Research article

Development of high-concentration labeled colloidal gold immunochromatographic test strips for detecting african swine fever virus p30 protein antibodies

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ABSTRACT

African Swine Fever (ASF), caused by the African swine fever virus (ASFV), has inflicted significant economic losses on the pig industry in China. The key to mitigating its impact lies in accurate screening and strict biosecurity measures. In this regard, the development of colloidal gold immunochromatographic test strips (CGITS) has proven to be an effective method for detecting ASFV antibodies. These test strips are based on the ASFV p30 recombinant protein and corresponding monoclonal antibodies. The design of the test strip incorporates a high-concentration colloidal gold-labeled p30 recombinant protein as the detection sensor, utilizing Staphylococcal Protein A (SPA) as the test line (T line), and p30 monoclonal antibody as the control line (C line). The sensitivity and specificity of the test strip were evaluated after optimizing the labeling concentration, pH, and protein dosage. The research findings revealed that the optimal colloidal gold labeling concentration was 0.05 %, the optimal pH was 8.4, and the optimal protein dosage was 10 μg/mL. Under these conditions, the CGITS demonstrated a detection limit of 1:512 dilution of ASFV standard positive serum, without exhibiting cross-reactivity with antibodies against other viral pathogens. Furthermore, the test strips remained stable for up to 20 days when stored at 50 °C and 4 °C. Comparatively, the CGITS outperformed commercial ELISA kits, displaying a sensitivity of 90.9 % and a specificity of 96.2 %. Subsequently, 108 clinical sera were tested to assess its performance. The data showed that the coincidence rate between the CGITS and ELISA was 93.5 %. In conclusion, the rapid colloidal gold test strip provides an efficient and reliable screening tool for on-site clinical detection of ASF in China. Its accuracy, stability, and simplicity make it a valuable asset in combating the spread of ASF and limiting its impact on the pig industry.

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1. Introduction

African Swine Fever (ASF) is an acute and highly contagious hemorrhagic disease that affects pigs worldwide, caused by the African swine fever virus (ASFV). Since its initial outbreak in China in August 2018, ASF has inflicted significant economic losses on the Chinese pig industry [1]. Unfortunately, the absence of an effective and safe vaccine makes it essential to develop a simple, rapid, specific, and sensitive serological detection method for ASFV antibodies. Such a method plays a critical role in the prevention and control of ASF [2].

Currently, laboratory diagnostics are the primary means of testing, with PCR-based methods and ELISA being the most widely employed techniques [3–6]. However, these methods have limitations that require laboratory-based procedures. Hence, there is a pressing need for a more efficient and practical point-of-care testing (POCT) method that can serve as a supplement to laboratory testing and for clinical screening of ASF. In the realm of diagnostic methods for detecting ASFV, PCR stands out as one of the most effective and widely recognized assays endorsed by the World Organization for Animal Health (WOAH). This method involves the detection of specific viral nucleic acid fragments, enabling confirmation of the presence of ASFV in infected pigs [7]. The PCR assay offers numerous advantages, including rapidity, high sensitivity, specificity, and minimal sample purity requirements [8,9]. As a result, during the initial two years of the virus's prevalence in China, identifying positive infections using PCR proved to be a valuable strategy for curbing the spread of ASF [3]. However, recent developments in China have raised concerns, as some gene-deficient strains of ASFV have been detected [10]. These strains may pose challenges to the accuracy and reliability of traditional PCR-based diagnostics, necessitating ongoing research and adaptation of diagnostic protocols to tackle these emerging variations effectively [11, 12]. These strains show intermittent shedding or remain non-shedding. Detecting positive pigs through antigen testing using PCR is not feasible. However, pigs generate antibodies that persist long after one week of infection with ASFV. Consequently, identifying positive herds through antibody testing has emerged as an effective strategy in pig farms across China. Currently, ELISA is employed for antibody detection, providing valuable evidence to ascertain the virus's spread [13–15]. Nonetheless, the utility of ELISA kits is restricted by the availability of equipment and skilled personnel, making their widespread implementation in all farming facilities challenging. Additionally, reliance on third-party laboratories for testing is often necessary. Thus, there is a pressing need for a convenient and rapid POCT method for ASF, enabling pig farmers to swiftly and effortlessly determine whether their pigs are infected with ASFV.

The colloidal gold immunochromatographic assay (GICA) emerges as a promising solution to this problem [16]. This convenient technique allows for qualitative detection of both antigens and antibodies without necessitating specialized skills or expensive complex equipment. Consequently, it stands out as one of the ideal technologies for POCT in ASF diagnosis. With GICA, pig farmers can access a user-friendly and efficient testing approach that can be implemented on-site. This empowers them to quickly assess the health status of their herds, enabling timely and targeted intervention strategies to mitigate the virus's spread and minimize economic losses. However, it should be noted that due to the speed of GICA, their sensitivity and specificity are relatively weaker compared to laboratory testing. Therefore, they are more suitable for initial screening purposes. In conclusion, the development and widespread adoption of the GICA represent a significant step forward in ASF diagnosis for pig farmers in China.

