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Development and validation of a lateral flow immunoassay using colloidal gold for the identification of feline herpesvirus type 1

Jianzhong Wang[1*†], Jicheng Qiu[2†], Yiduo Liu[3†], Xianglin Ma[4], Xiaojie Wu[4], Xiaoguang Li[4] and Wei Mao[5*]

Abstract

Feline herpesvirus type 1 (FHV-1) is a common and highly contagious pathogen in domestic cats that causes upper respiratory tract infections and ocular diseases. Accurate and rapid diagnosis of FHV-1 infections is essential for effective disease management and control. In this study, we developed an immunochromatographic lateral flow (ICLF) assay for the rapid and accurate detection of FHV-1-specific antibodies. The assay was founded upon the successful expression and purification of a 26 kDa recombinant glycoprotein B-glycoprotein D (gB-gD) fusion protein, which served as the primary antigen for the test. Rigorous testing for specificity and cross-reactivity confirmed the strip's ability to exclusively detect FHV-1 antibodies, even in the presence of a variety of other feline viruses. The assay demonstrated excellent precision, reproducibility across dilutions, and long-term stability, retaining efficacy for 24 months during storage. Furthermore, clinical sample analysis revealed exceptional sensitivity (97%) and specificity (100%). In conclusion, the ICLF strip developed in this study represents a reliable, highly specific, and stable diagnostic tool for the rapid detection and management of FHV-1 infections in cats.

KeywordS Feline herpesvirus type 1, Immunochromatographic lateral flow strip, gB-gD fusion protein, Rapid detection

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†Jianzhong Wang, Jicheng Qiu and Yiduo Liu contributed equally to this work.

*Correspondence:
Jianzhong Wang
jianzhongwang@cau.edu.cn; wjz2020@foxmail.com
Wei Mao
maowei2014@imau.edu.cn
[1] Shanxi Key Laboratory for Modernization of TCVM, College of Veterinary Medicine, Shanxi Agricultural University, Taigu 030801, China
[2] Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China
[3] Department of Animal Science and Technology, Beijing Vocational College of Agriculture, Beijing 102442, China
[4] Hangzhou Evegen Biotech Co., Ltd, Hangzhou 310018, Zhejiang Province, China
[5] Key Laboratory of Clinical Diagnosis and Treatment Techniques for Animal Disease, Laboratory of Veterinary Clinical Pharmacology, College of Veterinary Medicine, Inner Mongolia Agricultural University, No. 306, Zhaowuda Road, Saihan District, Hohhot 010018, China

Introduction

Feline herpesvirus type 1 (FHV-1), a member of the *Herpesviridae* family and the *alpha-Herpesvirinae* subfamily, primarily causes highly contagious upper respiratory tract infections in domestic cats (Li et al. 2022). This virus poses a significant threat to the health of domestic, stray, and wild felines, particularly affecting feline juveniles (Cohn 2011; Radford, Afonso, & Sykes, 2021; Meng Yang et al. 2024a, b). Serological studies indicate that up to 97% of cats have been exposed to the virus, with more than 80% of these cats becoming persistently infected. Additionally, approximately 45% of infected cats shed the virus in response to stress (Shi et al. 2022). Infected cats commonly exhibit symptoms such as depression, reduced appetite, respiratory distress, coughing, sneezing, conjunctivitis, increased ocular and nasal discharge, and occasionally, primary viral pneumonia or systemic

disease, particularly in young or immunocompromised animals (Jiao et al. 2024; Munks et al. 2017; Synowiec et al. 2023; M. Yang et al. 2024a, b). Infected pregnant cats may lack overt respiratory symptoms but risk fetal death or miscarriage. Kittens born to infected mothers may exhibit drowsiness, weakness, and respiratory symptoms, resulting in lower survival rates (Oliveira et al. 2018). During the acute phase of infection, infected cats become significant sources of viral transmission, shedding large amounts of the virus in their secretions. Additionally, recovered cats or those vaccinated against FHV-1 can still shed the virus, increasing the risk of transmission. FHV-1 is known to establish latent infections following recovery from the initial acute phase. This latent infection can persist within the host, making disease diagnosis and prevention challenging (Maggs 2005). Therefore, the development of reliable methods for FHV-1 detection is crucial for effective disease control.

