

IMMUNOLOGY, HEALTH AND DISEASE

Development of a colloidal gold immunochromatographic strip for rapid detection of *Riemerella anatipestifer* in ducks

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ABSTRACT *Riemerella anatipestifer* is one of the major bacterial pathogens of ducks and causes significant economic losses in poultry agriculture. Usually, methods for detecting *R. anatipestifer* infection need specialized equipment and highly skilled personnel. In this study, a novel colloidal gold immunochromatographic strip was developed for rapid detection of *R. anatipestifer* in ducks. The monoclonal antibodies 2D5 and 2A6 against *R. anatipestifer* were used as colloidal gold-labeled protein and capture protein, respectively, to recognize the bacteria in tryptic soy broth medium culture and in hearts of infected ducks. The goat anti-mouse IgG antibody was labeled on nitrocellulose membrane as a control for C line. The labeling pH was optimized as 10.0, and the concentration of 2D5 labeled to colloidal gold particles

was optimized as 18 µg/mL. The strip specifically detected serotypes 1, 2, and 10 *R. anatipestifer* strains and showed no cross-reaction with *Escherichia coli*, *Salmonella enterica*, and *Pasteurella multocida* strains. The sensitivity of the strip for detecting *R. anatipestifer* was 1.0×10^6 colony forming unit. The strips remained stable for up to 8 mo at 4°C, and the detection can be completed within 15 min. The strip can detect *R. anatipestifer* in hearts of the ducks experimentally infected with *R. anatipestifer* but not infected with *E. coli*, which were also confirmed with bacterial isolation followed by multiplex polymerase chain reaction. These results suggested that the strips are reliable methods for identification of *R. anatipestifer* in laboratories and in duck farms.

Key words: *Riemerella anatipestifer*, colloidal gold, monoclonal antibody 2D5, monoclonal antibody 2A6, immunochromatographic strip

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INTRODUCTION

Riemerella anatipestifer is a gram-negative, nonspore forming, rod-shaped bacterium, which belongs to *Flavobacteriaceae* rRNA superfamily V (Subramaniam et al., 1997). *R. anatipestifer* causes septicemic and exudative diseases in poultry, resulting in major economic losses to the duck industry. *R. anatipestifer* predominantly infects ducks and, less frequently, geese and turkeys (Leavitt and Ayroud, 1997). Ducks of 2 to 5 wk old are typically affected. At least 21 serotypes of *R. anatipestifer* have been isolated (Bruner and Fabricant, 1954; Sandhu and Harry, 1981; Pathanasophon et al., 1995; Pathanasophon et al., 2002), of them, serotypes 1, 2, and

10 are the most prevalent in China (Zhai et al., 2013). Symptoms of *R. anatipestifer* infection (Rubbenstroth et al., 2009) are similar to other bacterial infections in ducks, including *Escherichia coli*, *Salmonella enterica*, and *Pasteurella multocida*. By pathological feature, it is difficult to diagnose *R. anatipestifer* infection.

Clinical and laboratory methods for diagnosing *R. anatipestifer* infection is mostly based on plate culture, polymerase chain reaction (PCR) (Qu et al., 2006), loop-mediated isothermal amplification (Han et al., 2011), enzyme-linked immunosorbent assay (ELISA) (Lobbedey and Schlatterer, 2003), gel diffusion precipitin test, and slide agglutination tests antisera (Pathanasophon et al., 2002). However, these methods are tedious or require expensive equipment and highly skilled personnel. In addition, they are not appropriate for field diagnoses. Therefore, an efficient, rapid, specific, and easily performed method for diagnosis of *R. anatipestifer* infection is critically needed for field use.

The colloidal gold immunochromatographic strip (CGIS) assay is a rapid, reliable, easy, economical, and

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instrument-free analytical method. The analysis can be completed in 20 min without sophisticated equipment. The method has been widely used to detect multiple antigens for instance hormones, antibodies in serum (Zhao et al., 2017), viruses (Sun et al., 2018), bacteria (Wen-de et al., 2017), parasites (Zhuo et al., 2017), biotin (Yao et al., 2017), microelement (Fu et al., 2017), and drug residue (Sakamoto et al., 2017). In this study, a CGIS based on monoclonal antibodies against *R. anatipestifer* outer membrane protein A (OmpA) was developed to rapidly detect *R. anatipestifer* in ducks. The strip was specific for *R. anatipestifer*, and it had no cross reaction with other poultry bacterial pathogens. The sensitivity of strip met the detection requirements, and the whole test needs 10–15 min. It had a potential application prospect in the diagnosis of *R. anatipestifer* infection.

MATERIALS AND METHODS

Ethics Statement

Animal experiment was approved by the Institutional Animal Care and Use Committee of Shanghai Veterinary Research Institute, the Chinese Academy of Agricultural Sciences (approval no. Shvri-SZ-20191012-01). One-day-old Cherry Valley ducks were obtained from the ZhuangHang Duck Farm (Shanghai, China) and housed in cages under a controlled temperature of 28°C to 30°C under biosafety conditions, with water and food *ad libitum*.

