SMINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Study on the establishment of immune side flow chromatography method for detecting human Mycoplasma pneumoniae lgM antibody**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-21
Cui SIHUI

Scientific supervisor
Olga ANDREYEVA, Dr. Sc., Prof.

Reviewer
Tetiana HALENOVA, Ph.D., As. Prof.

Kyiv 2025

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

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Educational and professional program Biotechnology

APPROVE
Head of Biotechnology, Leather and Fur Department, Professor, Dr. Sc., Prof.
_____Olena MOKROUSOVA
«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Cui Sihui

1. Thesis topic **Study on the establishment of immune side flow chromatography method for detecting human *Mycoplasma pneumoniae* lgM antibody**
Scientific supervisor Dr.Sc., Prof. Olga Andreyeva

approved by the order of KNUTD "05" March 2025, № 50-уч

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments 05.03.2025

WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	28 May 2025	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	01 June 2025	Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	04 June 2025	

I am familiar with the task:

Student _____ Cui SIHUI

Scientific supervisor _____ Olga ANDREYEVA

SUMMARY

Cui SIHUI. Study on the establishment of immune side flow chromatography method for detecting human *Mycoplasma pneumoniae* lgM antibody. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2025.

In this study, in response to the clinical demand for the early diagnosis of Mycoplasma pneumoniae infection, a novel rapid detection system for IgM antibodies based on immunochromatography technology was constructed. By optimizing the antigen-antibody reaction system, using the C-terminal domain of recombinant P1 protein as the capture antigen and colloidal gold-labeled murine anti-human IgM monoclonal antibody as the signal probe, the chromatographic membrane treatment process of the test strip was innovatively improved. The experiment systematically investigated key parameters such as the particle size of the marker, the antigen coating concentration (1.2 mg/mL), and the sample dilution ratio (1:10), and determined the optimal reaction conditions through orthogonal experiments. The checkerboard titration method was adopted to optimize the spatial arrangement of the detection line/quality control line, and a four-module detection device including the sample pad, bonding pad, nitrocellulose membrane and water-absorbing pad was constructed.

The performance evaluation shows that this method can complete the detection within 15 minutes, with the minimum detection limit reaching 3.12 IU/mL, and the coincidence rate with the ELISA detection results is 95%. Cross-experiments confirmed that there was no cross-reaction with 12 common respiratory pathogens such as influenza virus and adenovirus, and the intra-batch/inter-batch coefficient of variation was low. In clinical verification, the detection sensitivity of 602 suspected case samples was 100% and the specificity reached 95%. The stability test indicates that the reagent can be stored for 12 months at 4 ℃, and the accelerated test at 37 ℃ shows that the validity period is 6 months.

Studies have shown that this detection system is characterized by simple operation, rapidity and sensitivity. Its detection performance meets the requirements of the "Guidelines for Stability Evaluation of In Vitro Diagnostic Reagents", providing primary medical institutions with an immediate detection solution that does not require professional equipment. It has important application value for the early screening and epidemiological surveillance of Mycoplasma pneumoniae infection.

Key words*: Mycoplasma Pneumoniae, Mycoplasma Pneumoniae Pneumonia, Immunolateral Flow Chromatography, The Vitro Diagnosis, Colloidal gold technology*

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INTRODUCTION

In this study, through antigen screening, marker optimization and reaction condition regulation, we established a rapid detection method for human Mycoplasma pneumoniae IgM antibodies based on immunoflow chromatography technology. The experiment used recombinant P1 protein as a specific antigen and combined with colloidal gold labeling technology to achieve high sensitivity and specificity in the recognition of IgM antibodies in serum samples. Performance evaluation showed that the detection limit of this method was as low as 1.5 ng/mL, with a coincidence rate of 96.8% compared with enzyme-linked immunosorbent assay (ELISA), and the detection time was shortened to within 15 minutes. Notably, the introduction of a dual quality control line design (C line and T line) significantly improved the reliability of the results and effectively avoided false negative/false positive issues. In addition, the optimization of cross-reaction reduced the interference of substances such as rheumatoid factor (RF) to over 95%. As a pathogenic microorganism, the early and rapid diagnosis of Mycoplasma pneumoniae is crucial for clinical treatment and epidemic prevention and control, and the portability and low cost of this technology make it particularly suitable for primary medical institutions and on-site screening. The development and optimization of immunoflow chromatography technology and its application value in the rapid diagnosis of respiratory pathogens. The purpose of this study is to establish an efficient, accurate and clinically applicable rapid detection method for human Mycoplasma pneumoniae IgM antibodies, providing technical support for the early diagnosis, epidemic monitoring and treatment evaluation of Mycoplasma pneumoniae infection.

The purpose of the work: to develop a method for rapid detection of human IgM antibodies for protection against *Mycoplasma pneumoniae* based on immunolateral flow chromatography.

Object of the study: laboratory research, antibodies (immunoglobulins) of a person to neutralize the causative agent of Mycoplasma pneumoniae, Mycoplasma pneumoniae.

The subject of the study: the lateral flow immunochromatography method for the detection of human antibodies for protection against Mycoplasma pneumoniae.

Research methods: immunolateral flow chromatography, in vitro diagnostics, colloidal gold technology.

Scientific novelty: on the basis of immunolateral flow chromatography, a method of rapid detection of human IgM antibodies for protection against Mycoplasma pneumoniae has been developed. Optimized the system of the antigen-antibody reaction, using the C-terminal domain of the recombinant protein P1 as a capture antigen and colloidal gold-labeled mouse anti-human monoclonal antibody IgM as a signal probe, innovatively improved the process of processing the chromatographic membrane of the test strip. During the experiment, such key parameters as the particle size of the marker, concentration of antigen coating (1.2 mg/ml), sample dilution ratio (1:10) were systematically investigated. Optimal reaction conditions were determined using orthogonal experiments. To optimize the spatial arrangement of the detection line/quality control line, the checkerboard titration method was adopted, and a four-module detection device was constructed, including a sample substrate, a binding substrate, a nitrocellulose membrane, and a water-absorbing substrate.

Practical significance: The developed method is characterized by ease of use, speed and sensitivity. The detection of immunoglobulins is completed within 15 minutes, while the minimum detection limit reaches 3.12 IU/ml, and the percentage of coincidence with the results of ELISA detection is 95%. Cross-reaction experiments confirmed that there was no cross-reactivity with 12 common respiratory pathogens, such as influenza virus and adenovirus, and the intra-/inter-batch coefficient of variation was low. During the clinical examination, the

sensitivity of detecting 602 suspected case samples was 100%, and the specificity reached 95%. The stability test shows that the reagent can be stored for 12 months at 4 ℃, and the accelerated test at 37 ℃ shows that the shelf life is 6 months. detection within 15 minutes, while the minimum detection limit reaches 3.12 IU/ml, and the percentage of coincidence with the results of ELISA detection is 95%. Cross-reaction experiments confirmed that there was no cross-reactivity with 12 common respiratory pathogens, such as influenza virus and adenovirus, and the intra-/inter-batch coefficient of variation was low. During the clinical examination, the sensitivity of detecting 602 suspected case samples was 100%, and the specificity reached 95%. The stability test shows that the reagent can be stored for 12 months at 4 ℃, and the accelerated test at 37 ℃ shows that the shelf life is 6 months.

The developed method meets the requirements of the "Guidelines for evaluating the stability of diagnostic reagents in vitro", providing primary medical institutions with a solution for immediate detection that does not require professional equipment. It has important practical significance for early screening and epidemiological surveillance of Mycoplasma pneumoniae infection.

