AMB Express

ORIGINAL ARTICLE Open Access

Rapid detection of African swine fever virus by a blue latex microsphere immunochromatographic strip

Yanli Hu[1,2,3], Yingjun Xia[1,2,3], Huiwen Mo[1,2,3], Li Zhang[1,2,3], Wenjian Cao[1,2,3], Rui Fang[1,2,3] and Junlong Zhao[1,2,3*]

Abstract

African swine fever (ASF) is a contagious viral disease in pigs, caused by the African swine fever virus (ASFV). Currently, there are no effective vaccines and drugs for ASFV, making diagnostic methods crucial for the prevention and control of ASF. In this study, the ASFV p30 protein was successfully expressed using a prokaryotic expression system and used as an immunogen to prepare monoclonal antibody (mAb) 2A5. The antigenic epitope recognized by the mAb 2A5 was identified as ^{58}VKYDIVKSARIYAGQGY^{74}. Then, a blue latex microsphere immunochromatographic strip method for detecting ASFV antigens was established. Wherein a rabbit polyclonal antibody (pAb) against p30 stored in our laboratory was coupled with blue latex microspheres to prepare an immune probe, with mAb 2A5 as the test line and goat anti-rabbit IgG as the control line. The immunochromatographic strip exhibited excellent sensitivity, with a minimum detection limit of 10 ng/mL for the p30 protein. It demonstrated good specificity, showing no cross-reactivity with PRRSV, PCV2, PPV, and PEDV. Moreover, it maintained good stability and could be stored for at least 100 days at 37 °C. The concordance rate between the immunochromatographic strip and qPCR test kit was 92.2%. The immunochromatographic strip method can meet the requirements of clinical testing and provide technical support for ASFV diagnosis.

Keywords African swine fever virus, p30, Monoclonal antibody, Antigenic epitope, Immunochromatographic strip

Introduction

African swine fever (ASF) is an infectious viral disease in pigs caused by the African swine fever virus (ASFV) (Galindo and Alonso 2017). ASFV, with a long history, was first discovered in Kenya in 1921 and initially remained prevalent only in Africa (Arzt et al. 2010). Later, ASF outbreaks occurred in other regions. In 2018, the first ASF outbreak in China occurred on a farm in Shenbei District of Shenyang, Liaoning Province. Subsequently, ASF cases were reported in multiple provinces, causing significant economic losses to China (Zhou et al. 2018). The ASFV strain initially emerged in China as genotype II strain, which was highly virulent and resulted in large numbers of pig deaths (Ge et al. 2018). In 2020, a lower virulent natural mutant of genotype II ASFV was

*Correspondence:
Junlong Zhao
zhaojunlong@mail.hzau.edu.cn
[1]National Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, China
[2]Key Laboratory of Preventive Veterinary Medicine in Hubei Province, Wuhan 430070, Hubei, China
[3]Key Laboratory of Animal Epidemical Disease and Infectious Zoonoses, Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, Hubei, China

discovered in China (Sun et al. 2021b). Subsequently, in 2021, two non-hemadsorbing genotype I ASFV strains, namely HeN/ZZ-P1/21 and SD/DY-I/21, were obtained from Henan and Shandong province, respectively (Sun et al. 2021a). Recently, recombinant strains of ASFV containing genotypes I and II were found in Chinese pigs (Zhao et al. 2023). The prevalence of ASFV in China is constantly changing, increasing the difficulty of preventing and controlling ASF.

ASFV encodes over 200 proteins, among which the p30 protein is an early expressed protein encoded by the CP204L gene (Liberti et al. 2023). The p30 protein is typically produced 2 to 4 h after ASFV infection and is maintained during the entire course of the infection (Hu et al. 2024). The p30 protein primarily plays a role in the virus's entry into cells, exhibits strong antigenicity, and can stimulate the host to produce neutralizing antibodies (Chen et al. 2022; GomezPuertas et al. 1996). Currently, the p30 protein is widely used in serological diagnosis and serves as a good diagnostic target (Cubillos et al. 2013; Gómez-Puertas et al. 1998).

At present, there are no effective vaccines and treatment drugs for ASFV, making diagnostic methods essential for preventing and controlling ASF. The main approach to diagnosing ASFV is through laboratory testing, in which PCR and ELISA are the most commonly used techniques (Gallardo et al. 2019). These methods require specific equipment and skilled personnel, making them unsuitable for on-site testing. However, immunochromatographic strip testing is simple to perform, requires no equipment, and is suitable for on-site detection, making it a good preliminary screening method (Pang et al. 2023). Various ASFV immunochromatographic strip detection methods have been reported, including ASFV antigen detection methods and ASFV antibody detection methods. Mainly, immunochromatographic strip methods have been established using proteins such as p72, p30, p54, and their monoclonal antibodies (mAbs). A study utilized colloidal gold-labeled p30 protein and p72 protein mixed in a 1:1 ratio as the gold-labeled antigen to establish a dual immunochromatographic strip capable of concurrently identifying antibodies against ASFV p30 and p72 proteins (Wan et al. 2021). Additionally, a fluorescent microsphere immunochromatographic strip antibody detection method was established using truncated p54 protein and mouse IgG bound to fluorescent microspheres as immunoprobes (Li et al. 2020). A method for detecting ASFV antigens was developed using two mAbs targeting p30, utilizing a colloidal gold immunochromatographic strip technique (Zhang et al. 2021). A different method using a colloidal gold immunochromatographic strip for detecting ASFV antibodies was established, employing the p30 protein along with a monoclonal antibody (mAb) against p30 (Liu et al. 2024).

In this study, we generated a mAb using recombinant protein p30 as an immunogen and identified the antigenic epitope that the mAb recognizes. Subsequently, we established a blue latex microspheres immunochromatographic strip method for detecting ASFV antigens, using both a mAb and a polyclonal antibody (pAb) against the p30 protein. This method is suitable for rapid on-site detection, providing valuable technical support for ASFV diagnosis.

Materials and methods

Reagent, serum, animal

Freund's complete and incomplete adjuvants, as well as the HAT and HT culture medium supplements, along with polyethylene glycol 1450 (PEG 1450), were sourced from Sigma-Aldrich (Louis, USA). The Protein G resin and the kit for identifying mouse mAb subtypes were purchased from Biodragon (Beijing, China). Latex microspheres were purchased from VDOBIOTECH (Jiangsu, China). Goat anti-rabbit IgG (purified) was obtained from Solarbio (Beijing, China). Nitrocellulose membranes (Sartorius CN 95) were purchased from Shanghai Jie Yi Biotechnology Co., Ltd (Shanghai, China). Sample pads (RB65), conjugate pads (DL42), absorbent paper (CH-27), and PVC backing plate (SM31-40) were acquired from Shanghai Jinbiao Biotechnology Co., Ltd (Shanghai, China). 4-Morpholineethanesulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-Hydroxysuccinimide (NHS) were purchased from Aladdin (Shanghai, China). Ni–NTA resin-based column was purchased from Qianchun Biotechnology Co., Ltd (Jiaxing, China). The ASFV qPCR kit was purchased from Qingdao Lijian Bio-Tech Co., Ltd (Shandong, China. Approval Number: Veterinary Drug Registration 154028912).

The positive sample for PRRSV, PCV2, PPV, and PEDV, as well as clinical samples, were provided by Wuhan KeWeiChuang Biological Technology Co., Ltd. The ASFV standard positive serum and standard negative serum were donated by Qigai He Research Group of Huazhong Agricultural University.

Female BALB/c mice at 6 weeks and 10 weeks of age were purchased from the Experimental Animal Center of Huazhong Agricultural University.