Bacterial Strains and Reagents

Sources of bacterial strains employed in this study were listed in Table 1. *R. anatipestifer* and *P. multocida* strains were grown at 37°C in tryptic soy broth medium (Difco, Franklin Lakes, NJ) or *tryptic soy agar medium* (Difco). *E. coli* and *S. enterica* strains were cultured at 37°C in Luria broth (Difco).

Sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) and hydrogen chloroauric acid (HAuCl_4) were purchased from Sigma (St. Louis, MO). The IgG chromatographic column were purchased from General Electric Company (Boston, MA). Polyvinyl chloride (PVC) baseboard, bibulous paper (H5072), glass fiber membrane, and nitrocellulose membrane (CN membrane, sartorius CN95) were purchased from Shanghai JieYi Biotech Co. (Shanghai, China). All chemicals were analytical grade, and used as received.

Recombinant *R. anatipestifer* OmpA Protein and Anti-OmpA Monoclonal Antibody

Recombinant *R. anatipestifer* OmpA was prepared in our laboratory previously (Zhang et al., 2018). Briefly, the *ompA* gene was cloned from *R. anatipestifer* strain CH3, and the recombinant OmpA was expressed, purified, and used as an immunization antigen. For preparation of anti-OmpA monoclonal antibody (MAb), BALB/c mice were immunized with the recombinant

OmpA at a dose of 500 µg per mouse for 3 times at 2-wk intervals, and the hybridoma technique was performed for the MAb development. Positive clones were screened using indirect ELISA and subcloned 3 times. The hybridoma cells producing anti-OmpA MAb 2D5 and 2A6 were obtained and used to develop the colloidal gold immunochromatographic strips. Antibody titers of 2D5 and 2A6 ascites fluids were detected by ELISA.

The specificity of anti-OmpA MAb was verified by Western blot (Chen et al., 2019). Whole-cell bacteria of *R. anatipestifer* strains CH3, NJ3, and Hxb2, *E. coli* strains E937, DE719, and APEC01, *S. enterica* strains CVCC 519 and CVCC 3384, and *P. multocida* strain CVCC 493 were respectively mixed with loading buffer, boiled for 10 min, and loaded on the gel wells for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The separated bacterial proteins were then transferred onto nitrocellulose membranes, blocked using phosphate-buffered saline (PBS) containing 5% nonfat milk at room temperature for 2 h, washed thrice with PBS containing 0.05% Tween 20 for 10 min. Ascites fluid 2D5 and 2A6 were respectively (diluted with PBST at a ratio of 1:4,000) added for overnight incubation at 4°C. After 3 washes, HRP-conjugated goat anti-mouse IgG (Abcam, Cambridge Biomedical Campus, Cambridge, UK, 1:20,000 diluted) was added for 1.5 h incubation at 37°C. Then, after 3 washes, the membrane was visualized using a Tanon 5200 automatic chemiluminescence image analysis system (Tanon, Shanghai, China). Monoclonal antibody 2D5 and 2A6 were then purified from ascites fluid using affinity chromatography column (HiTrap protein G of General Electric Company) for development of colloidal gold immunochromatographic strip.

Preparation of Colloidal Gold Suspension

All glassware soaked in aqua regia [HNO_3/HCl (v/3v)], cleaned with ultra-pure water, and dried before use in drying oven. All solutions were filtered with a 0.22 µm filter (Zhou et al., 2009). Colloidal gold nanoparticles with a mean diameter of 22 nm were prepared according to the methods (Hou et al., 2015). Briefly, 400 mL of 0.01% HAuCl_4 solution was boiled thoroughly, then 8 mL of 1% trisodium citrate was added in flask under continuous stirring. The suspension turned black first and then wine red in 3 min. The liquid was boiled for another 10 min and gradually cooled. Colloidal gold solution was stored at 4°C. Colloidal gold particles were characterized by transmission electron microscopy (Tecnai G2; FEI, GG Eindhoven, The Netherlands) and Synergy H1 microplate reader (Bio-Tek, Winooski, VT).

Preparation of Colloidal Gold-MAB Conjugate

The optimum pH and antibody quantity for colloidal gold-MAB conjugation were tested. For testing the optimum conjugation pH, 7 Eppendorf tubes containing 1 mL colloidal gold solution each were adjusted for their

Table 1. Strains used in this study.

