MINISTRY 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 a paper lateral flow immunoassay for human *Helicobacter pylori* antigen detection**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
BEBT-21
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APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
_____Olena MOKROUSOVA
«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Qu Jianying

1. Thesis topic **Study on the establishment of a paper lateral flow immunoassay for human *Helicobacter pylori* antigen detection**

Scientific supervisor Ph.D., Assoc. Prof. Olena Okhmat

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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)		
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)		Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)		

I am familiar with the task:

Student _____ Qu Jianying

Scientific supervisor _____Olena OKHMAT

Abstract

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Helicobacter pylori (Hp) infection is a major culprit leading to chronic gastritis, gastric ulcers, and even gastric cancer. Early detection helps facilitate timely clinical treatment. To improve the sensitivity, specificity, and stability of traditional colloidal gold lateral flow immunoassay (LFIA) for Hp antigen detection, it is necessary to enhance the colloidal gold labeling process and optimize the chromatographic system. In this study, colloidal gold particles of different sizes were prepared using the citrate reduction method, and 20 nm colloidal gold particles were ultimately selected as the labeling carrier to maintain high antibody activity while ensuring coupling efficiency. The optimized colloidal gold labeling process was as follows: coating particles were dispersed with K_2CO_3 solution (2% mass fraction, pH 12.5), followed by adding antibodies and shaking to form coating complexes. The complexes were suspended and stored in a resuspension buffer containing 0.05 mol/L PB (pH 7.4), 5% sucrose, 1% BSA, and 0.1% Tween-20.

Key words: Helicobacter pylori; colloidal gold; lateral flow immunoassay (LFIA); detection optimization; early screening

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INTRODUCTION

As a core technology of point-of-care testing (POCT), lateral flow immunoassay (LFIA) offers numerous advantages. LFIA directly achieves rapid diagnosis; it eliminates potential sample interference by simplifying sample pretreatment steps and introducing samples into the detection device. The overall technology is relatively simple, with an extremely straightforward detection process that requires only a few steps, reducing operational errors and ensuring the accuracy of detection results. Additionally, LFIA has low detection and reagent costs, simple experimental procedures, and does not require specialized personnel for operation-only personnel to monitor project progress—thus significantly reducing costs. Its relatively low detection cost facilitates the promotion and application of this testing project. However, how to improve the detection accuracy of LFIA remains an urgent issue. To address this, this paper aims to enhance detection accuracy by exploring the impact of various materials on detection results.

CHAPTER 1
LITERATURE REVIEW

1.1 RESEARCH OBJECTIVES AND MAIN CONTENT OF THIS PAPER

With the development and progress of society, people's attention to life and health has increasingly grown. As a major disease threatening human health, cancer has ruthlessly claimed numerous lives, making early diagnosis particularly significant. At the same time, while the rapid development of transportation has brought convenience, it has objectively accelerated the spread of human infectious diseases. How to conveniently and quickly test for the presence of *Helicobacter pylori* in the body has become a public concern. The most economical and convenient current method is to use antigen-based lateral flow immunoassay for rapid detection of *Helicobacter pylori*. Ensuring the stability and sensitivity of test strips is crucial for the experiment. This paper aims to study the stability of test strips under different material parameters and determine the most suitable values for practical production and daily use. Typically, the following three methods are used for measurement.

The aim of this study is to construct a high-performance LFIA method for Hp antigen detection by optimizing the colloidal gold labeling process and chromatographic system. The specific objectives include: (1) enhancing the antigen-antibody coupling efficiency by regulating the particle size and surface charge of colloidal gold; (2) optimizing the antibody coating concentration and chromatographic membrane materials to reduce non-specific adsorption.

1.2 MAIN RESEARCH CONTENT

Preparation and Characterization Optimization of Colloidal Gold Nanoparticles

Colloidal gold particles with different particle sizes (10 nm, 20 nm, 40 nm) were prepared by the sodium citrate reduction method. Uniform particle size and highly stable particles were screened as labeling carriers through ultraviolet-visible spectrophotometry, transmission electron microscopy (TEM), and Zeta potential analysis. The effects of different pH values (4.5–8.5) and ionic strengths (NaCl concentrations of 0.01–0.1 mol/L) on the coupling efficiency of colloidal gold-antibody were studied to determine the optimal labeling conditions (e.g., pH=7.0, NaCl concentration 0.05 mol/L). The Bradford method was used to measure the antibody protein concentration before and after coupling, calculate the labeling rate and binding constant, and optimize the labeling process to preserve antibody active sites.

Optimization Design of Chromatographic System

The chromatographic flow rate and protein binding capacity of nitrocellulose membrane (NC membrane), cellulose acetate membrane, and nylon membrane were compared to screen the matrix materials suitable for Hp antigen detection. The antibody coating concentrations (5–20 μg/mL) of the test line (T-line) and control line (C-line) and the working concentration of colloidal gold-labeled antibody (1:50–1:200 dilution) were optimized by the checkerboard titration method. The specificity of the system was verified by blocking tests (using antigens of gastrointestinal bacteria such as Escherichia coli and Campylobacter jejuni as interferents). Bovine serum albumin (BSA) and polyvinylpyrrolidone (PVP) were introduced as blocking agents, and the buffer formulation (containing 0.1% Tween-20 and 5% sucrose) was optimized to reduce background signals.

1.3 OVERVIEW OF LATERAL FLOW IMMUNOASSAY (LFIA) TEST STRIPS

1.3.1 INTRODUCTION TO LATERAL FLOW IMMUNOASSAY TEST STRIPS

(1) Basic Composition of Lateral Flow Immunoassay (LFIA) Test Strips

The core structure of an LFIA test strip consists of five functional components: the sample pad (made of glass fiber or fiber membrane), conjugate pad, nitrocellulose (NC) membrane (containing the test line "T-line" and control line "C-line"), absorbent pad (made of cellulose), and polyvinyl chloride (PVC) backing card. These components are precisely assembled with approximately 2 mm overlapping regions between adjacent parts, a design that utilizes capillary siphonage to ensure stable migration of the sample solution across functional zones. The detection process begins when the sample solution is applied to the sample pad. Driven by capillary force, the solution first wets the conjugate pad, dissolving pre-coated detection reagents (such as labeled antibodies or antigens) [9][10][11] to form a reaction mixture. The mixture then migrates to the NC membrane: when passing through the T-line, the analyte undergoes an immunoassay with immobilized specific capture reagents, forming and enriching signal complexes; unbound excess reagents continue to flow forward, [123]binding to immobilized quality control reagents at the C-line to verify the validity of the detection process.