FHV-1 is an enveloped double-stranded DNA virus. Viral envelope glycoproteins are essential for virus adsorption, invasion, and intercellular spread, and they can also stimulate host cells to produce neutralizing antibodies. Eight types of envelope glycoproteins of FHV-1, namely, gB, gC, gD, gE, gG, gH, gI, and gL, have been identified. These envelope glycoproteins play crucial roles in the recognition of host cell receptors by viruses, the mediation of cell fusion, virus replication, and infection processes (Maeda, K et al., 1998). Among them, four glycoproteins, gB, gD, gH, and gL, are essential for virus replication and infection and are thus referred to as essential glycoproteins. The gB protein, a highly conserved envelope glycoprotein, shows relatively high homology among the constituent proteins of different herpesvirus strains. The gD envelope glycoprotein is one of the major components of the herpesvirus envelope. It plays an important role in virus replication and can also trigger humoral and cellular immunity (Shiau A L et al., 2001). The FHV-1 gD protein can adsorb to feline cell lines but not to cells from dogs, chickens, or pigs (Maeda K et al., 1998), suggesting that the gD protein plays an important role in the host selection of virus infection. Additionally, the FHV- 1 gD protein, the equine herpesvirus gD protein (Mccollum et al. 1956), and the canine herpesvirus gD protein can agglutinate only the red blood cells of their respective hosts, indicating that differences in the biological functions of the gD gene in the herpesvirus family may endow each herpesvirus with unique characteristics.

Inspired by these advantages, the principal antigenic epitopes of the gB and gD proteins were selected, thereby generating a novel fusion antigen protein for the clinical diagnosis of FHV-1. The gB and gD proteins are the primary immunogenic antigens of feline herpesvirus and are highly conserved. Therefore, the fusion expression of the major antigenic epitopes of the gB and gD proteins can not only increase the sensitivity of diagnosis but also reduce cross-reactivity with other pathogens. Characterized by strong specificity, this approach holds great clinical significance and broad application prospects. On the other hand, traditional diagnostic methods such as virus isolation and immunofluorescence antibody (IFA) tests have been used, but these techniques suffer from limitations, including lower sensitivity, time consumption, and susceptibility to interference from biological matrices (Maggs 2005; Maggs et al., 1999). The enzyme-linked immunosorbent assay (ELISA) has also been developed for FHV-1 detection, utilizing infected cell lysates (Munks et al. 2017) or nucleocapsid protein (Lappin, Andrews, Simpson, & Jensen, 2002). However, the need for specialized equipment and trained personnel limits the widespread use of these assays. Polymerase chain reaction (PCR) remains the gold standard for FHV- 1 detection, offering high sensitivity and accuracy. Various PCR techniques have been employed to detect FHV- 1 DNA in different sample types (Cao et al., 2021; Maggs & Clarke, 2005; Mazzei et al., 2019). Nevertheless, PCR is time-consuming, expensive, and requires specialized equipment.

Given these limitations, the development of rapid, high-performance diagnostic methods for FHV-1 is imperative for effective prevention and treatment. Colloidal gold chromatographic detection, a common diagnostic method in veterinary practice, offers advantages in terms of speed, efficiency, and cost-effectiveness (Kim et al., 2014; Takano et al., 2014). In this study, as shown in Fig. 1, we developed a colloidal gold chromatographic assay in combination with a fusion protein for the detection of FHV- 1 antibodies in feline secretions, providing a rapid and reliable tool for the clinical diagnosis of FHV-1.

Results

Expression and purification of the recombinant gB-gD fusion protein

The prokaryotic expression plasmid pET-30a-gB-gD was introduced into *Escherichia coli* BL21(DE3) competent cells for protein expression. Following induction with IPTG, successful expression of the gB-gD fusion protein was achieved within 4 h, as shown in Fig. 2. The molecular weight of the expressed fusion protein was approximately 26 kDa. After confirming successful protein expression, the gB-gD fusion protein was extracted and purified through sonication and subsequent purification steps. The purity of the isolated protein was verified via SDS–PAGE. The SDS–PAGE results, depicted in Fig. 2A, confirmed the presence of a single band corresponding to the 26 kDa molecular weight of the gB–gD fusion protein.

