloidal selenium demonstrates significant potential for the rapid and reliable detection of NT-proBNP in biological samples. The specific testing procedure for colloidal selenium test strips is shown in Figure 2A. The interpretation of test results is shown in Figure 2B. The method exhibits robust plasma signal detection and exceptional colloidal stability, with a visual detection limit of 250 pg/mL, which can meet clinical demand. This study paves the way for rapid, sensitive, and convenient POCT of NT-proBNP in the clinic.

Materials and Methods

Materials and Instruments

Bovine serum albumin (BSA),PEG20000, trehalose and phosphate-buffered saline (PBS) from YuanYe Biological Co., Ltd. Selenium sulfide(Na_2SeO_3), ascorbic acid(Vc), and sodium dodecyl sulfate were purchased from Shanghai Aladdin Biological Co., Ltd. The mouse anti-human NT-proBNP mab-Clone-1,mouse anti-human NT-proBNP mab-Clone-2

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antibodies, and recombinant human source NT-proBNP antigen were purchased from Nanjing BaiKang Bio-Tech Co., Ltd. Goat anti-mouse IgG was purchased from Hangzhou Qitai Biotech Co., Ltd. Phosphate-buffered saline (PBS) was purchased from Jiangsu KeyGEN BioTECH Co., Ltd. All other reagents were analytical grade and were used without further purification. XYZ three-dimensional spray coating apparatus and automatic cutting machine were supported by Shanghai Jinbiao Biological Technology Co., Ltd. The absorbent pad, nitrocellulose (NC) membrane, plastic packing and sample pad were obtained from SARTORIUS Germany.

Preparation of Colloidal Selenium

Firstly, 0.1M SDS, 0.32M Vc and 0.04M Na_2SeO_3 were prepared separately, filtered and set aside. 17mL of ultrapure water was poured into a clean beaker fitted with an appropriately sized clean rotor, then added 100mg of PEG-20000 to the small beaker and stirred at 600rpm for 10 minutes under ambient conditions, then added 1mL of 0.1M SDS and mixed continuously for 20 minutes under ambient conditions then added 1mL of 0.04M Na2SeO3 and continued mixing for 20 minutes under ambient conditions, and finally added 1mL of 0.32M Vc to create a redox environment. At this time, the color of the solution in the beaker gradually changed from colorless to orange, and colloidal selenium was obtained after mixing for 20 minutes under ambient conditions. The chemical equation is shown in Figure 3.

Characterization of Colloidal Selenium

According to the principle of dynamic light scattering method, the particle size of colloidal selenium was determined and plotted by aspirating 1 mL of colloidal selenium solution in a cuvette using a Zetasizer Nano-ZS90 analyzer from Malvern Instruments, UK. To obtain the absorption spectrum of colloidal selenium, 1 mL of colloidal selenium solution was aspirated in a cuvette and the absorption spectrum was obtained by sweeping at room temperature with an external visible spectrophotometer (cat # 8453; Agilent Technologies, Santa Clara, CA, USA). In order to clearly observe the morphology of colloidal selenium and antibody-coated colloidal selenium, the morphology of the synthesized colloidal selenium and antibody-coated colloidal selenium was observed by transmission electron microscopy (TEM) (HT7700; Hitachi Ltd., Tokyo, Japan) using a charge-coupled device (CCD) camera at an accelerating voltage of 100 kV.

Preparation of Colloidal Selenium Labeled NT-proBNP Detection Antibody

The labeling procedure for the NT-proBNP mab-Clone-1 protein coupled with colloidal selenium follows the following steps. First, 1 mL colloidal selenium solution was adjusted to pH 5.5 with 0.1M K_2CO_3 solution, then 0.2 mg/mL NT-proBNP mAb-Clone 1 protein solution 13 μL was added to PBS and incubated for 20 min. Added 10% BSA 100μL, incubate for 20 min, centrifuge at 8500 rpm at 4°C for 15 min. Pipetted the supernatant with a pipette gun and redissolved with 50μL resolution (3% BSA, 5% sucrose, 5% trehalose, 0.125%Tween-20,0.1%P300 0.01M Tris-HCl, pH 4.5).

Preparation for LFIA

LFIA was prepared as described below and consists of an absorbent pad, a sample pad, a conjugate pad, a plastic wrap, and an NC membrane. The conjugate pads were treated with buffer (5% sucrose, 5% D-Trehalose anhydrous, 3% BSA, and 0.1% P300, dissolved in 0.01 M Tris-HCl; pH=6.5) and dried at 40 °C for 2 h. In a similar manner, the sample pad was handled with buffer (5% sucrose, 1% trehalose, 0.3% PVP K30, 0.2% BSA and 0.1% P300 in 0.01M Tris-HCl) and dried at 40 °C for 2 h. Using XYZ three-dimensional film spraying instrument, the capture antibody (NT-proBNP mab-Clone-1) and goat anti-mouse IgG antibody were drawn on the T line and C line with 7 mm interval respectively, and the capture antibody (NT-proBNP mab-Clone-1) (1.5 mg/mL) and goat anti-mouse antibody (1.7 mg/mL) were spotted onto

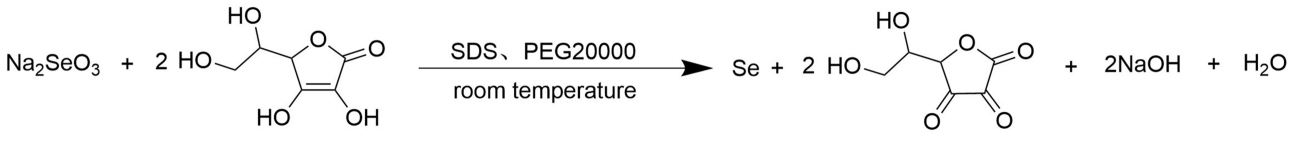

Figure 3 Equations for the synthesis of colloidal selenium nanoparticles.