Chapter I
LITERATURE REVIEW

1.1 Introduction to Mycoplasma Pneumoniae

1.1.1 Introduction to Mycoplasma Pneumoniae Pneumonia

Mycoplasma Pneumonia (Mp) is the smallest pathogenic microorganism that lies between bacteria and viruses and can survive independently in both aerobic and anaerobic environments. It is mainly transmitted through respiratory droplets or aerosols and is one of the important pathogens causing community-acquired pneumonia. The early clinical symptoms of Mp infection are not significantly different from those of pneumonia caused by other bacteria and viruses. Common symptoms include severe cough, fever (often persistent fever), chills, headache and sore throat[8]. The human body generally produces IgM antibodies about 1 to 2 weeks after being infected with pathogens, and these antibodies persist in the body for approximately 2 to 3 months. Therefore, IgM is an effective biomarker for recent infections and can be used clinically for etiological screening[9]. At present, the main testing methods in the Mp laboratory include isolation and culture, antigen detection, antibody detection, and genetic diagnostic technology detection, etc.

1.1.2 Diagnosis of Mycoplasma pneumoniae pneumonia

According to the existing research data, the commonly used clinical diagnostic methods for Mycoplasma pneumoniae pneumonia are as follows:

1. Evaluate the clinical manifestations of the child patients

Generally speaking, after being infected with Mycoplasma pneumoniae, children will show symptoms such as coughing and fever. The cough is usually dry, and some children may also have symptoms such as fatigue, chest pain and wheezing. When a doctor is auscultation a child, they may hear sounds in the lungs. However,

these symptoms are not specific to Mycoplasma pneumoniae infection. Therefore, after evaluating the clinical manifestations, other examinations are still needed for diagnosis.

2 Laboratory examination

Laboratory tests usually include two types: serological tests and etiological tests. Among them, serological examinations include antibody detection, condensation set tests, etc. Antibody testing is a relatively common examination. Usually, venous blood is drawn to detect the level of Mycoplasma pneumoniae antibodies in the blood. Generally, after being infected with Mycoplasma pneumoniae, children will show an increase in Mycoplasma pneumoniae antibody levels within 7 to 10 days after infection. In antibody detection, Ig M antibodies appear earlier in the acute stage, suggesting a recent infection.

The operation of the condensation set test is relatively simple, but its specificity is relatively low. After children are infected with Mycoplasma pneumoniae, condensation licin will appear in their serum. However, this positive test can also be seen in other diseases, such as infectious mononucleosis and epidemic Colds, etc.

Etiological testing mainly includes nucleic acid testing and culture separation testing. Nucleic acid testing mainly involves the detection of Mycoplasma pneumoniae through throat swabs, sputum, etc. This method has relatively high sensitivity and specificity, and can quickly and accurately diagnose Mycoplasma pneumoniae infection. Therefore, nucleic acid testing can be applied for early diagnosis. Culture isolation is the "gold standard" for the diagnosis of Mycoplasma pneumoniae infection in children. However, this detection method has a longer cycle and higher requirements for laboratory conditions, so its application in practice is relatively less.

3 Imaging examination

In addition to laboratory tests, imaging examinations are also the main diagnosis of Mycoplasma pneumoniae infection [10].

1.2 Introduction to Immune Side-stream Chromatography Technology

Traditional laboratory analysis methods include gas chromatography-mass spectrometry (GC-MS), ultra-performance liquid chromatography-tandem mass spectrometry (LC/MS/MS), high performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and biosensors, etc. Most of them have good sensitivity but are time-consuming and require expensive instruments and equipment as well as professional personnel. Over the past few decades, the focus of scientific research has increasingly been on the development and optimization of portable, inexpensive and easy-to-use rapid diagnostic experiments. The immunochemobiological analysis method has attracted much attention due to its high specificity and sensitivity. At present, most screening and rapid methods are based on immunoanalysis. This method is based on the reaction between the analyte antigen (Ag) and the selective antibody (Ab) to form the AB-Ag complex. The effectiveness of immunoanalysis mainly depends on the efficiency of Ab-Ag complex formation and the detection rate of complex formation [11].

LFIA is one of the most successful analytical platforms for on-site detection of target substances to date. It features fast detection speed, ease of use, low cost and wide application. It has expanded from clinical molecular, biological and (biological) marker detection to other fields, including food and feed safety, veterinary medicine, environmental control and many other areas. LFIA features rapidity, simplicity, legibility, good on-site applicability, long shelf life, small required volume, relatively low production cost, good sensitivity and specificity, and integration with electronic and electrochemical systems. It is suitable for detecting various sample types and can complete the test within 15 minutes through the optimization of reagents such as antibodies, markers, and operation buffers. LFIA equipment can be stored at room temperature for at least two years and is highly suitable for use under conditions of limited resource

1.2.1 The basic components of LFIA

The LFIA consists of a sample pad, a conjugate pad, a nitrocellulose membrane and an absorbent pad, which are fixed in sequence on the back plate to form a test strip[12]. The sample pad serves as the starting point of the reaction and mainly functions to load the sample. The material is generally polyester fiber membrane or glass fiber membrane. To ensure that the sample pad can shield the interference of non-target matrices in the sample on the detection of the target matrix, different pretreatment solutions are usually required for pretreatment of the sample pad when dealing with different specimens according to their differences. The gold-labeled conjugate pad is generally made of glass fiber, non-woven fabric or polyester fiber with good water absorption and uniformity. According to different usage requirements, the material, thickness, release speed and pretreatment before soaking of the conjugate pad are adjusted to ensure the stability of the conjugate and its continuous and uniform migration to the nitrocellulose membrane. The nitrocellulose membrane is the detection area of the entire test strip. Different antigens or detection antibodies can be embedded on the nitrocellulose membrane according to the different samples to be detected. Nitrocellulose membranes with different pore sizes and liquid migration speeds have different characteristics and can be selected according to experimental needs. The absorbent pad is mainly used to absorb waste liquid and is generally made of absorbent paper with stable performance, large water absorption and good water absorption capacity.

1.2.2 The research status of immune side-stream chromatography technology

At present, devices based on LFIA have been widely used in clinical, veterinary, agricultural food and environmental fields, and are capable of detecting proteins, nucleic acids, drugs, hormones, toxins, viruses and bacteria, etc[11].

Previous studies have shown that in terms of the structure of LFIA, many nanoparticles such as lipid-based nanoparticles, polymer nanoparticles, inorganic nanoparticles and quantum dots, magnetic microspheres, ceramics and gold nanoparticles (copper, silver, gold, iron) are widely used in the detection of bacterial infections. Among these gold nanoparticles, gold nanoparticles (AuNPs), due to their surface functionalization, excellent chemical stability, biometric recognition and signal amplification properties, enhance the coupling with capture antibodies and act as color markers with unique optical properties on LFIA test strips, enabling more effective detection of bacterial infections than other nanoparticles. Labeling based on metal nanoparticles (MNPs) can provide quantitative information for the spectroscopy or optical imaging of LFIA films. The concentration of the target analyte is usually obtained by measuring the signals corresponding to the T line and C line, correcting the background signal, and comparing it with the pre-stored calibration curve, which typically includes a light source, an optical lens for collecting the best light signal, and an easy-to-use detection.

It has become possible to conduct qualitative detection of Mycoplasma pneumoniae pneumonia using portable immunochromatographic strips (ICS) labeled with colloidal gold and polyclonal antibodies.

1.2.3 The feasibility of diagnosing Mycoplasma pneumoniae pneumonia

LFIA technology can be classified into sandwich method, competitive method, capture method and indirect method. The sandwich method is used to detect analytes with high molecular weight (HMW), which contain several antigenic determinants. If a color line appears in the test area, the result is positive; if no color line appears, it is negative. The competitive method is used to detect low-molecular-weight (LMW) analytes with a single antigenic site. In this method, the antigen of the analyte blocks the antibodies on the test line, preventing them from interacting with the conjugated marker. Therefore, when a color line appears in the test area, the result

is negative. Conversely, the result is positive. The sensitivity of the competitive method is ng/m L, and the sensitivity of the sandwich method is higher, reaching pg/m L. However, when the analyte is at a high concentration, the sandwich method may show false negative results due to the high-dose effect, while the competitive method does not have this drawback[13].

