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# *Study on the Process and Reagents of Carboxyl Latex Microsphere Coupled Chitosanase 3-Like Protein 1 Antibody*

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***Keywords:*** Chitosanase 3-Like Protein 1; Scattering Transmission Latex Enhanced Immunoblotting Method; Reagent Development and Evaluation

***Abstract:*** A method for quantitatively measuring the content of chitosanase 3-like protein 1 (CHI3L1) in human serum, applicable to both fully automatic biochemical analyzers and specific protein analyzers, has been developed. This method can assist in diagnosing liver diseases such as cirrhosis and liver fibrosis. The method's performance was evaluated to ensure it meets clinical diagnostic requirements. Using chemical covalent coupling technology, CHI3L1 monoclonal antibodies were covalently coupled with carboxylated latex microspheres. By optimizing coupling conditions, such as the antibody-to-microsphere ratio, activator concentration, and coupling buffer solution, the antibodies were fixed and uniformly coated on the microspheres' surface. This resulted in an immunogel system with specific steric hindrance effects and antigen-binding activity. The optimal gel turbidity reagent preparation process was then selected. The method's detection limit, sensitivity, precision, recovery, linear range, clinical reportable range, biological reference interval, and interference resistance were evaluated. The results were compared with those from chemiluminescent immunoassays on fully automatic biochemical analyzers and specific protein analyzers. Using an antibody-microsphere ratio of 40μg/mg, an activator concentration of 1-ethyl-(3-dimethylaminopropyl)carbodiimide (1-ethyl-(3-dimethylaminopropyl)carbodiimide) at 30μg/mg, and a coupling buffer of 20 mmol/L 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid buffer, the optimal immunogelatin reagent was obtained. Performance tests showed a detection limit of 5 ng/mL, a precision of less than 3%, a linear range of up to 1200 ng/mL, and an average recovery rate of 97.3%. Hemoglobin, triglycerides, bilirubin, and rheumatoid factor in the sample did not significantly interfere with the CHI3L1 test results. The overall agreement between the reagent and chemiluminescence immunoassay on biochemical analyzers and specific protein analyzers was 93.17% and 93.65%, respectively. The linear regression equations were y1 = 0.9809x1 + 2.0764 and y2 = 0.9608x2 + 2.2587, with correlation coefficients R2 of 0.9975 and 0.9969, indicating good correlation between the reagent and chemiluminescence results on both types of instruments. The process developed in this study can serve as a preparation condition for the CHI3L1 reagent, providing a more stable and efficient method for CHI3L1 clinical testing, which is significant for the diagnosis and monitoring of related diseases. In-depth research on the coupling process provides important theoretical foundations and practical references for the development of biomarker detection reagents.

## 1. Introduction

Chitinase-3-like protein 1 (CHI3L1) was first identified in 1992 by Johansen JS and colleagues[1] in the supernatant of human osteosarcoma cell line MG-63. The gene encoding this protein is located on human chromosome 1q32, consisting of 10 exons and 9 introns. The CHI3L1 protein, encoded by this gene, consists of 383 amino acids and has a relative molecular weight of approximately 40kDa, hence it is also known as YKL-40 [2]. Extensive research data indicates that CHI3L1 plays a crucial regulatory role in inflammation, tumors, and immune responses, particularly in the diagnosis of liver fibrosis or cirrhosis. The detection of CHI3L1 has gained increasing clinical attention [3-5]. In particular, the serum concentration of CHI3L1 in patients with cirrhosis, especially those with decompensated cirrhosis, is significantly higher than in healthy individuals, making CHI3L1 a highly promising serum marker for the early diagnosis and staging of liver fibrosis[8-11].Currently, common clinical methods for detecting CHI3L1 include enzyme-linked immunosorbent assay (ELISA), immunochromatography, and chemiluminescent immunoassay. This study aims to develop an automated, high-throughput, and highly specific and sensitive latex-enhanced immunoturbidimetric method, which can simultaneously measure scattering turbidity and transmittance turbidity. The latex-enhanced immunoturbidimetric assay involves chemically covalently coupling antibodies to latex microspheres. This method detects antigens by utilizing the changes in the light signal of the latex microspheres during antigen-antibody reactions. It is characterized by its simplicity, rapid detection, high-throughput automation, strong specificity, and high sensitivity, making it widely used in clinical testing.