Prokaryotic expression of the p30 protein

The full-length sequences of CP204L gene from ASFV isolate Pig/HLJ/2018 (GenBank: MK333180.1) in the NCBI database was synthesized by Tsingke Biotech Co., Ltd. The preceding 570 bp of the CP204L gene was inserted into the pET-30a (+) vector. The constructed recombinant plasmids were transformed into *Escherichia*

coli BL21 (DE3) cells for protein expression. The specific expression conditions for the p30 protein involved inducing the *E. coli* at 18 °C with 1 mmol/L isopropyl β-D-1-thiogalactopyranoside (IPTG) for 14–16 h. Soluble proteins were subsequently purified using a Ni–NTA resin affinity column.

Preparation of monoclonal antibody

All animal experiments were approved by the Laboratory Animals Research Centre of Hubei Province and the Ethics Committee of Huazhong Agricultural University, and all procedures were carried out in accordance with the relevant ethical guidelines and regulations. The preparation of mAbs was carried out as described previously (Zhang et al. 2024). One week after the third immunization, the antibody titers in the serum of immunized mice were determined by indirect ELISA. When the antibody titer exceeded $1:10^4$, a booster immunization was administered via intraperitoneal injection of 50 μg of protein. Three days after the booster immunization, PEG 1450 was used to fuse the spleen cells from immunized mice with myeloma cells SP2/0 at a ratio of 10:1. Hybridoma cells that secrete anti-p30 antibodies were screened using the indirect ELISA, and then the stable hybridoma cell line secreting anti-p30 mAb was obtained through four rounds of subcloning using the limiting dilution method (Greenfield 2019). Subsequently, antibodies were produced in large quantities using in vivo mouse induction, and the collected mouse ascites were purified using Protein G to obtain high-purity mAbs. The antibody subtype was identified using a mouse mAb subtype identification kit, in accordance with the provided instructions.

Chromosome analysis of hybridoma cells

Chromosome analysis of hybridoma cells was performed with improvements based on existing methods (Yuan et al. 2012). When the hybridoma cells reached the logarithmic growth phase, colchicine was added to a final concentration of 0.4 μg/mL, and the cells were cultured for an additional 4 h. Meanwhile, myeloma cells SP2/0 were set up as a control. Finally, chromosomes with good dispersion were selected for observation and analysis under a microscope.

Indirect ELISA

The p30 protein was diluted to a concentration of 4 μg/mL in carbonate buffer, then added at 100 μL per well to a 96-well plate and incubated at 4 °C overnight. The plate was then blocked with 150 μL of 1% BSA at 37 °C for 1 h. After blocking, the primary antibody was added, and the plate was incubated at 37 °C for 30 min. Next, 100 μL of HRP-conjugated goat anti-mouse IgG (diluted 1:5000) was added to each well, and the plate was incubated at 37 °C for 30 min. The plate was then incubated in the dark at room temperature for 10 min with the chromogenic substrate. Finally, 0.25% hydrofluoric acid solution was added to stop the reaction, and the absorbance was measured at 630 nm.

Western blot

Firstly, equal amounts of proteins were separated by SDS-PAGE, then transferred to a 0.45 μm PVDF membrane for Western blot analysis. The membrane was blocked with 5% skimmed milk at room temperature for 2 h. Then, the membrane was incubated overnight at 4 °C with the primary antibodies. The primary antibodies were anti-p30 mAb (diluted 1:4000), ASFV positive serum (diluted 1:5000), ASFV negative serum (diluted 1:5000), and His-tag monoclonal antibody (diluted 1:5000). Subsequently, the membrane was incubated with secondary antibodies at room temperature for 1 h. The secondary antibodies were HRP-conjugated goat anti-mouse IgG (diluted 1:5000) and HRP-conjugated goat anti-swine IgG (diluted 1:5000). Finally, the membrane was visualized using a bioanalytical imaging system.

Epitope mapping

In order to localize the antigenic epitopes recognized by the mAb, a series of p30 truncated proteins were designed, as shown in Fig. 5A. The target fragments were amplified according to specific primers (Table S1), and then cloned into the pET-30a (+) expression vector. The recombinant plasmids were transformed into *Escherichia coli* BL21 (DE3) cells to obtain recombinant proteins. Subsequently, the reactivity between the mAb and recombinant proteins was analyzed using Western blot and indirect ELISA to localize the specific antigenic epitopes.

Preparation of immunoassay probe

The blue latex microspheres were conjugated with the antibody to form the immunoassay probe according to the instructions, and the detailed steps can be found in the supplementary materials.

Assembly of immunochromatographic strip

The sample pad was cut into strips measuring 18 mm in width, soaked in sample pad treatment solution (containing 1% BSA, 2.5% sucrose, 0.3% PVP-K30, 0.5% Tween-20 in 0.01 M PB buffer) for 30 min, and then dried at 37 °C. The conjugate pad was cut into strips measuring 6 mm in width, soaked in conjugate pad treatment solution (containing 1% BSA, 2.5% sucrose, 0.3% PVP-K30 in 0.01 M PB buffer) for 30 min, and then dried at 37 °C. The antibody was coated onto the nitrocellulose membrane (NC membrane) using the XYZ-3060 three-dimensional dispensing platform. The anti-p30 mAb was applied as the Test (T) line, positioned 1 cm from the leading edge

of the NC membrane, while the goat anti-rabbit IgG served as the Control (C) line, positioned 0.5 cm from the T line. The NC membrane was subsequently incubated at 37 °C for 16 h to dry. The immunochromatographic strip was assembled as follows: NC membrane, conjugated pad, sample pad, and absorbent pad were pasted onto a PVC backing plate, overlapping each component by 2 mm. The immunochromatographic strip was cut into a width of 4 mm using a CM-4000 cutting machine.

The testing procedure and result determination

The test sample was introduced into the sample well at a volume of 100 µL, then waited for 10 min before recording the test results. A blue color on both the T line and the C line indicates a positive result. If the T line is colorless and the C line shows blue, the result is negative. If neither the T line nor the C line shows color, or only the T line is blue, the result is considered invalid. In positive results, a lighter blue color on the T line indicates a weak positive result, while a darker blue color on the T line indicates a strong positive result.

Performance of immunochromatographic strip

Sensitivity is a key indicator for evaluating the detection system of the immunochromatographic strip. The p30 protein was gradient-diluted with 0.01 M PBS to concentrations of 5.145 µg/mL, 2.57 µg/mL, 1.29 µg/mL, 0.64 µg/mL, 0.32 µg/mL, 0.16 µg/mL, 80 ng/mL, 40 ng/mL, 20 ng/mL, 10 ng/mL, and 8 ng/mL. Detection was performed on the above diluted samples, and the lowest sample concentration that can yield a positive result is defined as the detection limit.

Specificity is also a key indicator for evaluating the detection system of the immunochromatographic strip. Positive samples containing PCV2, PRRS, PPV, and PEDV were selected for testing, with ASFV-positive serum serving as the positive control and ASFV-negative serum as the negative control.

A batch of immunochromatographic strips was prepared and stored at 37 °C, with tests conducted at various times to verify their stability. The stability of the strip was evaluated using one sample of ASFV-positive serum and one sample of ASFV-negative serum. The positive serum was diluted with 0.1 M PBS at dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32, while the negative serum remained undiluted.

Detection of clinical samples

The immunochromatographic strip was utilized to analyze 333 clinical samples in total, which included 70 whole blood samples, 94 serum samples, 149 swab samples, 3 semen samples, 6 gauze samples, and 11 environmental samples. Among them, whole blood samples were diluted 1:2 with 0.01 M PBS, while the remaining samples were not diluted. All these clinical samples were identified using a qPCR kit. The results from the immunochromatographic strips were compared with those from the qPCR kit.