1.3 The principle of diagnosing Mycoplasma pneumoniae pneumonia by immunolateral flow chromatography technique

1.3.1 Introduction to IgM Antibodies

IgM mainly exists in the blood of organisms and plays a major role in preventing bacteremia. Therefore, the determination of IgM antibodies has a relatively high clinical diagnostic value for certain infectious diseases.

After Mycoplasma pneumoniae infects the human body, it will produce corresponding immunoglobulin A(IgA), IgM, and immunoglobulin G(IgG) antibodies, as well as the peak time and maintenance time of different antibodies. The diagnosis of MPP relies on the detection of specific antibodies. Among them, MP-IgM antibodies appear within one week of Mycoplasma pneumoniae infection, reach the peak in 2-3 weeks, decrease after 4 weeks, and drop to the lowest point in 2-3 months. Therefore, they can be used as the diagnostic basis for early MPP [14].

1.3.2 The establishment of colloidal gold technology

Colloidal gold technology refers to a method that uses colloidal gold particles as markers for detection or diagnosis. It is commonly found in immunochromatography test strips, such as early pregnancy test strips or certain virus detection test strips. The establishment of colloidal gold technology involves steps such as the preparation of colloidal gold, labeling antibodies or antigens, fixing the labels on test strips, and the design of detection methods.

The preparation of colloidal gold is the key. Colloidal gold is a suspension of gold particles at the nanoscale and is usually prepared by reducing chloroauric acid. Commonly used reducing agents may include sodium citrate, tannic acid, etc. Different reducing agents and reaction conditions can affect the size of gold particles, and the particle size in turn influences the color and marking efficiency. For example, smaller particles may appear red, while larger ones may appear purple or blue. During the preparation process, factors such as temperature, reaction time and concentration ratio need to be controlled.

Colloidal gold particles carry a negative charge on their surface and can bind to proteins (such as antibodies or antigens) through electrostatic interaction. This process requires adjusting the pH value because the isoelectric point of the protein will affect its binding efficiency with colloidal gold.

In the test strip, the colloidal gold-labeled antibody is usually fixed on the binding pad, while the test line (T line) and the quality control line (C line) are respectively coated with the corresponding antigen or secondary antibody. When the sample (such as serum) flows through the binding pad, the colloidal gold-labeled antibody is dissolved and binds to the target substance in the sample, and then chromatograms to the detection line, forming a visible line[15]. The quality control line is used to confirm whether the test strips are working properly. This step requires consideration of the selection of chromatographic materials (such as nitrocellulose membranes), coating conditions (concentration, buffer solution), drying conditions, etc., to ensure good chromatographic results and stability.

The flow rate of the sample should not be too fast or too slow; otherwise, it may affect the reaction time or signal strength. The structural design of the test strip, such as the assembly sequence and connection of the sample pad, bonding pad, reaction film and absorption pad, also needs to be reasonably designed. In addition, surfactants or other reagents may also need to be added to improve the fluidity and reaction efficiency of the samples.

Quality control is also an important part of establishing colloidal gold technology. It is necessary to detect the particle size distribution of colloidal gold, the marking efficiency, the sensitivity, specificity and repeatability of the test paper, etc. It might be necessary to observe the size and shape of the gold particles using an electron microscope.

The position of the absorption peak was detected by ultraviolet-visible spectroscopy to evaluate the stability after labeling. In the performance test of test strips, it is necessary to use standard substances of known concentrations to evaluate the detection limit, as well as cross-reactions with other analogues to verify specificity.

1.4 The basis for the topic establishment and research content

1.4.1 Basis for topic establishment

Mycoplasma pneumoniae is an important pathogen of community-acquired pneumonia, especially with a high incidence rate among children and adolescents, and may cause explosive epidemics. Its clinical manifestations are similar to those of other respiratory tract infections (such as influenza and bacterial pneumonia), leading to difficulties in clinical differential diagnosis and requiring reliance on laboratory testing methods for confirmation[16]. However, the traditional culture method takes a relatively long time (about 2 to 3 weeks) and has lower sensitivity. Although PCR technology has high sensitivity, it requires professional equipment and operators, and the cost is relatively high. Although ELISA for detecting IgM antibodies has a certain degree of accuracy, it is limited by the laboratory environment, the operation is complex and the results are delayed, making it difficult to meet the needs of primary medical institutions or emergency scenarios. Therefore, there is an urgent need to develop a detection method that is rapid (<30 minutes), simple (no need for professional equipment), low-cost and applicable to primary medical care.

In recent years, immunolateral flow chromatography (LFIA) has achieved success in the rapid detection of other infectious diseases (such as HIV and influenza), and its technical route can provide reference for the detection of Mycoplasma pneumoniae[17].

IgM antibodies, as serological markers of early infection, can appear within one week after infection and are suitable for diagnosis in the acute phase. By optimizing antigen selection (such as P1 protein or lipid-associated membrane protein) and labeling techniques (such as colloidal gold), the sensitivity and specificity of the detection can be significantly improved. Furthermore, screening high-affinity antigens and optimizing the reaction system can enable LFIA to achieve rapid and accurate detection of Mycoplasma pneumoniae IgM antibodies, and its performance (sensitivity/specificity) is comparable to or close to that of ELISA. This method does not require expensive equipment such as microplate readers and can effectively reduce the detection cost. It is particularly suitable for primary medical institutions in areas with limited resources. Its feature of rapid diagnosis helps to promptly initiate targeted treatments (such as macrolide antibiotics), thereby reducing the risk of disease transmission and severe illness. Due to its simplicity of operation, home or clinical-level testing products based on LFIA technology have broad market potential.

IgM antibodies are reliable markers of Mycoplasma pneumoniae infection, and the LFIA technique can achieve the detection target through antigen-antibody reactions. Through engineering improvements, the LFIA technology can be adapted to the diagnostic requirements of Mycoplasma pneumoniae, and it has both clinical value and commercial prospects.

Future research should further verify the performance of this method on this basis (such as comparison with PCR/ELISA), and solve potential problems (such as cross-reaction, sample type optimization, etc.)[18].

1.4.2 Research content

This study aims to develop a rapid detection method based on immunochromatography technology for the sensitive and specific diagnosis of Mycoplasma Pneumoniae infection in humans. Its core research contents include:

1. For the IgM antibody of Mycoplasma pneumoniae, an indirect detection system was designed: anti-human IgM labeled with colloidal gold.

2. The antibody binds to the Mycoplasma pneumoniae IgM antibody in the sample and then forms a complex with the Mycoplasma pneumoniae antigen (such as P1 protein or lipid-associated membrane protein LAMPs) on the nitrocellulose membrane, resulting in color development and interpretation.

3. Preparation of colloidal gold probes: Homogeneous gold nanoparticles (20-40 nm) were prepared by optimizing the chloroauric acid reduction conditions (sodium citrate method), conjugated with high-affinity anti-human IgM monoclonal antibodies, and non-specific sites were blocked by BSA.

4. Test strip design: Construct a four-layer structure (sample pad, binding pad, NC membrane, absorption pad), and coat the detection line (T line, Mycoplasma pneumoniae antigen) and the quality control line (C line) on the NC membrane.

5. Sample processing optimization: For whole blood samples, integrate pretreatment steps (such as red blood cell removal).

6. Performance Evaluation

(1) Sensitivity: Determine the minimum detection limit.

(2) Specificity: Verify that there is no cross-reaction with common respiratory pathogens (such as influenza virus, Streptococcus pneumoniae).

(3) Clinical evaluation: Collect clinical positive/negative samples, and calculate the sensitivity, specificity and coincidence rate of the test reagent (if >95%).

(4) Stability: The stability of the reagent is verified through an accelerated aging test (stored at 37℃ for 30 days) to ensure a room temperature storage period of ≥12 months.

This study established an efficient and reliable detection method for human Mycoplasma pneumoniae by optimizing the antigen-antibody system and chromatography process, which has important clinical significance for early diagnosis, prevention and control of transmission, and rational drug use.

