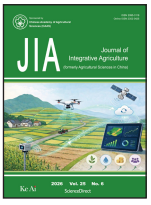
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LETTER

Rapid and ultrasensitive point-of-care detection of ASFV antibodies using p30-Fc-labeled nanoparticle-based fluorescence strip-assisted portable immunosensor

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Highlights

- A portable immunosensor device assisted by p30-Fc-labeled nanoparticle-based fluorescence strips was developed for the rapid detection of African swine fever virus (ASFV) antibodies in the field.
- The fluorescent test strip prepared in this study has the advantages of low cost, high specificity and sensitivity, good repeatability and stability.
- The fluorescent test strip detection method in this study has extremely high sensitivity and antibodies can be detected four days after ASFV infection, which has high potential application value in the monitoring and control of African swine fever.

African swine fever (ASF) is an acute and highly contagious hemorrhagic disease caused by the African swine fever virus (ASFV) that affects the swine industry worldwide (Zhong *et al.* 2021). This virus is a large enveloped icosahedral double-stranded DNA virus belonging to the genus Asfivirus of the Asfivirus family (Digiaro *et al.* 2024). Strengthening biosafety, developing early detection and diagnostic products, improving detection capacity and expanding detection scope are the key aspects of ASF prevention and control (Sun *et al.* 2021). ASFV p30 protein is one of the inner capsule membrane proteins of ASFV and one of the most antigenic proteins (Wu *et al.* 2020). The p30 protein, encoded by the ASFV CP204L gene, is often expressed 2 to 4 h after ASFV infection, and its synthesis is reduced after 12 h, which can induce the production of neutralizing antibodies (Kazakova *et al.* 2017). This protein, which is an important structural protein of ASFV, can be first expressed and secreted in the early stage of ASFV infection, and remains detectable during the entire course of the infection (Noll *et al.* 2024). The p30 antibody can be detected as early as 8 days after infection with ASFV in pigs. These characteristics indicate that p30 is an ideal antigen for serological diagnosis and immunological detection (Hang Vu *et al.* 2023; Gong *et al.* 2024).

To date, there have been no effective vaccines or therapeutic drugs for the prevention and control of ASF, so early surveillance is one of the most important solutions for its prevention and control (Dixon *et al.* 2020). At present, laboratory diagnosis is the main means of detection, and PCR-based methods and Enzyme-Linked Immunosorbent Assay (ELISA) are the most widely used technique. However, these methods have limitations and require laboratory-based procedures. With the emergence of low-virulence natural mutant strains of ASFV in China, ASF presents the characteristics of being persistent, non-lethal and with subacute or chronic onset, and ASFV infection causes a strong humoral immune response in pigs and lasts for a long time (Liu *et al.* 2024). While it is difficult to detect the virus in the blood of recovered pigs infected with ASFV, the blood contains a high level of antibodies, which makes research on serum antibody detection methods for ASF particularly important for the prevention, control and purification of ASF (Teklue *et al.* 2020). Nevertheless, the use of ELISA Kits is limited by equipment and skilled personnel, making their widespread implementation in farming facilities challenging. In addition, it is often necessary to rely on third-party laboratories for testing. Fluorescence colloidal gold

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immunochromatography is a promising method to solve this problem. This convenient technology allows for field testing of antigens and antibodies without the need for specialized skills or expensive complex equipment (Di Nardo *et al.* 2021). Therefore, it is one of the ideal techniques for the initial diagnosis of ASF in the field. This enables farmers to quickly assess the health of their herds, enabling timely and targeted measures to mitigate the spread of ASFV and minimize economic losses.