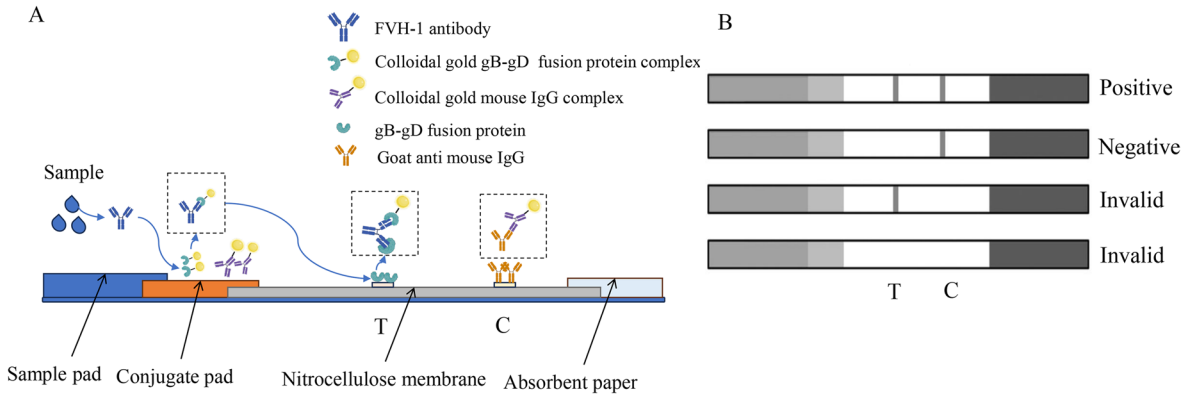

Fig. 1 The structural composition and detection principle of the FVH-1 antibody ICLF strip. **A** Schematic diagram of the colloidal gold test strip. **B** Reaction principle and criteria for interpreting the results of the strip

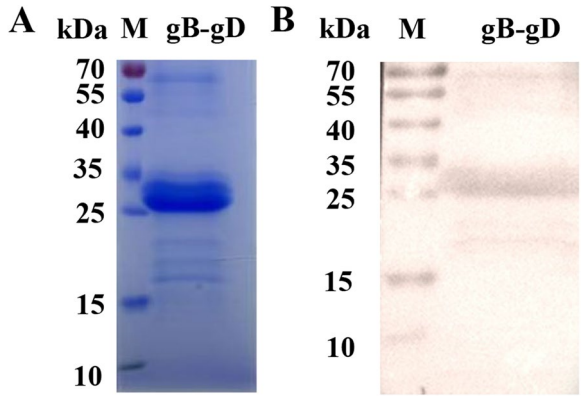

Fig. 2 SDS-PAGE and Western blotting analysis of the purified fusion protein. **A** SDS-PAGE detection of the gB-gD protein. **B** Western blotting with mouse anti-FHV–1 serum confirmed the presence of the recombinant gB-gD fusion protein. M, protein molecular weight marker

Western blotting analysis revealed that the gB-gD fusion protein clearly reacted with anti-FHV-1 serum (Fig. 2B).

Preparation of colloidal gold conjugates

In the novel detection method, the isolated gB-gD fusion protein and goat anti-mouse IgG serve as key components, acting as the test and control lines, respectively.

In negative feline secretion samples, only the control line displayed a distinct purple-red band, confirming the functionality of the detection system. This visual signal serves as an essential reference. In contrast, when positive feline secretion samples were tested, both the test and control lines presented a vivid purple–red color, clearly indicating a positive result. This dual-line appearance signifies the successful detection of the targeted antibodies, confirming their binding to the gB-gD fusion protein on the test line.