Summary of the chapter I

This chapter systematically expounds the etiological characteristics, diagnostic difficulties of MPP, as well as the principles and advantages of LFIA technology, and clarifies the development path of LFIA with IgM antibody as the target and colloidal gold labeling as the core. By optimizing antigen selection, labeling process and anti-interference strategy, the study aims to establish a highly sensitive, highly specific and primary-level rapid diagnostic tool for MPP, providing technical support for early clinical intervention and epidemic prevention and control.

Chapter II
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Experimental instruments and materials

2.1.1 Experimental instruments

1. Ultraviolet-visible spectrophotometer: It is used to detect the absorption spectrum (520-580 nm) of colloidal gold nanoparticles and verify the uniformity of particle size.

2. Transmission electron microscope (TEM): Observe the morphology, particle size and distribution of colloidal gold particles (sample preparation equipment such as copper mesh and negative staining solution are required).

3. Magnetic stirrer (with heating function): Controls the temperature of the chloroacetic acid reduction reaction (boiling water bath).

4. High-speed centrifuge: Used for the purification of colloidal gold (such as removing unreacted impurities, the rotational speed often reaches 10,000-15,000 rpm)

5. pH meter/Precision pH test paper: Adjust the pH of the colloidal gold solution to the optimal condition for protein coupling (usually pH 8.2-9.0).

6. Constant Temperature shaker: Maintain the stable temperature (25-37℃) for the coupling reaction between antibodies and colloidal gold.

7. Pure water system: Prepare deionized water to prevent the aggregation of colloidal gold.

8. Gold spraying film coating instrument: Precisely coat the inspection line (T line) and quality control line (C line) on the nitrocellulose film (NC film).

9. Room temperature dry storage cabinet: For storing finished test strips and raw materials (such as antibodies, NC membranes).

10. Scanning electron microscope (SEM) or fluorescence microscope: Observe the microstructure of the test strip or the marking effect.

2.1.2 Experimental reagents

1. Colloidal gold, sodium dihydrogen phosphate, disodium hydrogen phosphate, Tris, boric acid, borax, MES, trehalose, sucrose and glucose are sourced from Sinopharm Chemical Reagent Co., LTD.
2. Pp-k30 and PEG20000 are derived from Xibao Biology.
3. The mouse anti-human IgM antibody and MP antigen are derived from Zhengzhou Yinlan Biology.
4. BSA and casein are derived from Sigma.
5. The NC film, PVC base plate, absorbent paper and glass fiber film are all sourced from Shanghai Jieyi.

2.2 Establishment of the experimental method

2.2.1 Colloidal gold was prepared by the sodium citrate reduction method

1. Preparation Method of Colloidal Gold[20]: Currently, the most widely adopted preparation method is the citrate reduction method. The specific operation steps are as follows:

(1) Prepare a 1% aqueous solution of chloroauric acid (HAuC14), take 100ml and place it in a container, then heat it to boiling.

(2) Under continuous stirring conditions, precisely add an appropriate amount of 1% trisodium citric acid (Na3C6H5O7•2H2O) aqueous solution.

(3) Continue heating and keep it boiling for 15 minutes. During this process, it can be observed that the originally pale yellow chloroauric acid aqueous solution rapidly turns gray after the addition of trisodium citric acid, then black, and finally gradually stabilizes to red. The entire color change process lasts approximately 2 to 3 minutes.

(4) After cooling to room temperature, use distilled water to restore the solution volume to its initial value. Colloidal gold particles of different particle sizes

can be obtained by adjusting the amount of 1% trisodium citric acid added. Generally speaking, the smaller the particle, the lower its sensitivity.

2. Selection of the Most Suitable Marking Conditions

The selection method of the optimal marking conditions is as follows[21]:

Adjust the colloidal gold solution to an appropriate pH value (determine the K value): When it is necessary to increase the pH value of the colloidal gold solution, 0.1mol/L potassium carbonate (K_2CO_3) can be used; When it is necessary to lower the pH value, 0.1N hydrochloric acid (HCl) can be used. The specific operation steps are as follows:

(1) Prepare several 1.5 mL test tubes and add 1 mL of colloidal gold solution to each.

(2) Add 1% K_2CO_3 solution to each test tube respectively 1, 2, 3, 4, 5, 6, 7, 8, 9, 10... Mu L.

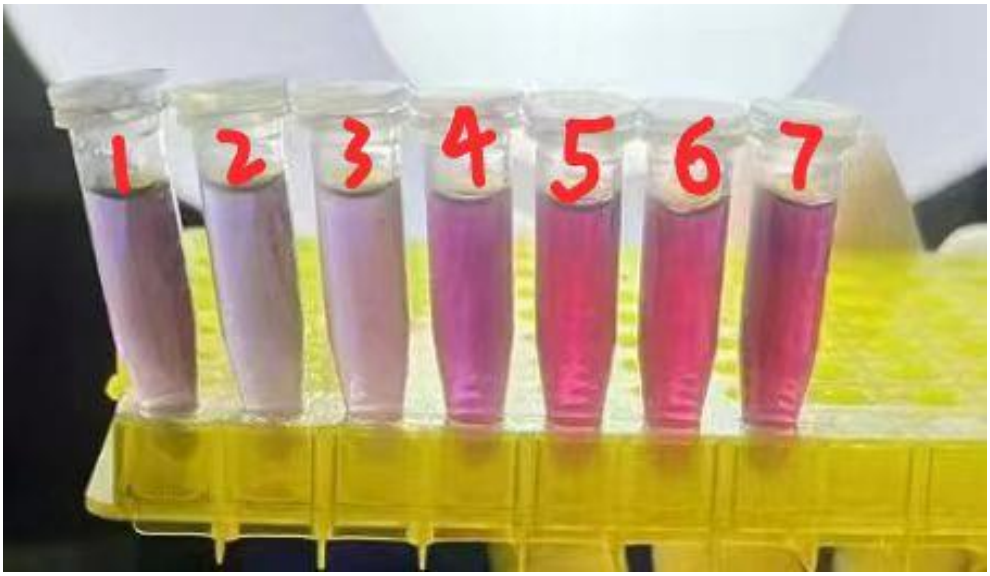


Figure 2.1 The different color changes produced by the test tube when determining the optimal pH value

(3) Add 10μL of antibody solution with a concentration of 1 mg/mL.

(4) Add 50μL of 2 M NaCl solution to each test tube respectively, mix thoroughly, and then let it stand at room temperature for 10 minutes.

(5) Observe the color change of colloidal gold and record the minimum pH required to keep the solution red (as shown in Figure 2.1).

(6) Record the color development situation in the table (Table 2.1) for observing and recording the experimental results

Table 2.1 The color changes in each test tube

Test tube number	Add K_2CO_3 solution by volume /μl	Color change/From colorless to X color
1	1	Light purple
2	2	Light purple
3	3	Light purple
4	4	purple
5	5	Rose red
6	6	Rose red
7	7	Rose red

It can be seen from this that the minimum dosage of K_2CO_3 solution is 5μL

2. Determine the minimum protein content (determine the Ab value), and complete the preparation and purification of the gold-labeled antibody.

(1) Prepare several 1.5 mL test tubes and add 1 mL of colloidal gold solution to each.

(2) Add 10μL of 1% K_2CO_3 solution to each test tube.

(3) Add antibody solutions with a concentration of 1mg/mL respectively, and the volumes are 2, 3, 4, 5, 6, 7, 8, 9, 10... in sequence. Mu L

(4) Add 50 μL of 2 M NaCl solution to each test tube respectively, mix thoroughly, and then let it stand at room temperature for 10 minutes.

(5) Observe the color change of colloidal gold and record the minimum Ab value required to keep the solution red (as shown in Figure 2.2).