As the first site for sample entry, the sample pad is made of glass fiber or fiber membrane. Its core function is to uniformly disperse the sample onto the conjugate pad, rapidly absorb the chromatographic fluid to promote efficient binding between the analyte and reagents, and reduce sample matrix interference through material properties. In practice, the sample pad is often pre-treated with proteins, surfactants, buffer solutions, etc.: these additives adjust sample viscosity, appropriately reduce solution migration speed in the test strip, and prolong the reaction time between the analyte and detection reagents in the T-line and conjugate pad regions, thereby significantly enhancing detection sensitivity.[45]

The conjugate pad shares similar material composition with the sample pad, typically using fiber membrane or glass fiber and requiring pre-treatment. Its core function is to load signal reporters (such as colloidal gold particles, fluorescent microspheres, etc.) immobilized with antibodies or other biorecognition molecules. During detection, after the conjugate pad absorbs water via capillary action, it must ensure complete release of the loaded signal reporters and their migration with the

liquid to the NC membrane. Therefore, the conjugate pad is required to have low nonspecific adsorption and excellent reagent release performance to avoid signal molecule residue or nonspecific binding that could affect results.[67]

The NC membrane serves as the core site for immunoassays, with two critical pre-coated functional lines: the T-line (test line) and C-line (control line).[89] The T-line identifies analytes by immobilizing specific capture reagents (such as antigens or antibodies), and its color development indicates the presence of the target analyte; the C-line immobilizes universal quality control reagents to verify effective operation of the detection process and ensure normal chromatographic migration. The NC membrane requires high-efficiency protein immobilization capacity and hydrophilicity to ensure specific immunoreactions between signal reporters and analytes at the T-line, generating clear signals. [1011]Additionally, the pore size of the NC membrane directly affects liquid chromatography speed: larger pores accelerate liquid migration but shorten reaction time; smaller pores slow flow velocity, prolonging analyte-T-line interaction. Precise regulation of NC membrane pore size optimizes the matching between detection sensitivity and reaction kinetics.[1213]

The absorbent pad is primarily characterized by its water absorption capacity, with the main role of sustaining liquid migration via capillary siphonage to ensure unidirectional and stable chromatography. Meanwhile, it effectively controls liquid flow to prevent sample backflow or retention-induced nonspecific binding and false-positive results. This flow regulation mechanism is a critical factor for result accuracy, particularly in complex sample detection, where the absorbent pad's stable water absorption performance is essential for reducing background interference.[1415]

Flow-injection chromatography (Lateral flow chromatographic assay) is a chromatographic technique that uses capillary forces to drive samples through the reaction matrix, achieving separation based on the differential migration speeds of components (Fig. 1.1).

assembly	material quality	key parameter	Function priority
Sample pads	Glass fiber / fiber membrane	Water absorption> 150μL/cm²/min	Matrix interference elimination
Combine cushions	Polyester fiber / glass fiber	Non-specific adsorption <5%	Marker release efficiency
NC membrane	cellulose nitrate	Aperture 8-12μm (conventional detection	Protein fixation ability
Water-absorbing pads	High density cellulose	Water absorption> 300μL/cm²	Siphonic pull persistence

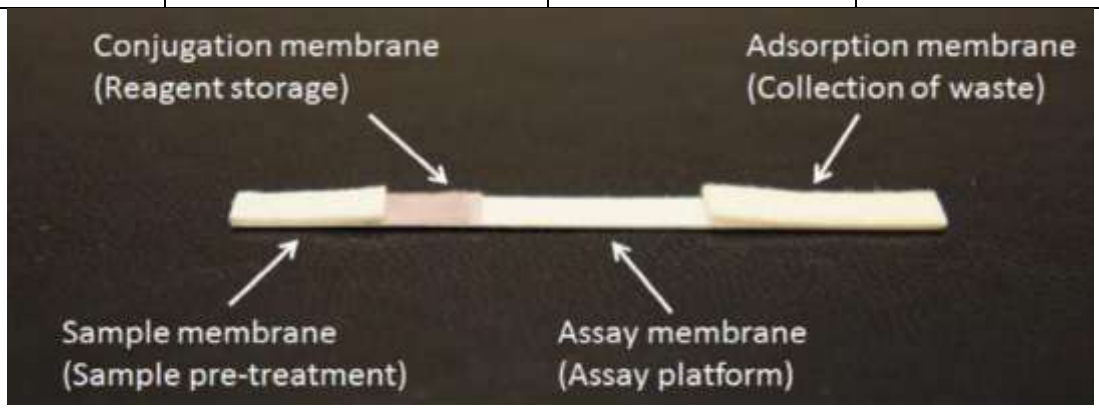

Figure 1.1 – Schematic diagram of LFIA test strip (reference 1)
carbon point nanospheres

The core apparatus consists of four parts: a sample pad, a binding pad, a chromatographic membrane, and an absorbent pad.[1617] The sample pad is a processed fiber membrane that can quickly absorb the sample and evenly distribute it; the binding pad is typically made of fiber membrane or glass wool and preloaded with labeled materials (such as gold-labeled antibodies). During detection, these labeled materials specifically bind to target substances in the sample, forming detectable complexes; the chromatographic membrane is

primarily nitrocellulose membrane, with test lines (T lines) and control lines (C lines) printed on it. Immune reactions capture the labeled complexes and produce color changes to observe the results; the absorbent pad is composed of absorbent paperboard, which ensures continuous detection by directing the sample laterally along the chromatographic membrane through capillary action. This technique, due to its low cost, ease of operation, speed, and good stability, has become a simple, rapid, and sensitive method for detecting foodborne pathogens, tumor markers, heavy metals, pesticides, and other substances. As one of the key factors affecting the sensitivity of flow-injection chromatography, colloidal gold-labeled materials are currently the most widely used because they do not require auxiliary instruments to read data

Detection mode of lateral flow immunochromatographic test strip[181920]

(A) Sandwich mode

The sandwich model (LFIA test strip) is commonly used to detect larger targets with multiple antigen sites (such as pathogens, proteins, viruses, etc.). The principle is based on the characteristic that the target can bind to two different antibodies simultaneously: the T line on the NC membrane is pre-coated with "capture antibody." When a positive sample solution is added to the sample pad, it quickly penetrates the binding pad and combines with the labeled detection antibody (coupled signal reporter such as colloidal gold, fluorescent microspheres), forming a "detection antibody-target" complex. As the mixed fluid flows toward the NC membrane, the capture antibody specifically recognizes and binds to the target in this complex, causing the signal reporter to accumulate and color at the T line. Any excess "detection antibody-target" complex or free detection antibody continues to migrate to the C line, where it is captured by the antibody fixed at the C line, forming a control signal. The coloration at the C line confirms the effective operation of the test strip. For negative samples, since there is no target present, the labeled detection antibody cannot form a complex and will only be captured and colored at the C line, while the T line shows no signal. Therefore, under this model,

both the T line and the C line show positive results; if only the C line shows color, it indicates a negative result.