## 2. Materials and methods

### 2.1 Materials

#### 2.1.1 Main reagents and materials

Carboxylated latex microspheres (Japan Synthetic Rubber Co., Ltd., P0113); CHI3L1 monoclonal antibody (Xuxiang Bio, R20241106); 1-Ethyl-(3-Dimethylaminopropyl)carbodiimide (Merck, 100112503); 4-Hydroxyethylpiperazine ethanesulfonic acid (Aladdin, K2217971); 2-Morpholine ethanesulfonic acid (Merck, 1002120247); trimethylenemethane (Feijin Bio, 20240802); all other chemical reagents are of analytical purity and were purchased from Sinopharm Chemical.

#### 2.1.2 Instruments and equipment

Ultrasonic cell crusher (Lianjing Electronics JY92-11DN); pH meter (Ohhaus a-AB33PH); fully automatic biochemical analyzer (Hitachi 7080) and fully automatic specific protein analyzer (Unide PA100); constant temperature shaker (Super ZD-85A).

### 2.2 Methodology

#### 2.2.1 Preparation of immunorubber

Take an appropriate amount of carboxylated latex microspheres and resuspend them in coupling buffer (20mmol/L pH7.0 Hepes buffer) to achieve a concentration of 1% (w/v). Add an appropriate amount of 1-ethyl-(3-dimethylaminopropyl)carbodiimide to the microsphere suspension, ensuring the final concentration of 1-ethyl-(3-dimethylaminopropyl)carbodiimide reaches 30μg/mg of microspheres. Stir the mixture in a 37℃ constant temperature stirrer for 30 minutes to fully activate

the carboxyl groups. Next, add the activated carboxylated latex microspheres to the diluted antibody solution and stir at 37℃ for 90 minutes. After the coupling reaction is complete, add an appropriate amount of blocking buffer (50mmol/L pH7.4 glycine buffer containing 10% bovine serum albumin) to the reaction system and stir at 37 ℃ for 60 minutes to block any unreacted active groups.

### 2.2.2 Preparation of latex turbidity reagent

The closed immunomicrospheres are diluted to 1mg/mL (microspheres/volume) using a microsphere dilution buffer (containing 5% trehalose, 1% bovine serum albumin, 0.5% Tween-20, and 0.1% preservative in a 50mmol/L pH7.4 Tris buffer) to form Reagent 2. Reagent 1 consists of a 50mmol/L pH7.4 trimethylamine buffer, which includes 2% sodium chloride, 1% polyethylene glycol 8000,1% blocking agent, 0.1% Tween-20, and 0.1% preservative. Reagent 1 and Reagent 2 together form the CHI3L1 latex immunoturbidimetric assay reagent.

### 2.2.3 Performance evaluation

We conduct tests according to relevant industry standards and operating methods.

## 2.3 Statistical methods

SPSS 25.0 and EXCEL 2016 were used to analyze the results.

# 3. Results and analysis

## 3.1 Optimization of immunorubber coupling system

### 3.1.1 Optimization of antibody-microsphere ratio

The activated carboxyl latex microspheres were added to the diluted antibody solution in varying proportions (20μg/mg, 30μg/mg, 40μg/mg, 50μg/mg, 60μg/mg) of the antibody. The mixture was stirred at 37℃ for 90 minutes. After coupling is complete, the microspheres are blocked and tested using an automated biochemical analyzer.

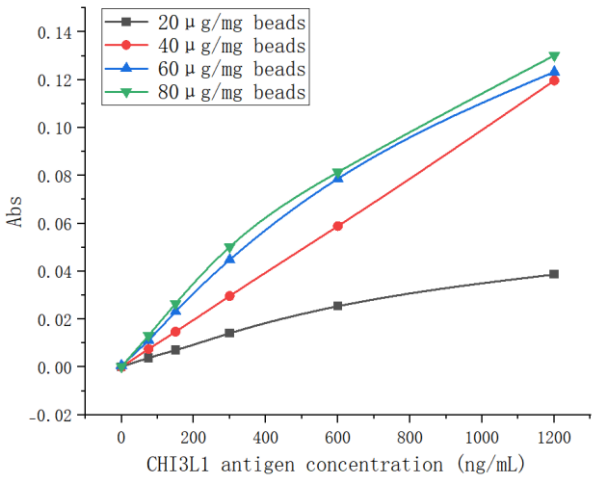


Figure 1 Effect of coupling with different antibody dosage

The coupling effect varies with different antibody-to-microsphere ratios. As the ratio increases, the binding capacity of the latex microspheres gradually improves. When the ratio is 40μg/mg, the amount of bound antibodies reaches a saturation point. Further increasing the antibody

concentration does not significantly enhance binding efficiency and may lead to antibody waste and system instability. Therefore, the optimal ratio of antibody to microsphere is set at 40μg/mg (Figure 1).