Results

Prokaryotic expression of p30 protein

Using the synthetic CP204L gene as a template, the target fragment was amplified with specific primers, resulting in a target gene size of approximately 570 bp, consistent with the expected size (Fig. 1A). Subsequently, it was successfully cloned into the pET-30 (+) expression vector. The constructed recombinant plasmid was then transformed into BL21 (DE3) cells and expressed successfully. The p30 protein was expressed in both the supernatant and precipitate fractions. The soluble protein was purified using a Ni–NTA resin affinity column, yielding a highly pure protein with an approximate size of 35 kDa (Fig. 1B). Western blot analysis revealed that the purified

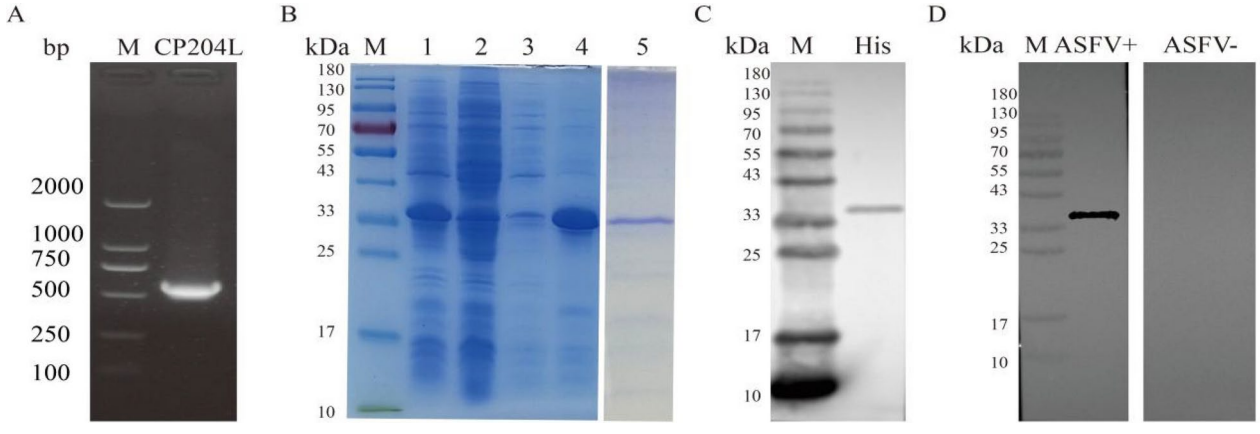

Fig. 1 Expression and Identification of p30 Protein. **A** PCR amplification of CP204L gene; **B** SDS-PAGE analysis of the p30 protein; M: Marker; 1: Induced Escherichia coli; 2: Uninduced Escherichia coli; 3: Supernatant; 4: Precipitate; 5: Purified p30 protein; **C** Western blot analysis of the purified p30 protein using His-tag mAb; **D** Western blot analysis of the purified p30 protein using the ASFV positive serum and negative serum

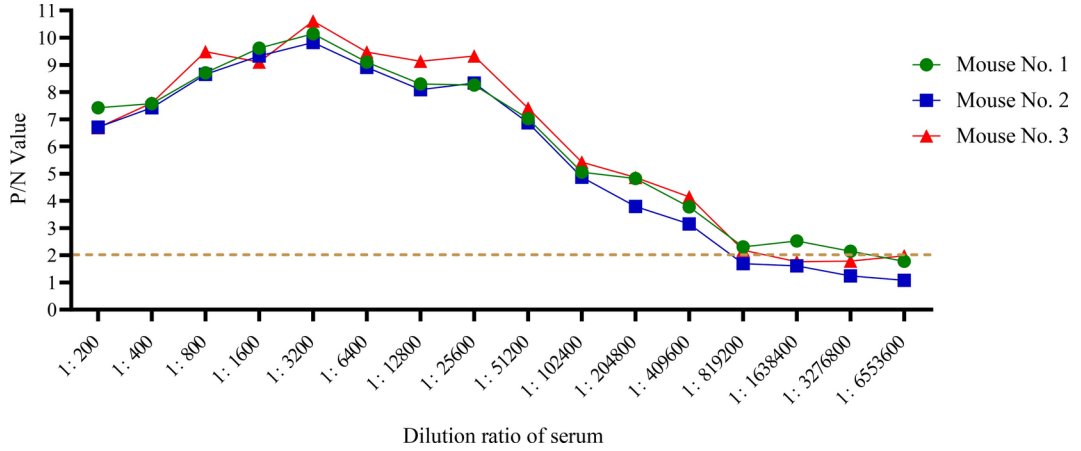

Fig. 2 Determination of antibody titer in mice immunized with p30 protein

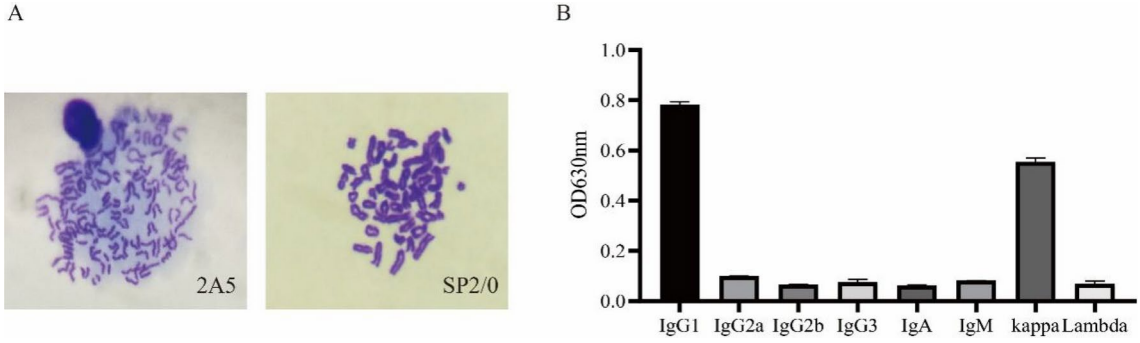

Fig. 3 Identification of hybridoma cells. **A** Chromosomal analysis of the hybridoma cells; **B** The monoclonal antibody subtype detection results

p30 protein reacted with the His-tag mAb, showing a single band (Fig. 1C). Additionally, the p30 protein exhibited specific reactivity with ASFV-positive serum but not with ASFV-negative serum (Fig. 1D). These findings indicate successful expression of the p30 protein and its excellent immunogenicity.

Screening of positive hybridoma cells

The antibody titer of mice immunized with the p30 protein was determined, with a titer of $1{:}4.096 \times 10^5$ (Fig. 2), indicating a highly efficient antibody response in the immunized mice. We selected mouse No. 3 for the next experiment. After fusion of mouse spleen cells with SP2/0 myeloma cells, a large number of fused hybridoma cells were observed on the 8th day. Positive hybridoma cell lines producing antibodies against p30 were screened using the indirect ELISA method. After four rounds of subcloning, one anti-p30 hybridoma cell line named 2A5 was finally selected.

Identification of hybridoma cells

Chromosomal analysis of hybridoma cells provides an objective indicator to determine whether the cells are genuine hybridomas. Additionally, chromosomal analysis of hybridoma cells is significant for understanding the ability of hybridoma cells to secrete monoclonal antibodies. Chromosomal analysis of the hybridoma cells revealed that the hybridoma cells 2A5 had approximately 100 chromosomes, which is significantly higher than the chromosome count in the myeloma cells SP2/0 (Fig. 3A). Additionally, this count was close to the total sum of chromosomes from both parental cell types, indicating successful fusion. Furthermore, the chromosome distribution of the hybridoma cells 2A5 was relatively concentrated and their morphology was good, indicating that these cells have good genetic stability and can secrete antibodies effectively.

The mouse mAb subtype identification kit was utilized to determine the antibody subtype, the results indicated that the heavy chain of mAb secreted by anti-p30 hybridoma cells 2A5 was of the IgG1 type, while the light chain was identified as kappa type (Fig. 3B).