Based on the above, we developed a novel fluorescent colloidal-gold test strips (fluorescent test strips) containing p30-Fc protein and Staphylococcal Protein A (SPA) for ASFV antibody detection (Fig. 1-A). The p30-Fc fusion protein of ASFV was expressed using the baculovirus expression system, then purified with Ni-NTA affinity chromatography. Finally, the p30-Fc fusion protein was identified using SDS-PAGE and WB. The concentration of p30-Fc protein was determined and stored at –80°C. The sample pad, binding pad, nitrocellulose membrane and absorbent pad were superimposed onto a polyvinyl chloride (PVC) bottom plate in sequence to obtain fluorescent test strips for detecting the African virus. After assembly, the sample was added to the sample pad (Fig. 1-B and C). After reacting for 10–15 min, the fluorescent test strips detector was used to detect the fluorescence value. The minimum detection value of the machine in this test was 8 — that is, when it is less than 8, it is judged as negative, when it is equal to 8, it is judged as suspicious and needs to be tested again, and it is positive if the value is greater than 8.

ASFV-positive serum was diluted at 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:128, 1:2,560 and 1:5,120 to detect the sensitivity of the fluorescent test strips. For the 1:5,120 serum, the T-line band disappeared under the UV lamp and the test value was negative (Fig. 1-D and E). The darker the band color observed under the UV lamp, the higher the detection value. The fluorescent test strips detection method constructed in this study has ultra-high sensitivity (1:2,560), which indicates that this method also has higher sensitivity when compared with the traditional colloidal gold test strip detection method.

The specificity of fluorescent test strips was tested using antisera of other closely related porcine viruses, including ASFV-, Porcine reproductive and respiratory syndrome virus (PRRSV)-, Japanese encephalitis virus (JEV)-, Getah virus (GETV)-, Classical swine fever virus (CSFV)-, Porcine circovirus type 2 (PCV2)-, Porcine Parvovirus (PPV)-, Pseudorabies virus (PRV)-positive, and ASFV-negative serum samples. The results showed that the fluorescent test strips could only detect ASFV-positive serum samples, and bands appeared on the T-line under the ultraviolet lamp, and there was no cross-reaction with other antisera (Fig. 1-F and G). The fluorescent test strips constructed in this experiment uses high-purity-specific protein to capture antibodies in serum, so it has high specificity.

To assess the reproducibility of the FICT within and between runs, six standard serum samples were tested using different batches of fluorescent test strips. The analysis was performed using the coefficient of variation (CV). The experimental results showed that the maximum CV of the intra-test repeatability test was 6.25% (Table 1), and the maximum CV of the inter-test repeatability test was 5.06% (Table 1), both of which were <10%. These results demonstrate that the fluorescent test strips detection method has good repeatability. The stability of fluorescent test strips was tested with an accelerated stability test. The results showed that under ultraviolet light, 10 ASFV-positive sera showed two lines (C-line and T-line), while only one line (C-line) was found in ASFV-negative sera. This indicates that the fluorescent test strips remained stable at 37°C for 30 days. Therefore, the method presented in this study has good stability (Fig. 1-H and I).

Fluorescent test strips and an ELISA Kit for Antibody Detection Against ASFV (Luoyang Putai Biological Technology Co. Ltd., China) were used to evaluate whether the former was suitable for the detection of ASFV antibody in clinical applications. A total of 100 clinical samples were tested, the results of the two detection methods were compared, and the coincidence rate was calculated. A total of 26 positive and 74 negative ASFV samples were detected using commercial ASFV Antibody ELISA Kits. A total of 27 positive ASFV and 73 negative ASFV samples were detected using fluorescent test strips. The coincidence rate of the fluorescent test strips and ASFV ELISA Antibody Kit was 98% (Appendix A).

Serum samples on the 0th, 2nd, 4th, 6th and 8th days after ASFV infection were detected using the ELISA Kit for Antibody Detection Against ASFV (Luoyang Putai Biological Technology Co. Ltd., China) and the fluorescent test strips constructed in this study. Serum samples obtained before challenge (day 0) were negative for ASFV antibody with both assays. Two detection methods were used to test the serum samples on the second day after ASFV infection, and the results showed that the ASFV antibodies were all negative. The fluorescent test strips constructed in this study was used to detect the serum samples on the 5th day after ASFV infection, and three of them were positive for ASFV antibody, while the ELISA Kit test results showed that the serum samples on the 5th day after ASFV infection were all negative. Serum samples on the 6th and 8th days of ASFV infection were detected using these two methods. All serum samples were positive for ASFV antibodies (Fig. 1-J and K; Appendix B). The detection results of the fluorescent test strips constructed in this study showed that they were positive as early as 4 days post-infection (dpi), while the ELISA Kit for Antibody Detection Against ASFV (Luoyang Putai Biological Technology Co. Ltd., China) detection results indicated that they were positive as early as 6 dpi. This suggests that the fluorescent test strips constructed in this study can detect ASFV antibodies earlier than the ELISA Kit for Antibody Detection Against ASFV (Luoyang Putai Biological Technology Co. Ltd., China).