### 3.1.2 Influence of activator concentration

The ratio of fixed antibody to microspheres was 40μg/mg, and 1-ethyl-(3-dimethylaminopropyl) carbodiimide with different concentrations (20μg/mg, 30μg/mg, 40μg/mg) was used to activate the microspheres. Other conditions were unchanged, and the coupling effect was observed.

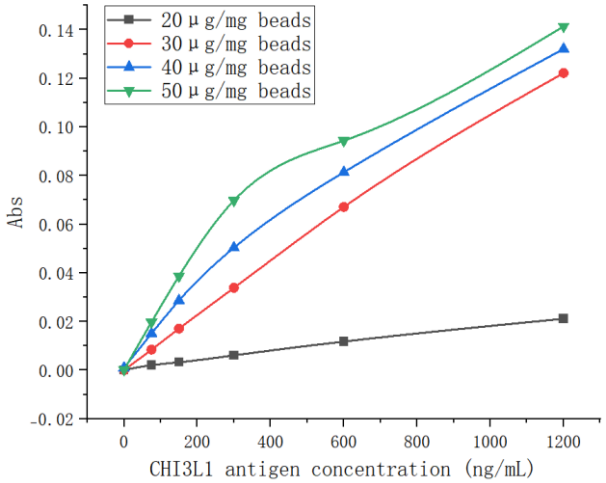


Figure 2 Effect of coupling with different amounts of activators

When the concentration of 1-ethyl-(3-dimethylaminopropyl) carbodiimide is 30 μg/mg, the carboxyl groups on the microsphere surface are fully activated, resulting in the highest coupling efficiency between the antibody and the microspheres. If the concentration is too low, the carboxyl groups are not fully activated, leading to a lower coupling efficiency. Conversely, if the concentration is too high, it may introduce excessive side reactions, which can negatively impact the coupling effect (Figure 2).

### 3.1.3 Influence of coupling buffer type

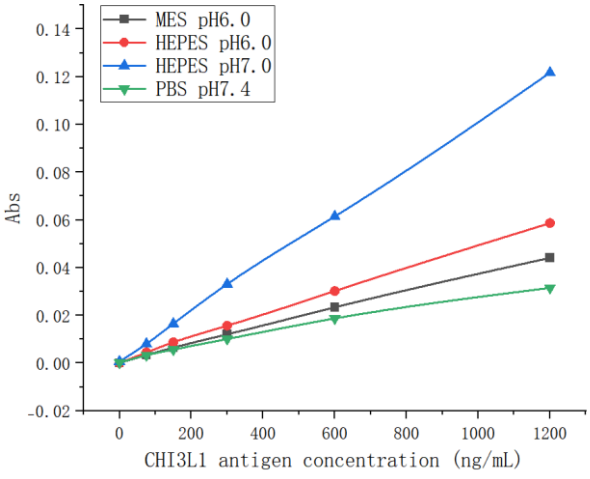


Figure 3 Coupling effect of different coupling buffer

The experimental results indicate that when the coupling buffer is 20 mmol/L pH7.0 4-hydroxyethylpiperazine ethanesulfonic acid, the antibody-cysteine coupling effect is optimal, resulting in immunogel with good sensitivity and linearity. However, when using 20mmol/L pH6.0 2-morpholin ethanesulfonic acid or 20mmol/LpH6.5 4-hydroxyethylpiperazine ethanesulfonic acid as the coupling buffer, microspheres tend to aggregate during the coupling process, possibly due to the pH of the system. Using 20mmol/L pH7.4 phosphate buffer as the coupling buffer resulted in too low a coupling efficiency, failing to meet the required reaction degree. Therefore, this experiment uses 20mmol/L pH7.0 4-hydroxyethylpiperazine ethanesulfonic acid as the coupling buffer (Figure 3).