Preparation of monoclonal antibody

Using the method of inducing ascites in mice to produce a substantial amount of antibody, followed by purification of the ascites using Protein G affinity purification method, the obtained antibody was named mAb 2A5. The SDS-PAGE results showed clear bands corresponding to the heavy and light chains of mAb 2A5, indicating

that the mAb is of high purity (Fig. 4A). Subsequently, the specificity of the purified mAb was assessed using Western blot analysis, and the results showed that the mAb 2A5 specifically bound to the p30 protein, indicating excellent specificity (Fig. 4B). The antibody titer of mAb 2A5 was measured using an indirect ELISA, with a titer of $1{:}1.024 \times 10^6$ (Fig. 4C).

Epitope mapping

To identify the antigenic epitope recognized by the mAb 2A5 against p30, Western blot analysis and indirect ELISA were employed to detect the truncated protein of p30. The p30 protein was subjected to four rounds of truncation expression (Fig. 5A). In the first round of truncation, the full-length p30 protein was segmented into four proteins (1–98 aa, 1–108 aa, 59–158 aa, and 109–190 aa), named p30-1, p30-2, p30-3, and p30-4, respectively. Both Western blot and indirect ELISA results demonstrated that the mAb 2A5 reacted with p30-1, p30-2, and p30-3, indicating that the antigenic epitope recognized by the mAb 2A5 lay within the 1–108 aa (Fig. 5B**,** Table 1). Then, in the second round of truncation, p30 was truncated to 75–190 aa, 90–190 aa, and 1–94 aa, respectively named p30-5, p30-6, and p30-7. Both Western blot and indirect ELISA identification results showed that the mAb 2A5 reacted only with p30-7, indicating that the antigenic epitope recognized by the mAb 2A5 was located between 1 and 74 aa (Fig. 5C**,** Table 1). In the third round of truncation, to determine the specific location of the N-terminal antigenic epitope, the p30 protein was truncated to 1–55 aa, 1–56 aa, 1–57 aa, and 1–58 aa, respectively named p30-8, p30-9, p30-10, and p30-11. Among them, p30-10 was not expressed. Both Western blot and indirect ELISA identification results showed that the mAb 2A5 reacted only with p30-11, indicating that the N-terminal antigenic epitope was located at the 57th or 58th amino acid (Fig. 5D, Table 1). In the fourth round of truncation, to determine the specific location of the C-terminal antigenic epitope, the p30 protein was truncated to 70–190 aa, 71–190 aa, 72–190 aa, 73–190 aa, and 74–190 aa, named p30-12, p30-13, p30-14, p30-15, and p30-16, respectively. Both Western blot and indirect ELISA results showed that the mAb 2A5 reacted well with p30-12, p30-13, p30-14, p30-15, and p30-16, confirming that the C-terminal antigenic epitope was located at the 74th amino acid (Fig. 5E**,** Table 1). Finally, it was determined that the mAb 2A5 recognized the antigenic epitope of the p30 protein as 58–74 aa, with the amino acid sequence being ^{58}VKYDIVKSARIYAGQGY^{74}.

To further verify the antigenic epitope, the identified antigenic epitope amino acid sequence was synthesized, and the reactivity of the synthesized peptide was detected by indirect ELISA. The indirect ELISA results showed that the peptide reacted well with mAb 2A5, p30 immune mouse serum, and ASFV positive serum (Table 2), indicating that the synthesized peptide sequence contains the antigenic epitope recognized by monoclonal antibody 2A5 for p30.

The p30 protein sequences of 32 strains of ASFV were downloaded from the NCBI database (Table S2), encompassing various genotypic strains. Analyzed the conservation of the p30 antigenic epitopes identified by the mAb 2A5 using Jalview software (Fig. 6). The analysis revealed that the antigenic epitope of p30 differed by one amino acid residue from 5 strains of genotype I viruses (the 67th amino acid). Furthermore, it differed by two amino acid residues from the following strains: genotype IX strain R35 and genotype X strain HINDE I (the 64th and 65th amino acid), genotype XV strain TAN/01/1, genotype XI strain KAB/62, genotype XII strain MZI/92/1, genotype XIV strain NYA/1/2, genotype XIII strain SUM/1411, genotype XVI strain TAN/03/2 (the 64th and 68th amino acid). The antigenic epitope sequence was highly conserved in genotype II strains (Belgium 2018/1, Georgia 2007/1, Wuhan 2019-1, Kyiv/2016/131, Timor-Leste/2019/1), genotype I and genotype II

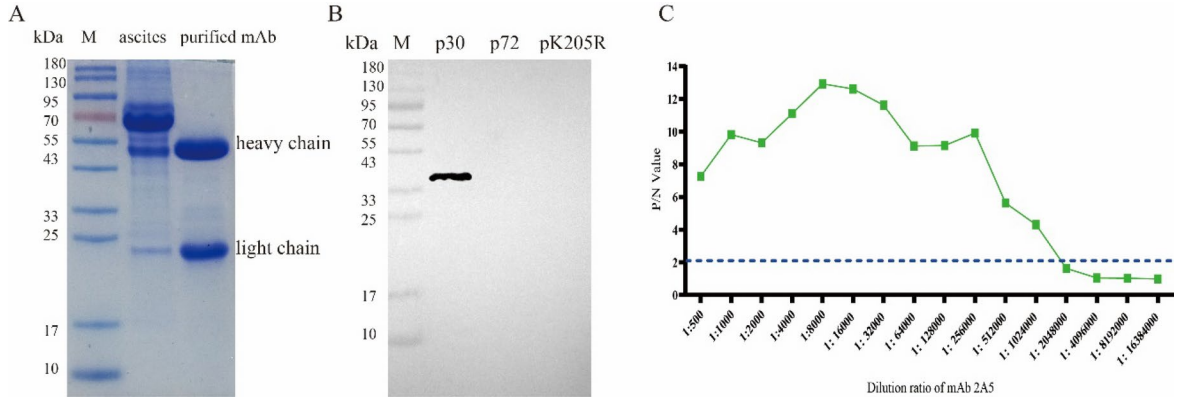

Fig. 4 The purified mAb 2A5 was identified by SDS-PAGE and Western blot. **A**The purified mAb 2A5 was identified by SDS-PAGE; **B** Western blot was used to assess the specificity of mAb 2A5; **C** The antibody titer of mAb 2A5 was measured using an indirect ELISA