the NC membrane at a concentration of 0.9 μL/cm and 0.8 μL/cm, respectively, and goat anti-mouse antibody (1.7 mg/mL) were respectively spotted into the NC membrane at a concentration of 0.9 μL/cm and 0.8 μL/cm to form the T line and C line. The NC membrane was dried at 40 °C under a vacuum for 2 h. Subsequently, the NC membrane, sample pad and absorbent pad were fixed on a plastic substrate and cut into 4 mm wide strips of immunochromatographic test paper using a guillotine cutter model ZQ2002. The re-dissolved colloidal selenium antibody conjugate was added to the coupling pads and dried in an oven at 40 °C for 20 minutes, then the coupling pads were mounted on the test strips and the samples were added to observe the color development of the C line and T line.

Preparation of NT-proBNP Standard Sample

A series of standard NT-proBNP antigen solutions at different concentrations were prepared by adding recombinant NT-proBNP protein to 0.01M PBS with target concentrations of 0 (as a negative control), 0.25, 1, 5, 10, 50 ng/mL.

Optimization of Test Strip Processes and Conditions

In order to achieve the maximum signal, a series of conditions were optimized. According to our previous work, factors such as the pH of coupling and resuspension, the quantity of redissolved solution Tween-20,the volume of antibody and the probe had an important effect on the signal intensity. These key factors were optimized as follow. Different antibody proteins have different isoelectric points, so the pH of the coupling environment affects the efficiency of the coupling of colloidal selenium to antibodies. Screening of pH value of labeled antibody: 1 mL of colloidal selenium was added to a 1.5 mL centrifuge tube, and 0,4,8,16,24,32 μL of 0.1 M K_2CO_3 was added to adjust the PH value of the colloidal selenium solution, and mixed well. According to the preparation method of colloidal selenium-labeled NT-proBNP mab-Clone-1 antibody described above, the conjugated antibodies under different PH conditions were obtained. Add 2, 2.5, 3 μg of NT-proBNP mAb-Clone 1 Antibody, respectively, and incubate for 20 minutes with slight shaking every 2 minutes. Conjugated antibodies labeled with different amounts of antibodies were obtained according to the preparation method of colloidal selenium-labeled NT-proBNP mab-Clone 1 antibody described above. Screening of PH and Tween-20 content of the compound dissolution solution, 50 μL of compound dissolution solution (3% BSA, 5% sucrose, 5% trehalose, 0.01% P300, 0. 01M Tris-HCl, Tween-20 content (0, 0.125%, 0.25%, 0.5 0.25%, 0.5%, 1%, 2%); re-dissolution solution (3% BSA, 5% sucrose, 5% trehalose, 0.01% P300 0.01M Tris-HCl, Tween-20 content 0.125%, pH (3.5, 4.5, 5.5, 6.5, 7.5, 8.5). Equal amounts of antigen were added to the test strips under different screening conditions to observe the color development of C and T lines under different conditions and to select the optimal conditions. We systematically investigated the PEG 20000 to SDS ratio in colloidal selenium formulations. Using dynamic light scattering (hydrodynamic diameter and PDI) and zeta potential measurements, we characterized colloidal formulations with PEG 20000 to SDS mass ratios of 0.25:20, 0.5:20, 1:20, 2:20, and 4:20.

Determination of the Limit of Detection (LOD)

The visual limit of detection (LOD) refers to the minimum analyte concentration at which a detectable marker is visibly observed in the detection zone and can be reliably differentiated from a control sample.[27,28] To evaluate the sensitivity of the biosensor developed here, we tested several concentrations of NT-proBNP antigen standard solutions to determine the visual LOD.

Determination of the Sensitivity and Cross-Reactivity of Colloidal Selenium Test Strip

After screening the aforementioned relevant conditions, we obtained the optimal color development conditions. A batch of conjugated antibodies was prepared under these optimal conditions and assembled into test strips following relevant procedures. NT-proBNP protein was sequentially diluted in PBS to concentrations of 50, 10, 1, 0.3, 0.125, and 0 ng/mL. These antigen concentrations were sequentially applied to the test strips, and the color development of the C line and T line was observed. The OD_T and OD_C values were then measured using a handheld reader. To validate the specificity of the colloidal selenium test strips, we collected troponin, phospholipase A2, CRP protein, BNP, and cTnl. After diluting them to the same concentrations, they were simultaneously tested, and their color reactions were observed.

Repeatability (Intra-Batch Variation) and Inner-Batch Variation

To verify the validation and stability of the LFIA, 3 test strips were randomly selected from the same batch while other 3 test strips were randomly selected from three different batches and tested with standard NT-proBNP solution.[29]

High Dose Hook Effect

When the antigen concentration exceeds a certain threshold, the signal value of the T-line does not increase with increasing antigen concentration. This phenomenon is known as the high-dose hook (HOOK) effect and often exceeds the detection limit, making the correlation value unquantifiable. In this experiment, three high-dose NT-proBNP standard samples of 250, 500, and 1000 ng/mL were selected to test the prepared test strips and to determine the limits of the HOOK effect.

Stability Study

Assemble the labeled selenium markers onto the test strips and store at 4°C for one month. Perform sample testing at 0, 7, 14, and 30 days using PBS as the sample.