Strains	Description	Source or reference
CH3	<i>R. anatipestifer</i> strains, serotype 1	(Hu et al., 2010)
NJ3	<i>R. anatipestifer</i> strains, serotype 2	(Hu et al., 2010)
HXB2	<i>R. anatipestifer</i> strains, serotype 10	(Hu et al., 2010)
E937	APEC strain, serotype O78	(Wang et al., 2016)
DE719	APEC strain, serotype O2	(Wang et al., 2016)
APEC01	APEC strain, serotype O1	(Johnson et al., 2007)
CVCC 519	<i>S. enterica</i> serovar pullorum	CVCC ¹
CVCC 3384	<i>S. enterica</i> serovar typhimurium	CVCC
CVCC 493	<i>Pasteurella multocida</i>	CVCC

¹CVCC, Chinese Veterinary Culture Collection Center, Beijing, China.

pH with addition of 6, 8, 10, 12, 14, 16, and 18 μL of 1 mol/L K_2CO_3 , which pH were determined as 8.4, 8.6, 9.2, 9.5, 10.0, 10.2, and 10.5, respectively. After that, 12 μL of 2D5 protein (1.0 mg/mL) was added into each of the tubes with sufficient mingling and then left static for another 15 min at room temperature. Conjugation of colloidal gold to antibodies was examined using a Synergy H1 microplate reader (Bio-Tek) as described (Xu et al., 2017). The OD values were measured at 400–700 nm, and the optimum pH of the colloidal gold solution was analyzed by absorption curve. The optimum antibody quantity of the conjugation was tested using 7 Eppendorf tubes containing 1 mL colloidal gold

solution each, with adjusted optimum pH. Then, 3 μg , 6 μg , 9 μg , 12 μg , 15 μg , 18 μg , and 21 μg of 2D5 protein was added, respectively, into each Eppendorf tubes. After keeping static 30 min at room temperature, 100 μL of 10% NaCl was added into per Eppendorf tubes. Finally, the solution was maintained static 30 min at room temperature, and the OD value was measured using a Synergy H1 microplate reader (Bio-Tek) at 400 to 700 nm. The optimum 2D5-labeled dose of the colloidal gold particles was analyzed by absorption curve.

The colloidal gold-MAb conjugation was then performed using optimum pH and antibody quantity. Briefly, 2D5 protein (1.0 mg/mL, 360 μL) was added