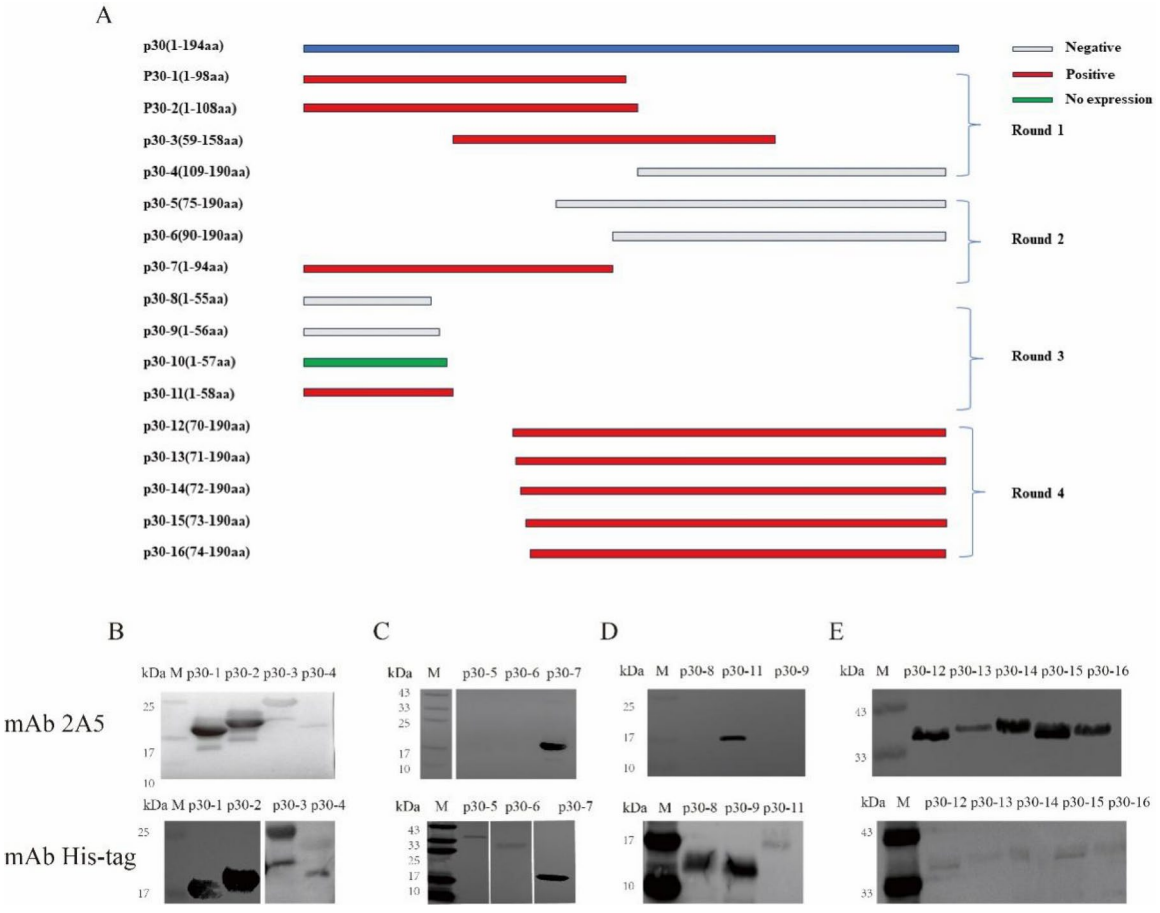

Fig. 5 Localization of mAb 2A5 recognizing the antigen epitope. **A** Schematic representation of truncated p30 protein; Using Western blot to identify **B** the first-round truncated protein, **C** the second-round truncated protein, **D** the third-round truncated protein, **E** the fourth-round truncated protein, with the identification results of His-tag monoclonal antibody serving as the positive control

Table 1 Results of the indirect ELISA for identifying mAb 2A5 recognizing the antigenic epitope (OD_{630nm})

Proteins	OD_{630nm}				
	mAb 2A5	Serum with antibodies targeting p30 protein (BALB/c mice)	Serum from healthy BALB/c mice	ASFV-positive serum	ASFV-negative serum
p30-1	1.161	1.15	0.088	0.929	0.186
p30-2	1.137	1.149	0.077	0.985	0.153
p30-3	0.894	0.938	0.078	0.936	0.132
p30-4	0.098	1.031	0.071	1.169	0.126
p30-5	0.122	0.871	0.056	1.141	0.156
p30-6	0.061	0.785	0.058	1.132	0.146
p30-7	0.885	0.822	0.081	0.986	0.105
p30-8	0.083	0.853	0.080	0.524	0.185
p30-9	0.100	0.871	0.088	0.542	0.225
p30-11	0.860	0.932	0.091	0.921	0.275
p30-12	0.835	0.837	0.068	0.655	0.129
p30-13	0.746	0.844	0.085	0.693	0.126
p30-14	0.688	0.849	0.083	0.669	0.132
p30-15	0.750	0.905	0.082	0.747	0.155
p30-16	0.617	0.779	0.047	0.658	0.101

Table 2 The results of the indirect ELISA detection of the p30 antigenic epitope peptide (OD_{630nm})

Samples	OD_{630nm}			
	First test	Second test	Third test	Average value
mAb 2A5	0.412	0.401	0.399	0.404
Serum with antibodies targeting p30 protein (BALB/c mice)	0.566	0.581	0.602	0.583
Serum from healthy BALB/c mice	0.038	0.039	0.041	0.039
ASFV-positive serum	0.876	0.891	0.882	0.883
ASFV-negative serum	0.050	0.050	0.058	0.053

recombinant strain Pig/Henan/123014/2022, genotype V strain MK-200, genotype VI strain MOZ/94/1, genotype XVIII strain NAM-95/1/P, genotype XXI strain RSA-96/1/P, genotype XX strain RSA/95/1, genotype XIX strain RSA/96/3, genotype VII strain RSA/98/1, genotype IV strain RSA/99/1/W, genotype III strain SPEC-257, genotype XVII strain Zim-MVM-90/1, genotype XXII strain Spec-245, and genotype VIII strain Malawi/1978, with a sequence similarity of 100%.

Optimization of conditions for immunochromatographic strip

We established an immunochromatographic strip method for detecting ASFV antigen using anti-p30 rabbit pAb and anti-p30 mAb 2A5, with the pAb stored in our laboratory. The main parameters of the immunochromatographic strip method were optimized. Blue latex microspheres with a diameter of 369 nm were chosen as the solid-phase carrier. Blue latex microspheres were then conjugated with 20 μg of the pAb to prepare the immunological probe. During the conjugation process, a pH 6.5, 0.05 M borate solution was selected as the coupling buffer, and the coupling lasted for 3 h. Then, a blocking solution (containing 1% BSA, 0.24% ethanolamine, in pH 6.5, 0.05 M borate solution) was applied for 1 h to block the latex microspheres. The mAb 2A5 was