(b) interfering mode

The competition mode of the LFIA test strip is divided into direct competition and indirect competition. It is commonly used to detect small molecules such as pesticides, veterinary drugs, mycotoxins, and heavy metals (since they have only one antigenic determinant, making it impossible for two antibodies to bind simultaneously). In the indirect competition mode, the antigen serves as the target: when a negative sample solution is added to the sample pad, the labeled antibody is dissolved on the binding pad and migrates with the sample flow to the NC membrane, where it is specifically captured by the capture reagent on the T line (such as a target protein conjugate), causing the T line to color. Any excess labeled antibody continues to migrate to the C line, where it is captured by the fixed goat or rabbit anti-mouse IgG secondary antibody, forming a control signal. If the sample contains the target, it will compete with the capture reagent fixed on the T line for a limited amount of labeled antibody. The higher the concentration of the target, the fewer labeled antibodies are captured by the T line, resulting in a weaker signal. Regardless of whether the target is present, the C line always colors to confirm the validity of the test strip. Therefore, under this mode, both the T line and the C line color negative (no target) or the C line colors while the T line remains colorless (target present). The detection target in the direct competition mode is an antibody or other recognition biomolecule. The principle is that when the sample solution flows through the test strip, the target antibody competes with the labeled antibody for binding to the fixed coated antigen on the T line (the target antibody can specifically recognize this coated antigen). The difference in competitive binding between the two achieves the detection.

1.3.2 APPLICATION FIELDS OF LATERAL FLOW IMMUNOCHROMATOGRAPHIC TEST STRIPS

Lateral flow immunoassay (LFIA) test strips have been widely used in a number of key areas due to their advantages of simple operation, rapid detection, low cost and no need for professional equipment, and have become one of the core technologies of point-of-care rapid testing (POCT) (cited 2 R&D gold nanorods labeling)

In the medical field, LFIA is commonly used to detect diseases. For rapid screening of infectious diseases, the sandwich method is employed, targeting specific antigens or antibodies of pathogens such as SARS-CoV-2, influenza virus, HIV, Treponema pallidum, and Mycobacterium tuberculosis, enabling early diagnosis of infection. This method is particularly suitable for primary healthcare facilities and initial screening at epidemic sites. Tumor marker detection involves capturing specific proteins like alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) released by tumor cells, aiding in the early detection of cancer and postoperative monitoring. Combined with portable readers, quantitative analysis can also be achieved. Pregnancy and reproductive health testing, using a classic example of pregnancy test strips to detect HCG hormone, employs the sandwich method to quickly determine pregnancy status. This method has been extended to include sex hormone tests for LH and FSH, guiding reproductive cycle monitoring. (Citation 3 based on smart terminals)

In agriculture, the LFIA is commonly used to check pesticide residues. The detection of pesticides and veterinary drug residues employs a competitive model targeting small molecule pollutants such as organophosphorus pesticides and sulfonamide veterinary drugs, meeting the on-site rapid screening needs for agricultural products, livestock products, and aquatic products, effectively controlling the risk of excessive drug residues in food; mycotoxin and biological toxin detection involves testing for aflatoxin B1, vomitoxin, and tetrodotoxin, preventing moldy foods and toxic animal and plant products from entering the market, ensuring the safety of the food supply chain; foodborne pathogen detection

can quickly identify E. coli O157:H7, Salmonella, Listeria, and other foodborne pathogens, suitable for monitoring microbial contamination during food processing, preventing mass food poisoning incidents.

In addition, in the work of pollution and environmental governance in public areas, portable detection methods are used for water quality and soil pollutants. These methods enable on-site rapid testing of heavy metals such as lead, mercury, and cadmium, organic pollutants like polycyclic aromatic hydrocarbons, and algal toxins, providing data support for the formulation of emergency response measures and ecological restoration projects in case of environmental pollution incidents. In biosafety and counter-terrorism emergency work, multiple fluorescence PCR, immunochromatography, and other methods are employed to quickly identify and trace anthrax bacilli, ricin, and other biological agents. This allows for rapid response and activation of corresponding prevention and control mechanisms after public safety incidents occur, offering strong technical support for event warnings and prevention efforts, thereby minimizing the risk of biological threats.

In the field of animal disease diagnosis, advanced methods such as real-time fluorescent PCR and immunochromatographic test strips have been applied in livestock disease control. For highly pathogenic pathogens that severely threaten the health of poultry and livestock, such as avian influenza virus, foot-and-mouth disease virus, and African swine fever virus, nucleic acid testing and antigen-antibody screening have been conducted, establishing a comprehensive and multi-dimensional detection platform. The detection technology boasts high sensitivity and specificity, allowing for rapid and accurate identification of pathogens within farms. This not only enables timely acquisition of data on pathogen genotyping during outbreaks but also allows for early warning of potential infection risks through regular testing, thereby establishing a dynamic disease prevention system for farming enterprises. Taking the prevention and control of African swine fever as an example, real-time detection of infected pigs' blood and environmental swabs can promptly interrupt transmission chains; scientifically implementing purification plans can significantly reduce the loss rate

of pig herds, ensuring the safety of livestock production and building a robust biosafety barrier for the healthy and steady development of the industry. For the identification of animal-derived components, methods such as ELISA antigen detection and PCR species-specific gene amplification are used to accurately identify bovine and ovine, horse, and poultry proteins in food and feed. In response to illegal issues in the meat processing industry, such as horse meat adulteration with beef and fox meat passing off as mutton, the DNA or protein samples are extracted, and the immune reactions of corresponding species-specific antibodies or comparisons between different gene sequences are used to identify trace amounts of animal components present at one-thousandth levels. It has already been able to combat the fraud of "selling dog meat under the sign of mutton" in food regulation and establish traceability testing for feed ingredients according to standards, preventing the risk of zoonotic diseases such as mad cow disease caused by the addition of ruminant-derived components in feed at the source. The two technologies, one before the other, serve as the technical guardian of livestock health and safety and the frontline safety barrier for food ingredient regulation, providing technical support for the high-quality development of animal-derived industries.