Performance of the FHV-1 antibody ICLF (immunochromatographic lateral flow)strips

To evaluate the specificity of the ICLF strip, a critical factor in preventing false positives due to interference from endogenous secretion components, 20 negative secretion samples were tested from various sources, including ocular, nasal, and anal samples from different cats. As shown in Fig. 3, a distinct and uniform purple–red band appeared at the control (C) line on all test strips, confirming the functionality of the kit. Importantly, compared with the positive sample, no corresponding purple–red band was observed at the test (T) line for any of the negative samples in any of the strips. This absence of coloration at the T line confirms that the endogenous components from the feline secretions do not trigger a reaction, ruling out interference and ensuring that the test strip does not yield false-positive results. These findings collectively underscore the high specificity of the test for detecting FHV-1 in cat secretions.

To ensure accurate detection and avoid misclassification due to potential cross-reactivity with other feline viruses, we conducted a comprehensive cross-reactivity assessment. This involved testing a variety of positive samples, including those for feline giardia, feline leukemia virus (FeLV), *Toxoplasma gondii*, feline panleukopenia virus (FPV), feline immunodeficiency virus (FIV), and Feline Coronavirus (FCoV), along with FHV-1-negative samples. All sample statuses were verified via PCR. The results of this evaluation, depicted in Fig. 4, revealed a clear purple–red reaction at the C line for all the samples, confirming the validity of the control. Notably, none of the samples, including those positive for feline giardia, FeLV, *Toxoplasma gondii*, FPV, FIV, or FCoV, presented any detectable purple–red coloration at the T line. These results definitively confirm the absence of cross-reactivity, further validating

Fig. 3 Specificity evaluation of the FVH-1 antibody ICLF strips in different cat secretions. (ES: eye specimen; NS: nasal cavity specimen; NA: anus specimen)

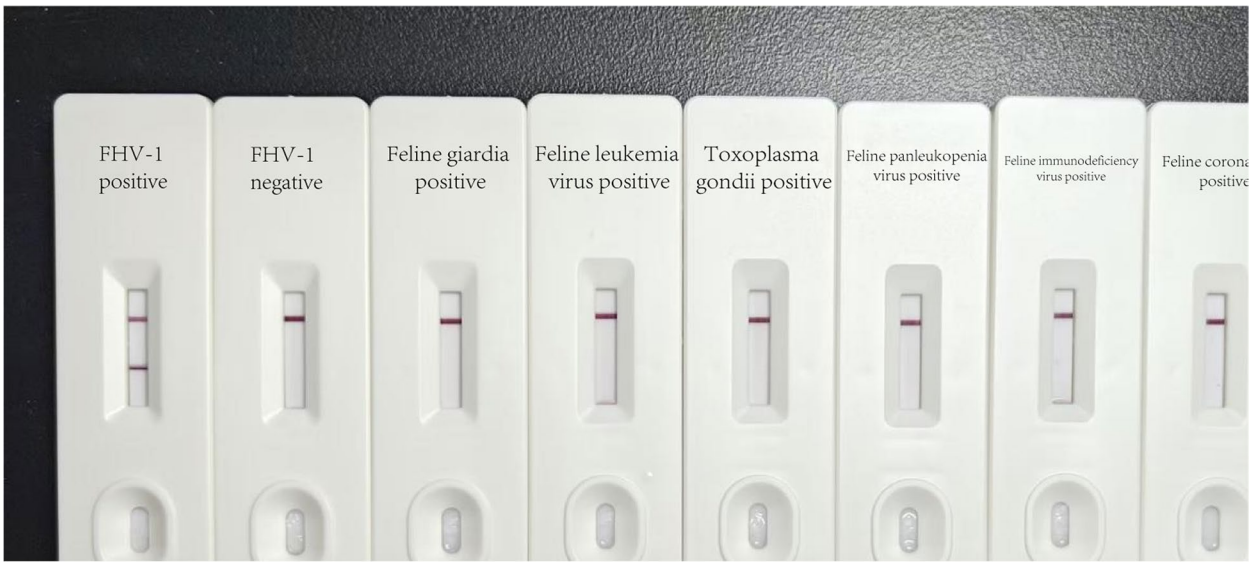

Fig. 4 Evaluation of the cross-reactivity of the FVH-1 antibody ICLF strips

the specificity of the test strip. According to Fig. 5, the T line can be clearly observed on the test line of the 1:8 dilution, whereas only one red band can be noted on the test line of the 1:16 dilution.