The p30 protein, encoded by the ASFV CP204L gene, plays a crucial role as a phosphorylated protein secreted early during viral infection, and it is located in the viral inner envelope [17]. Given its significance in ASFV entry, it has become a widely utilized target in the detection of ASF [18]. Previously, an indirect ELISA was successfully developed based on the recombinant protein p30, enabling the detection of ASFV antibodies in serum or oral fluid specimens [19]. More recently, advancements have been made using gold-labeled ASFV p30 and p72 proteins to create colloidal gold test strips. These innovative test strips demonstrated a remarkable sensitivity of 1:256 dilution for positive serum, showcasing their effectiveness in detecting ASFV infection [20]. In this study, mAbs specific to the ASFV p30 protein were preparation and purification as control line antibody. The p30 protein labeled with high-concentration colloidal gold particles (0.05 %) as a detection sensor. This combination served as the foundation for developing colloidal gold test strips with heightened sensitivity. These test strips were designed to effectively detect antibodies against ASFV in clinical sera from pigs. The implementation of these colloidal gold test strips has the potential to provide critical technical support in controlling the spread and prevalence of ASF in China. The high sensitivity of this method enables early and accurate detection of ASFV antibodies, allowing for timely intervention and preventive measures in affected pig populations.

In conclusion, the development of colloidal gold test strips based on monoclonal antibodies to ASFV p30 protein and recombinant antigens is a significant advancement in ASF screening. This technology offers a sensitive and efficient means to detect ASFV antibodies in clinical pig sera, aiding in the prompt containment and control of ASF in China.

2. Materials and methods

2.1. Reagents, sera and mice

The His-tagged Ni affinity purification columns and antibody affinity chromatography reagent kits were purchased from GE HealthCare (Chicago, IL, USA). Freund's complete and incomplete adjuvants, HAT culture medium supplement, HT culture medium supplement, 1640 culture medium, fetal bovine serum (FBS), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (StLouis, MO, USA). The mouse monoclonal antibody subtype identification kit was purchased from Proteintech (Chicago, IL, USA). Staphylococcus protein A (SPA), nitrocellulose membranes (pall 120), sample pads (SB08), conjugate pads (8975), absorbent paper (H-1), and PVC backboards (JB6) were acquired from Shanghai Jining Biotechnology Co., Ltd. (Shanghai, China). Colloidal gold was obtained from LingSi Biotechnology Co., Ltd. (Zhengzhou, China). ASFV standard positive and negative sera were purchased from

Pudao (Beijing) Standard Technology Co., Ltd. (Beijing, China). The ASFV serum ELISA test kit was purchased from ID.vet (Montpellier, France). CSFV-, FMDV-, PRRSV-, PRV-, and PCV2-positive sera were preserved by the lab and clinical samples were collected from several pig farms in Shandong Province. The SPF-grade BALB/c mice purchased from Jiangsu Huachuangxinnuo Pharmaceutical Technology Co., Ltd. housed at the Experimental Animal Center of Nanjing Agricultural University. The immunization and ascites preparation experiments were conducted in compliance with the relevant regulations on experimental animal welfare ethics, under the supervision and inspection of the committee and laboratory managers.

2.2. Expression and purification of ASFV p30 recombinant protein

The CP204L gene of ASFV Pig/HLJ/2018 strain [21] (GenBank: MK333180.1) was synthesized by Genscript Biotech Co., Ltd. (Nanjing, China) and inserted into the plasmid pET-28a. A recombinant plasmid pET-28a-p30 was constructed and transformed into Escherichia coli BL21(DE3) cells. After induction with IPTG, the bacteria were sonicated to obtain the recombinant p30 protein of ASFV. Subsequently, the protein was identified using SDS-PAGE and Western blotting. The identified protein was purified using an affinity chromatography column with His-tag purification. The purified protein was dialyzed to remove excess salts. The purified and desalted protein was used for the preparation of monoclonal antibodies and subsequent labeling with colloidal gold.

2.3. Preparation of ASFV p30 monoclonal antibodies

To prepare monoclonal antibodies (mAbs), the previously expressed and identified p30 recombinant protein was used as the antigen. The antigen was emulsified with Freund's complete adjuvant and incomplete adjuvant at a 1:1 ratio. Six-week-old BALB/c mice were immunized three times with 100 μg of the antigen per mouse. Spleen cells from mice with high antibody titers were collected along with myeloma cells (SP2/0) for cell fusion. The fusion was performed in HAT medium. The hybridoma cells were then screened using HT medium to obtain stable and high antibody-secreting cell lines [22].