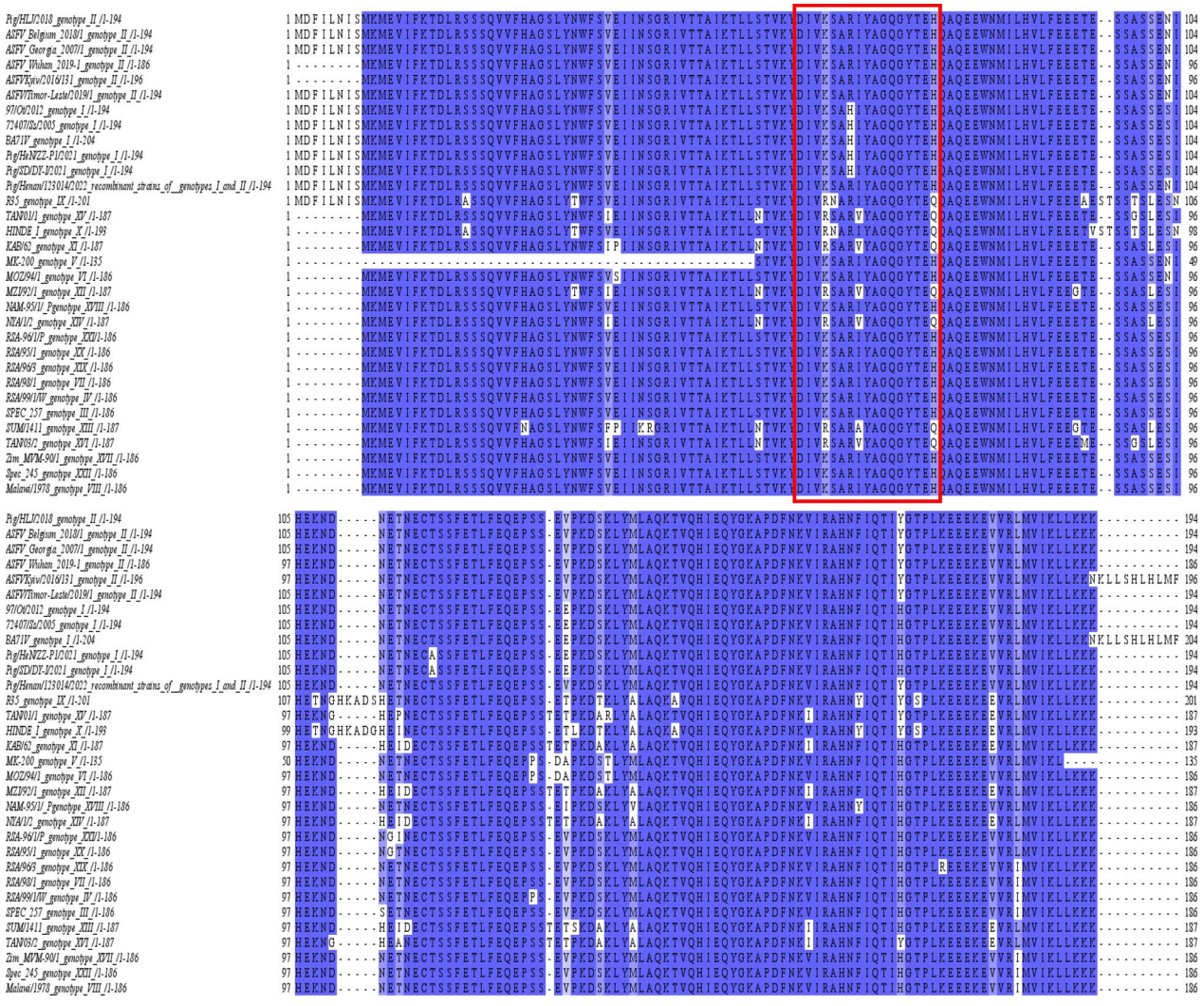

Fig. 6 Analysis of sequence conservation for the identified linear B-cell epitope

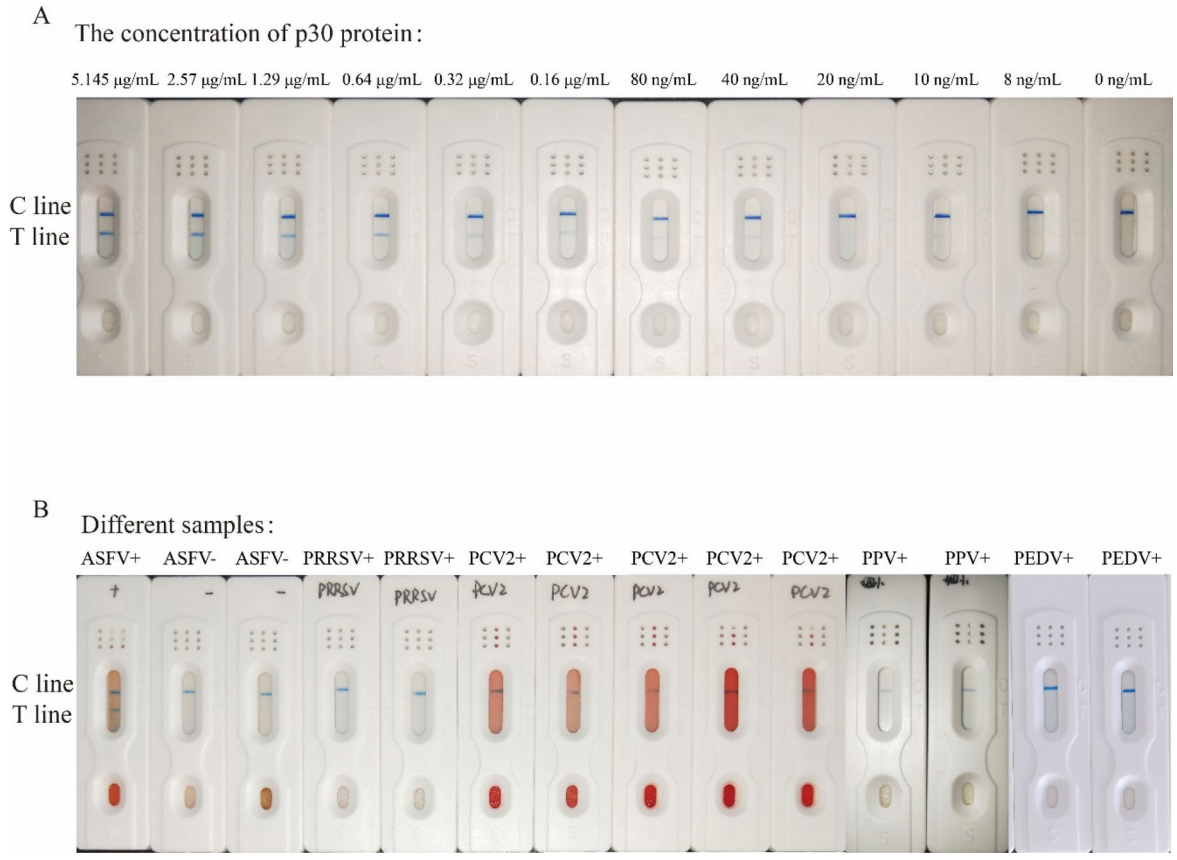

Fig. 7 Verification of sensitivity and specificity of immunochromatographic strip. **A** Verification of sensitivity of immunochromatographic strip, the lowest detection limit of the strip was established at 10 ng/mL; **B** Verification of specificity of immunochromatographic strip, the test strip did not exhibit cross-reactivity with PCV2, PRRSV, PPV, and PEDV

Table 3 The stability of immunochromatographic strip stored at 37 ℃

	Storage time							
	0 d	14 d	28 d	42 d	56 d	70 d	84 d	100 d
ASFV positive serum (1:2)	+	+	+	+	+	+	+	+
ASFV positive serum (1:4)	+	+	+	+	+	+	+	+
ASFV positive serum (1:8)	+	+	+	+	+	+	+	+
ASFV positive serum (1:16)	+	+	+	+	+	+	+	+
ASFV positive serum (1:32)	+	+	+	+	+	+	+	+
ASFV negative serum(undiluted)	–	–	–	–	–	–	–	–

+: positive; –: negative

coated on NC membrane as the T line of the immunochromatographic strip, and the optimal concentration of the T line was determined to be 1 mg/mL. Goat anti-rabbit IgG was immobilized on NC membrane as the C line of the test strip, with the optimal concentration for the C line determined to be 1 mg/mL (Fig. S1).

Performance of immunochromatographic strip

The p30 protein was diluted to different concentrations and detected using the prepared immunochromatographic strips to validate the sensitivity. According to the detection results, the lowest detection limit of the test strip was established at 10 ng/mL (Fig. 7A). Different antigen-positive samples were tested using the prepared immunochromatographic strips to verify the specificity. The results indicated that the strip did not exhibit cross-reactivity with PCV2, PRRSV, PPV, and PEDV, indicating good specificity (Fig. 7B). After 100 days of storage at 37 °C, the strips could still differentiate between positive and negative results. As the storage time increased, the color of the T line and C line became lighter, but the sensitivity of the strip remained basically unchanged (Table 3 and Fig. S2).

Table 4 The immunochromatographic strip was compared with the qPCR kit

		The immunochromatographicstrip		Concordance rate
		Positive	Negative	
The commercial qPCR kit	Positive	127	14	92.2%
	Negative	12	180	

Detection of clinical samples

A total of 333 clinical samples were tested using both the commercial qPCR kit and the established immunochromatographic strip method, with the test results shown in Table S3. Based on the results, it was found that the detection performance of the immunochromatographic strip varied depending on the sample type. The immunochromatographic strip detected 70 whole blood samples, with 4 false negatives and 1 false positive. For 94 serum samples, there were 6 false negatives and 1 false positive. When testing 149 swab samples, there were 4 false negatives and 8 false positives. Due to the small sample size of semen, gauze, and environmental samples, the results were not representative and were not evaluated. Overall, the detection performance was better for whole blood and serum samples.

When comparing the two methods, the commercial qPCR kit detected 141 positive samples and 192 negative samples, while the established immunochromatographic strip method detected 139 positive samples and 194 negative samples, resulting in a concordance rate of 92.2% (Table 4). The established immunochromatographic strip method shows promising application prospects and is suitable for on-site testing.