To assess the precision and reproducibility of the ICLF strips, a series of positive samples diluted at various concentrations (1:2, 1:4, and 1:8) were tested. As shown in Table 1, all samples tested positive

Fig. 5 Detection limit evaluation of the FVH-1 antibody ICLF strips. (The red rectangular frame indicates the detection limit of the test strip.)

Table 1 Precision and reproducibility evaluation of the FVH- 1 antibody ICLF strip

Source	Dilution	Number of determinations	Results #Neg/#Pos	Precision (%)
Eyes	1:2	20	0/20	100
	1:4	20	0/20	100
	1:8	20	0/20	100
Nasal cavities	1:2	20	0/20	100
	1:4	20	0/20	100
	1:8	20	0/20	100
Anus	1:2	20	0/20	100
	1:4	20	0/20	100
	1:8	20	0/20	100

consistently across five sessions. This indicates outstanding 100% interbatch precision across a wide range of sample matrices, further highlighting the reliability and consistency of the test strips. In addition, eight negative serum samples were collected and tested via a test strip. The results revealed that all the samples included only one control (C) line (data not shown). This further showed the reliability of the test strip.

The stability of the strips was thoroughly evaluated by testing negative samples (assay buffer), weakly positive samples (1:8 dilution), and strongly positive samples (1:2 dilution) stored at temperatures between 15-25°C for up to 24 months. The results, summarized in Table 2, revealed that after 24 months of storage, a clear red line consistently appeared on the T line for both weakly positive and strongly positive samples. The negative samples yielded consistent negative results. These findings provide strong evidence that the FHV-1 antibody ICLF strip maintains exceptional stability and reliability, even after prolonged storage for up to 24 months.

Table 2 Stability of the strips with different storage times determined by EIAV-positive control serum

Time (month)	Negative specimen (assay buffer)			Weak positive (1:8)			Positive (1:2)		
	1	2	3	1	2	3	1	2	3
24	-	-	-	+	+	+	+	+	+
21	-	-	-	+	+	+	+	+	+
18	-	-	-	+	+	+	+	+	+
15	-	-	-	+	+	+	+	+	+
12	-	-	-	+	+	+	+	+	+
9	-	-	-	+	+	+	+	+	+
6	-	-	-	+	+	+	+	+	+
3	-	-	-	+	+	+	+	+	+

Analysis of the clinical samples

The clinical samples were tested via the FHV-1 antibody ICLF strip to assess its reliability in clinical diagnostics. The cats ranged in age from 2 months to 10 years, with a median age of 2.5 years. Immunization status was documented, revealing that 62% of the cats had received routine vaccinations, whereas 38% were either unvaccinated or had an unknown vaccination history. The sources of the cats were diverse, including 45% from pet owners, 30% from animal shelters, 20% from stray populations, and 5% from breeding facilities. As shown in Table 3, out of the 100 clinical samples confirmed as positive by RT-PCR, the ICLF strip correctly identified 97 as positive. In contrast, among the 102 clinical samples confirmed as negative by RT-PCR, the ICLF strip yielded no false positives. This resulted in an impressive sensitivity rate of 97%, demonstrating the strip's ability to accurately detect true positive cases. Additionally, the assay exhibited a perfect specificity rate of 100%, confirming its ability to correctly identify true negative cases without any false positives. To reduce the interference of high antibody concentrations in the sample, the data showed that for serum samples with low antibody concentrations, the sensitivity rate of the test strip could also reach 100% (Figure s1).

Discussion

Since its isolation and identification in 1958, FHV-1 has become widely prevalent across the global feline population, representing a significant threat to both domestic and wild cat species. It is considered one of the most important pathogens affecting feline health. Currently, there is no universally approved vaccine for FHV- 1, and existing vaccines offer only partial protection, failing to provide complete prevention of the disease. As such, the development of a rapid, efficient, and highly sensitive diagnostic method is essential for preventing and managing FHV-1 infections in feline populations.