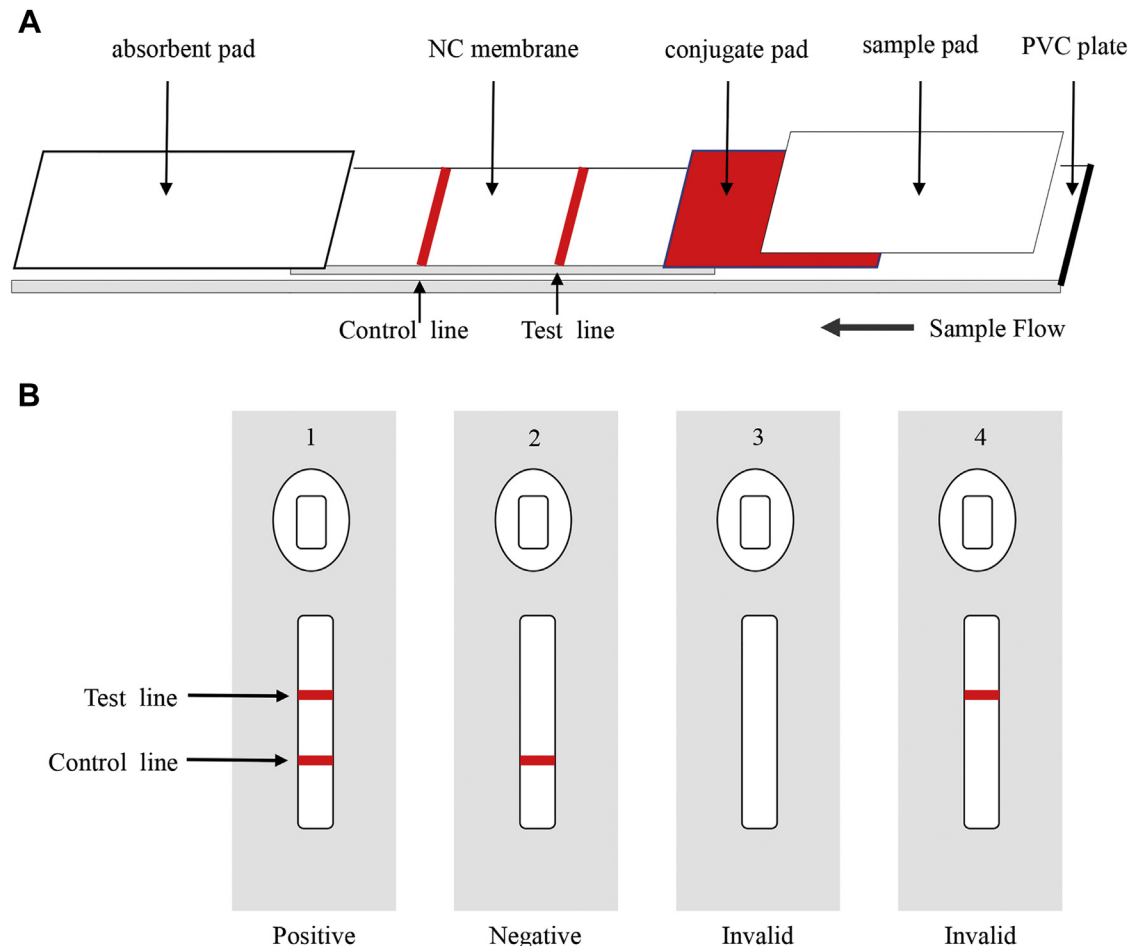


Figure 1. Schematic diagram of immunochromatographic strips. (A) The immunochromatographic strip has 5 main components: sample pad, conjugate pad, nitrocellulose membrane, absorbent pad and PVC plate. (B) The positive, negative and invalid results were shown. Abbreviation: PVC, polyvinyl chloride.

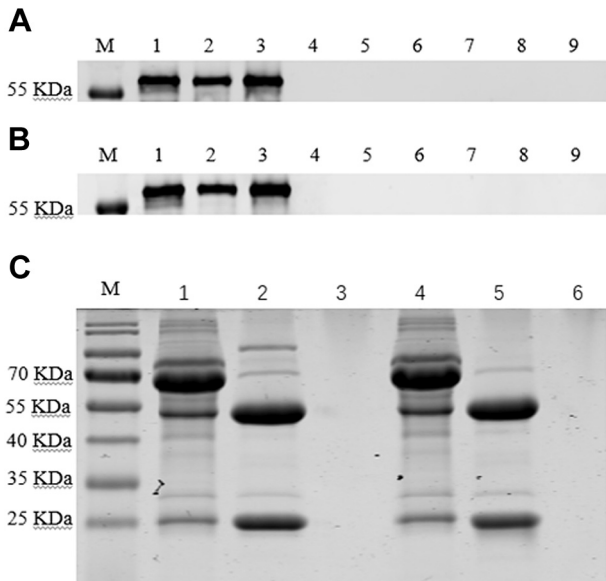


Figure 2. Characterization of the specificity and purification of the MAb 2D5 and 2A6. (A, B) Western blot analysis of the specificity of MAb 2D5 (A) and 2A6 (B). Lane M: protein marker; Lanes 1-3: *R. anatispestifer* serotype 1, 2, and 10 strains CH3, NJ3, and Hxb2, respectively; Lanes 4-6: *Escherichia coli* strains E937, DE719, and APEC01, respectively; Lane 7-8: *S. enterica* strain CVCC 519 and CVCC 3384, respectively; Lane 9: *P. multocida* strain CVCC 493. C: SDS-PAGE analysis of the MAb 2D5 and 2A6. M: protein markers; Lane 1: 2D5 ascites fluid. Lane 2: purified protein 2D5. Lane 4: 2A6 ascites fluid. Lane 5: purified protein 2A6. Lanes 3 and 6: blank. Abbreviation: MAb, monoclonal antibodies.

into 20 mL of colloidal gold solution (adjusted to optimum pH 10.0), and the mixture was stirred vigorously for 30 min. Then, 4 mL 10% of bovine serum albumin was added to the solution with vigorously stirring for 30 min. Next, the solution was centrifuged at 12,000 rpm for 15 min, and the colloidal gold-MAb (2D5 protein) was re-suspended with 10 mL of re-suspended solution. After washed 3 times, the colloidal gold-MAb (2D5 protein) was re-suspended with 2 mL of re-suspended solution. Finally, the absorption curve was measured using a Synergy H1 microplate reader at 400 to 700 nm (Wang et al., 2014).

Preparation of Colloidal Gold Immunochromatographic Strips

Assembly of the immunochromatographic strip was shown in Figure 1. The CGIS has 5 main components: sample pad, conjugate pad, nitrocellulose membrane, absorbent pad, and PVC plate.

The sample pad was treated with 0.01 M PBS solution (pH 7.4) containing 0.2% Tween-20 and dried at 37°C for 4 h. Then, the sample pad was stored in a desiccator at 4°C.

The conjugate pad was saturated with blocking solution of 0.01 M PBS (pH 7.4) containing 2% BSA, 0.5% PEG 200, 0.5% Tween 20, 0.5% polyvinylpyrrolidone K30, and 0.02% sodium azide (pH 7.4) and dried at 37°C. The concentration of colloidal gold-MAb (2D5 protein) conjugate solution was adjusted to OD = 2.5

on a Synergy H1 microplate reader at 530 nm and jetted onto the conjugate pad. The conjugate pad was dried at 37°C for 4 h and stored in a desiccator at 4°C.

Respectively, MAb 2A6 (1.5 mg/mL) and the goat anti-mouse antibody (Boster Biotech Co. Wuhan, China, 1.5 mg/mL) were sprayed onto nitrocellulose membrane in volumes of 1.5 μ L/cm in 2 discrete zones: 1 for test line (T line) and the other for control line (C line). The nitrocellulose membrane was dried at room temperature and stored in a desiccator at 4°C.

