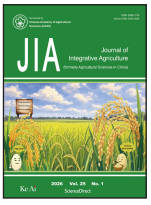
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LETTER

Point-of-care antigen detection for porcine deltacoronavirus: Colloidal gold and fluorescent immunochromatographic test strips

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Highlights

- By conjugating the same anti-N monoclonal antibody (mAb4-mAb1) with colloidal gold or fluorescent microspheres, this study developed two rapid point-of-care antigen immunochromatographic strips for the detection of porcine deltacoronavirus.
- The fluorescent microsphere-based lateral flow test strip demonstrated a sensitivity of $10^{1.7}$ $TCID_{50}$/0.1 mL, which is fourfold higher than that of the colloidal gold-based assay.

Porcine deltacoronavirus (PDCoV) is a recently identified enteric coronavirus that causes an acute infectious disease in piglets, leading to diarrhea, vomiting, dehydration, and mortality (Hu *et al.* 2015). In recent years, its incidence has escalated, resulting in a global epidemic that has caused substantial economic losses to the swine industry (Dong *et al.* 2015). Beyond swine, PDCoV has been shown to infect various animal species, including mice (Zhang *et al.* 2022), calves (Jung *et al.* 2017), and chickens (Liang *et al.* 2019). Notably, Lednicky *et al.* (2021) reported the detection of PDCoV nucleic acid in plasma samples from three children with acute undifferentiated febrile illness in Haiti. This finding suggests that PDCoV may cross species barriers and potentially become the eighth coronavirus capable of infecting humans (Zhai *et al.* 2022), posing a significant threat to public health. Early and accurate diagnosis is crucial for timely pharmacological intervention to mitigate disease progression in affected pigs and reduce transmission, thereby safeguarding herd health and minimizing breeding costs (Zhou *et al.* 2024). Therefore, developing rapid, easy-to-use, and reliable diagnostic methods is essential for effective disease prevention and control (Turlewicz-Podbielska *et al.* 2021).

PDCoV is a single-stranded positive-sense RNA virus belonging to the family Coronaviridae, genus *Deltacoronavirus*. Its relatively low genomic stability makes it prone to mutations during transmission and replication, which enhances its transmissibility and evolutionary potential, thereby introducing significant and unpredictable risks (Kong *et al.* 2022). The genome of PDCoV is approximately 25 kb in length, encoding four primary structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (He *et al.* 2023). The highly immunogenic N protein, the most abundantly expressed viral protein in infected cells, is an ideal target for detecting virus-specific antibodies and diagnosing PDCoV infection (Lee and Lee 2015).

Immunochromatography is an innovative diagnostic tool based on specific antigen-antibody interactions. Commonly used detection reagents include colloidal gold and fluorescent microspheres (Xie *et al.* 2014). Compared to conventional laboratory diagnostic methods, colloidal gold test strips offer advantages such as simplicity, rapid results, and easy visual interpretation, making them highly suitable for rapid disease diagnosis(Wang *et al.* 2021). Fluorescent microsphere immunochromatography employs fluorescent lanthanide microspheres as detection reagents, with results observed under ultraviolet illumination, providing significantly higher sensitivity compared to colloidal gold strips (Zhang *et al.* 2023). In this study, we developed two rapid antigen

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detection methods for immunochromatographic strips targeting the conserved N protein of PDCoV: colloidal gold test strips (Fig.1-A) and fluorescent test strips (Fig.1-B).

First, the PDCoV-N protein was purified using a nickel affinity column and verified by SDS-PAGE. The results showed that the N protein was present in both the supernatant and inclusion bodies, with a higher expression level in the supernatant. This indicates that the purified PDCoV-N protein is soluble (Appendix A-a).Following immunization of 6-wk-old BALB/c mice with the N protein as the antigen, four monoclonal antibodies were successfully generated and purified. The integrity of these monoclonal antibodies was confirmed (Appendix A-b), revealing that their heavy chains belonged to the IgG1 isotype and their light chains were of the kappa type (Appendix A-c). Additionally, the antibody titers reached up to 1:102,400 (Appendix A-d). Subsequently, Western blot analysis demonstrated that each monoclonal antibody specifically recognized the N protein (≈45 kDa) of PDCoV in infected LLC-PK1 cells (Appendix A-e).