1.3.3 COMMON MATERIALS OF LATERAL FLOW IMMUNOCHROMATOGRAPHIC TEST STRIP

The immunochromatographic assay (LFIA) using colloidal gold nanoparticles (AuNPs) as markers, due to its excellent stability and high detection speed, remains a core technology in the field of bioanalysis (Citation 4: Flow Chromatography Immunoassay Applied to SARS-CoV-2). It is based on the principle of antigen-antibody specificity, where AuNPs are conjugated with antibodies to form labeled probes. Capillary action facilitates the migration of the sample from one end of the chromatographic membrane to the other, allowing for the detection of the presence or absence of the target substance in the sample. The observable result is a visible band. Due to their nanoscale size (typically 10-60 nm),

AuNPs can be used to label antibodies without deactivating them, and they can produce strong red visual signals through surface plasmon resonance phenomena. This eliminates the need for complex equipment; results can be observed directly with the naked eye under normal natural light. Therefore, it significantly reduces the cost of testing.

AuNPs is a probe with excellent chemical stability and resistance to photodegradation, which prevents agglomeration or de-labeling during storage. It can be stored at room temperature for extended periods without issues, significantly enhancing its portability and extending the shelf life of reagent kits. Compared to enzyme labeling and fluorescence labeling methods, AuNPs-labeled LFIA does not require chromogenic agents or excitation light sources, thus avoiding the problems of enzyme activity being affected by temperature and fluorescence signals being easily interfered with by background. This makes it particularly suitable for applications such as field sites, primary hospitals, or rapid food screening. Additionally, the detection process is simplified: samples can be collected and dropped immediately, and under capillary action, they can be carried to the chromatographic membrane coated with target lines and control lines specific to the corresponding antigens/antibodies. The binding reaction between the target substance and the labeled probe, signal formation, and output all occur within 15-30 minutes, greatly reducing the testing time. To improve the accuracy and sensitivity of the test technology, it is essential to select appropriate colloidal gold, determine the minimum protein content, and choose the gold-labeled antibody.

1.4 OVERVIEW OF *HELICOBACTER PYLORI*

1.4.1 INTRODUCTION OF *HELICOBACTER PYLORI*

Helicobacterpylori (Helicobacterpylori, *H. pylori*) is one of the most significant clinical pathogens colonizing the human stomach, predominantly during childhood. Once it establishes itself on the gastric mucosal epithelium, almost all infected individuals will develop varying degrees of chronic active

gastritis, which can progress to peptic ulcers, dyspepsia, gastric mucosa-associated lymphoid tissue lymphoma, gastric cancer, and some extraintestinal diseases, particularly various autoimmune disorders. Besides directly producing virulence factors such as urease that harm the body, it also causes changes in bacterial toxins within the body, thereby damaging the health. Clinical trials have confirmed that eradicating Hp can block its continuous damage to the gastric mucosa. Eradication therapy can also be performed for asymptomatic carriers, reducing the risk of disease onset. Clinically, Hp clearance mostly involves antibiotic treatment; however, due to improper use of antibiotics, resistance to metronidazole, clarithromycin, and levofloxacin has been increasing year by year. In China, resistance to metronidazole exceeds 50%, while resistance to levofloxacin and clarithromycin both exceed 30%, consistent with earlier research data. Resistance leads to a decreasing eradication rate over time and makes re-resistant cases more likely after initial treatment failure, especially in the first-time treated population. From this, it can be seen that the guidelines recommend that individuals in high-drug-resistant areas and those with relevant indications undergo a drug sensitivity test before receiving first-line routine anti-Hp eradication therapy. Based on the results of the drug sensitivity test, appropriate anti-Hp drugs should be selected. This is one effective way to improve the success rate of Hp eradication and also helps reduce the irrational use of antibiotics and control the spread of drug resistance.

1.4.2 HARM OF *HELICOBACTER PYLORI*

Helicobacter pylori (Hp), classified as a Group I carcinogen by the International Agency for Research on Cancer, has an infection mechanism closely related to its pathogenic hazards: Hp breaks down urea using urease to produce ammonia and simultaneously secretes cytotoxins. This not only triggers systemic inflammation and immune responses that damage the gastric mucosa and affect gastrointestinal hormone secretion, leading to irreversible damage to the gastric mucosa, but also can induce extragastric diseases due to excessive immune

reactions. Clinical data show that Hp infection almost invariably leads to chronic active gastritis in all infected individuals, and is directly associated with peptic ulcers, gastric cancer, and gastric lymphoid tissue lymphoma, increasing the risk of gastric cancer by 4 to 6 times. The 2019 "Expert Consensus Opinion on *Helicobacter pylori* Eradication and Gastric Cancer Prevention and Control in China" clearly states that Hp infection is the primary cause of gastric cancer in China, with approximately 90% of non-cardia gastric cancers being related to it. Timely eradication of Hp can effectively reduce the incidence of gastric cancer, highlighting the critical importance of early intervention in the prevention and control of digestive tract diseases. Moreover, due to its exceptional survival capabilities, high transmissibility, and extreme drug resistance, *Helicobacter pylori* (Hp) is regarded as a "superbug" in the biological community. As one of the common chronic infections in humans, although the Hp infection rate in China has been declining with improved hygiene standards, it remains as high as 40% to 50%, affecting about 600 million people. Therefore, timely detection and treatment of *Helicobacter pylori*-induced harm to the human body are extremely important.

Conclusions to chapter 1

1. This text systematically introduces the lateral flow immunoassay (LFIA) test strip technology and the related research on *Helicobacter pylori*. Firstly, the basic composition of LFIA test strips includes five functional components: the sample pad (made of glass fiber or fiber membrane), which uniformly disperses samples, absorbs chromatographic fluid, and reduces matrix interference through pre-treatment with proteins or surfactants; the conjugate pad (similar to the sample pad in material), which loads signal reporters (such as colloidal gold particles) and requires low nonspecific adsorption to ensure complete reagent release; the nitrocellulose (NC) membrane, the core site for immunoassays, with T-line (for analyte detection) and C-line (for quality control), whose pore size (8–12 μm) affects migration speed and reaction time; the absorbent pad (high-density cellulose), which maintains unidirectional flow and prevents backflow to avoid

false positives; and the PVC backing card for structural support. The detection process relies on capillary siphonage, with sample solution migrating through each pad to form reaction complexes at the NC membrane, where T-line and C-line signals indicate analyte presence and assay validity, respectively.

2. LFIA employs two main detection modes: the sandwich mode, suitable for large analytes (e.g., pathogens, proteins), where the target binds to two antibodies to form a complex captured by the T-line, with the C-line verifying validity (both lines colored for positive, only C-line for negative); and the competition mode (direct/indirect), used for small molecules (e.g., pesticides), where the target competes with immobilized antigens or antibodies for labeled reagents, resulting in weaker T-line signals with higher target concentrations while the C-line always confirms validity.