The PVC plate and absorbent pad need no pretreatment. Successively, nitrocellulose membrane, conjugate pad, and sample pad were installed onto PVC plate. Finally, the strips were cut into 0.38 cm width and stored in a desiccator at 4°C for detection of *R. anatispestifer* in ducks and in culture.

Specificity, Sensitivity, and Stability of Colloidal Gold Immunochromatographic Strip

R. anatispestifer strains CH3 (serotype 1), NJ3 (serotype 2), and HXb2 (serotype 10) and *E. coli*, *S. enterica*, and *Pasteurella* strains were used to evaluate the specificity of colloidal gold immunochromatographic strip. All tested strains were diluted to 10^9 colony forming units (CFU)/mL with 0.01 M PBS solution (pH 7.4), and 100 μ L of sample was added to the sample pad. Phosphate buffered saline solution (0.01 M, pH 7.4) was used as the blank control. Meanwhile, all strains were detected by multiplex PCR (Wei et al., 2013) to confirm the specificity of the colloidal gold immunochromatographic strips.

R. anatispestifer strains CH3, NJ3, and HXb2 were diluted, respectively, to 1×10^8 , 1×10^7 , 1×10^6 , and 1×10^5 CFU/mL, and each 100 μ L of the samples were added to the sample pad. Meanwhile, all strains were detected by multiplex PCR to measure the sensitivity of the colloidal gold immunochromatographic strips.

Then, the strips were stored in a desiccator at 4°C for 8 mo, and the sensitivity and specificity of the immunochromatographic strips were tested at 2-mo interval during the storage at 4°C. Multiplex PCR was used as the parallel control to determine the stability of the strips.

Analysis of Duck Samples

Fifteen 14-D Cherry Valley ducks were randomly divided into 5 groups, each group contained 3 ducks for experimentally bacterial infection. The ducks in groups 1 to 4 were intramuscular injected with *R. anatispestifer* CH3, NJ3, HXb2, and *E. coli* DE719 strains at 10 median lethal dose, respectively. The ducks in group 5 was used as the negative control. The ducks were closely monitored daily, and the heart samples from the dead ducks were collected and homogenized for testing using the immunochromatographic strip, bacterial isolation, and multiplex PCR analysis. Briefly, the heart samples were collected

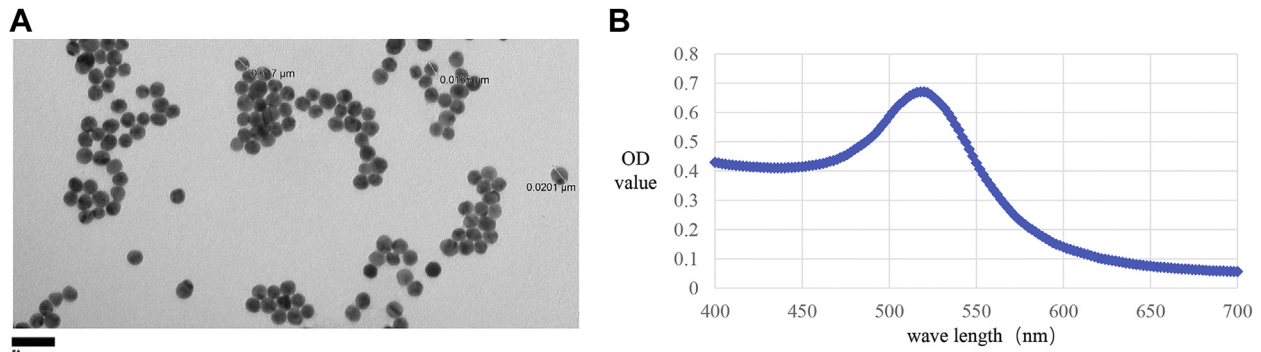


Figure 3. Preparation of colloidal gold solution. (A) Transmission electron microscopy image of colloidal gold nanoparticles, particles were shown roundness with an average diameter of 20 ± 5 nm. (B) Spectra absorption curve of colloidal gold solution, maximum absorption peak of spectra absorption curve is 0.68 at 518 nm.

and weighed, 2 g of each sample were put in 5 mL tubes, respectively, and then 1 mL PBS and 5 small steel balls at 3 mm in diameter (Tianjin feida steel ball Co. Ltd., Tianjin, China) were added for a 10 min-vortex stirring at room temperature. Then, allow the solution to stand for 10 min at room temperature, 100 μ L supernatant was then collected and added to the sample pad. The results were showed after 10 to 15 min. Synchronously reveal of red color T line and C line on the strip was recorded as positive result. Only display of red color C line on the strip was recorded as negative result. Absence of red color T line and C line on the strip was recorded as inefficient result (Figure 1). To confirm the results of immunochromatographic strip, the bacterial isolation was performed as described (Subramaniam et al., 1997; Tsai et al., 2005) followed with multiplex PCR identification using *R. anatipestifer* 16S rRNA, *P. multocida* KMT1, *S. enterica* inv A, and *E. coli* pho A as the targets (Wei et al., 2013).