The envelope glycoprotein gB is FHV-1's primary immunogen. It plays a key role in triggering a robust host immune response, leading to the production of neutralizing antibodies. Additionally, the gB protein is highly homologous across various herpesvirus strains, underscoring its high degree of conservation (Maeda et al. 1993; Tan et al. 2020). This conservation makes gB a prime target for antibody detection assays. Similarly, the gD protein, which is composed of 374 amino acids, is highly conserved and serves as the primary immunogen for herpesviruses(Maeda et al. 1998; Shi et al. 2022). It effectively initiates the host immune response, stimulating the production of neutralizing antibodies. As a result, the gD protein has been utilized in the development of diagnostic reagents (Liu et al. 2019), subunit vaccines (Maeda et al. 1998), nucleic acid vaccines, and anti-gD antibody therapeutic drugs (Wu et al. 2022). Both gB and gD proteins are the primary immunogenic antigens of feline herpesvirus and are highly conserved. Their ability to elicit immune responses, leading to the production of neutralizing antibodies, makes them critical for immune recognition. Therefore, incorporating the key antigenic epitopes of both gB and gD proteins in diagnostic assays not only enhances sensitivity but also reduces the likelihood of cross-reactivity with other pathogens.

The ability to rapidly diagnose FHV-1 onsite is crucial for early detection, timely treatment, and prevention of further transmission. Currently, the use of gold immunochromatographic test strips is the only available method for swift detection of the virus because of their rapidity, time efficiency, and convenience. In this study, fusion proteins were engineered using the highly conserved gB and gD proteins of FHV-1 as detection and capture antibodies. These proteins were employed to develop a colloidal gold immunochromatographic assay for the detection of FHV-1 antibodies in cat secretions. Our results demonstrate that this detection method can be used to visually diagnose FHV-1 antibodies in various cat secretion samples within just 10 min.

Following extensive validation, the colloidal gold test kit developed in this study exhibited excellent specificity and accuracy, effectively detecting both strongly positive and weakly positive samples. Additionally, the kit exhibited outstanding reproducibility when stored at room temperature for up to 24 months. On the basis of these findings, we are confident that our developed detection method can accurately identify FHV-1 antibodies in cat secretions. This conclusion was further supported by the analysis of more than 200 clinical samples. However, this study has limitations. The clinical testing results may not fully reflect the seropositivity of FHV-1 antibodies in pet cats across the broader population. Several factors limit the generalizability of these findings, including the exclusive use of samples from pet hospitals in central cities, the relatively small sample size, and the lack of representation across different age groups. Therefore, the results of this study may not provide a comprehensive

Table 3 Clinical serum sample detection via the FHV-1 antibody ICLF strips

Result		RT–PCR Test	
		Positive	Negative
Feline herpesvirus type-1 antigen rapid test	Positive	97	0
	Negative	3	102
Relative sensitivity		97%	
Relative specificity		100%	

representation of the overall seropositivity of FHV-1 antibodies in cats.

Conclusions

This study developed a rapid colloidal gold immunochromatographic assay for detecting FHV- 1 antibodies in cat secretions, achieving 97% sensitivity and 100% specificity. The results are obtained within 10 min, making it suitable for onsite diagnosis. The assay demonstrated excellent stability, remaining effective for up to 24 months at room temperature. Clinical validation with over 200 samples confirmed its reliability in detecting FHV-1 in veterinary settings. While the assay shows promise, expanded studies with diverse populations are warranted. Further studies with larger and more diverse sample populations are needed to fully assess its broader applicability.