Discussion

Currently, there are no vaccines and therapeutic drugs available for ASFV (Wang et al. 2022). Hence, an efficient and rapid detection method is crucial for the prevention and control of ASF. The pathogen diagnosis of ASFV is the most direct method, and conducting on-site testing using immunochromatographic strip allows for timely results, enabling farmers to take measures early on to minimize losses.

In this study, we generated a mouse mAb against p30 and identified the antigenic epitope recognized by the mAb 2A5 as ^{58}VKYDIVKSARIYAGQGY^{74}, which differs from previously reported epitopes. Currently reported p30 antigenic epitopes identified using monoclonal antibodies mainly include regions such as 12-18aa, 16-48aa, 61-93aa, 84-91aa, 96-105aa, 116-125aa, 122-128aa, 144-154aa, 146-160aa, 164-170aa, 171-180aa, and 176-185aa (Murgia et al. 2018; Petrovan et al. 2019; Tian et al. 2024; Wu et al. 2020; Zhang et al. 2021; Zhou et al. 2022, 2023). This study identified the antigenic epitope of p30 as 58-74aa using the mAb 2A5. Analysis of the conservation of this antigenic epitope revealed that it has relatively high conservation with prevalent strains in China, indicating that the detection method established using the mAb 2A5 against p30 is suitable for detecting ASFV in China.

The immunochromatographic strip method is simple to operate, provides rapid results, and is suitable for on-site testing, showing promising application prospects. This study established a dual-antibody sandwich immunochromatographic strip method for detecting ASFV antigen. As only one monoclonal antibody was prepared, we utilized the mAb 2A5 along with the existing pAb in our laboratory to establish the dual-antibody immunochromatographic strip detection method. Blue latex microspheres were chosen as the solid-phase carrier, conjugated with the pAb against ASFV p30 to prepare the immune probe. The mAb 2A5 against ASFV p30 was utilized as the T line, while goat anti-rabbit IgG was employed as the C line, both fixed on the NC membrane.

The labeling materials used in immunochromatographic strip are mainly categorized as colorimetric, fluorescent, and others. Colorimetric labeling materials do not require any instruments and allow direct result observation, making them ideal for clinical on-site testing. Although colorimetric labeling materials have slightly lower sensitivity, their visual characteristics make them widely applicable. Colorimetric labeling materials include colloidal gold, colloidal silver, colored latex microspheres, nanocarbon, nano-selenium, colored silica nanoparticles, composite nanomaterials, and ultramarine pigments, among others. Among these, colloidal gold is the most widely used labeling material (Zhang et al. 2018). This study opted for blue latex microspheres as the labeling material, which offers several advantages over traditional colloidal gold. Firstly, the preparation process of latex microspheres is mature, ensuring stability across different batches (Yu et al. 2022). Secondly, latex microspheres exhibit strong tolerance, withstanding a wide pH range of buffer solutions and high salt ion concentrations. Thirdly, the coupling of latex microspheres is more robust, relying on the covalent coupling between carboxyl groups on the latex microspheres and amino groups on proteins (Garcia et al. 2020; Liang et al. 2022).

A study reported the development of a colloidal gold strip detection method utilizing two mAbs against p30, with the lowest detection limit of the strip being 72 ng/mL (Zhang et al. 2021). Additionally, the VDRG® ASFV Ag Rapid Kit was developed using two mAbs against the p30 protein, achieving a minimum detection limit of 11.5 ng (Hang Vu et al. 2023). The latex immunochromatographic strip method developed in this study demonstrates slightly higher sensitivity compared to the

colloidal gold strip method utilizing two mAbs against p30 mentioned above. In the later stages, it may be possible to improve the sensitivity of the immunochromatographic strip by attempting to screen more mAbs.

The immunochromatographic strip method established in this study is simple to operate, fast in detection, and cost-effective, making it highly suitable for on-site testing. Compared to traditional colloidal gold, the blue latex microspheres selected in this study as the labeling material for the preparation of the immunochromatographic strips offer greater stability. The mAb 2A5 used in the preparation of the test strips recognizes an antigen epitope that differs from previously reported mAbs, providing a new option for ASFV antigen detection. Moreover, the immunochromatographic strip method established in this study still has limitations, and further improvements could be made in the future. Firstly, enhancing sensitivity by further screening mAbs against ASFV and pairing them with the mAb 2A5 to develop the strip method. Secondly, improving the sample pad of the strip to make it more suitable for detecting whole blood samples without any preprocessing, thus making the detection process more convenient.

In this study, we prepared a mAb 2A5 against p30 and identified the antigenic epitope recognized by mAb 2A5 as ^{58}VKYDIVKSARIYAGQGY^{74}. Based on the anti-p30 mAb 2A5 and anti-p30 pAb, we established a latex microsphere immunochromatographic strip method for detecting ASFV antigens. The immunochromatographic strip method was demonstrated to have excellent detection performance, with good sensitivity, specificity, and stability. The concordance rate between the immunochromatographic strip method and the qPCR assay kit for clinical sample detection was 92.2%. The immunochromatographic strip method is capable of meeting clinical testing requirements and has promising application prospects, providing a new choice for on-site detection of ASFV.

Abbreviations

ASF	African swine fever
ASFV	African swine fever virus
mAb	Monoclonal antibody
mAbs	Monoclonal antibodies
pAb	Polyclonal antibody
PEG 1450	Polyethylene glycol 1450
MES	4-Morpholineethanesulfonic acid
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
NHS	*N*-Hydroxysuccinimide
PRRSV	Porcine reproductive and respiratory syndrome virus
PCV2	Porcine circovirus type 2
PPV	Porcine parvovirus
PEDV	Porcine epidemic diarrhea virus
IPTG	Isopropyl β-D-1-thiogalactopyranoside

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13568-025-01857-x .

Supplementary Material 1.

Author contributions

Yanli Hu: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Validation, Visualization, Writing-original draft. Yingjun Xia: Investigation, Visualization. Huiwen Mo: Resources, Validation. Li Zhang: Data curation. Wenjian Cao: Investigation. Rui Fang: Project administration, Supervision. Junlong Zhao: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing-review & editing.

Funding

This work was supported by the STI2023-Major Projects (Project 2023ZD0404301) and the Fundamental Research Funds for the Central Universities in China (Project 2662020DKPY016).

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request. All data are available within the manuscript and supplementary materials.

Declarations

Ethics approval and consent to participate

All animal experiments were performed with the approval of the Laboratory Animals Research Centre of Hubei Province and the Ethics Committee of Huazhong Agricultural University (permit number: SCXK(E)-2020-0019).

Conflict of interest

The authors declare that they have no competing interests.

Received: 5 October 2024 / Accepted: 27 February 2025
Published online: 15 March 2025

References

Arzt J, White WR, Thomsen BV, Brown CC (2010) Agricultural diseases on the move early in the third millennium. Vet Pathol 47:15–27

Chen XN, Chen XJ, Liang YF, Xu SJ, Weng ZJ, Gao Q, Huang Z, Zhang GH, Gong L (2022) Interaction network of African swine fever virus structural protein p30 with host proteins. Front Microbiol 13:971888

Cubillos C, Gómez-Sebastian S, Moreno N, Nuñez MC, Mulumba-Mfumu LK, Quembo CJ, Heath L, Etter EMC, Jori F, Escribano JM, Blanco E (2013) African swine fever virus serodiagnosis: A general review with a focus on the analyses of African serum samples. Virus Res 173:159–167

Galindo I, Alonso C (2017) African swine fever virus: a review. Viruses 9:103

Gallardo C, Fernández-Pinero J, Arias M (2019) African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. Virus Res 271:197676

Garcia VS, Guerrero SA, Gugliotta LM, Gonzalez VDG (2020) A lateral flow immunoassay based on colored latex particles for detection of canine visceral leishmaniasis. Acta Trop 212:105643