RESULTS

Identification of MAb Against *R. anatipestifer*

Antibody titers of 2D5 and 2A6 ascites fluids were higher than 1:256,000 by ELISA. Western blot analysis showed that MAb 2D5 and 2A6 reacted specifically

with *R. anatipestifer* serotypes 1, 2, and 10 strains but not with *E. coli*, *S. enterica*, and *P. multocida* strains (Figures 2A, 2B). The 2D5 and 2A6 ascites fluids were purified using affinity chromatography column and analyzed by SDS-PAGE. The result showed 2 specific protein bands, suggesting MAb 2D5 and 2A6 have high purity (Figure 2C).

The MAb 2D5 and 2A6 showed high affinity to *R. anatipestifer*. The MAb 2D5 was conjugated to colloidal gold as labeled protein in control line. The MAb 2A6 was spray onto nitrocellulose membrane as the capture protein in the test line.

Characterization of the Colloidal Gold Particles

The colloidal gold solution was prepared with chemical method using chloroauric acid (HAuCl_4). Images of transmission electron microscope indicated that the colloidal gold particles were spherical with an average diameter of 20 ± 5 nm and well dispersed (Figure 3A), and the maximum OD value peak of spectra absorption curve was 0.68 at 518 nm (Figure 3B), indicating that the solution was stable and reliable. Thus, the solution met the needs of colloidal gold for probes.

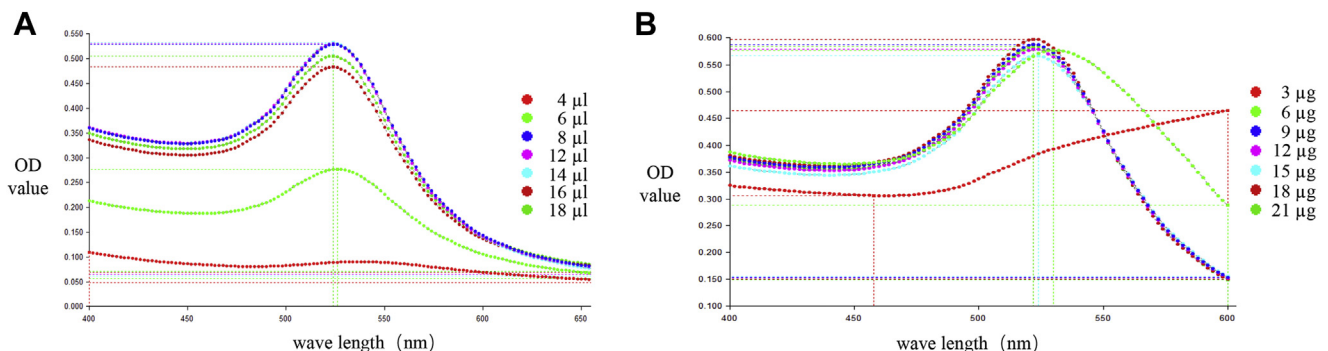


Figure 4. Optimal conditions of colloidal gold probes. (A) The optimum pH of spectra absorption curve, maximum absorption peak of spectra absorption curve (azury) is 0.57 added 14 μ L of 0.2 mol/L K_2CO_3 (pH = 10.0). (B) The optimum 2D5-labeled dose of spectra absorption curve, maximum absorption peak of spectra absorption curve (red) is 0.59 added 18 μ g of 2D5 protein.