Methods

Experimental materials

The pET30a-gB-gD recombinant plasmid was constructed by Sangon Biotech Co., Ltd. (Shanghai, China). Goat anti-mouse IgG and mouse IgG were obtained from Arista Biologicals, Inc. (Pennsylvania, USA). Kanamycin and isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Chloroauric acid, proclin 300, and Tris were obtained from Sigma–Aldrich (St. Louis, USA). The conjugate pad, sample pad, absorbent paper, and nitrocellulose (NC) membranes were procured from Equibox Biotech Co., Ltd. (Guangdong, China), while polyvinyl chloride (PVC) sheets were supplied by Shanghai Zhuo Yue Technology Co., Ltd. (Shanghai, China). Ultrapure water was obtained from a Millipore system (MA, USA). All other reagents were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Informed consent was obtained from all feline guardians prior to sample collection. The study was approved by Shanxi Agricultural University and conducted in accordance with the institution's Animal Ethics Committee guidelines. The study was evaluated and approved by Shanxi Agricultural University, 22402-23-D-021. All owners were informed of the use of the samples.

Expression, purification, and characterization of the gB-gD fusion protein

The gB-gD fusion protein was designed by selecting hydrophilic segments from the FHV-1 glycoproteins gB and gD on the basis of sequence analysis from NCBI GenBank (gB gene: YP_003331552.2; gD gene: YP_003331589.1) and ExPASy-ProtScale predictions. These segments were fused via a GSGSG linker peptide, resulting in a recombinant sequence aimed at increasing solubility and expression. Therefore, the amino acid sequence of the gB-gD recombinant fusion protein is as follows:

MSTRGDLGKRRRGSRWQGHSGYFRQRCFFP-SLLGIAATGSRHGNGSSGLTRLARYVSFIWIVL-FLVGPRPVEGQSGSTSEQPRRTVATPEVGGTP-PKPTTGSGSGSGSEDSKRSNDSRGESSGPNWI-DIENYTPKNNVPIIISDDDVPTAPPKGMNNQSV-VIPAIVLSCLIIALILGVIYYILRVKRSRSTAYQQLPI-IHTTHHP.

The gB-gD fusion was expressed in *Escherichia coli* BL21 (DE3), and HisSep Ni-NTP agarose resin was used to purify the protein. To obtain high-purity fusion protein, elution buffers of 50 mM Tris–HCl, 0.2 M NaCl, and imidazole at concentrations ranging from 0.05 M to 0.5 M (specifically 0.05 M, 0.1 M, 0.2 M, and 0.5 M) with a pH of 8.0 were used. The elution buffer containing 0.5 M imidazole was subsequently collected. Finally, the collected elution buffer containing the fusion protein was dialyzed to remove imidazole and exchanged into phosphate buffer (PBS, pH 7.4) for subsequent use. The purified gB-gD protein was verified by SDS–PAGE and western blotting with mouse anti-FHV- 1 serum.

Preparation of colloidal gold and its conjugate

Colloidal gold and its conjugates were synthesized following a modified protocol from prior studies (Wang Y., et al. 2014; Zou, S., et al. 2021). Colloidal gold was prepared by boiling a solution of 4 mL of 10% chloroauric acid in 1000 mL of ultrapure water, followed by the addition of 6 mL of 10% trisodium citrate under stirring until the color changed, indicating the formation of colloidal gold. The solution was cooled, filtered through a 0.22 μm filter, and stored at 4°C in a dark bottle for stability.

For the conjugates, the pH of the colloidal gold solution was adjusted to 9.5 for the gB-gD fusion protein conjugate and to 7.0 for the mouse IgG conjugate using 0.2 M K_2CO_3. Each conjugate was formed by adding isolated proteins to adjusted colloidal gold, followed by stabilization with BSA and subsequent centrifugation to isolate the conjugate. The conjugates were resuspended in gold label dilution buffer and stored at 2–8°C in the dark.

Assembly of FHV- 1 antibody immunochromatographic strips

As illustrated in Fig. 1A, a sandwich lateral flow immunochromatographic assay was designed for the detection of FHV-1 antibodies. To establish the test line (T-line), the purified gB-gD fusion protein was diluted to a concentration of 0.9 mg/mL using a spotting diluent prepared with 50 mM Tris and 2% sucrose, pH 8.5, and applied to the NC membrane. Concurrently, the C line was formed by applying goat anti-mouse IgG diluted to a concentration

of 0.3 mg/mL with the same diluent. The NC membrane was then dried overnight at 37°C. The colloidal gold conjugates of mouse IgG and gB-gD fusion protein were separately applied to the conjugate pad and dried for 4 h at 37°C. The structure and reaction principle of the test strip are shown in Fig. 1. After drying, the assembled components, including the NC membrane, conjugate pad, sample pad, and absorbent paper, were cut into 3 mm wide strips, as shown in Fig. 1A, and then mounted onto a PVC backing.