## 3.2 Performance evaluation

### 3.2.1 Detection limit

Zero concentration calibrators were taken and measured 20 times, and the mean (x) and standard deviation (s) were calculated. The detection limit was calculated as 5ng/mL by X+3SD, which could meet the needs of clinical detection for low concentration samples.

### 3.2.2 Precision

The quality control samples at the concentration levels of 75ng/mL and 300ng/mL were measured respectively. One batch was measured every day, and each batch was tested three times, and the monitoring lasted for 28 days. The mean (x) and standard deviation (SD) were calculated respectively. The test mean of each day was within the range of x±2SD, indicating that the system had good repeatability and stability (Figure 4).

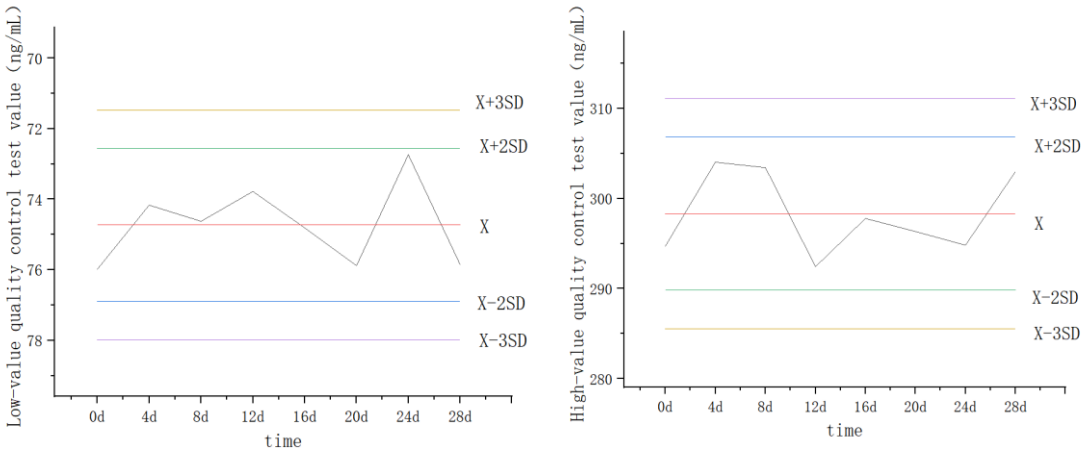


Figure 4 Intermittent precision test results

### 3.2.3 Linear range

Take one sample of low concentration L (approximately 5ng/mL) and one sample of high value H (approximately 1200ng/mL). Prepare at least 10 samples of different concentrations from the high and low concentration samples in a proportional manner. CHI3L1 concentration is arranged at equal intervals, with each sample tested three times. The theoretical concentration ( $X_i$ ) is used as the independent variable, and the mean of the test results ( $Y_i$ ) is used as the dependent variable. Calculate the correlation coefficient (r) of the linear regression equation. The estimated value $\hat{Y}_i$ is obtained by substituting $X_i$ into the linear regression equation. The relative deviation (RD) and

absolute deviation (AD) between $\hat{Y}_i$ and $Y_i$ are also calculated. The linear regression equation is y=0.9903x+2.3636, the linear correlation coefficient R2 is 0.9999, the linear relative deviation ranges from-3.47% to 1.08%, and the linear absolute deviation ranges from (-6.88 to 6.46) ng/mL. This indicates that within this linear range, the test results show a good linear relationship with the sample concentration, making it suitable for accurate detection of clinical samples(Figure 5).