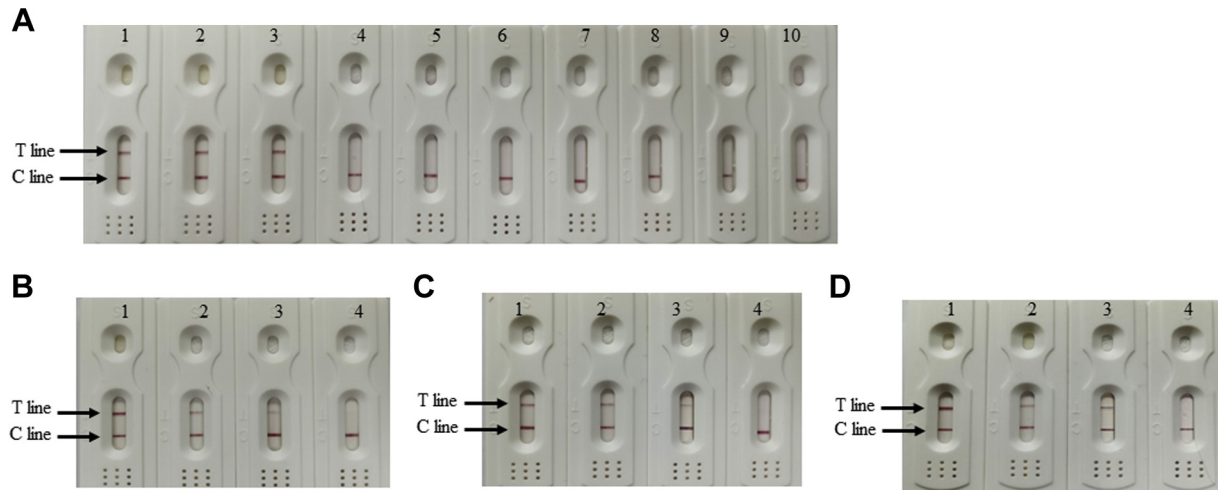


Figure 5. Specificity and sensitivity of colloidal gold immunochromatographic strip. (A) Specificity testing. Lanes 1-3: *R. anatipestifer* serotypes 1, 2, and 10 strains CH3, NJ3, and Hxb2, respectively; lanes 4-6: *Escherichia coli* strains E937, DE719, and APEC01, respectively; lanes 7-8: *S. enterica* strains CVCC 519 and CVCC 3384, respectively; 9: *P. multocida* strain CVCC 493. (B–D) Sensitivity testing for *R. anatipestifer* serotypes 1, 2, and 10 strains CH3, NJ3, and Hxb2, respectively. Lanes 1-4: bacterial CFU at 10^8 , 10^7 , 10^6 , and 10^5 , respectively.

Optimal Conditions of Colloidal Gold Probes

The optimal pH value and dose of 2D5 for preparing colloidal gold probes were measured using different concentrations of K_2CO_3 and 2D5 protein in the reaction system. The maximum peak of spectra absorption curve for optimal pH determination was pH 10.0 (Figure 4A) and for optimal 2D5-labeled dose determination was 18 $\mu\text{g}/\text{mL}$ (Figure 4B). Therefore, the optimum pH and the optimum 2D5-labeled dose for colloidal gold particles were determined as pH 10.0 and 18 $\mu\text{g}/\text{mL}$. The colloidal gold probes were prepared under optimal conditions (pH 10.0, 18 $\mu\text{g}/\text{mL}$, 2% BSA) and concentrated to OD = 2.5 at 530 nm with resuspended solution.

Specificity, Sensitivity, and Stability of Colloidal Gold Immunochromatographic Strip

R. anatipestifer serotypes 1, 2, and 10 strains and other bacterial species were used to confirm the specificity of the strip. The result showed that the strip appeared as 2 red lines (test line and control line) when the sample containing the *R. anatipestifer* bacteria, whereas the strip appeared single red lines (control line) when the sample containing other bacterial species than the *R. anatipestifer* bacteria (Figure 5A), suggesting the strip has high specificity for detection of

R. anatipestifer but not cross-reaction with other bacterial species.

Different bacterial CFU samples of *R. anatipestifer* strains were prepared to determine the sensitivity of the strip. The results showed that the minimum detectable bacterial amount was 10^6 CFU for *R. anatipestifer* strains CH3 (Figure 5B), NJ3 (Figure 5C), and HXb2 (Figure 5D).

The same batches of colloidal gold immunochromatographic strip were stored at 4°C. Specificity and sensitivity of the strip were detected at 2-mo intervals for 8 mo. The result showed that specificity and sensitivity of the strip did not change. Therefore, the strips stored at 4°C were stable at least 8 mo (Table 2).

Detection of Animal Samples

Experimentally infected ducks in groups 1 to 4 with intramuscular injection of *R. anatipestifer* CH3, Yb2, HXb2, and *E. coli* DE719 strains exhibited symptoms of perihepatitis and pericarditis. The strip detection results of heart samples from the ducks infected with *R. anatipestifer* CH3 (Figure 6A), Yb2 (Figure 6B), HXb2 (Figure 6C) infected ducks presented positive results with 2 lines. The heart samples from the ducks with *E. coli* DE719 infection (Figure 6D) or no infection (Figure 6E) showed negative results with 1 line. All tests were finished within 10 to 15 min, and the results were consistent with the multiplex PCR analysis (Table 3).

Table 2. The stability of colloidal gold immunochromatographic strip.

Storage time (mo)	Detection limit (CFU/strip)	Positive samples	Negative samples
0	10^6	+	–
2	10^6	+	–
4	10^6	+	–
6	10^6	+	–
8	10^6	+	–

+, positive result; –, negative result.