Collection and Application of NT-proBNP Sample from Clinical Patients

All 30 serum samples were obtained from patients diagnosed with heart failure based on pathological and clinical evaluations at Taixing People's Hospital. This study strictly adheres to the ethical principles outlined in the Declaration of Helsinki and has been approved by the Medical Ethics Committee of Taixing People's Hospital (Ethics Approval No.: LS2024031-2). Written informed consent was obtained from all participants. All samples were diluted 1:1 with PBS and stored at 4°C.

Results

Characterization of Colloidal Selenium

The optical properties and colloidal characteristics of colloidal selenium are crucial for the stability and sensitivity of LFIA detection. Meanwhile, the shape and size of colloidal selenium have a very strong influence on colloidal stability, optical absorption intensity, surface plasmon resonance (SPR) band and color. In this study, colloidal selenium were synthesized by ascorbic acid reduction method.colloidal selenium had maximum SPR absorptions at 300 nm.The average particle size of colloidal selenium is 69.6nm and polydispersity indices is 0.023. As shown in Figure 4A and B. Photographs of colloidal selenium and antibody-bound colloidal selenium were taken by transmission electron microscopy, and it was found that compared to colloidal selenium, antibody-bound colloidal selenium was covered with a protein halo on the outside, as shown in Figure 4C and D. To demonstrate that the binding of colloidal selenium to the antibody was by means of intermolecular forces such as electrostatic adsorption rather than covalent binding, the colloidal selenium was analyzed by using X-ray Photoelectron Spectroscopy (XPS), and after fitting the high-resolution Se3d spectra, as shown in Figure 4E and F. two major peaks were observed, Se3d5/2 at 54.7 eV and Se3d3/2 at 55.5 eV two major peaks, further indicating that the antibody-bound colloidal selenium nanoparticles are zero-valent selenium. Antibody binding to colloidal selenium is not in the form of covalent bonding, but through intermolecular forces such as electrostatic adsorption.

In order to determine whether the colloidal selenium was successfully bound to the antibody, we performed mapping energy spectrum scanning of TEM on the antibody-bound colloidal selenium. As shown in Figure 5A–E, the presence of both N and O elements proved that the antibody was successfully bound to the colloidal selenium.In order to investigate the process of antibody response to antigenic stimuli, it is of interest to measure the kinetics of the immune response. Standard antigens of 0.0.5,2 and 10 ng/mL were prepared, 80 μL drops were aspirated and added to the sample pads, and the OD values of the test strips were measured with a colloidal gold quantimeter at one-minute intervals starting from the first minute and recorded, as shown in Figure 5F.