In this study, the ASFV structural protein p30 and IgG Fc fusion protein (p30-Fc) were used as antigens and combined with fluorescent microspheres as tracers, and the SPA double-sandwich fluorescent test strip was prepared for the specific detection of anti-ASFV antibodies. The results demonstrated that the fluorescent test strips prepared in this study have a high sensitivity to the ASFV antibody, with a sensitivity of 1:2,560. They also have high specificity for the ASFV antibody

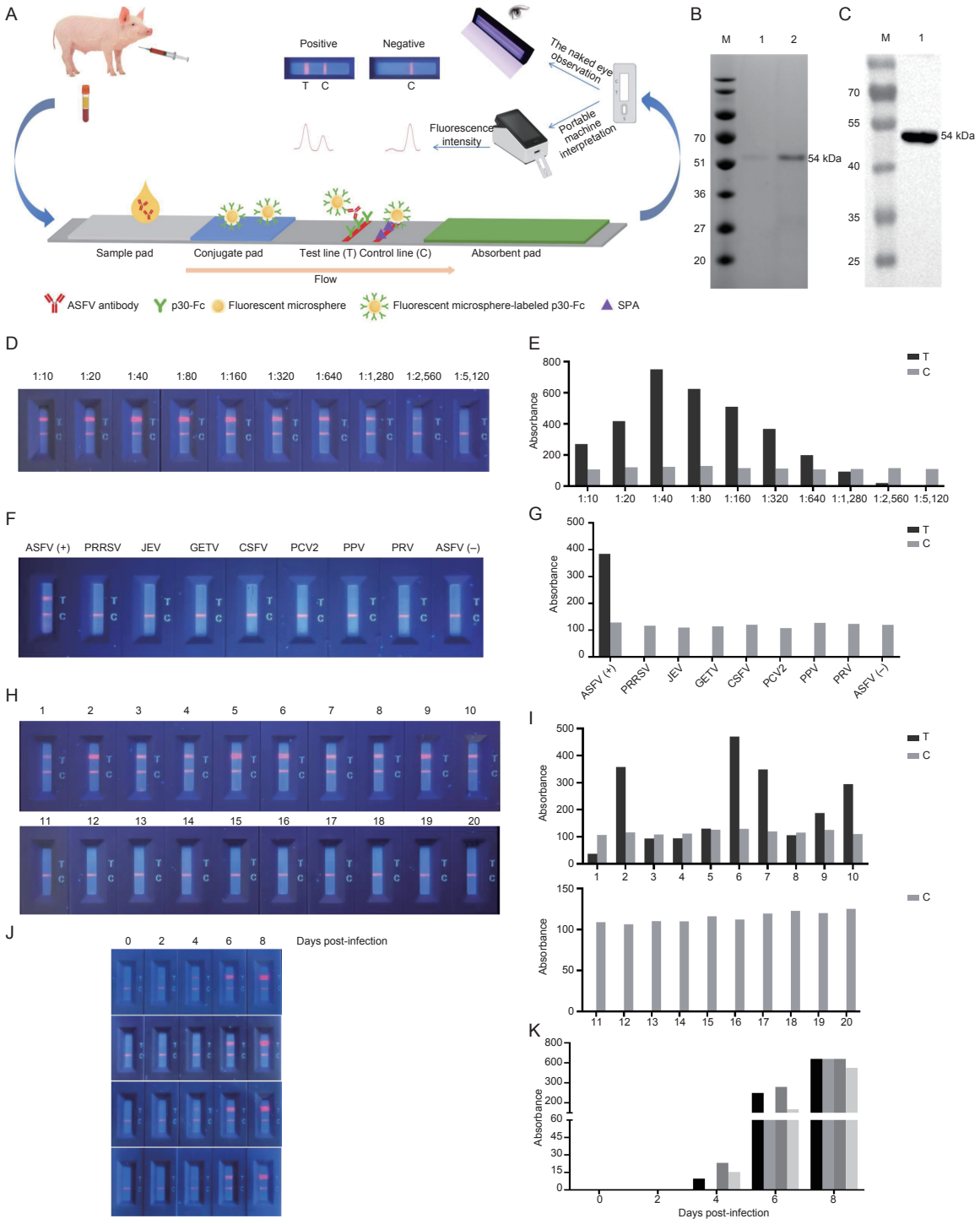


Fig. 1 Construction and performance evaluation of a fluorescent lateral flow Immunoassay for African swine fever virus (ASFV) antibody detection based on p30-Fc fusion protein. A, design process and schematic diagram of fluorescent test strips for African swine fever virus antibody. After collecting pig serum, the prepared fluorescent test strips were added and reacted for 15 min. Then, the test results were observed using an ultraviolet lamp. Portable detection devices can also be used to assess the fluorescence value of the test strips. If the test result is less than 8, it is judged as negative; if it is greater than 8, it is judged as positive. B, purification of p30-Fc protein. M, marker; 1, p30-Fc-1; 2, p30-Fc-2. C, expression of p30-Fc proteins was detected with anti-His monoclonal antibodies. M, marker; 1, p30-Fc. D, the sensitivity of the fluorescent test strips was detected against various dilutions of ASFV-positive serum from 1:10 to 1:5,120, and the highest dilution detected with this method was 1:2,560. F, the specificity of fluorescent test strips was detected against ASFV-positive and -negative serum, and Porcine reproductive and respiratory syndrome virus (PRRSV)-, Japanese encephalitis virus (JEV)-, Getah virus (GETV)-, Classical swine fever virus (CSFV)-, Porcine circovirus type 2 (PCV2)-, Porcine Parvovirus (PPV)-, Pseudorabies virus (PRV)-positive