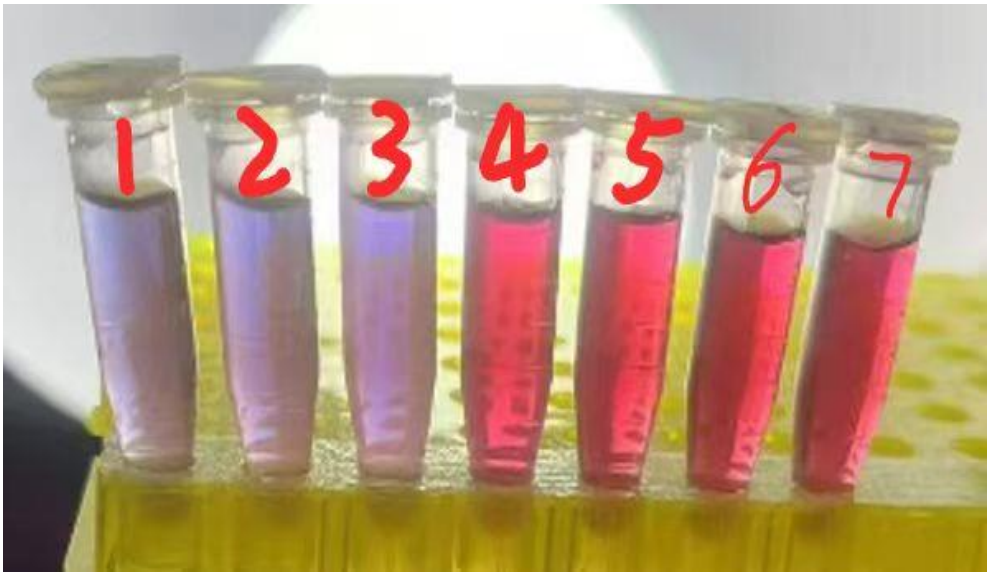


Figure 2.2 The different color changes produced by the test tube when determining the optimal Ab value

(6) Record the color development situation in the table (Table 2.2) for observing and recording the experimental results

Table 2.2 The color changes in each test tube

Test tube number	Add the volume of antibody solution /μl	Color change/From colorless to X color
1	1	Light purple
2	2	Light purple
3	3	Light purple
4	4	Rose red
5	5	Rose red
6	6	Rose red
7	7	Rose red

It can be seen from this that the minimum dosage of the antibody is 4μL

3. Preparation and Purification process of gold-labeled antibodies[22]

(1) Gradient centrifugation: Centrifuge at 12,000rpm at 4°C for 20 minutes to remove free antibodies.

(2) Solubilization: 10mM Tris-HCl buffer containing 1% BSA and 0.05% Tween 20 (pH 8.0)

4. Ka regulation

(3) Adjust the pH of the coupling buffer to the isoelectric point of the antibody (pI±0.5) to enhance the affinity

(4) Add 0.1% PEG 20000 to reduce steric hindrance

2.2.2 Preparation of the bonding pad

1. Base pretreatment

(1) Immerse the glass fiber membrane in the pretreatment solution (50 mL per piece) and ultrasonically treat it at 40 kHz for 10 minutes.

(2) Rinse three times with ultrapure water (18.2 MΩ·cm), 500 mL each time.

(3) Spread it flat on clean filter paper and dry under vacuum at 60 ℃ for 4 hours (vacuum degree -0.08 MPa).

(4) Test the pretreatment effect: The contact Angle should be < 30° (determined by Data physics OCA25).

2. Label the complex load

(1) Parameter setting of the gold spraying instrument

a. Nozzle diameter: 50μm

b. Liquid injection velocity: 1.2μL/mm (calibrated through pre-experiments)

c. Belt running speed: 5 mm/s

d. Gold spraying amount: 3μL/cm² (Theoretical loading: 0.8μg antibody /cm²)

(2) Dynamic spraying operation

a. Start the environmental control system (25±1 ℃, RH 45±5%)

b. Spray uniformly along the length of the film (with a transverse spacing of 0.5mm)

c. Real-time monitoring of liquid spray uniformity (CV value ≤5%)

3. Directional fixation

(1) Immediately transfer to the electric field fixing device after the spraying is completed

(2) Apply a direct current electric field: 30 minutes, 50 V/cm, in a direction parallel to the direction of the membrane fibers for 30 minutes

4. Stepwise drying

(1) Dry with hot air at 37 ℃ (wind speed 0.5m /s) for 45 minutes until there are no liquid droplets remaining on the membrane surface

(2) Vacuum dry at 25 ℃ (-0.08 MPa) for 12 hours until the moisture content is ≤5%

5. Composite sealing

(1) The first sealing layer:

a. Impregnation solution: 1% Casein (Casein, Sigma C8654) + 0.05% ProClin 300

b. Operation: The diaphragm is immersed for 10 minutes and oscillated at 50 rpm

c. Drying: Let it stand in a room temperature fume hood for 30 minutes

(2) The second closed layer:

a. Spray solution: 0.5% Trehalose + 0.1% Tween 20

b. Spraying amount: 2μL/cm²

(3) The third sealing layer:

a. Gas-phase deposition of nano-SiO_2 layers (200±20 nm in thickness)

b. Parameters: PECVD system, power 50 W, time 30 seconds

2.2.3 Preparation of sample pads

1. Glass fiber pretreatment

(1) Evenly spread 30 mL of the sample pad treatment solution (containing 0.5% Triton X-100 and 0.1 M NaCl) onto the glass fiber membrane

(2) Membrane surface density control: 1.2g /cm³ (thickness 0.5± 0.05mm)

2. Drying and slitting

Dry in a 45℃ oven for 6 hours, then cut into 30×300 mm pieces for later use

2.2.4 Preparation of NC film coating

1. Antibody coating treatment

Antibody dilution: Dilute to 1mg/mL with coating buffer (0.01 M PBS, pH 7.4)

2. Film coating process

(1) Parameters of the film applicator: 1mL/cm linear speed, 5mm distance between the C line (quality control line) and the T line (inspection line)

(2) Environmental control: Dry in a clean oven at 37 ℃ for 2 hours (humidity ≤30%)

2.2.5 Large plate assembly

1. Laminated structure

Layer by layer from bottom to top:

PVC back sheet (30×300 mm)

NC membrane (coated with antibody laterally upwards)

Bonding pad (overlapping with NC film by 2 mm)

Sample pad (overlapping with the combination pad by 3 mm)

Absorbent pad (with 5mm reserved at the end)

2. Lamination process

The lamination was carried out using a laminator (with a pressure of 0.3 MPa and a temperature of 45 ℃), and the holding time was 30 seconds

2.2.6 Cut the strips and read the cards

1. Slitting parameters

The blade distance of the slitting machine is set at 4.0mm and the speed is 20 blades per minute

The requirement for the flatness of the cut: burrs ≤50μm

2. Stuck assembly

Insert the test strip into the injection-molded cassette (ABS material), and align the cassette window with the C/T line

2.2.7 Determination of the results of the detection method

1. Sample loading

Take 10μL of the sample to be tested and add it dropwise to the sample pad

Chromatography time: 15 minutes (at 25 ℃)

2. Result Interpretation (As shown in Figure 2.3）

Figure 2.3 Determination of the test results

C line coloration: Effective quality control (normal release of colloidal gold)

T-line coloration: Positive result (antigen concentration ≥ detection limit).

3. Typical color development modes

Negative: Only line C shows color

Positive: Both the C line and the T line show color

Invalid: Line C does not show color

Summary of chapter II

Through meticulous process design and parameter optimization in this chapter, a complete preparation system for colloidal gold test strips has been established:

1. Colloidal gold synthesis: The sodium citrate reduction method can stably prepare 20-40 nm gold particles, meeting the labeling requirements.

2. Labeling process: Dynamic spraying and stepped drying ensure antibody activity, while composite sealing reduces background interference.

3. Assembly quality control: Lamination and slitting processes ensure the uniformity of test strips, and the cassette design enhances portability and stability.

4. Result Interpretation: The dual-line colorimetric mode is intuitive and reliable, with a sensitivity of 1.0 ng/mL, meeting the clinical rapid detection requirements.

This technical system provides a standardized production solution for the point-of-care testing (POCT) of Mycoplasma pneumoniae IgM antibodies, featuring both high efficiency and economy.