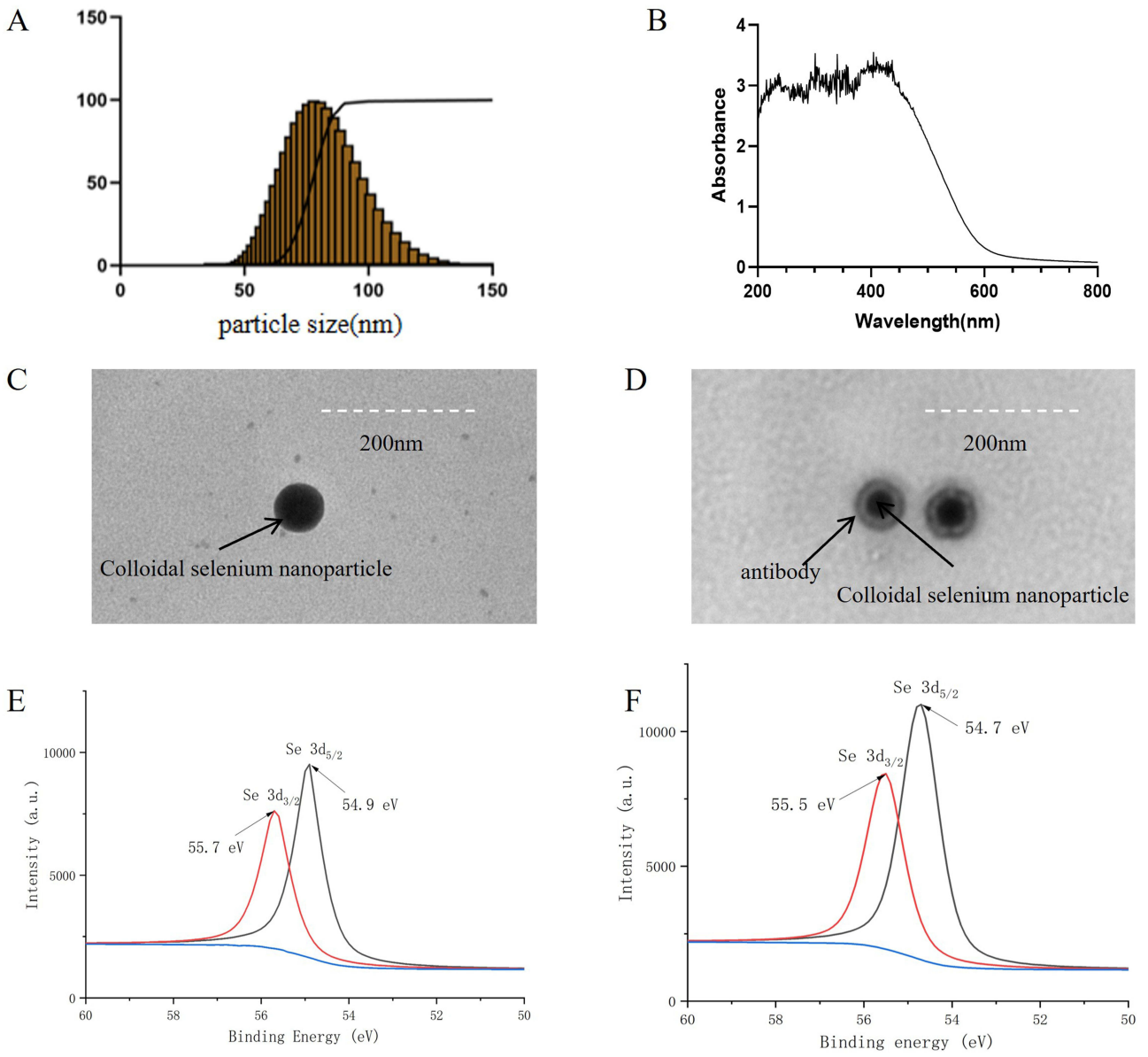

Figure 4 (**A**) Size distribution of the colloidal selenium. (**B**) the absorption spectrum of the colloidal selenium. (**C**) TEM image of the colloidal selenium. (**D**) TEM image of the antibody-labeled colloidal selenium. (**E**) XPS high-resolution spectra of colloidal selenium Se3d. (**F**) XPS high-resolution spectra of antibody-bound colloidal selenium Se3d.

Optimization of Test Strip Processes and Conditions

LFIA performance was evaluated based on test strip T/C signal intensity and storage stability. Considering particle size, PDI value, optimal zeta potential, and optimal T/C signal intensity of test strips, the PEG 20000:SDS ratio of 1:20 was ultimately selected as the optimal formulation (Table 1). Different antibody proteins have different isoelectric points, so the pH of the coupling environment affects the efficiency and effectiveness of the coupling of colloidal selenium to antibodies.[30,31] The NT-proBNP monoclonal antibody labelled with colloidal selenium was synthesized using a conventional one-pot synthesis[32] method as described above. By investigating the effect of varying potassium carbonate volumes and comparing the color development of T-line and C-line reactions, we determined the optimal pH range (5, 6, 7, 8, 9, 10) for antibody-colloidal selenium binding, as shown in Figure 6A and F. The study indicated that an antibody binding environment with a pH of 8 yielded the best results. This is because pH 8 is close to the isoelectric point of the NT-proBNP monoclonal antibody clone 1 protein, facilitating its binding to colloidal selenium. The antibody addition

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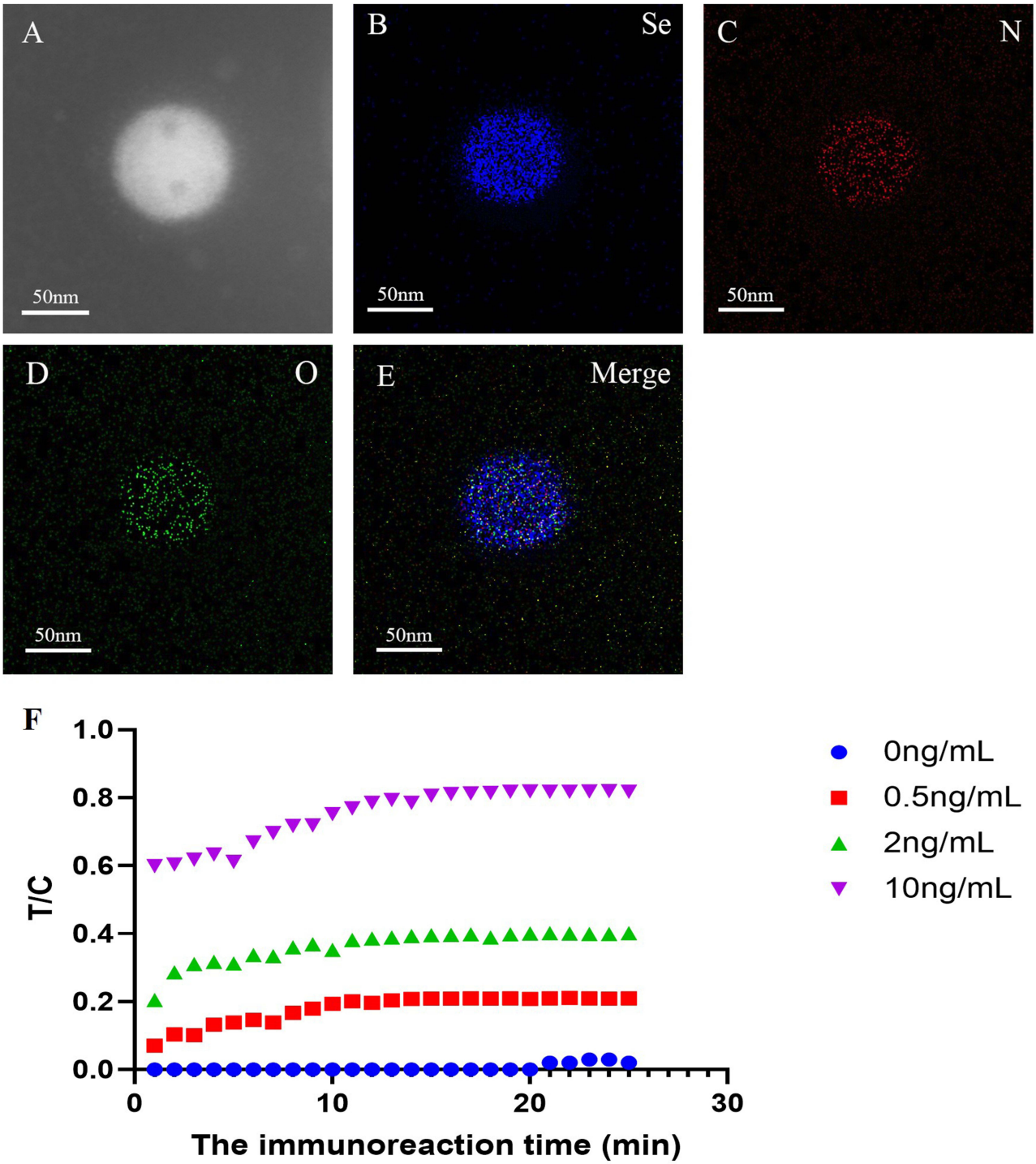