The supernatant from the hybridoma cells was subsequently used for indirect immunofluorescence assay and Western blotting against ASFV-infected porcine alveolar macrophages (PAMs) and lysates to verify their reactivity with viral proteins. The antibody subtype was determined using an antibody subtype identification kit. The cell supernatant containing mAb was purified using an antibody affinity chromatography kit, and the purified mAb was used as the C-line on the test strip.

2.4. Western blotting

The purified p30 protein and cell culture supernatants collected from ASFV-infected PAMs as samples were subjected to SDS-PAGE and then transferred to itrocellulose membranes. The membrane was incubated with cell culture supernatants and then washed three times for 10 min each with PBST. The membrane was subsequently dried, treated with enhanced chemiluminescence (ECL) detection reagents (GE Healthcare), and exposed.

2.5. Indirect immunofluorescence assay (IFA)

ASFV-infected and uninfected PAMs were fixed with 4 % paraformaldehyde and washed three times with PBS. Then, 100 μL of hybridoma cell supernatant was added to each well and incubated overnight at 4 °C, followed by three washes. Next, 50 μL of goat anti-mouse IgG-FITC (1:200) was added to each well, and incubated at 37 °C in the dark for 2 h, followed by three washes. Finally, the fixed cells were stained with DAPI. The results were observed under a fluorescence microscope.

2.6. Optimizing labeling concentration of colloidal gold

To determine the optimal labeling concentration of colloidal gold, we used 7 acid-washed vials and added a certain amount of pH 9.1 citrate-borate buffer and 1 % high-concentration 40 nm colloidal gold to each vial, resulting in colloidal gold concentrations of 0.01 %, 0.05 %, 0.10 %, 0.05 %, and 0.20 %. Subsequently, 10 μg of p30 recombinant protein was added to each vial and labeled for 1 h at 4 °C. The labeled colloidal gold was then diluted to 0.01 % using citrate-borate buffer, and 200 μL was taken to measure the absorbance spectrum in the 400–600 nm wavelength range. The optimal labeling concentration was determined based on the absorbance peak.

2.7. Optimizing labeling pH for p30 protein

To determine the optimal labeling pH of colloidal gold, we diluted the high-concentration colloidal gold to the optimal labeling concentration using citrate-borate buffer solutions at pH 7.8, 8.4, 8.8, and 9.1. Next, 10 μg of p30 recombinant protein was added to each colloidal gold solution and labeled for 1 h at 4 °C. The labeled colloidal gold was then diluted to 0.01 % using citrate-borate buffer, and 200 μL was taken to measure the absorbance spectrum in the 400–600 nm wavelength range. The optimal labeling pH was determined based on the absorbance peak [23].

2.8. Optimizing the dose of colloidal gold labeling protein

To determine the optimal labeling dose of colloidal gold, we diluted the high-concentration colloidal gold to the optimal labeling concentration using the buffer solution at the optimal labeling pH. Then, different dose of p30 recombinant protein (0, 2, 4, 6, 8, and 10 µg/mL) were added to separate aliquots of colloidal gold and labeled for 1 h at 4 °C. Afterward, 10 % NaCl solution was added to each labeled colloidal gold solution, and the color change was observed. The lowest protein amount that did not result in color change was determined as the optimal protein quantity for labeling [24].

2.9. Assembly of the test strip

The first step in assembling the test strip is to prepare the conjugate of colloidal gold. The preparation process was as follows: The high-concentration colloidal gold was diluted to the optimal labeling concentration using the buffer solution at the optimal pH. Then, p30 recombinant protein was added dropwise to the colloidal gold. After thorough mixing of the protein and colloidal gold, the mixture was labeled for 1 h at 4 °C. Subsequently, 100 µL/mL of 10 % BSA was added to the mixture, resulting in a final concentration of 1 % BSA. The mixture was incubated for 30 min for blocking. Following that, the mixture was centrifuged at 4 °C (1000×g) for 5 min, and the supernatant was discarded. The pellet was then resuspended in a rehydration buffer (containing 0.1 % casein, 0.5 % Tween-20, and 5 % sucrose in 0.01 M pH 8.0 Tris-HCl) to a final volume of 1/10 of the original volume.