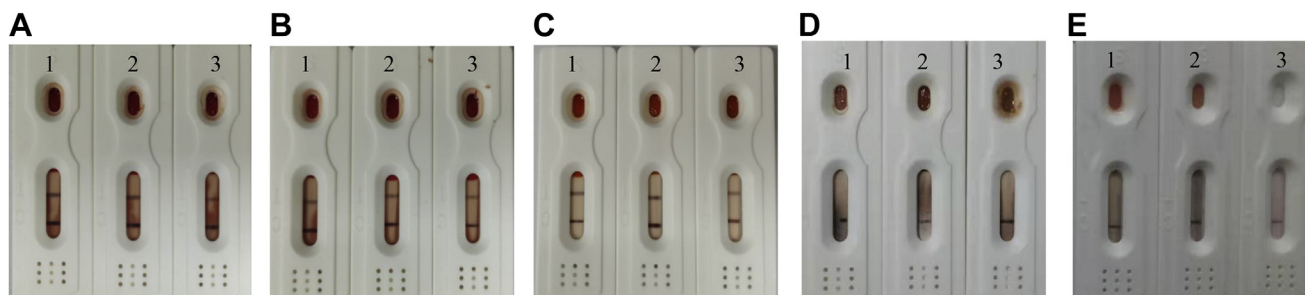


Figure 6. Diagnosis of samples from experimentally infected ducks. (A) heart samples from ducks with *R. anatipestifer* CH3 infection; (B) heart samples from ducks with *R. anatipestifer* NJ3 infection; (C) heart samples from ducks with *R. anatipestifer* HXb2 infection; (D) heart samples from ducks with *E. coli* DE719 infection; (E) heart samples from ducks without infection. Lanes 1-3 indicated 3 samples from ducks in the same group.

DISCUSSION

The ducks infected with *R. anatipestifer* usually show clinical symptoms of lethargy, diarrhea, and respiratory and nervous (Leibovitz, 1972; Leavitt and Ayroud, 1997). The symptoms are similar to clinical signs infected other bacteria in ducks. Vaccine could be used to control *R. anatipestifer* infection (Liu et al., 2013; Chu et al., 2015), and antibody level was used to evaluate the effect of immunization with ELISA (Huang et al., 2011). It is difficult to distinct antibody levels in vaccinated and wild-type *R. anatipestifer*-infected ducks. These factors cause great difficulties and challenges for the prevention and treatment of *R. anatipestifer* epidemics. Thus, an efficient method to detect *R. anatipestifer* is one of the important measures to control the spread of this bacterial contagious disease.

Primary methods for clinical diagnosis of bacterial infection include bacterial isolation and identification, real-time PCR (Zhang et al., 2017), multiplex PCR (Wei et al., 2013), loop-mediated isothermal amplification (Han et al., 2011), ELISA, and agglutination test. Advantages of the above diagnostic methods are higher sensitivity, specificity, and accuracy, but the above

diagnostic methods usually require either long-time, or special equipment, expensive reagents, skillful staff, several hours, and only be operated in laboratories. Those methods cannot meet clinical diagnoses needs. Therefore, we developed a colloidal gold immunochromatographic strip for detection of *R. anatipestifer* in duck, which is rapid, reliable, simple, economical, and instrument-free detection.

In this study, the developed strip was based on monoclonal antibodies against *R. anatipestifer* OmpA. Outer membrane protein A have the characteristics of high immunoreactivity and can be used in development of diagnostic tools to detect *R. anatipestifer* infections of all serotypes (Subramaniam et al., 2000). Therefore, anti-*R. anatipestifer* OmpA MAbs 2D5 and 2A6 were selected, purified, and served as colloidal gold-labeled protein and capture protein. Detection result showed that the strip reacted with *R. anatipestifer* strains but not with *E. coli*, *S. enterica*, and *P. multocida* strains.

In conclusion, a rapid, simple, and specific diagnostic colloidal gold immunochromatographic strips was developed to detect *R. anatipestifer* in ducks using monoclonal antibodies. The strip showed high sensitivity, specificity, and stability for detecting *R. anatipestifer*

Table 3. Detection of heart samples with the colloidal gold immunochromatographic strip and multiplex PCR.

Heart samples from ducks infected with	Detection results		
	Strip	Multiplex PCR	
		<i>R. anatipestifer</i>	<i>E. coli</i>
<i>R. anatipestifer</i> CH3	+	+	-
<i>R. anatipestifer</i> CH3	+	+	-
<i>R. anatipestifer</i> CH3	+	+	-
<i>R. anatipestifer</i> NJ3	+	+	-
<i>R. anatipestifer</i> NJ3	+	+	-
<i>R. anatipestifer</i> NJ3	+	+	-
<i>R. anatipestifer</i> HXb2	+	+	-
<i>R. anatipestifer</i> HXb2	+	+	-
<i>R. anatipestifer</i> HXb2	+	+	-
<i>E. coli</i> DE719	-	-	+
<i>E. coli</i> DE719	-	-	+
<i>E. coli</i> DE719	-	-	+
No infection	-	-	-
No infection	-	-	-
No infection	-	-	-

+, positive result; -, negative result.

in duck. The diagnostic strip is also indicated for diagnosis of *R. anatipestifer* infection using heart tissue samples and can be applied in clinical detection of *R. anatipestifer*. Additional optimization will be accomplished to improve detection sensitivity and accuracy for testing tissue samples.

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