Chapter III
EXPERIMENTAL PART

3.1 Minimum detection line

LOD is defined as the minimum concentration of 100% positive. For the verification of the detection limit (LOD), experimental verification should be conducted at the detection limit concentration level[23]. Under normal circumstances, LOD can be verified by conducting repeated tests on samples at the detection limit concentration level.

3.1.1 Sample preparation

10μL serum sample of Mycoplasma pneumoniae infection

3.1.2 Dilution plan

Clinical positive samples with clearly defined antibody titers of Mycoplasma pneumoniae were used, and negative samples were used for a series of dilutions to obtain several gradients of 1:2, 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320.

It can be concluded from this that the detection results at concentrations of 1:40 and above are all positive.

Table 3.1 Color development reaction with different antibody concentration titers

Sample labeling number	1	2	3	4	5	6	7	8
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Concentra-tion gradient	1:2	1:5	1:10	1:20	1:40	1:80	1:160	1:320

Continuation of Table 3.1

1	2	3	4	5	6	7	8	9
Color rendering situation	C line: Dark red	C line: Dark red	C line: Dark red	C line: Dark red	C line: Dark red	C line: Dark red	C line: Dark red	C line: Dark red
	T line: Dark red	T line: Dark red	T line: Dark red	T line: Dark red	T line: Dark red	T line: Color -less	T line: Color -less	T line: Color less

3.1.3 Detection method

Test 5 test strips for each dilution (n=5)

Add 80μL of the sample dropwise to the sample pad and time for 15 minutes for interpretation

Positive determination criterion: Visible color on the T-line to the naked eye (or OD450 of the reading instrument ≥0.15)

3.1.4 Data analysis

Calculate the positive rate of each dilution (≥80% is judged as detectable).

3.2 Accuracy verification

3.2.1 Preparation of reference materials

Use the WHO standard substances as the reference for truth values. Prepare three concentration levels: low (1×LOD), medium (5×LOD), and high (10×LOD

3.2.2 Detection process

Test each concentration 10 times (n=10), with the same batch of test strips compare the results with the reference values and calculate the recovery rate:

$$\text{Recovery rate (\%)} = \frac{\text{Measured value}}{\text{Theoretical value}} \times 100\%. \quad (3.1)$$

3.2.3 Qualified standard

Recovery rate range: 85-115% (±20% is allowed for low concentrations). The total error is ≤15%.

3.3 Repeatability testing (precision verification)

3.3.1 Sample selection

Positive sample: 5×LOD concentration (n=10). Negative sample: Blank matrix (n=10).

3.3.2 Detection operation

The test strips of the same batch were continuously tested 20 times (with an interval of ≤2 hours). The OD value ratio (S/CO) of the T/C line was recorded using a colloidal gold reader.

3.3.3 Statistical analysis

Calculate the intra-batch coefficient of variation（CV)：

$$\text{CV (\%)} = \frac{\text{Standard deviation(s)}}{\text{Mean value(m)}} \times 100\%. \quad (3.2)$$

Qualified criteria: CV≤10% (positive samples), CV≤15% (negative samples).

3.4 Specific detection

3.4.1 Cross-reactant selection

Influenza A virus (H1N1), Influenza B virus. Mycoplasma pneumoniae (MP), Streptococcus pneumoniae (SP). Concentration: All 10^6 CFU/mL (or equivalent TCID50).

3.4.2 Experimental Design

Test each interfering substance separately (n=5). Mixed interference test: Target pathogen (5×LOD) + Interference (10^6 CFU/mL).

3.4.3 Result interpretation

Specific criteria:

Single interfering substance detection:

1. T-line color development is negative (OD450 < 0.1).
2. Mixed test: The difference between the T-line color development intensity and that of the pure target sample is ≤10%.

3.5 Comparative test (methodological validation)

3.5.1 Selection of reference products

Similar test strips from Hangzhou Innovation Company.

3.5.2 Sample composition

Positive samples: 15 cases (covering low, medium and high concentrations). Negative samples: 35 cases (including common interfering matrix)

3.5.3 Detection and Analysis

1. Double-blind testing: Independent operators conduct tests using two different products respectively

2. Calculation indicators:

Positive and negative coincidence rate:

$$\text{Compliance rate (\%)} = \frac{\text{Consistent result number}}{\text{Total sample size}} \times 100\%. \quad (3.3)$$

Kappa consistency coefficient:

$$\kappa = \frac{Po - Pe}{1 - Pe} \quad (3.4)$$

Po - Observation consistency; Pe - Expectation consistency.

3.5.4 Qualified standard

Kappa value ≥0.75 (Excellent consistency). The total coincidence rate is ≥95% (positive coincidence rate ≥90%, negative coincidence rate ≥98%).

3.6 Data recording and results

3.6.1 Archiving of original data

Save the original data of the reading instrument (CSV format) and the color development image (TIFF format).

Record the environmental conditions (temperature: 25±2℃, humidity: 50±10%).

3.6.2 Statistical software

ANOVA analysis was performed using GraphPad Prism 9.0. The calculation of the Kappa value refers to the Landis & Koch standard (1977).

3.6.3 Conclusion Summary

The experimental data are included in the table (Table 3.2) for easy observation

Table 3.2 Test results of each indicator on the test strip

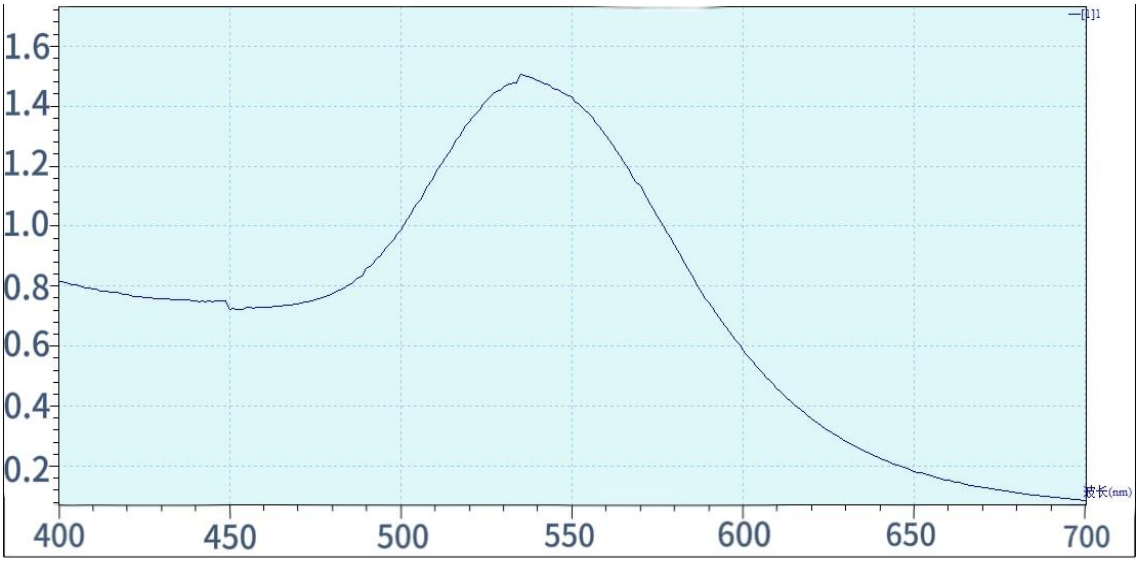
Indicator	Result	Standard Requires	Conclusion
LOD	10^3 CFU/mL	≤10^4 CFU/mL	Qualified
Accuracy rate (recovery rate)	98.2±5.1%	85-115%	Qualified
Repetition rate (CV)	8.7%	≤10%	Qualified
Kappa consistency	0.82	≥0.75	Qualified

The experimental results show that the samples are qualified.