Subsequently, the various consumables were prepared as follows: The sample pad, conjugate pad, and absorbent pad were cut according to the dimensions of 1.7 cm × 30 cm, 0.5 cm × 30 cm, and 1.7 cm × 30 cm, respectively. Then, the sample pad and conjugate pad were soaked in a solution of 0.01 M PBS containing 1 % BSA for 5 min, followed by drying at 37 °C for 3 h. The rehydrated colloidal gold-protein conjugate was applied onto the conjugate pad at a volume of 25 µL/cm and dried at 37 °C for 3 h. The proteins were dispensed onto the nitrocellulose (NC) membrane at a rate of 1 µL/cm using a striping machine, with SPA as the test line (T line) and the p30 monoclonal antibody as the control line (C line). Afterward, the PVC backing card was taken out and a 2.5 cm × 30 cm NC membrane was attached to it. The absorbent pad was placed on top, overlapping with the NC membrane by approximately 2 mm. Next, the dried conjugate pad was attached onto the NC membrane, overlapping by approximately 1 mm. Finally, the sample pad was attached onto the conjugate pad, overlapping by approximately 1 mm. The assembled sample pad, conjugate pad, NC membrane, and absorbent pad were trimmed into strips of 4 mm width using the PVC backing card. They were secured with a plastic housing, and the test strip was ready for use.

2.10. Evaluation of the test strip

To objectively evaluate the performance of the test strip, we verifed its specifcity, sensitivity, and stability. Firstly, the test strips were individually tested using ASFV-, CSFV-, FMDV-, PRRSV-, PRV-, and PCV2-positive sera as test samples to test their specifcity. Secondly, the standard negative serum was used as a negative control. ASFV standard positive serum was diluted from 1:16 to 1:2048
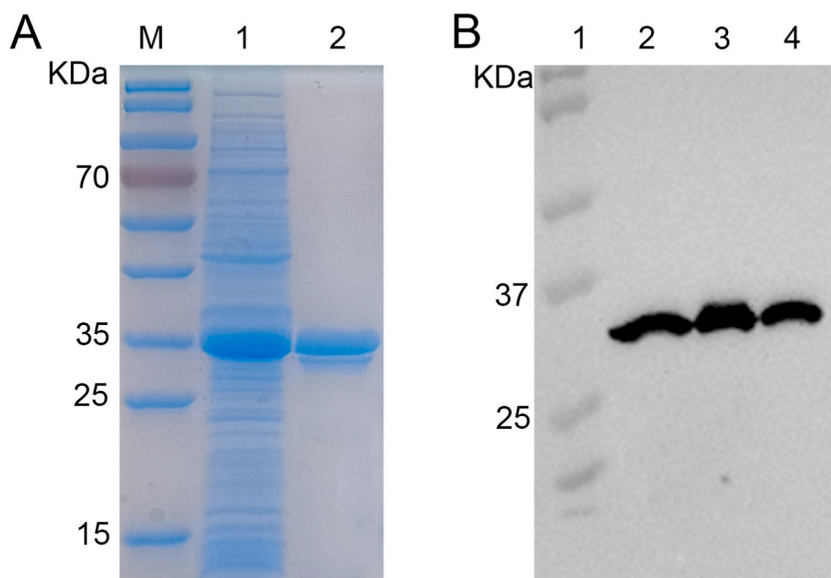

Fig. 1. Purification and reactivity Western blotting identification of p30 recombinant protein. A: Purified p30 recombinant protein before and after purification. M: Protein Marker; 1: expression of p30 recombinant protein in whole bacteria,2:purified p30 recombinant protein. B: WB identification of the reactivity of p30 recombinant protein with anti-His tag antibody. 1:pET-28a, 2: PET-28a-p30 Inclusion bodies expressed protein, 3: PET-28a-p30 supernatant expressed protein,4: PET-28a-p30 whole bacterial protein.

in PBS containing 0.5 % Tween-20. The test strips were then tested using the diluted ASFV serum samples, with the dilution buffer used as a negative control. The highest dilution factor at which the appearance of the T-line could be observed was recorded to determine the detection limit of the test strips. Thirdly, the test strips were placed in a 50 °C drying oven to accelerate degradation, while also storing them in a 4 °C refrigerator. On the 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 16th, 18th, and 20th days, the bands were removed and tested with positive and negative serum to evaluate their stability.

2.11. Detecting the clinical samples

A total of 108 clinical serum samples collected from some pig farms in Shandong province were diluted in PBS containing 0.5 % Tween-20. These diluted samples were then tested using CGITS to validate the usability in clinical sample detection. Alternatively, the same samples were tested using the commercial kit ELISA kit (ID.vet, France) to calculate the specificity and sensitivity of the test strips.

3. Results

3.1. Expression and purification of ASFV p30 recombinant protein

Based on the meticulous analysis of SDS-PAGE, the observed size of the p30 recombinant protein was approximately 30 kDa (Fig. 1A), confirming its identity and integrity. Furthermore, Western blotting was performed by using ASFV positive serum and anti-His antibody, the result showed that this protein had excellent reactivity and specificity (Fig. 1B).