Four purified monoclonal antibody strains, numbered 1 to 4, were paired in all possible combinations, with each antibody alternately serving as the colloidal gold-labeled antibody or the capture antibody, resulting in 12 unique pairings. Antibody pairing experiments showed that only the mAb2-4 and mAb4-1 combinations yielded positive results at the same antigen concentration. Comparing these two combinations revealed that mAb4-1 produced a significantly stronger signal (Appendix B-a). Consequently, mAb4 was selected as the colloidal gold-labeled antibody, and mAb1 was designated as the capture antibody. The labeling efficiency of mAb4 was evaluated at varying concentrations. At 60 μg mL^{-1}, the colloidal gold solution maintained a stable pink hue, whereas a lighter red color was observed at 30 μg mL^{-1} (Appendix B-b). Therefore, 60 μg mL^{-1} was identified as the optimal concentration for labeling. For colloidal gold test strips, both the test (T) line (coated with mAb1) and control (C) line (using commercial goat anti-mouse antibody) were evaluated across a concentration gradient of 0.5, 0.8, 1.0, 1.5, and 2.0 mg mL^{-1}. Results showed that color development intensity at 1.0, 1.5, and 2.0 mg mL^{-1} was significantly higher compared to 0.5 and 0.8 mg mL^{-1} (Appendix B-c and d). Balancing cost and performance, 1.0 mg mL^{-1} was selected for both T-line and C-line coating.

The same monoclonal antibodies (mAb4 and mAb1) were applied to fluorescent test strips. Labeled antibody concentrations of 20, 40, 80, 125, and 160 μg mL^{-1} were tested. Under negative control conditions, C-line signals were consistently observed across all groups with no detectable T-line signals, although C-line intensity at 20 μg mL^{-1} was notably attenuated compared to higher concentrations. At the same antigen concentration, both the T-line and C-line developed normally at 20, 40, and 80 μg mL^{-1}. However, at 125 and 160 μg mL^{-1}, the T-line developed normally while the C-line failed to develop, likely due to competitive inhibition between the C-line and T-line (Appendix B-e). Consequently, 40 μg mL^{-1} was selected as the optimal concentration for labeled antibodies for the fluorescent test strips. Additionally, the color development time for positive results was monitored following the application of the antigen to the sample pad. Both colloidal gold and fluorescent test strips detected positive results within 5 min (Appendix B-f and g), satisfying clinical requirements for rapid detection.

The PDCoV CC23 strain ($10^{3.8}$ $TCID_{50}$ 0.1 mL^{-1}) was serially diluted twofold, and the diluted samples were analyzed using RT-qPCR. The detection limit of RT-qPCR was determined to be $10^{1.1}$ $TCID_{50}$ 0.1 mL^{-1} (Appendix C). The colloidal gold test strips were subsequently tested with the diluted samples. Positive signals began to weaken at $10^{2.3}$ $TCID_{50}$ 0.1 mL^{-1} and became barely visible at $10^{2.0}$ $TCID_{50}$ 0.1 mL^{-1} (Fig.1-C), indicating the detection limit for the colloidal gold test strip is $10^{2.3}$ $TCID_{50}$ 0.1 mL^{-1}.

During the early stages of viral infection, the viral load is generally low, necessitating the use of highly sensitive detection methods (Marthaler *et al.* 2014). To address this challenge, we developed fluorescent test strips. The PDCoV solution ($10^{3.8}$ $TCID_{50}$ 0.1 mL^{-1}) was serially diluted twofold, and the sensitivity of the test strips was evaluated. Positive bands were clearly visible at $10^{2.0}$ $TCID_{50}$ 0.1 mL^{-1}, weakly positive at $10^{1.7}$ $TCID_{50}$ 0.1 mL^{-1}, and absent at $10^{1.4}$ $TCID_{50}$ 0.1 mL^{-1} (Fig.1-D). Therefore, the detection limit of the fluorescent test strip was determined to be $10^{1.7}$ $TCID_{50}$ 0.1 mL^{-1}.