antibodies porcine serum. Except for the ASFV-positive serum, there were no bands on the T-line of the other test serum strips. H, stability of the fluorescent test strips. E, G, and I, the fluorescent test strips of the test line (T-line) and control line (C-line) of the lateral flow assay. J, real detection figures of ASFV antibodies based on lateral flow assay. The ASFV serum on days 0, 2, 4, 6 and 8 of ASFV infection was detected using fluorescent test strips. K, the p30-Fc fluorescent test strips of the test line (T value) of the lateral flow assay.

Table 1 The results of the repeating test

Sample number	The result of intra			The result of inter		
	Average value	Standard deviation	Coefficient variation (%)	Average value	Standard deviation	Coefficient variation (%)
1	373.95	13.83	3.70	121.44	6.14	5.06
2	176.27	11.01	6.25	123.23	3.62	2.93
3	115.64	5.60	4.84	263.28	11.52	4.38
4	125.13	4.79	3.83	399.76	27.01	6.75
5	106.68	1.91	1.79	203.99	6.90	3.38
6	94.06	1.75	1.86	38.81	1.81	4.66

and no cross-reaction with other porcine virus antibodies. Meanwhile, the results of the fluorescent test strips were in strong agreement with those of the commercially available ASFV Antibody ELISA Kits (98%). Antibodies can be detected four days after ASFV infection, allowing the proposed test to be used as an early diagnostic method.

All the serum sources used in this article are listed in Appendix C.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Ethical statements

This study did not involve animal experiments. All pig serum samples used in this study were archival specimens obtained from existing sample repositories or previously collected clinical isolates. No live animals were used, handled, or euthanized for the purpose of this research.

Appendices associated with this study are available at https://doi.org/10.1016/j.jia.2025.11.028

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