3. Widely applied across fields, LFIA excels in point-of-care testing (POCT) due to its simplicity, rapidity, and low cost. In medicine, it detects infectious diseases (e.g., SARS-CoV-2, HIV), tumor markers (e.g., AFP, CEA), and pregnancy (HCG) via the sandwich mode, with portable readers enabling quantitative analysis. In agriculture, it uses the competition mode to screen pesticide/veterinary drug residues (e.g., organophosphates, sulfonamides), mycotoxins (e.g., aflatoxin B1), and foodborne pathogens (e.g., E. coli O157:H7) to ensure food safety. For environmental monitoring, it rapidly tests heavy metals (e.g., lead, mercury) and organic pollutants in water/soil, supporting emergency responses. In biosafety, it identifies biological agents (e.g., anthrax, ricin), while in animal health, it diagnoses livestock diseases (e.g., avian influenza, African swine fever) and detects animal-derived components in food/feed to prevent adulteration and zoonotic risks (e.g., mad cow disease).

4. Colloidal gold nanoparticles (AuNPs) are pivotal LFIA materials, offering stability, rapid detection (15–30 minutes), and visual readability via surface plasmon resonance (red signals visible to the naked eye), eliminating the need for complex equipment. With nanoscale size (10–60 nm), AuNPs label antibodies without deactivation, resist photodegradation, and store at room temperature,

enhancing portability and shelf life. Compared to enzyme/fluorescence labeling, they avoid temperature interference and background noise, fitting field and rapid screening applications, though optimizing AuNP size, protein conjugation, and antibody selection is critical for sensitivity.

5. Regarding *Helicobacter pylori* (Hp), a major gastric pathogen colonizing humans (often in childhood), it causes chronic active gastritis, peptic ulcers, gastric cancer, and extraintestinal disorders via virulence factors like urease and cytotoxins. Classified as a Group I carcinogen, Hp is linked to 90% of non-cardia gastric cancers in China, increasing gastric cancer risk by 4–6 times. Despite declining infection rates (40–50% in China, affecting ~600 million), its high transmissibility and drug resistance (metronidazole >50%, clarithromycin/levofloxacin >30%) necessitate pre-treatment drug sensitivity testing to improve eradication success and reduce antibiotic misuse. Eradication prevents mucosal damage and lowers disease risk, underscoring the urgency of early detection and intervention in digestive health management.

6. In summary, LFIA test strips represent a versatile POCT technology with broad applications in health, agriculture, and environment, while Hp research highlights the critical need for targeted detection and resistance management to combat its severe public health impact.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

This study aims to construct a high-performance LFIA method for Hp antigen detection by optimizing the colloidal gold labeling process and chromatographic system. The specific objectives include: (1) improving antigen-antibody coupling efficiency by regulating the particle size and surface charge of colloidal gold particles; (2) reducing non-specific adsorption by optimizing antibody coating concentration and chromatographic membrane materials.

2.1 MATERIALS

2.1.1 MAIN EXPERIMENTAL REAGENTS

The experimental reagents include buffers (colloidal gold, sodium dihydrogen phosphate, disodium hydrogen phosphate, Tris, boric acid, borax, MES), sugars (trehalose, sucrose, glucose), macromolecules (PVP-K30, PEG20000), biological materials (antibodies, BSA, casein, positive reference samples for each project, negative reference samples, samples, etc.), and excipients and consumables (NC membrane, PVC plate, absorbent paper, glass fiber membrane).

2.1.2 MAIN EXPERIMENTAL INSTRUMENTS

The equipment needed is pure water system, centrifuge, magnetic heating stirrer, UV-visible spectrophotometer, ultrasonic cleaner, gold spraying film instrument, colloidal gold reader.

2.2 METHOD

2.2.1 PREPARATION OF REFERENCE DISK

Take soybean-sized feces and add 10 times the volume of PBS buffer. Vortex and oscillate for 10 minutes, then centrifuge (5,000 rpm, 5 minutes) to collect the supernatant. Digest with proteinase K (50 μg/mL, 37℃,30 minutes) to inactivate enzyme activity, filter, and store for later use. Select positive samples as p1-4,

negative samples as n1-4, and set up weakly positive samples L1-3 with antigen concentrations close to the detection threshold (Cut-off value)

2.2.2 ESTABLISHMENT OF PROCESS

(1) Colloidal gold preparation

The most commonly used preparation method is the citrate reduction method. The specific procedure is as follows: first prepare HAuCl4 of a 1% aqueous solution, take 100ml and heat to boiling, then accurately add a certain amount of 1% sodium thiosulfate (Na3C6H5O7·2H2O) aqueous solution while stirring. Continue heating and boiling for 15 min. At this point, a pale yellow chloroauric acid aqueous solution can be observed, which quickly turns gray after the addition of sodium citrate, then gradually changes to black, and finally stabilizes into red. The entire process takes about 2-3 minutes. After cooling to room temperature, restore to the original volume with distilled water. By adjusting the amount of 1% sodium thiosulfate, different particle sizes of colloidal gold can be obtained, and generally, the smaller the particle size, the lower the sensitivity.

(2) Colloidal gold labeled antibody

Our experiment requires selecting colloidal gold with the appropriate pH and determining the minimum protein content, choosing gold-labeled antibodies, and purifying them. First, we need to determine the suitable pH. Take several 1.5ml test tubes, add 1ml of colloidal gold each, and adjust the pH to 1% K_2CO_3 to 2, 3, 4, 5, 6, 7, 8, 9, 10.....ul. Add 1 mg/ml antibody at 10 μl per well, and then add 50 μl of a 2M NaCl solution per well. Mix and let sit at room temperature for 10 minutes, observing the color change of the colloidal gold and recording the lowest pH that remains red. Next, we need to determine the minimum protein content (Ab value). Take several 1.5ml test tubes, add 1ml of colloidal gold each, and add 1% K_2CO_3 X ul to each. Add 1 mg/ml of antibody at 2, 3, 4, 5, 6, 7, 8, 9, 10.....ul to each, and then add 50 μl of a 2M NaCl solution per well. Mix and let sit at room temperature for 10 minutes, observing the color change of the colloidal gold and recording the lowest Ab amount that remains red. Finally, we need to carry out the

purification step. Take several 15ml test tubes, add 5ml colloidal gold, add 1% K_2CO_3 X ul, and add 1mg/ml antibody Y ul. Mark at room temperature for 30 min. Add blocking solution (such as 1% BSA) 150 μl and block at room temperature for 30 min. Centrifuge at 10000r/min for 20 min, discard the supernatant, resuspend the colloidal gold with 500 μl of suitable suspension, and measure the OD value at 530 nm.