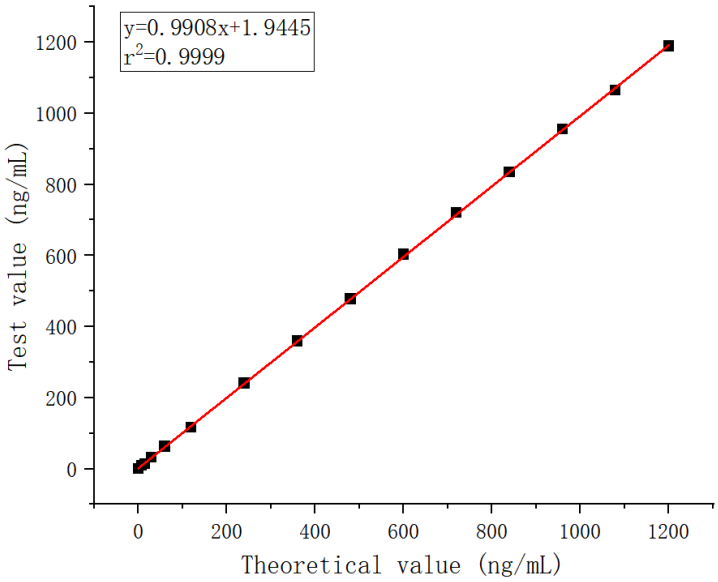


Figure 5 Results of linear range experiment

### 3.2.4 Clinical reportable range

A sample with a CHI3L1 concentration of 1200ng/mL was selected and diluted with physiological saline in a specific ratio. The original sample and each diluted sample were tested three times, and their average values were calculated. The test results were compared with the expected values, confirming that the upper limit of the clinically reportable range is as high as 36000ng/mL, meeting the clinical testing requirements for samples of different concentrations.

### 3.2.5 Recovery test

The recovery rate was between 95% and 100%, and the average recovery rate was 97.30%, indicating that the system had good accuracy (Table 1).

Table 1 Results of recovery test

| Sample book | Results of determination (ng/mL) | | | mean | theoretical value | percent recovery |
|---|---|---|---|---|---|---|
| 90% sample + 10% normal saline | 71.25 | 73.31 | 72.75 | 72.44 | - | - |
| 90% sample +10% 500ng/mL CHI3L1 | 125.11 | 127.24 | 125.28 | 125.88 | 130.83 | 96.21% |
| 90% sample +10% 1000ng/mL CHI3L1 | 176.45 | 178.14 | 176.54 | 177.04 | 180.83 | 97.90% |
| 90% sample +10% 2000ng/mL CHI3L1 | 269.12 | 275.17 | 273.04 | 272.44 | 280.83 | 97.01% |

### 3.2.6 Interference test

To evaluate the impact of interfering substances on test results, two clinical samples with different CHI3L1 concentrations (72ng/mL and 300ng/mL) were selected. Both samples showed no significant hemolysis, jaundice, or turbidity. In the experiment, interfering substances such as hemoglobin (Hb), bilirubin (Bil), triglycerides (TG), and rheumatoid factor (RF) were added to the samples. The samples were mixed in a 9:1 volume ratio with the interfering substances, and physiological saline was used as a control. Each interfering substance was diluted to three different levels, and each concentration of the mixed sample was tested three times, with the average values of each test being calculated. When the bilirubin (Bil) concentration reached 40mg/dL, the hemoglobin (Hb) concentration reached 200mg/dL, the triglycerides (TG) concentration reached 200mg/dL, and the rheumatoid factor (RF) concentration reached 200IU/ml, the test results showed no significant interference. This indicates that within this concentration range, the impact of these interfering substances on the test results is acceptable.

## 3.3 Comparison of results with chemiluminescence immunoassay under different conditions

Take more than 200 samples that have been tested using chemiluminescence immunoassay (CLIA). The sample concentrations should cover the linear range of this method. The reagents prepared using this method were used to simultaneously test these samples on both fully automatic biochemical analyzers and specific protein analyzers. In this study, the overall agreement rates of the reagents with CLIA on biochemical analyzers and specific protein analyzers were 93.17% and 93.65%, respectively. The linear regression equations were y1=0.9809x1+2.0764 and y2=0.9608x2+2.2587, and the correlation coefficients R ²were 0.9975 and 0.9969, indicating a good correlation between the results of this reagent and the chemiluminescence method on both types of instruments (Figure 6).

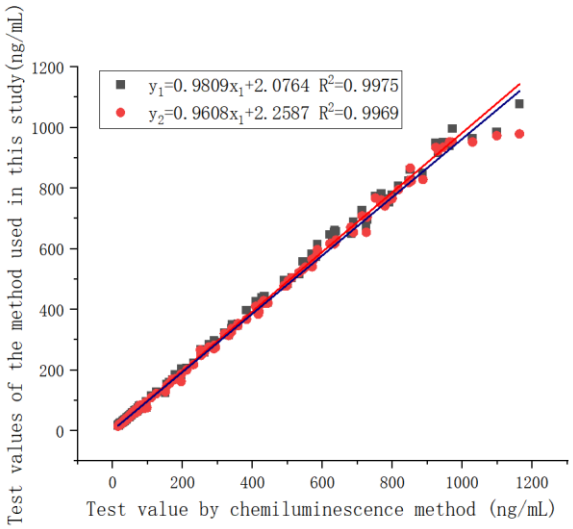


Figure 6 Experimental results of methodological comparison

## 4. Discussion

### 4.1 The importance of the antibody-to-microsphere ratio

The ratio of antibodies to microspheres is a critical factor affecting the coupling efficiency. If the ratio is too low, the binding sites on the microsphere surface cannot be fully utilized, resulting in