3.2. Preparation and purification of ASFV p30 monoclonal antibody

As shown in Fig. 2A, the striking observation was that the 2E monoclonal antibody (mAb) against p30 demonstrated remarkable specificity in recognizing ASFV-infected PAMs, as evidenced by the distinct red fluorescence signal. This fluorescence was observed to be dispersed and evenly distributed in the cytoplasm of the infected cells, indicating an efficient and widespread infection (Fig. 2B). In contrast, the uninfected PAMs did not exhibit any specific red fluorescence. Furthermore, the Western blotting results provided further validation of the p30 mAb's specificity (Fig. 2C). The mAb showed reactivity with both purified p30 protein and cell lysates from ASFV-infected cells. This successful reactivity confirms the mAb's ability to detect the p30 protein in different contexts, consolidating its utility in ASFV detection assays. To determine the subclass of the 2E mAb, an antibody subtyping kit was employed. The results conclusively revealed that the mAb belonged to the IgG1 subclass and possessed a kappa light chain type.

3.3. The optimal labeling concentration, pH, and protein dose of colloidal gold

Through careful observation of the absorbance spectra of colloidal gold diluted to 0.01 % after labeling at various concentrations, a significant finding emerged. The highest peak was consistently obtained at a colloidal gold labeling concentration of 0.05 % (Fig. 3A). Consequently, this specific concentration, 0.05 % colloidal gold, was deemed the optimal labeling concentration for the experiment.

To prepare the colloidal gold conjugates, a dilution process was carried out by reducing the high-concentration colloidal gold from 1 % to 0.05 % using a fixed pH buffer. Subsequently, equal amounts of p30 protein were introduced for labeling. The absorption spectra in the visible range were then carefully observed. The crucial observation was that there was a rightward shift of the absorption peak, indicating an increase in particle size following the protein labeling process. Remarkably, during this investigation, it was found that the colloidal gold conjugates displayed the most pronounced absorption peak at pH 8.4 (Fig. 3B). Based on these compelling findings, pH 8.4 was identified as the optimal labeling pH for the colloidal gold conjugates.
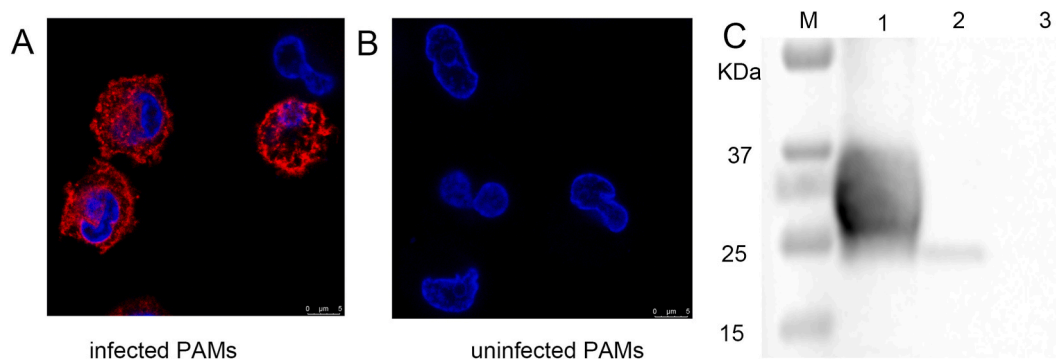

Fig. 2. p30 mAb IFA and Western blotting identification. A: IFA reactivity of p30 mAb with ASFV-infected PAMs. B: PAMs control. C: Reactivity identification of p30 mAb with p30 recombinant protein and ASFV-infected PAMs lysate. M: Prestained Protein Marker; 1: p30 recombinant protein, 2: cell culture supernatants collected from ASFV-infected PAMs, 3: negative control.
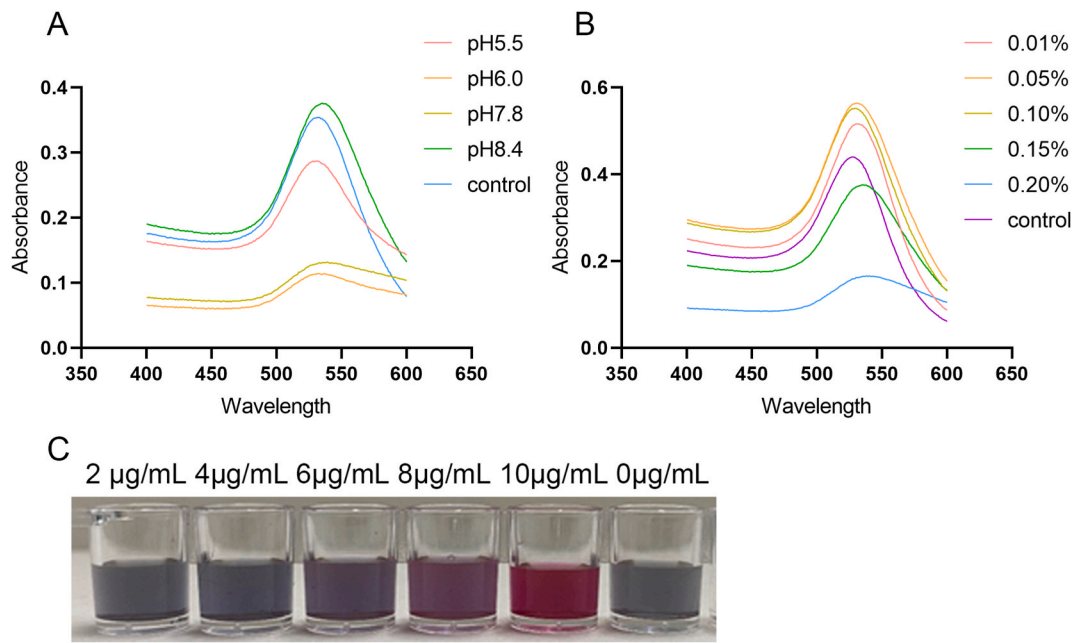
Fig. 3. Colloidal gold labeling concentration, pH, and protein dose. A: Colloidal gold labeling concentration; B: Colloidal gold labeling pH; C: Colloidal gold labeling protein dose. Plot the absorption spectrum of a solution in the range of 400–600 nm with wavelength (nm) on the X-axis and absorbance values on the Y-axis. The lowest protein amount that did not result in any color change was determined as the optimal protein amount.