(3) Combined pad treatment

First, select a glass fiber membrane with an appropriate pore size (9601, type), cut it to the width that matches the chromatography strip (usually 3 mm), and place it in a solution containing 0.05 mol/L Tris-HCl buffer (pH 7.5),5% trehalose, 0.1% Tween-20, and 1% BSA for 10 to 15 minutes to allow the membrane material to fully adsorb the protectant and surfactant to reduce non-specific binding; then lay the treated glass fiber membrane flat in a 37°℃ drying oven and dry for 2 to 3 hours

(4) Processing of antigen coating

Select a suitable NC membrane (Sartorius CN140 type, pore size 140 nm), cut into strips 2.5 to 3 cm wide, and place them on a clean bench for later use. Prepare the blocking solution using 0.05 mol/L Tris-HCl buffer (pH 8.0), add the target antigen (Hp urease UreA protein, concentration optimized by the square method, typically 0.5 to 2 mg/mL) and auxiliary components: add 5% methanol to reduce protein stability and enhance membrane adsorption, 0.1% Tween-20 to minimize non-specific binding, and 0.9% NaCl to maintain ion strength balance. The control line blocking solution uses goat anti-mouse IgG antibody (concentration 1 to 3 mg/mL) to ensure specific binding with the Fc segment of the labeled antibody on the pad. Spray the blocking solution uniformly over the designated area of the NC membrane at a rate of 1 to 2 μL/cm (the distance between the T-line and C-line is 5 mm), controlling the spray speed to 10 to 15 mm/s to avoid droplet spread or breakage. After spraying, dry the membrane strips in an oven at 37°℃ for 1 to 2 hours) for 30 minutes to ensure that the protein is fully fixed to the surface of the membrane fibers. Immediately after drying, perform the blocking treatment: soak

the membrane strips in a blocking solution containing 5% casein (0.05 mol/L PB, pH 7. 4) In the process, incubate at room temperature for 30 minutes to block unbound protein binding sites on the NC membrane, reducing background signals. After blocking, wash the membrane strips with deionized water 2-3 times (5 min each) to remove residual blocking agent, then place them in a 37℃ drying oven for secondary drying for 30 minutes to ensure complete dryness of the membrane surface. The treated NC membranes should be sealed in an aluminum foil bag containing desiccant and stored away from light at 4℃ or room temperature

(5) Sample pad treatment

Select a sample pad (Type 8950 glass fiber membrane), cut it to the width that matches the chromatography card strip (usually 4 mm), and place it in a petri dish containing the pre-treatment solution for soaking for 10-15 min to allow the membrane material to fully adsorb the components of the treatment solution. After soaking, lay the sample pad flat in a 37℃ drying oven to dry for 2-3 hours. Once dried, spray the specific blocking agent (such as anti-human IgG antibody, concentration 5-10 μg/mL) onto the pad.

(6) Large plate assembly

Prepare a clean and dry PVC base plate (thickness 0.3 mm). Apply acrylic adhesive or double-sided tape evenly in the central area of the base plate using an applicator, with the adhesive layer thickness controlled at 50 μm. Then, center the NC membrane (width 2.5-3 cm), which has been coated with antigen (T line) and goat anti-mouse IgG (C line), onto the adhesive layer of the base plate. Attach the pre-treated sample pad (Type 8964 glass fiber membrane) to the sample end of the NC membrane (usually the left end), ensuring an overlap of 2-3 mm between the sample pad and the NC membrane. Compact the area using a film press (pressure 0.1-0.2 MPa, temperature 30-40°℃) to enhance adhesion. Attach absorbent paper (width 5-8 cm) to the other end of the NC membrane, with an overlap of 2-3 mm, and ensure that the absorbent paper extends 1-2 cm beyond the edge of the base plate. Above the overlapping area between the NC membrane and the sample pad, attach a colloidal gold conjugate pad (glass fiber membrane loaded with colloidal

gold-antibody complexes), ensuring an overlap of 1-2 mm between the conjugate pad and the NC membrane, so that the labeled substance can directly contact the antigen on the NC membrane after release.

test method

Add 10ul sample to the sample hole, then add 100ul dilution solution, chromatography for 10-15min, and determine the results

2.3 PROCESS PERFORMANCE ANALYSIS

2.3.1 MINIMUM DETECTION LIMIT

Positive and negative samples with clearly defined *H. pylori* antibody titers were diluted in a series of gradients (1:2, 1:5, 1:10,1:20,1;40) to ensure that the positive samples could be detected.

2.3.2 REPETITIVE TESTING

P1 and p2 were tested 20 times in duplicate, and the coefficient of variation CV was calculated by using a colloidal gold reader

Calculate coefficient of variation (CV)

(1) Calculate the average value (X^-) $X^-=n\sum i=1nXi(n=20)$

(Xi is the T line value, C line value or T/C ratio of each test)

(2) Calculate the standard deviation (SD) $SD=n-1\sum i=1n(Xi-X^-)2$

(3) Calculate the coefficient of variation (CV) $CV=X^-SD\times100\%$

Use a pipette to add the same volume of sample, such as 100 μL, to the reagent well and start the timer. Wait for the reaction to complete according to the specified time in the reagent manual, which is about 15 minutes. Immediately after the reaction, place the reagent into the colloidal gold reader to measure the absorbance values (OD) of the T line and C line. Repeat this process 20 times, and replace the reagent strip with a new one each time to avoid cross-contamination.

2.3.3 COMPARISON TEST

Select 40 positive samples and 50 negative samples. Use real-time fluorescent quantitative PCR. The same operator, under identical experimental conditions, will use the chromatographic reagent to be validated in parallel with the reference method for the same sample. Record the results of the chromatographic reagent (T-line color intensity, which can be semi-quantitatively graded as "-," "±," "+," "++," "+++"). A T-line color intensity ≥ the critical value is considered positive. Calculate the Kappa value.

Conclusions to chapter 2

To achieve this goal, the paper proposes to use a set of research methods. Namely: Preparation of reference disks Preparation of colloidal gold-labeled antibodies Processing of conjugate plates Processing of antigen plates Processing of sample plates Assembling of large plates.

CHAPTER 3

EXPERIMENTAL PART

3.1 ESTABLISHMENT OF PROCESS

3.1.1 SCREENING OF DIFFERENT NC FILMS

Four different types of NC film (CN90, CN110, CN120 and CN140) were selected to test the reference disk, and CN140 was selected as the most suitable (Fig. 3.1).