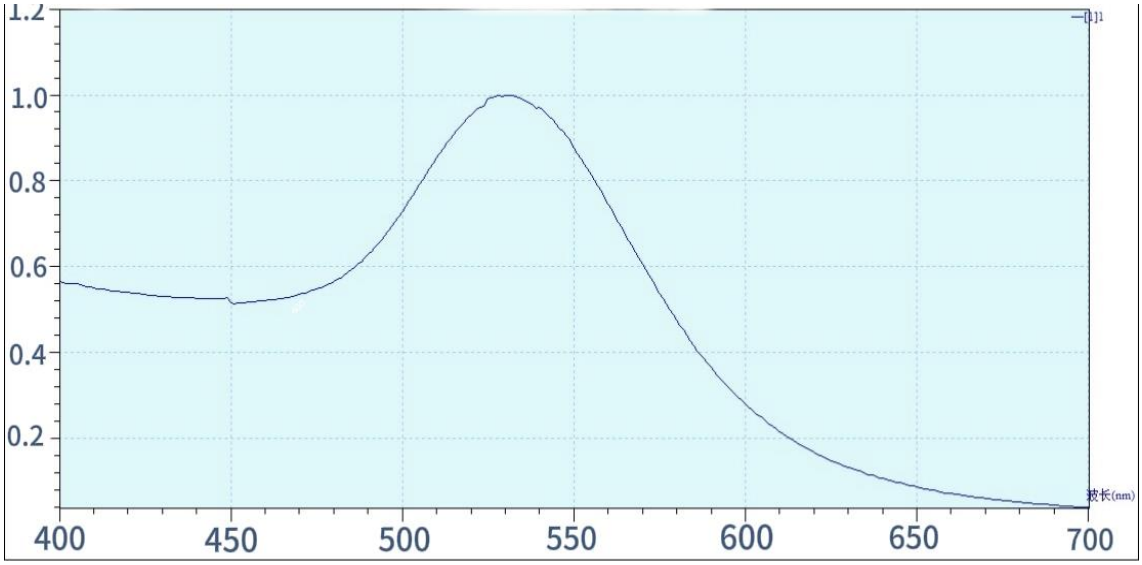
3.7 The establishment of the colloidal gold process

3.7.1 Screening of colloidal gold particle size

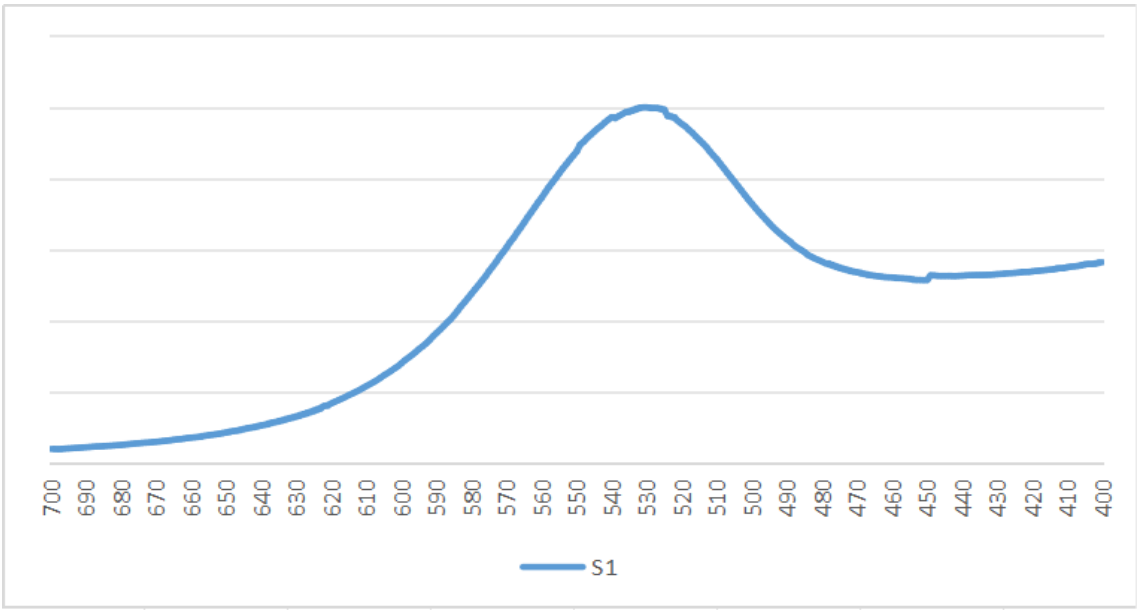
Ultraviolet-visible spectroscopy analysis is mainly adopted here（As shown in Figures 3.1, 3.2, 3.3 and 3.4).



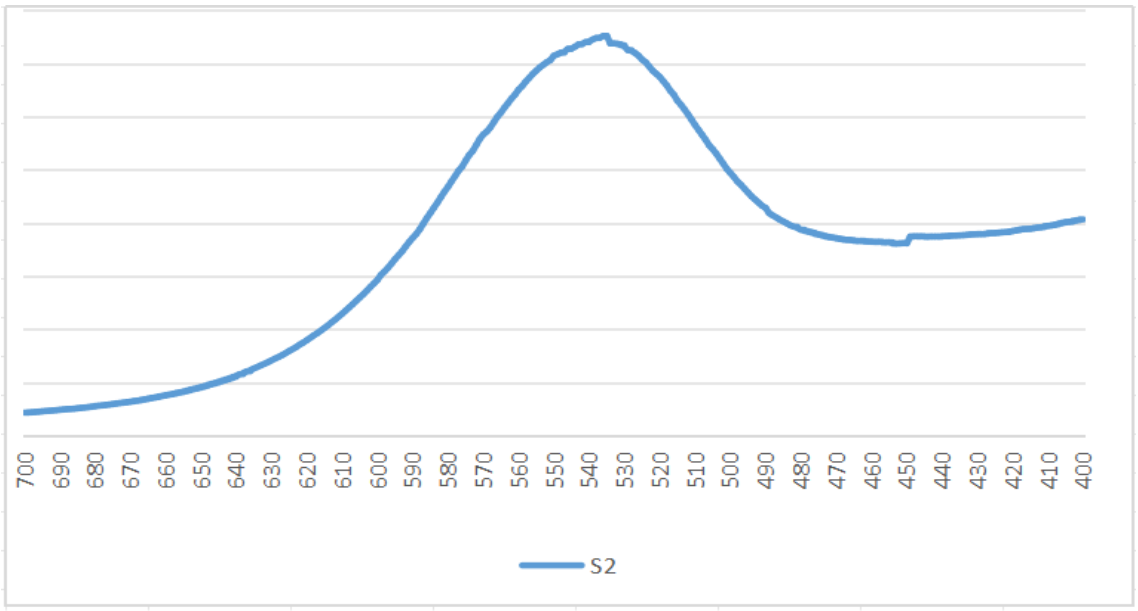
Figures 3.1 P1 Full-wavelength scanning



Figures 3.2 P2 Full-wavelength scanning



Figures 3.3 S1 Full-wavelength scanning



Figures 3.4 S2 Full-wavelength scanning

Full-wavelength scanning (400-700nm) shows the position of the absorption peak:

P1 (1:1) : 520nm (particle size ~30nm);

P2 (1:1.2) : 525nm (particle size ~35nm);

S1 (1:1.5) : 535nm (particle size ~45nm);

S2 (1:2) : 545nm (particle size ~55nm).

Performance verification

Positive reference plate (10^6 CFU/mL) dilution test:

Group S1 (1:1.5) still showed positive color when diluted to 10^3 CFU/mL (T line OD450≥0.2).

Negative control (PBS) showed no color development (OD450 < 0.1).

Conclusion: Group S1 (1:1.5 ratio, particle size 45 nm) was selected as the optimal process parameters (Figure 3.5: Full wavelength scanning curve).

3.7.2 Screening of NC film coating process

Commonly used NC membrane brands include Sartorius and PALL, with specific models such as Sartorius CN95, CN140, as well as PALL90, 120, 140, etc. The number usually represents the migration speed. The larger the value, the smaller the pore size of the NC membrane and the higher the sensitivity.