Specificity testing showed that the colloidal gold and fluorescent test strips reacted exclusively with PDCoV and did not cross-react with porcine hemagglutinating encephalomyelitis virus (PHEV), porcine circovirus type 2 (PCV2), porcine circovirus type 3 (PCV3), pseudorabies virus (PRV), senecavirus A (SVA), bovine herpesvirus 4 (BoHV-4) (Fig.1-E and F), confirming excellent specificity. Stability testing was conducted on strips stored for 30, 60, 90, 120, and 180 d. Regardless of the production batch, all test strips exhibited normal color development over a period of 180 d (Fig.1-G and H), indicating excellent stability.

A total of 89 intestinal and fecal samples from diarrheic piglets were analyzed using colloidal gold strips, fluorescent test strips, and RT-qPCR. Among these samples, 28 tested positive using colloidal gold strips, 30 using fluorescent test strips, and 31 using RT-qPCR. When RT-qPCR was used as the gold standard, the concordance rates were 90.32% (28/31) for colloidal gold strips and 96.77% (30/31) for fluorescent test strips (Table 1). Both methods exhibited high sensitivity; however, the fluorescent test strips demonstrated superior sensitivity compared to the colloidal gold strips. Although the strips showed lower sensitivity than RT-qPCR, the high coincidence rates suggest that these strips can serve as viable options for rapid field detection. Among the two, the fluorescent test strip is the preferred choice due to its superior sensitivity, particularly in scenarios with low viral loads.

In this study, PDCoV N protein was chosen due to its high conservation and lack of cross-reactivity with other viruses, ensuring detection specificity (Wang *et al.* 2021). Clinically, other diarrheal viruses such as porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), swine acute diarrhea syndrome coronavirus (SADS-CoV), porcine enteric alphacoronavirus (PEAV) also cause gastrointestinal symptoms in piglets (Gao *et al.* 2024; Qi *et al.* 2024). Given the similar clinical presentations associated with these swine coronaviruses, differential diagnosis based solely on clinical symptoms is challenging. Future research