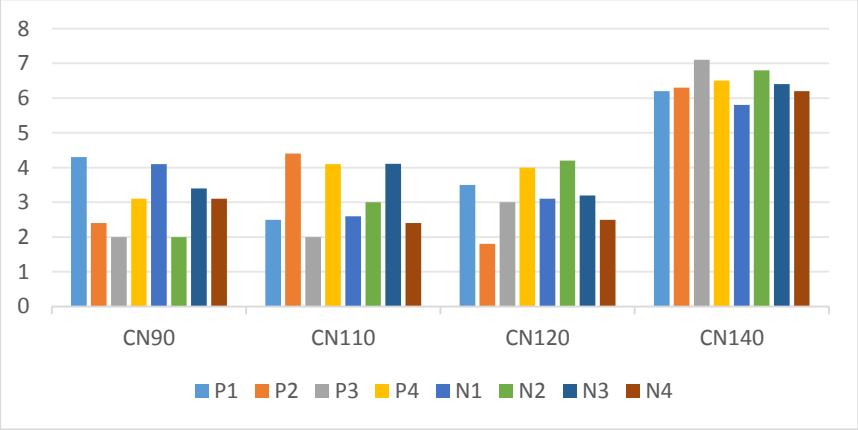

Figure 3.1 – Performance Results of Different NC Membranes

3.1.2 SCREENING OF DIFFERENT SAMPLE PADS

Three different types of sample pads (8950, 8964 and 8965) were selected to test the reference disk, and 8950 was selected as the most suitable (Fig. 3.2).

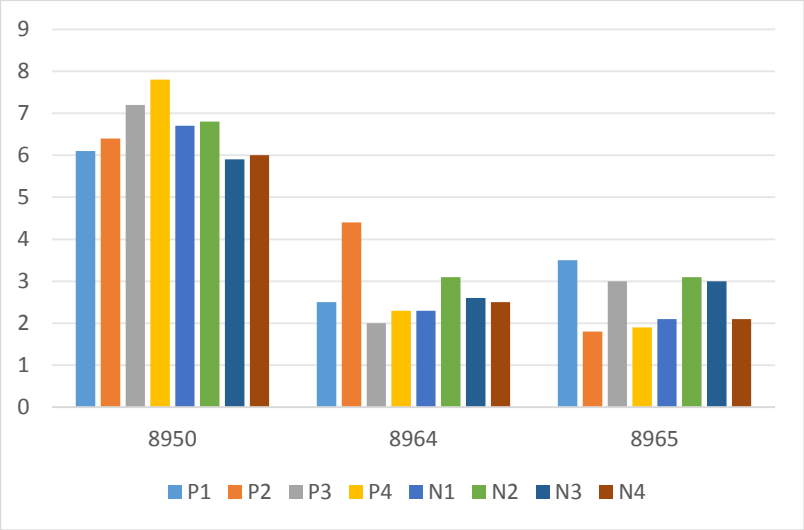

Figure 3.2 – Performance Results of Different sample pads

3.2 PERFORMANCE EVALUATION OF THE PROCESS

Minimum detection limit

Clinical positive samples and negative samples with a clear antibody titer of *Helicobacter pylori* were diluted to ensure that the positive test can be detected at any dilution ratio.

Repetitive testing

P1 and P2 were tested 20 times in repeated tests to calculate the coefficient of variation CV ≤ 15%.

Comparison test

Select 40 positive samples and 50 negative samples, calculate the yin-yang conformity rate is greater than or equal to 90%, Kappa value is 0.83, yin-yang conformity rate ((Po = 95.6%)), sensitivity (95%), specificity (96%) are all higher than 90%, meeting the performance requirements.

Performance index	bear fruit
Check for consistency	- 0.956
Expect consistency	- 0.506
Kappa price	- 0.83
Sensibility	- 0.95
specificity	- 0.96
positive predictive value	- 0.95
negative predictive value	- 0.96

3.3 CONCLUSIONS TO CHAPTER

1. Experimental Preparation

The experimental reagents include buffers (colloidal gold, sodium dihydrogen phosphate, disodium hydrogen phosphate, Tris, boric acid, borax, MES), sugars (trehalose, sucrose, glucose), macromolecules (PVP-K30, PEG20000), biological materials (antibodies, BSA, casein, positive/negative reference samples for each project, test samples, etc.), and excipients/consumables (NC membrane, PVC plate, absorbent paper, glass fiber membrane). The main instruments required are a pure

water system, centrifuge, magnetic heating stirrer, UV-visible spectrophotometer, ultrasonic cleaner, gold spraying film instrument, and colloidal gold reader. For reference disk preparation, soybean-sized feces are mixed with 10× volume of PBS buffer, vortexed and oscillated for 10 minutes, then centrifuged (5,000 rpm, 5 minutes) to collect the supernatant. The supernatant is digested with proteinase K (50 μg/mL, 37°C, 30 minutes) to inactivate enzymes, filtered, and stored. Positive samples (p1-4), negative samples (n1-4), and weakly positive samples (L1-3) with antigen concentrations near the detection threshold (Cut-off value) are selected.

2. Core Experimental Methods

① Colloidal Gold Preparation: The citrate reduction method is used. A 1% HAuCl4 aqueous solution (100 mL) is heated to boiling, and a precise amount of 1 % sodium citrate (Na3C6H5O7·2H2O) solution is added with stirring. Boiling continues for 15 minutes, during which the pale yellow solution turns gray, then black, and finally stabilizes as red. After cooling to room temperature, the volume is restored with distilled water. Varying the amount of sodium citrate adjusts colloidal gold particle size (smaller sizes generally reduce sensitivity).

② Colloidal Gold-Labeled Antibody:

Optimal pH determination: Add 1 mL colloidal gold to multiple test tubes, adjust pH with 1% K_2CO_3 (2–10 μL), add 10 μL 1 mg/mL antibody and 50 μL 2M NaCl, and record the lowest pH maintaining red color.

Minimum protein content (Ab value) determination: Add 1 mL colloidal gold to test tubes, adjust pH with K_2CO_3, add antibody (2–10 μL), and determine the lowest Ab amount keeping the solution red after NaCl addition.

Purification: Mix 5 mL colloidal gold with antibody (Y μL) after pH adjustment, incubate for 30 minutes, block with 150 μL 1% BSA for 30 minutes, centrifuge (10,000 rpm, 20 minutes), discard the supernatant, resuspend in 500 μL buffer, and measure OD at 530 nm.