Ge SQ, Li JM, Fan XX, Liu FX, Li L, Wang QH, Ren WJ, Bao JY, Liu CJ, Wang H, Liu YT, Zhang YQ, Xu TG, Wu XD, Wang ZL (2018) Molecular characterization of African swine fever virus, China, 2018. Emerg Infect Dis 24:2131–2133

GomezPuertas P, Rodriguez F, Oviedo JM, Ramirolbanez F, RuizGonzalvo F, Alonso C, Escribano JM (1996) Neutralizing antibodies to different proteins of African swine fever virus inhibit both virus attachment and internalization. J Virol 70:5689–5694

Gómez-Puertas P, Rodríguez F, Oviedo JM, Brun A, Alonso C, Escribano JM (1998) The African swine fever virus proteins p54 and p30 are involved in two distinct steps of virus attachment and both contribute to the antibody-mediated protective immune response. Virology 243:461–471

Greenfield EA (2019) Single-cell cloning of hybridoma cells by limiting dilution. Cold Spring Harb Protoc 2019 11:726–728

Hang Vu TT, Le VP, Jeong DG, Yeom M, Oh J, Kang B, Park S-K, Song D (2023) Establishment of a p30-based lateral flow assay for African swine fever virus detection. J Virol Methods 322:114823

Hu C, Li S, Zhou J, Wei D, Liu X, Chen Z, Peng H, Liu X, Deng Y (2024) In vitro SELEX and application of an African swine fever virus (ASFV) p30 protein specific aptamer. Sci Rep 14:4078

Li C, He X, Yang Y, Gong W, Huang K, Zhang Y, Yang Y, Sun X, Ren W, Zhang Q, Wu X, Zou Z, Jin M (2020) Rapid and visual detection of African swine fever virus antibody by using fluorescent immunochromatography test strip. Talanta 219:121284

Liang Z, Peng T, Jiao X, Zhao Y, Xie J, Jiang Y, Meng B, Fang X, Yu X, Dai X (2022) Latex microsphere-based bicolor immunochromatography for qualitative detection of neutralizing antibody against SARS-CoV-2. Biosensors 12:103

Liberti R, Colabella C, Anzalone L, Severi G, Paolo A, Casciari C, Casano A, Giammarioli M, Cagiola M, Feliziani F, Giuseppe A (2023) Expression of a recombinant ASFV P30 protein and production of monoclonal antibodies. Open Vet J 13:358–364

Liu H-C, Liu R-C, Hu M-R, Yang A-B, Wu R-H, Chen Y, Zhang J, Bai J-S, Wu S-B, Chen J-P, Long Y-F, Jiang Y, Zhou B (2024) Development of high-concentration labeled colloidal gold immunochromatographic test strips for detecting African swine fever virus p30 protein antibodies. Heliyon 10:e25214

Murgia MV, Mogler M, Certoma A, Green D, Monaghan P, Williams DT, Rowland RRR, Gaudreault NN (2018) Evaluation of an African swine fever (ASF) vaccine strategy incorporating priming with an alphavirus-expressed antigen followed by boosting with attenuated ASF virus. Adv Virol 164:359–370

Pang J, Tian X, Han X, Yuan J, Li L, You Y, Zhou Y, Xing G, Li R, Wang Z (2023) Computationally-driven epitope identification of PEDV N-protein and its application in development of immunoassay for PEDV detection. J Pharm Biomed Anal 235:115660

Petrovan V, Yuan F, Li Y, Shang P, Murgia MV, Misra S, Rowland RRR, Fang Y (2019) Development and characterization of monoclonal antibodies against p30 protein of African swine fever virus. Virus Res 269:197632

Sun E, Huang L, Zhang X, Zhang J, Shen D, Zhang Z, Wang Z, Huo H, Wang W, Huangfu H, Wang W, Li F, Liu R, Sun J, Tian Z, Xia W, Guan Y, He X, Zhu Y, Zhao D, Bu Z (2021a) Genotype I African swine fever viruses emerged in domestic pigs in China and caused chronic infection. Emerg Microbes Infect 10:2183–2193

Sun EC, Zhang ZJ, Wang ZL, He XJ, Zhang XF, Wang LL, Wang WQ, Huang LY, Xi F, Huangfu HY, Tsegay G, Huo H, Sun JH, Tian ZJ, Xia W, Yu XW, Li F, Liu RQ, Guan YT, Zhao DM, Bu ZG (2021b) Emergence and prevalence of naturally occurring lower virulent African swine fever viruses in domestic pigs in China in 2020. Sci China Life Sci 64:752–765

Tian P, Sun Z, Wang M, Song J, Sun J, Zhou L, Jiang D, Zhang A, Wu Y, Zhang G (2024) Identification of a novel linear B-cell epitope on the p30 protein of African swine fever virus using monoclonal antibodies. Virus Res 341:199328

Wan Y, Shi Z, Peng G, Wang L, Luo J, Ru Y, Zhou G, Ma Y, Song R, Yang B, Cao L, Tian H, Zheng H (2021) Development and application of a colloidal-gold dual immunochromatography strip for detecting African swine fever virus antibodies. Appl Microbiol Biotechnol 106:799–810

Wang ZY, Ai QY, Huang SL, Ou YT, Gao YZ, Tong TZ, Fan HY (2022) Immune escape mechanism and vaccine research progress of African swine fever virus. Vaccines Basel 10:344

Wu P, Lowe AD, Rodríguez YY, Murgia MV, Dodd KA, Rowland RR, Jia W (2020) Antigenic regions of African swine fever virus phosphoprotein P30. Transbound Emerg Dis 67: 1942–1953

Yu C, Wei Y, Zhang H, Liu J, Feng L, Liu C, Huang L (2022) Rapid detection of porcine circovirus type 2 by a red latex microsphere immunochromatographic strip. Appl Microbiol Biotechnol 106:5757–5769

Yuan J, Zhang C, Fang S, Zhuang Z, Ling S, Wang S (2012) A monoclonal antibody against F1–F0 ATP synthase beta subunit. Hybridoma (Larchmt) 31:352–357

Zhang D, Huang L, Liu B, Ni H, Sun L, Su E, Chen H, Gu Z, Zhao X (2018) Quantitative and ultrasensitive detection of multiplex cardiac biomarkers in lateral flow assay with core-shell SERS nanotags. Biosens Bioelectron 106:204–211

Zhang X, Liu X, Wu X, Ren W, Zou Y, Xia X, Sun H (2021) A colloidal gold test strip assay for the detection of African swine fever virus based on two monoclonal antibodies against P30. Adv Virol 166:871–879

Zhang J, Zhang K, Sun S, He P, Deng D, Zhang P, Zheng W, Chen N, Zhu J (2024) Specific monoclonal antibodies against African swine fever virus protease pS273R revealed a novel and conserved antigenic epitope. Int J Mol Sci 25:8906

Zhao D, Sun E, Huang L, Ding L, Zhu Y, Zhang J, Shen D, Zhang X, Zhang Z, Ren T, Wang W, Li F, He X, Bu Z (2023) Highly lethal genotype I and II recombinant African swine fever viruses detected in pigs. Nat Commun 14:3096

Zhou XT, Li N, Luo YZ, Liu Y, Miao FM, Chen T, Zhang SF, Cao PL, Li XD, Tian KG, Qiu HJ, Hu RL (2018) Emergence of African swine fever in China, 2018. Transbound Emerg Dis 65:1482–1484

Zhou GJ, Shi ZW, Luo JC, Cao LY, Yang B, Wan Y, Wang LJ, Song R, Ma Y, Tian H, Zheng HX (2022) Preparation and epitope mapping of monoclonal antibodies against African swine fever virus P30 protein. Appl Microbiol Biotechnol 106:1199–1210

Zhou J, Ni Y, Wang D, Fan B, Zhu X, Zhou J, Hu Y, Li L, Li B (2023) Development of a competitive enzyme-linked immunosorbent assay targeting the-p30 protein for detection of antibodies against African swine fever virus. Viruses 15:154

Publisher's Note