Figure 5 (**A**–**E**) Mapping in TEM, denote C, Se, N, O and merge, respectively, **(F)** Immunoreaction dynamics of OD_T/OD_C at different NT-proBNP concentration (0,0.5,2 and 10 ng/mL).

amount required screening: too little antibody resulted in insufficient binding and weakened color development; too much increased the cost of test strip preparation. As shown in Figure 6B and G, the addition of 2.5 micrograms of antibody produced the best results. Given that the pH of the resuspension solution affects test strip performance and that the surfactant Tween-20 influences the base color and color development of the test strip, screening was conducted for the pH of the resuspension solution and the Tween-20 concentration. As depicted in Figures 6C and H, adding SDS

Table 1 PEF20000/SDS Proportion Optimization

$n_{PEG20000}/n_{SDS}$	0.25:20	0.5:20	1:20	2:20	4:20
Particle size (nm)	146.7	85.7	69.6	126.3	203.1
PDI	0.258	0.146	0.023	0.241	0.351
Potential(mv)	−18.7	−23.5	−25.8	−19.3	−16.2
OD_T/OD_C(Antigen concentration 10ng/mL)	0.13	0.21	0.39	0.25	0.11

significantly improved false positive issues and produced a cleaner background. Results in Figures 6D and I indicate that the optimal detection performance is achieved when the resuspension solution pH is 8 and the Tween-20 concentration is 0.125%. As shown in Figures 6E–J, the optimal effect was achieved at a suspension pH of 8. Similarly, this study screened the impact of adding SDS during colloidal selenium synthesis on false positives.

Determination of the Cross-Reactivity and Sensitivity of Colloidal Selenium Test Strip

Different concentrations of NT-proBNP protein were simultaneously added to the sample pad. As the concentration decreased, the T line gradually faded. As shown in Figure 7A, when the T line concentration was 0.25 ng/mL, the T line exhibited nearly colorless development, indicating a detection limit of 0.25 ng/mL. No color development was observed in the T line after adding PBS, confirming the absence of false-positive reactions. In specificity testing (Figure 7B), no