To evaluate the stability of the colloidal gold conjugates, 10 % sodium chloride solution was added, followed by thorough mixing. Subsequently, color changes and sedimentation of colloidal gold were closely observed (Fig. 3C). The 0 µg/mL control exhibited a transformation into a purple-gray color, while the colloidal gold conjugates with protein doses of 2, 4, 6, and 8 µg/mL showed varying degrees of color change. Remarkably, the colloidal gold conjugates with a protein dosage of 10 µg/mL displayed a distinct and stable color change. Based on these compelling observations, the optimal protein dose for labeling was determined to be 10 µg/mL.
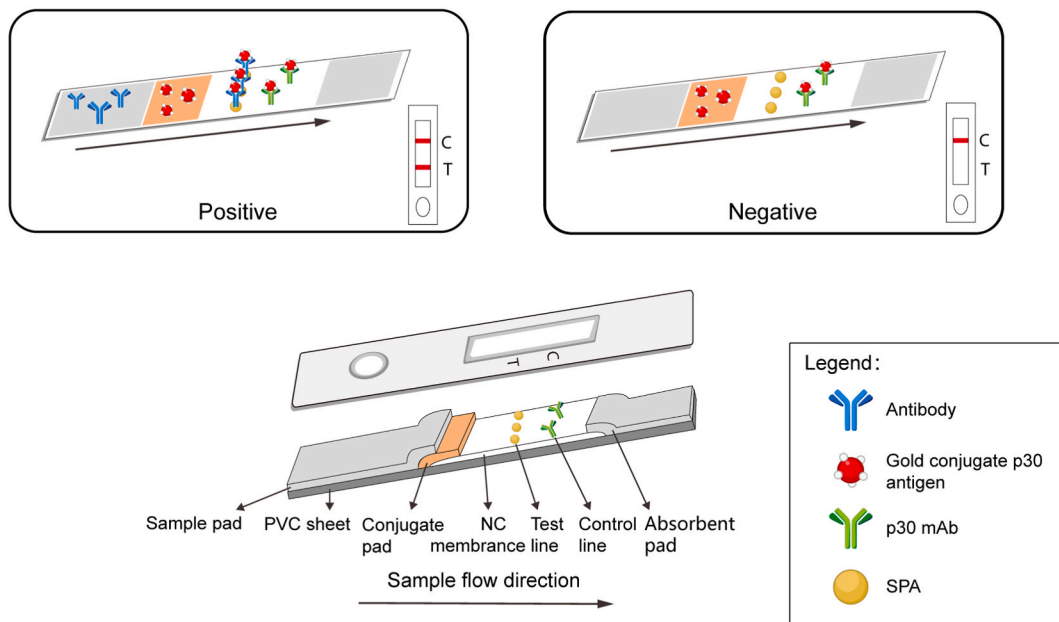

Fig. 4. Schematic diagram of CGITS result judgment.

3.4. Judgment of CGITS results

The test strip employs ASFV antibodies as the target, with colloidal gold-labeled p30 recombinant protein serving as the detection sensor. As shown in Fig. 4, the T line features the capture protein SPA, while the C line features the capture protein p30 mAb. A positive result is indicated when both the C line and T line are visible. Conversely, a negative result is determined when only the C line appears, and the T line is absent. If only the T line appears, and the C line is absent, the test is considered invalid.

3.5. Evaluation of CGITS

Using ASFV positive serum samples, as well as CSFV, FMDV, PRRSV, PRV, and PCV2 positive serum samples as test samples, it was observed that only the ASFV positive sample showed both the C line and T line on the test strip, while the other virus-positive sera and negative controls only displayed the C line (Fig. 5). This indicates that the developed test strip exhibits specificity and does not cross-react with other common viruses.