insufficient antibody binding and reduced detection sensitivity [12-13]. Conversely, if the ratio is too high, excess antibodies can lead to non-specific adsorption, destabilizing the system and wasting antibodies. This study found that optimizing the ratio to 40μg/mg ensures adequate antibody binding while maintaining system stability and sensitivity.

### 4.2 Influence of activator concentration on coupling effect

1-Ethyl-(3-dimethylaminopropyl) carbodiimide is a commonly used carboxyl activator, and its concentration directly affects the degree of carboxyl activation [13]. Activated carboxyl groups can then undergo covalent coupling with the amino groups on antibodies. If the activator concentration is too low, the carboxyl groups will not be fully activated, reducing coupling efficiency; conversely, if the concentration is too high, it may introduce excessive side reactions, damaging the microsphere surface structure and performance, thus affecting the coupling effect. This study determined that a concentration of 1-Ethyl-(3-dimethylaminopropyl) carbodiimide at 30μg/mg achieves the optimal coupling effect.

### 4.3 The role of the components of the coupling buffer

Different buffers have varying pH buffering ranges, and the antigen-antibody reaction typically requires specific pH conditions to achieve optimal results. If the pH of the buffer is not suitable, it can alter the charge state of the antigen or antibody, affecting their electrostatic interactions, which in turn reduces or destabilizes the formation of immune complexes, ultimately impacting the accuracy and sensitivity of the test. The ion composition and concentration in the buffer determine the ionic strength of the reaction system. The properties of the buffer also influence the surface charge, particle size distribution, and stability of the latex microspheres [13]. If the pH or ionic strength of the buffer is inappropriate, it can cause the latex microspheres to aggregate or precipitate, affecting their uniform dispersion during the reaction and leading to inaccurate turbidity measurements. For example, changes in pH can alter the charge state of the latex microspheres, causing them to repel or attract each other, thus affecting their dispersion; high ionic strength can compress the double layer on the surface of the latex microspheres, reducing their stability and leading to aggregation. The 50mmol/L pH7.4 4-hydroxyethylpiperazine ethanesulfonic acid buffer selected in this study significantly enhances the coupling efficiency of the antibodies.

### 4.4 Clinical application value of reagents

The immunogel turbidimetric reagent developed in this study, based on antibody-conjugated carboxyl latex microspheres, demonstrates excellent sensitivity, a wide linear range, precision, recovery rate, and resistance to interference. It shows good correlation with chemiluminescence immunoassay results, making it highly valuable for clinical testing in CHI3L1. This system can be used for the early diagnosis, monitoring, treatment evaluation, and prognosis assessment of liver fibrosis and cirrhosis, providing clinicians with more accurate and timely diagnostic information. It also aids in formulating personalized treatment plans, thereby improving patient outcomes and quality of life.

## 5. Conclusion

Following in-depth research and reports by scholars from various fields on chitosanase-like protein 1, relevant medical organizations in China have also included this protein as a key detection indicator [6-7]. They have developed clinical diagnostic reagents that are significant for the early

screening of liver fibrosis and cirrhosis. This study successfully optimized the process of coupling CHI3L1 antibodies with carboxylated latex microspheres, creating an excellent immunogel system. By optimizing key factors such as the ratio of antibodies to microspheres, the concentration of activators, and the composition of the coupling buffer, the study achieved efficient and stable antibody coupling. The immunogel system demonstrated excellent performance in detection, with good sensitivity, linearity, precision, recovery, and interference resistance. It also showed strong correlation with chemiluminescence immunoassay results, making it highly valuable for clinical applications. This new and reliable technical approach provides a significant advancement in the clinical testing of CHI3L1, potentially driving the development and innovation of related disease diagnosis technologies and contributing positively to improving patient outcomes and quality of life. Additionally, the technical approaches and methods used in this study offer valuable insights and references for the development of other biomarker detection reagents, with important theoretical and practical significance.

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