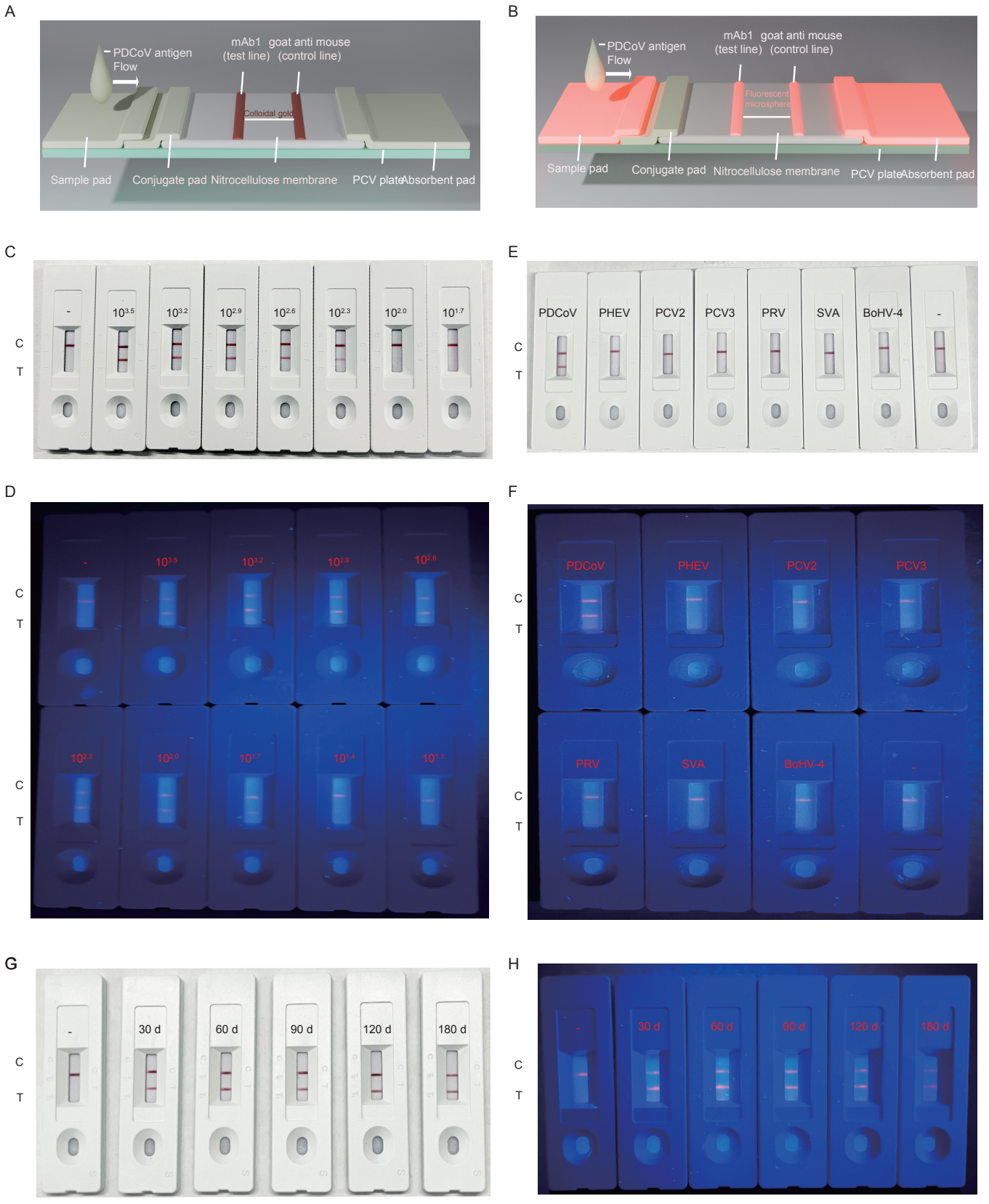


Fig. 1 Schematic representation of colloidal gold and fluorescent test strips and analysis of sensitivity, specificity and stability. A and B, schematic representation of the colloidal gold (A) and fluorescent (B) test strips. C and D, following continuous two-fold dilutions of the PDCoV solution ($10^{3.8}$ $TCID_{50}$ 0.1 mL^{-1}), the sensitivity of the colloidal gold test strips (C) and the fluorescent test strips (D) was evaluated at concentrations of $10^{3.5}$, $10^{3.2}$, $10^{2.9}$, $10^{2.6}$, $10^{2.3}$, $10^{2.0}$, $10^{1.7}$, $10^{1.4}$, and $10^{1.1}$ $TCID_{50}$ 0.1 mL^{-1}. E and F, detection of PDCoV, PHEV, PCV2, PCV3, PRV, SVA, and BoHV-4 was conducted to evaluate the specificity of the colloidal gold test strip (E) and fluorescent test strip (F). G and H, stability of colloidal gold (G) and fluorescent (H) test strips stored for 30, 60, 90,120, and 180 d was evaluated. PDCoV, porcine deltacoronavirus; C, control line; T, test line.

Table 1 Detection and analysis of clinical samples

Samples[1)]	RT-qPCR	Fluorescent test strips	Colloidal gold test strips
Positive	31	30	28
Negative	58	59	61
Coincidence rate	–	96.77% (30/31)	90.32% (28/31)

[1)] The coincidence rate represents the proportion of positive results obtained from colloidal gold and fluorescent test strip tests relative to the positive results confirmed by RT-qPCR.

should focus on developing multiplex diagnostic test strips capable of simultaneously detecting multiple diarrheal viruses.

In summary, we have successfully developed both colloidal gold and fluorescent immunochromatographic test strips for PDCoV detection. Notably, this is the first report of PDCoV antigen detection using fluorescent microsphere immunochromatography. Both test strips can produce results within 5 min and exhibit high sensitivity, strong specificity, and excellent stability. Additionally, they do not require sophisticated equipment, making them robust diagnostic tools for clinical applications.

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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

During the preparation of this manuscript, the authors used Youdao Dictionary to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

Ethical approval

All experiments involving animals were approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, Jilin University, China (permission number KT202302172). All applicable institutional and/or national guidelines for the care and use of animals were followed.

Appendices are associated with this study are available at https://doi.org/10.1016/j.jia.2025.08.001

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