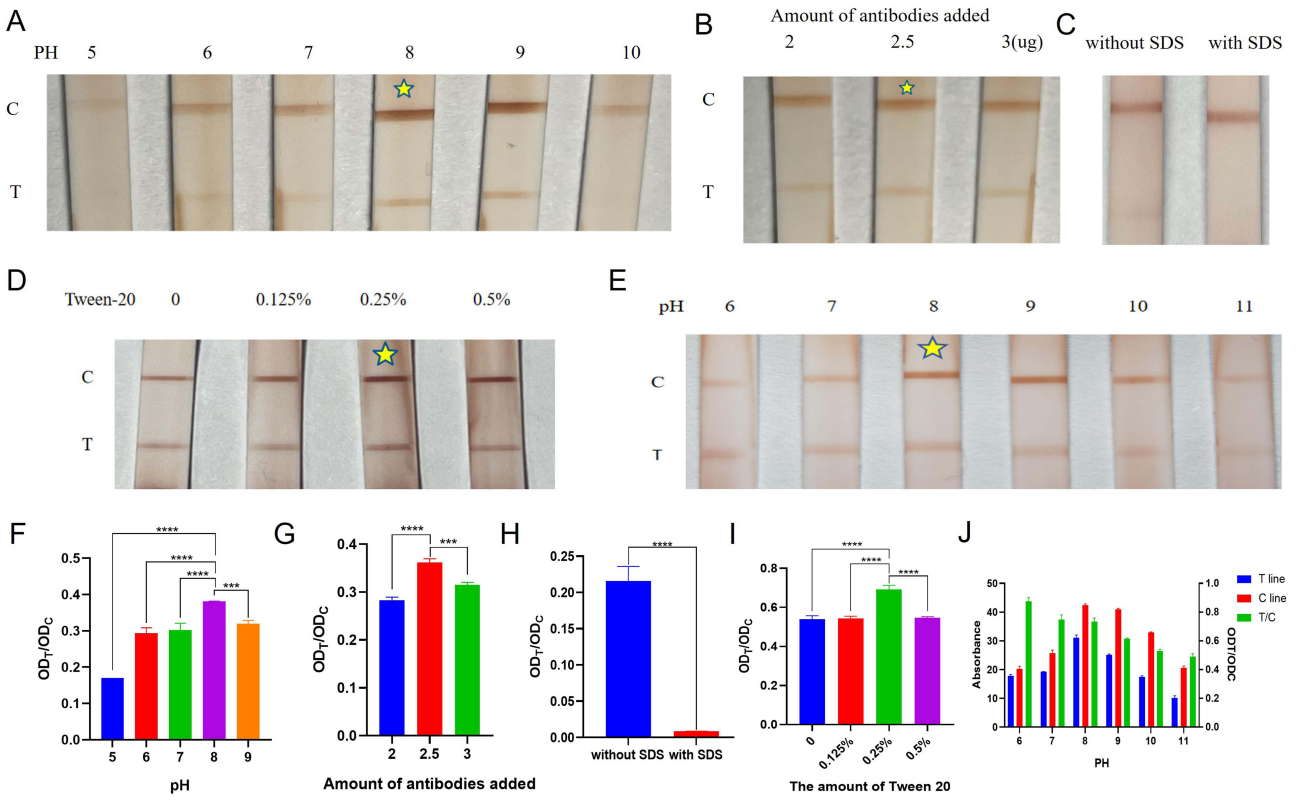

Figure 6 (**A**) Screen of antibody binding environment, the star indicates the optimal pH for antibody binding is 8. (**B**) Screen of antibody addition, the star indicates that the optimal antibody addition is 2.5 μg. (**C**) The effect of adding SDS, (**D**) Content screening of redissolved solution Tween-20, the star indicates that the optimal addition amount of Tween-20 to the redissolved solution is 0.25%. (**E**) pH screening of redissolved solution, the star indicates that the optimal pH for the redissolved solution is 8. (**F**) OD_T/OD_C values for different antibody binding environments, (**G**) OD_T/OD_C values for different antibody additions, (**H**) OD_T/OD_C values of with SDS and without SDS, (**I**) OD_T/OD_C values of redissolved solutions with different Tween-20 additions, (**J**) OD_T/OD_C values of redissolved solutions at different pH. (*** $p < 0.001$, **** $p < 0.0001$).

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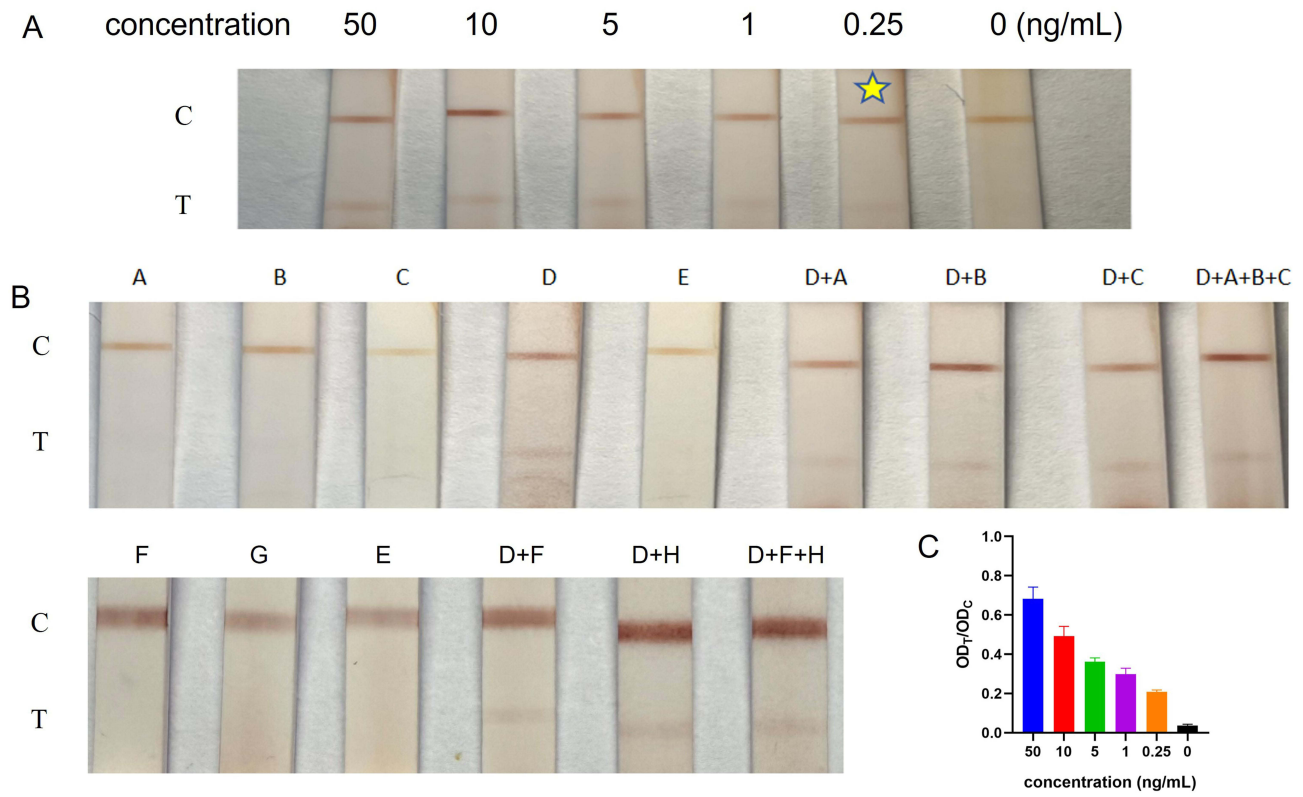

Figure 7 (**A**) Detection of NT-proBNP sensitivity, the star indicates that the sensitivity of this test strip for detecting NT-proBNP is 0.25 ng/mL. (**B**) Detection of NT-proBNP specificity, A is troponin, B is phospholipase A2, C is CRP, D is NT-proBNP, E is pbs, F is BNP, G is cTnl. (**C**) OD_T/OD_C values for different concentrations of NT-proBNP.

color development was observed for troponin, phospholipase A2, BNP, cTnl or CRP, while NT-proBNP exhibited color development, demonstrating the reagent's excellent specificity. When NT-proBNP was added simultaneously with troponin, phospholipase A2, BNP, cTnl or CRP, color development still occurred, confirming that NT-proBNP coloration is not interfered with by endogenous substances. The PBS negative control group showed no T-line, verifying the absence of false positives and the reliability of the results. Figure 7C further confirms the sensitivity of 0.25 ng/mL demonstrated in Figure 7A.