Furthermore, as the dilution ratio increases, the intensity of the C line gradually deepens. Conversely, the T line becomes lighter with increasing dilution ratio, but it can still be observed even at a dilution of 1:512 of the standard positive serum (Fig. 6). Therefore, the detection limit of the test strip is a 1:512 dilution of positive serum. It is important to note that at lower dilution ratios, the presence of a higher concentration of antibodies in the sample leads to relatively complete binding of the gold-labeled antigen, resulting in a lighter C line color.

The assembled test strips were placed in a 50 °C drying oven and a 4 °C refrigerator, remove them on days 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20, and use positive and negative serum for testing. As shown in Table 1, both positive and negative results were displayed correctly, indicating that the test strip can be stable stored at 50 °C and 4 °C for at least 20 days, with a certain degree of stability.

3.6. Testing clinical samples

A total of 108 clinical serum samples were tested using CGITS, along with simultaneous testing using the commercial ELISA kit. The detailed results of the tests was found in Table 2. The positive rate obtained from CGITS was 48.1 % (52/108), while the positive rate from ELISA was 50.9 % (55/108), the coincidence rate between the CGITS and ELISA was 93.5 % (101/108).

4. Discussion

In the face of the devastating impact of African Swine Fever (ASF) and the absence of safe and effective vaccines, the urgency to minimize its harm cannot be overstated. Precise and timely detection of ASFV has emerged as a paramount necessity. At present, ASFV detection methods predominantly fall into two categories: pathogen detection and serological detection, both performed in laboratory settings. For pathogen detection, the WOAH recommends using virus isolation, fluorescent antibody test (FAT) to detect viral antigens, or PCR and fluorescent quantitative PCR to detect viral genomic DNA. These methods offer high accuracy but are often limited by the need for specialized instruments and skilled personnel, and they typically rely on third-party laboratories, making them less feasible for on-site and rapid diagnostics [25]. Similarly, for serological detection, techniques such as IFA, ELISA, indirect immunoperoxidase test (IPT), and immunoblotting (IBT) are commonly used to detect ASFV antibodies. While these methods yield valuable information about the immune response, they also encounter the same challenges of instrument availability and trained personnel, hindering their widespread use as point-of-care tests. Besides, antibody detection can only identify infections with moderately virulent strains, as acute infections with highly virulent strains may lead to pig mortality before the development of antibodies. In countries where ASF is

Fig. 5. No cross reactivity of CGITS. Apply 100 μL of the sample onto the sample pad and wait for 15 min. Read the results, where the appearance of both the Control Line (C line) and Test Line (T line) indicates a positive reaction. If only the Control Line (C line) appears and the Test Line (T line) is absent, it indicates a negative result with no cross-reactivity.
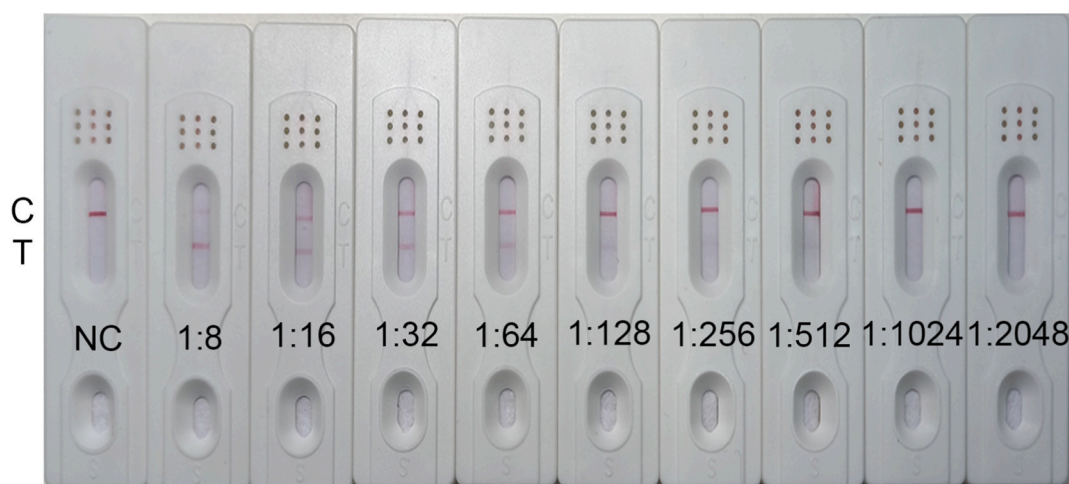
Fig. 6. The detection limit of CGITS. Add 100 µL of the sample onto the sample pad and wait for 15 min. After 15 min, read the results. The highest dilution at which the Test Line (T line) is still visible is considered the detection limit.