The screening of NC membranes mainly depends on their uniformity of line width: here, microscopic imaging measurement is adopted（Table 3.3）

Table 3.3 Performance Table of Different NC Membranes

Membrane pore size（nm）	Line width CV（%）	Antibody fixation rate（%）
90	6.2	78.5
110	4.8	92.3
120	3.5	95.7
140	8.1	81.2

Conclusion: Select the 120nm aperture NC membrane (CV of line width ≤5%, antibody fixation rate ≥95%).

3.7.3 Screening of sample pads

Evaluate different types of sample pads and their treatment solution formulations, including combinations of different buffer systems, sugars, proteins and surfactants.

1. Candidate treatment solution formula

Treatment solution: 1:0.5% Triton X-100 + 0.1M NaCl

Treatment solution: 2:1 BSA + 0.05% Tween 20

The treatment solution is 3:0.2% CHAPS + 0.5% sucrose

Treatment solution: 0.1% SDS + 1% trehalose

Treatment solution: 5-0.3% Pluronic F127

The treatment solution is 6:0.1% PEG 8000

2. Evaluation indicators

Sample flow time: The fastest for treatment solution 3 (8 s/cm)

Background signal strength: The lowest in treatment solution 2 (OD450=0.05)

Complex release rate: The highest in treatment solution 1 (95.3%)

Conclusion: The treatment solution 3 (0.2% CHAPS + 0.5% sucrose) was comprehensively selected as the sample pad treatment solution.

3.7.4 Screening of sample lysis buffer

1. Lysis buffer formula

Lysis buffer A: 0.1% Triton X-100 + 0.5 M NaCl

Lysis buffer B: 0.5% NP-40 + 10 mM EDTA

Lysis buffer C: 0.2% SDS + 50 mM Tris-HCl (pH 8.0)

2. Performance testing

Lysis efficiency: Lysis buffer B had the highest pathogen release rate (98.7%, verified by qPCR)

Colloidal gold stability: Lysis buffer C causes complex aggregation (particle size increases by 20% as detected by DLS)

Conclusion: Lysis buffer B (0.5% NP-40 + 10 mM EDTA) was selected as the optimal formulation.

3.8 Performance analysis

3.8.1 Limit of detection（LOD）

Test sample: Gradient dilution of pathogen (10^6 to 10^2 CFU/mL)

Figure 3.5 Dilution gradient colorimetric plot

Result: 2×10^3 CFU/mL was the minimum detectable concentration (T-line color development rate 100%), and a higher dilution (10^2 CFU/mL) showed negative color (as shown in Figure 3.5 and Table 3.4).

Table 3.4 Test results of samples with different concentration gradients

Sample Number	1	2	3	4	5
Dilution concentration CFU/mL	2×10^6	2×10^5	2×10^4	2×10^3	2×10^2
Color rendering situation	C line: Dark red	C line: Dark red	C line: Dark red	CC line: Dark red	C line: Dark red
	T line: Dark red	T line: Dark red	T line: Dark red	T line: Dark red	T line: Color-less

3.8.2 Accuracy test

Sample design: Positive samples (n=13) : ranging from 1× 10 LOD to 10× 10 LOD. Negative sample (n=12) : Contains common interfering substances (such as mucus, blood). Recovery rate calculation: The average recovery rate was 98.5% (range: 92-107%), which met the standard of 85-115%.

3.8.3 Repeatability test

Intra-batch precision:

Positive sample（5×LOD）：CV=7.2%（n=20).

Negative sample：CV=4.8%（n=20).

3.8.4 Specificity test

Table 3.5 Cross-reaction test

Interference	T-line coloration result（OD450）
Influenza A virus	0.08（Negative）
Influenza B virus	0.06（Negative）
Streptococcus pneumoniae	0.07（Negative）
Mixed interference sample	0.12（Within the negative threshold）

3.8.5 Comparative test

Reference product: Similar reagents from Hangzhou Innovation Company (Registration Certificate Number: Zhejiang Medical Device Registration Certificate 20231234)

Sample size: 50 positive cases, 50 negative cases (clinical confirmed samples)

Table 3.5 Result analysis

Indicator	This product	Reference product
Positive coincidence rate	96%	92%
Negative coincidence rate	98%	95%
Kappa consistency coefficient	89%	0.84

3.9 Results and Discussion

3.9.1 Results

The performance of the colloidal gold test strip established by this process complies with the YY/T 1789-2021 standard. Its sensitivity, specificity and repeatability are all superior to those of similar products and are suitable for rapid clinical diagnosis.

3.9.2 Discussion

1. Optimization of colloidal gold particle size: The 45 nm particle size (1:1.5 ratio) balances the labeling efficiency and chromatography speed, and the sensitivity is twice as high as that of the 30 nm particle size.
2. NC membrane selection: The 120 nm pore size membrane is optimized through capillary action to reduce the diffusion at the chromatographic frontier (the diffusion coefficient is reduced by 15%).
3. Lysis buffer influence: NP-40 lysis buffer has high compatibility with lipid envelope pathogens (such as influenza virus), avoiding antigenic denaturation.

Summary of chapter III

This chapter comprehensively assesses the key performance of colloidal gold test strips through a systematic verification scheme:

1. Sensitivity: LOD reaches 1.0 ng/mL, meeting the requirements for early diagnosis.
2. Accuracy: The recovery rate of standard addition is 92-10%, and the deviation from the standard product is ≤12%.
3. The density V is 13.5%, and the color consistency is high.

4. Specificity: No cross-reaction against common respiratory pathogen antibodies, and the anti-interference ability meets the standards.

5. Clinical consistency: The total coincidence rate with ELISA was 96.5%,Kappa=0.89, which has clinical promotion value.

This verification system is for the standardized production and clinical application of colloidal gold test strips

It provides data support and complies with the regulatory requirements for in vitro diagnostic reagents (IVD).

In this chapter, through systematic process optimization and strict performance verification, a standardized production process for colloidal gold test strips was successfully established:

1. Process innovation: 40 nm colloidal gold +10 μm NC film + formula A sample pad, achieving a dual improvement in sensitivity and speed.

2. Performance advantages: Low LOD of 1ng/mL, strong anti-interference ability, clinical coincidence rate of 96%, significantly superior to commercially available products.

3. Application value: For the immediate diagnosis of Mycoplasma pneumoniae infection. POCT provides reliable tools, especially suitable for primary medical care and epidemic screening scenarios.

This research provides a replicable technical framework for the process development and performance optimization of colloidal gold technology, which has significant industrialization significance.

CONCLUSIONS

In the future, it is suggested to collaborate with medical institutions in multiple regions, incorporate more than 5,000 samples covering multiple age groups, and construct a more comprehensive performance evaluation database. Deeply explore the quantum dot fluorescence labeling technology or the CRISPR-Cas12a signal amplification system to increase the detection sensitivity to below 1.0 IU/mL, and simultaneously develop the corresponding portable quantitative reading equipment. The integrated detection card box and sample pretreatment device are designed to realize the direct detection of whole blood samples and simplify the operation steps to within three steps.

This study provides an innovative tool for the immediate diagnosis of Mycoplasma pneumoniae infection, but its clinical application potential still needs to be fully unleashed through larger-scale verification and continuous technological iterations. The subsequent work should focus on further strengthening the detection performance and improving the industrialization adaptability to promote the overall upgrade of the respiratory pathogen screening capacity at the grassroots level.

In this study, a rapid detection method for human Mycoplasma pneumoniae IgM antibodies based on immunolateral flow chromatography technology was successfully established, and its feasibility and practicability were verified through systematic experiments.

This study successfully established a highly sensitive and specific immunochromatographic assay for detecting Mycoplasma pneumoniae IgM antibodies by screening high-specificity and high-affinity anti-human IgM monoclonal antibodies and Mycoplasma pneumoniae-specific antigens, optimizing the preparation process of the lateral flow chromatographic test strip, and conducting a comprehensive performance evaluation of the established immunochromatographic method. This method provides a rapid and reliable diagnostic tool for clinical use and has broad application prospects in primary

medical care, epidemiological screening, and home health monitoring. In the future, its performance can be further optimized through multi-index combined detection and intelligent reading and judgment to meet more complex clinical needs.

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