Repeatability (Intra-Batch Variation), Inner-Batch Variation, High Dose Hook Effect and Stability Study

As shown in Figure 8A, the test strips exhibit good repeatability. As shown in Figure 8B, the detection results from three different batches of test strips are nearly identical, demonstrating both good repeatability and minimal inter-batch variation. As shown in Figure 8C, starting from 250 ng/mL, the color intensity of the T line does not increase further with higher concentrations, indicating that the hook effect threshold is 250 ng/mL. As shown in Figure 8D, the measured cutoff value is 0.052. As shown in Figure 8E, stability testing of the test strip was conducted by performing false positive detection with PBS at days 0, 7, 15, and 30, demonstrating excellent stability of this test strip.

Test of Clinical Sample

Pipette 80mL of diluted clinical samples onto the assembled test strips and observe the color development. As shown in Figure 9 and Table 2, the test results from 30 samples demonstrate the clinical accuracy of this test strip.

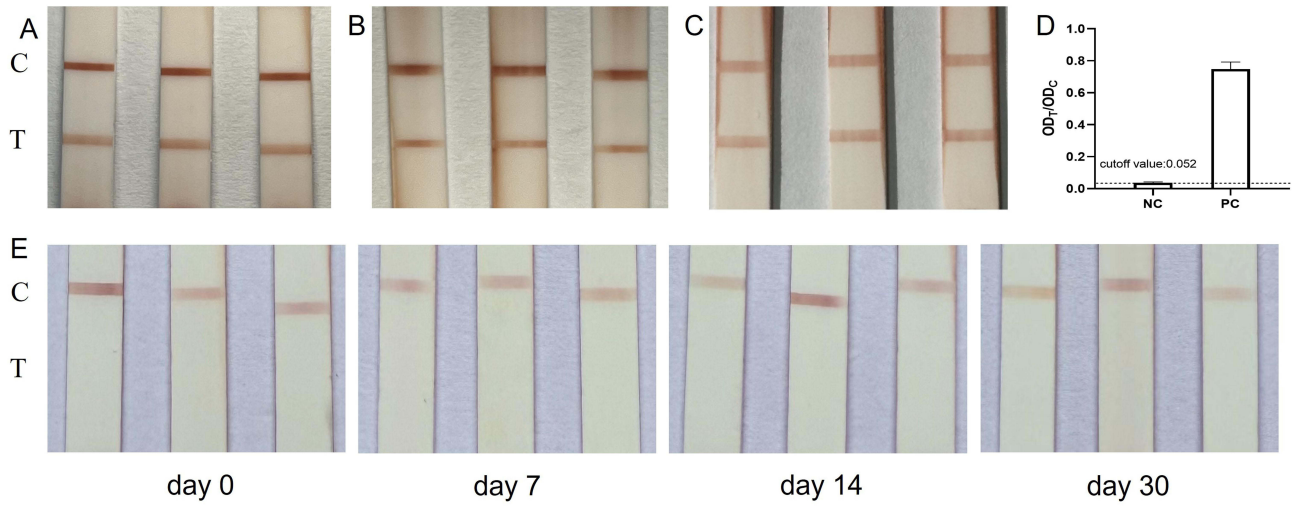

Figure 8 (**A**) Repeatability (**B**) Inner batch Variation (**C**) High dose hook effect (**D**) Cut off value (**E**) Stability Study.