Table 1
The stability test.

Temperature	2 d	4 d	6 d	8 d	10 d	12 d	14 d	16 d	18 d	20 d
4 °C	+	+	+	+	+	+	+	+	+	+
50 °C	+	+	+	+	+	+	+	+	+	+

Table 2
Detecting of clinical samples.

Assay		ELISA		Total
		Positive	Negative	
CGITS	Positive	50	2	52
	Negative	5	51	56
	Total	55	53	108

prevalent, the World Organisation for Animal Health recommends that confirmation of suspected cases of disease can be done using a standard serological test (ELISA), combined with an alternative serological test (IFAT, IPT, IBT) and an antigen-detection test. Therefore, diagnosis needs to be conducted in the laboratory, and CGITS, as screening tools on farms, can enhance the efficiency of diagnosis. Such a method would empower pig farmers and veterinarians to quickly identify antibodies against African Swine Fever virus, providing information for further diagnosis and laying the foundation for timely intervention and control.

Immunochromatographic analysis has emerged as a preferred technique for Point-of-Care Testing (POCT) due to its simplicity, speed, and accuracy [26]. Various materials, including colloidal gold, latex particles, quantum dots, colloidal carbon, and magnetic microspheres, can be utilized for immune labeling [27,28]. Among these options, colloidal gold stands out as a widely adopted choice in side flow immunochromatographic detection [29]. Its excellent stability, adaptability, and ease of result observation have made it a popular selection. However, traditional colloidal gold labeling has some limitations, prompting the need for refinement. Firstly, the large volume of the label can complicate handling and operation. Secondly, the low labeling efficiency necessitates a significant amount of protein, leading to increased costs and resource consumption. Lastly, colloidal gold can obstruct the pH meter electrode, affecting pH regulation accuracy [30]. To address these concerns, researchers have employed a novel approach, diluting high concentration colloidal gold with buffer solution. This innovative method not only overcomes the drawbacks but also offers additional benefits. By choosing an appropriate concentration of colloidal gold (0.05 %) and a pH of 8.4, the labeling efficiency improves significantly, while over-labeling issues are mitigated. Moreover, at a 0.05 % labeling concentration, the labeled volume comprises only 20 % of the 0.01 % labeling concentration, streamlining centrifugation and other operational processes. The use of buffer solutions for pH adjustment ensures immunity to colloidal gold interference. Furthermore, this optimized approach results in a remarkable 60 % reduction in the protein mass labeled at 0.05 % concentration compared to the conventional 0.01 % concentration, making it more cost-effective and resource-efficient. It is essential to note that for successful colloidal gold labeling, the salt ion concentration of the proteins employed should not exceed 0.01 M, as higher concentrations can disrupt the colloidal state of the gold particles, affecting the accuracy and reliability of the labeling process.

The ASFV p30 protein antibody emerges as an exceptional and well-supported detection target, as attested by previous studies [31, 32]. Building upon this strong foundation, our study thoughtfully selected the ASFV p30 protein antibody as the primary antibody detection target for pigs clinically infected with ASF. Through meticulous preparation and purification of proteins and monoclonal antibodies, coupled with meticulous optimization of gold labeling conditions, the immunochromatographic detection exhibited remarkable specificity and sensitivity. Sensitivity, which measures the ability to correctly detect positive results, stood at an impressive 90.9 % (50/55), while specificity, gauging the ability to correctly detect negative results, reached an outstanding 96.2 % (51/53). These compelling figures testify to the effectiveness and precision of our detection approach. Notably, when compared to other colloidal gold test strips designed for detecting ASFV p30 protein antibodies, the method established in our study stands out with double the detection limit [31]. Astonishingly, it successfully detected positive serum samples even at a dilution of 1:512, elevating the bar of detection capability to new heights.

Taken together, we have developed CGITS using high-concentration colloidal gold, with the ASFV p30 protein antibody as the target. It possesses the potential for clinical screening of African Swine Fever.

Ethics approval and consent to participate

The animal experiments conducted followed the requirements of the Ethics and Animal Welfare Review Committee at Nanjing Agricultural University, with approval number NJAU.No20220309035.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Huai-cheng Liu: Writing – review & editing, Writing – original draft, Investigation. **Rong-chao Liu:** Investigation. **Mei-rong Hu:** Resources. **Ao-bing Yang:** Resources. **Ren-hu Wu:** Investigation. **Yan Chen:** Investigation. **Jin Zhang:** Formal analysis. **Ji-shan Bai:** Resources. **Sheng-bo Wu:** Supervision, Investigation. **Jian-peng Chen:** Methodology, Formal analysis. **Yun-feng Long:** Supervision, Methodology. **Yan Jiang:** Supervision, Methodology. **Bin Zhou:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25214.

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