Results, Reaction Principles, and Performance of the ICLF Strip

The specificity, cross-reactivity, sensitivity, precision, and stability of the ICLF strips developed for the detection of FHV-1 antibodies were comprehensively evaluated to confirm their robust and sensitive detection capabilities. After adding the sample and incubating for ten minutes, the results are interpreted as shown in Fig. 1B: both the T and C lines, which are red in color, are positive for FHV-1 antibodies; only the C line, which is red in color, is negative; and the absence of coloration in both lines or a solitary red T line indicates an invalid sample.

Specificity was assessed by testing a series of 20 negative samples collected from various anatomical regions of feline subjects, including the ocular, nasal, and anal areas. Cross-reactivity was evaluated via a panel of clinically positive samples, which included FHV-1, FeLV, FPV, FCoV, FIV, *Toxoplasma gondii*, and feline giardia. These samples were confirmed to be positive through RT–PCR. Precision and reproducibility were evaluated by creating positive samples at various dilutions (1:2, 1:4, 1:8) via assay buffers, as well as negative samples (assay buffer). Each concentration was tested four times daily over five consecutive days, totaling 20 tests per concentration, with RT-PCR serving as the reference standard for comparison. To assess the stability of the ICLF strips under real-world conditions, negative (assay buffer), weakly positive (1:8 dilution), and positive (1:2 dilution) samples were stored at 15-25°C for 3, 6, 9, 12, 15, 18, 21, and 24 months. The stability of the strips was evaluated at each time point.

Analysis of the clinical samples

To assess the practicality and reliability of the ICLF strips developed in this study, clinical samples were tested in parallel with RT–PCR. A total of 202 samples from 21 different animal hospitals located in Shanxi, Beijing, Henan, and Zhejiang Provinces were analyzed. The performance and precision of the ICLF strips were rigorously evaluated in this clinical setting. The following metrics were used to assess the strip's accuracy:

- *Relative* sensitivity = (number of EIAV antibody ICLF strip positives/number of cELISA test positives) × 100%
- *Specificity* = (number of EIAV antibody ICLF strip negatives/number of cELISA test negatives) × 100%
- *Relative accuracy* = [(Both EIAV antibody ICLF strip and cELISA test positive) + (Both EIAV antibody ICLF strip and cELISA test negative)]/total number of samples.

Abbreviations

FHV- 1	Feline herpesvirus type 1
gB	Glycoprotein B
gD	Glycoprotein D
RT–PCR	Reverse transcription polymerase chain reaction
ICLF	Immunochromatographic lateral flow
ELISA	Enzyme-linked immunosorbent assay
IFA	Immunofluorescence
PCR	Polymerase chain reaction
FVR	Feline viral rhinotracheitis
vFeLV	Feline leukemia virus
FPV	Feline panleukopenia virus
FIV	Feline immunodeficiency virus
vFCoV	Feline coronavirus
BSA	Bovine serum albumin
PVC	Polyvinyl chloride

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-025-00170-1.

Supplementary Material 1. Figure S1.

Authors' contributions

Substantial contributions to the research and preparation of the manuscript, Jianzhong Wang, Jicheng Qiu, Yiduo Liu, Xianglin Ma and Xiaojie Wu; substantial contributions to the conception, revision, and critical evaluation of the content, Yiduo Liu, Xiaoguang Li and Wei Mao.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no conflicts of interest. Xianglin Ma and Xiaojie Wu are employees of Hangzhou Evegen Biotech Co., Ltd. Xiaoguang Li is an employer of Hangzhou Evegen Biotech Co., Ltd. No AI-assisted technologies were used in the generation of this manuscript.

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