③ Conjugate Pad Treatment: Select a glass fiber membrane (type 9601, appropriate pore size), cut to 3 mm width, soak in 0.05 mol/L Tris-HCl (pH 7.5)

containing 5% trehalose, 0.1% Tween-20, and 1% BSA for 10–15 minutes, then dry at 37°C for 2–3 hours.

④ Antigen Coating: Cut Sartorius CN140 NC membrane (140 nm pore size) into 2.5–3 cm strips. Prepare coating solutions: T-line with Hp urease UreA protein (0.5–2 mg/mL, optimized by square method) in 0.05 mol/L Tris-HCl (pH 8.0) with 5% methanol, 0.1% Tween-20, and 0.9% NaCl; C-line with goat anti-mouse IgG (1–3 mg/mL). Spray solutions at 1–2 μL/cm (5 mm between T/C-lines, spray speed 10–15 mm/s), dry at 37°C for 1–2 hours, block with 5% casein in 0.05 mol/L PB (pH 7.4) for 30 minutes, wash with deionized water 2–3 times, redry, and store sealed with desiccant at 4°C or room temperature.

⑤ Sample Pad Treatment: Cut type 8950 glass fiber membrane to 4 mm width, soak in pretreatment solution for 10–15 minutes, dry at 37°C for 2–3 hours, and spray with 5–10 μg/mL anti-human IgG antibody.

⑥ Large Plate Assembly: Apply 50 μm-thick acrylic adhesive on a 0.3 mm PVC base plate, center the NC membrane, attach the sample pad (type 8964, 2–3 mm overlap with NC membrane), absorbent paper (2–3 mm overlap, 1–2 cm extension beyond the plate), and conjugate pad (1–2 mm overlap with NC membrane). Press with a film press (0.1–0.2 MPa, 30–40°C) for adhesion. For testing, add 10 μL sample and 100 μL diluent to the sample well, allow chromatography for 10–15 minutes, and read results.

3. Process Performance Analysis

① Minimum Detection Limit: Serial dilute *H. pylori* antibody-positive and -negative samples (1:2, 1:5, 1:10, 1:20, 1:40) to determine the lowest detectable concentration of positive samples.

② Repetitive Testing: Test samples P1 and P2 in duplicate 20 times. Use a colloidal gold reader to measure T-line, C-line values, and T/C ratios. Calculate:

Average ($\bar{X}$) = $\Sigma Xi/n$ (n=20)

Standard deviation (SD) = $\sqrt{[\Sigma(Xi-\bar{X})^2/(n-1)]}$

Coefficient of variation (CV) = $(SD/\bar{X})\times 100\%$

Add 100 μL sample to the reagent well, start timing, react for 15 minutes, measure OD values in the reader, and replace strips for each test to avoid contamination.

③ Comparison Test: Use 40 positive and 50 negative samples with real-time fluorescent quantitative PCR. A single operator performs parallel testing with the chromatographic reagent and reference method under identical conditions. Record T-line color intensity (semi-quantitatively graded as "-", "±", "+", "++", "+++"), define ≥ critical value as positive, and calculate the Kappa value for consistency evaluation.

4. Process Establishment

During process establishment, four NC membrane types (CN90, CN110, CN120, CN140) were screened via reference disk testing, with CN140 selected as optimal for its suitability in immunoassay signal generation and liquid migration. Three sample pads (8950, 8964, 8965) were also evaluated, and 8950 was chosen for its superior sample dispersion, absorption, and matrix interference reduction. These component selections formed the foundational framework for the lateral flow immunoassay strip, ensuring compatibility and functionality for subsequent performance optimization.

5. Process Performance Evaluation

Minimum Detection Limit: Clinical *H. pylori* antibody-positive/negative samples were diluted to confirm detectability at all ratios, ensuring high sensitivity for low-concentration targets, critical for real-world pathogen detection.Repetitive Testing: Samples P1 and P2 were tested 20 times, yielding CV≤15%, verifying result stability and consistency, minimizing errors from operational/equipment variability, and ensuring reliability.Comparison Test: Using 40 positive and 50 negative samples, the test achieved a yin-yang conformity rate ≥90%, Kappa value 0.83, observation consistency \(P_o = 95.6\%\), sensitivity 95%, and specificity 96% (both > 90%). These metrics demonstrate strong agreement with reference methods (e.g., real-time PCR), accurately distinguishing sample types and meeting clinical performance requirements, validating the process’s effectiveness.

6. Summary and Application Value

By optimizing key components (CN140 NC membrane, 8950 sample pad) and evaluating performance (sensitivity, repeatability, consistency), the lateral flow immunoassay process for *H. pylori* detection was established. This technology supports rapid diagnosis, aids in antibiotic resistance management (reducing misuse), and serves as a model for LFIA applications in healthcare, agriculture, and environmental monitoring. Its core strength lies in component compatibility and rigorous performance testing, ensuring stability, accuracy, and practicality–ideal for POCT kit development and clinical deployment. This methodology also advances the standardization and industrialization of rapid detection technologies, enhancing pathogen detection efficiency and public health management, with far-reaching implications for disease prevention, control, and global health security.

CONCLUSIONS

Different particle sizes of colloidal gold prepared by the citrate reduction method were characterized through full-wavelength scanning and transmission electron microscopy. The results showed that colloidal gold with a particle size of 20 nm had a high coupling efficiency (labeling rate> 90%) and strong signal intensity when coupled with the Hp urease antibody. By optimizing the concentration of K2CO3 (0.02 mol/L) and the amount of antibody (10 μg/mL), a colloidal gold labeling system with good stability and high specificity was established. For samples with high migration rates (Type 8950), a slightly larger pore size NC membrane (Type CN140) should be used to balance the liquid flow rate and prevent insufficient antigen-antibody binding due to rapid migration. The stability of the colloidal gold-antibody complex was determined by selecting a resuspension buffer, which contained 0.05 mol/L PB (pH 7.4),5% sucrose, 1% BSA, and 0.1% Tween-20.

Due to the high rate of Hp resistance in clinical settings, predicting whether patients will develop Hp resistance based on nucleic acid testing is extremely challenging. We can address this issue through a "virus-resistance" combined test: using specific multi-group antibodies (for example, capable of binding both the Hp urease antigen and the clindamycin resistance-related gene mutation protein); labeling different antibodies with colloidal gold at various sizes (20nm and 40 nm) (wild-type antibodies and resistant mutant antibodies), respectively, to produce a color line that completes a series of tests. This method can achieve simultaneous infection and resistance typing, effectively addressing the high cost of blood use in clinical settings, and directly guiding clinical medication use, avoiding the problem of antibiotic misuse caused by empirical antibiotic treatment, aligning with the concepts of "precision medicine" and "antibiotic management."

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