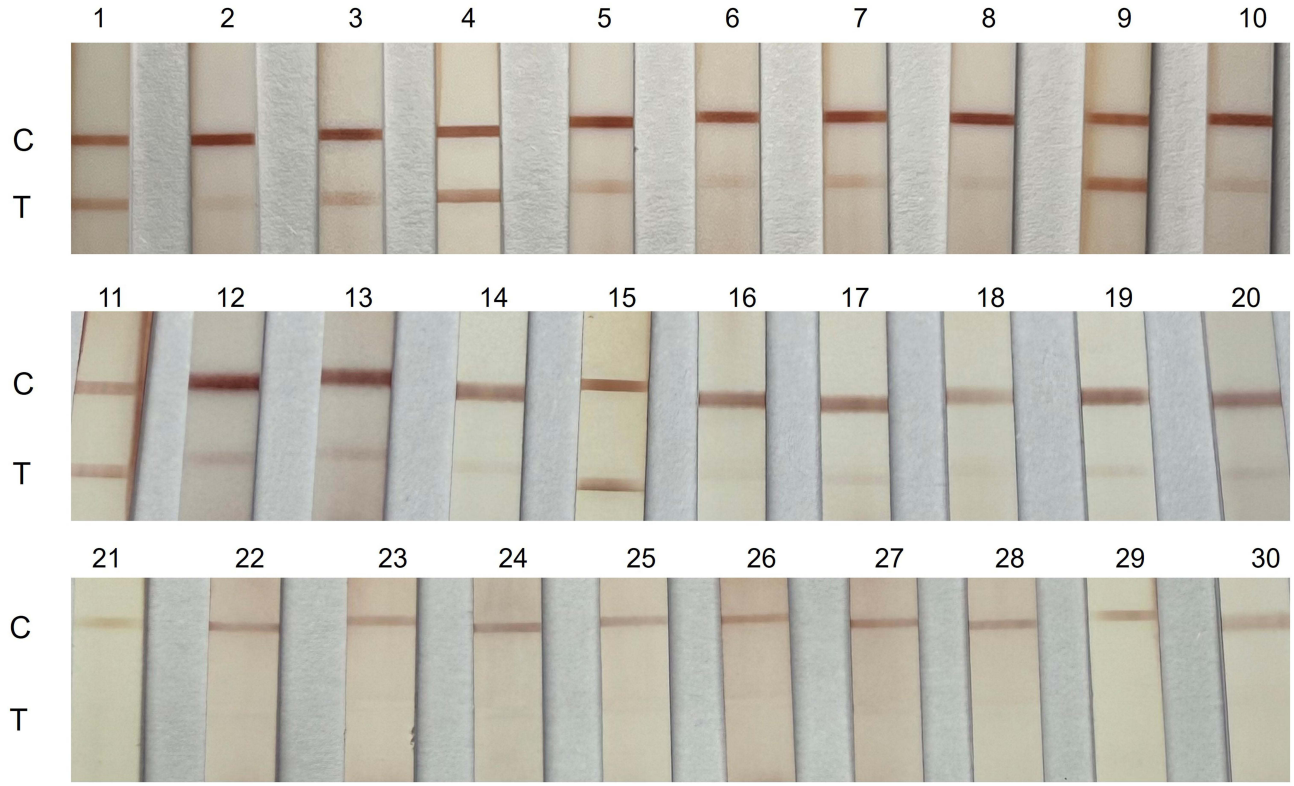

Figure 9 Detection clinic samples, NT-proBNP concentrations in samples 1 through 10 were in the order of 10192.8, 979.0, 6482.6, 17,308.6, 5012.9, 714.4, 1819.7, 684.3, 18,228.7, 1218.3, 12,537.5, 1254.2, 1138.2, 538.2, 10,284.3, 597.3, 942.1, 482.6, 1127.4, 1357.2, 58.1, 78.8, 99.2, 42.7, 105.8, 44.5, 84.1, 118.4, 102.4, 99.3 pg/mL.

Discussion

In this study, appropriate colloidal selenium particles were successfully prepared by using L-ascorbic acid as a reducing agent and PEG20000 as a stabilizing agent. The color of colloidal selenium is orange and transparent. It is mentioned in China HF Diagnosis and Treatment Guidelines 2024 that NT-proBNP is the main marker for the diagnosis of HF. When NT-proBNP is ≤0.3ng/mL, acute heart failure can be excluded; when NT-proBNP is less than or equal to 0.125ng/mL, chronic heart failure can be excluded. However, its sensitivity and specificity are lower than those in the diagnosis of

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Table 2 Information on Clinical Samples

Sample No.	Concentration of NT-proBNP (pg/mL) by ChemiLuminescence	Result
1	10,192.8	Positive
2	979.0	Positive
3	6482.6	Positive
4	17,308.6	Positive
5	5012.9	Positive
6	714.4	Positive
7	1819.7	Positive
8	684.3	Positive
9	18,228.7	Positive
10	1218.3	Positive
11	12,537.5	Positive
12	1254.2	Positive
13	1138.2	Positive
14	538.2	Positive
15	10,284.3	Positive
16	597.3	Positive
17	942.1	Positive
18	482.6	Positive
19	1127.4	Positive
20	1357.2	Positive
21	58.1	Negative
22	78.8	Negative
23	99.2	Negative
24	42.7	Negative
25	105.8	Negative
26	44.5	Negative
27	84.1	Negative
28	118.4	Negative
29	102.4	Negative
30	99.3	Negative

acute HF. Because the detection of NT-proBNP concentration is low, it is easy to appear false positive, so the synthesis method of colloidal selenium has been improved, because SDS is an anionic surfactant, which can reduce the interference of background impurities, reduce the production of false positive, so the amount of SDS in the synthesis, greatly reduce the occurrence of false positive. The detection limit reached 0.25ng/mL. Troponin, phospholipase A2, CRP, BNP and NT-proBNP were detected at the same concentration at the same time. Troponin, phospholipase A2, CRP and BNP were all negative, while others were positive, which proved the specificity of this colloidal selenium test strip for the detection of NT-proBNP. The content reported in this study demonstrates the potential for initial screening for HF in primary care settings as well as in-home testing.

Conclusion

Although the LFIA platform has been broadly applied in the prevention, diagnosis, treatment, and prognosis of a variety of diseases, there is no corresponding product on the market for point-of-care testing of HF, so it is significant to design a rapid, accurate and home-based immunochromatographic reagent for the detection of NT-proBNP for home testing of high-risk behavior groups and some medical institutions for the rapid initial screening of potential patients. In this study, colloidal selenium was synthesized by chemical reduction and PEG20000 was used as a stabilizer to improve the stability and accuracy of the test. Modifying the anionic surfactant SDS reduces background interference, thereby greatly reducing

the occurrence of false positives. The results showed that the detection sensitivity of the colloidal selenium LFIA platform was 250 pg/mL and meet the criteria for the diagnosis of acute HF in the Chinese Guidelines for the Diagnosis and Treatment of HF 2024.Besides, it had a good specificity, which Demonstrates that our designed colloidal selenium LFIA platform is a significant serum NT-proBNP detection method. Additionally, the colloidal selenium nanoparticle material is simple to synthesize and has a low manufacturing cost, and its developed LFIA platform assay is simple and accurate, and test strips for detecting HF have not yet appeared in the market, which means that the colloidal selenium test strips (colloidal selenium LFIA platform) have a great market prospect and competitive advantage.

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Disclosure

The authors report no conflicts of interest in this work.

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