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Immunochromatographic Strip Test for *Fasciola gigantica* Diagnosis Using Rabbit Polyclonal Antibodies against rFgSAP2

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Abstract

Saposin-like protein 2 (SAP2) plays a critical role in the survival of *Fasciola* species by disrupting the membranes of red blood cells. SAP2 is highly immunogenic and is abundantly present in the excretory-secretory (ES) antigens of both juvenile and adult stages of *Fasciola* spp. These characteristics make SAP2 a promising candidate for immunodiagnosis of both animal and human fasciolosis. Its strong antigenic properties also highlight its potential as a targeted biomarker for the detection of parasitic infections. Currently, the gold-standard diagnostic technique for *Fasciola* spp. infection involves the detection of parasite eggs through microscopic examination. However, this method has low sensitivity, particularly when egg counts are low, a situation commonly observed in chronic infections or following unsuccessful treatment. Therefore, this study aims to fine the alternative method, we developed and evaluated an immunochromatographic strip test using a rabbit polyclonal antibody (PoAb) against rFgSAP2 for the detection of fasciolosis. The recombinant *Fasciola gigantica* SAP2 (rFgSAP2) and rabbit PoAb against rFgSAP2 were purified and concentrated. The optimize pH and buffer were evaluated before constructing the immunochromatographic (IC) strip test. The IC strip test consists of rFgSAP2 conjugated with colloidal gold probes, protein A as a test line, and rabbit PoAb against rFgSAP2 as a control line. Both experimentally and naturally *Fasciola gigantica*-infected serum samples were tested using IC strips. Serum samples from thirty experimentally infected and thirty non-infected mice as well as from fourteen naturally infected and twelve healthy cattle were evaluated using IC strip test. The IC strips were dipped into the sample solution and observed the results within 15 min. These tests demonstrated sensitivity, specificity, accuracy, positive predictive value, negative predictive value, false-positive rate, and false-negative rate of 96.67%, 90.00%, 93.33%, 90.63%, 96.43%, 10.00% and 3.33%, respectively. Thus, the IC strip test could be used for the diagnosis of fasciolosis in cattle and humans in the future.

Keywords: Serological detection, Diagnosis, Immunochromatographic strip test, Fasciolosis, Saposin-like protein 2

Introduction

Fasciolosis is a disease prevalent in tropical regions, caused by infection with *Fasciola* spp., including *Fasciola gigantica* (*F. gigantica*) and *Fasciola hepatica* (*F. hepatica*), affecting both livestock and humans. Fasciolosis results in significant global economic losses estimated at approximately US$3 billion and causes a variety of clinical manifestations in livestock, including weight loss, anemia, liver disease, and, in cases of heavy infection, death [1-5]. The disease also affects human health, with at least 2.4 million people currently infected and over 180 million at risk of infection worldwide [4,5]. The negative impacts on livestock productivity, particularly reduced milk and meat yields, present significant challenges to agricultural sustainability in endemic areas [6]. Additionally, the public health significance of *F. gigantica* is emphasized by its zoonotic potential, as human infections frequently result from the consumption of contaminated aquatic

vegetation [7]. Hepatic dysfunction, biliary obstruction, and cirrhosis comprise the clinical spectrum of human fasciolosis, which extends from asymptomatic to severe complications [2]. Effective disease management requires a timely and precise diagnosis.

Fecal microscopy, a traditional method for detecting parasite eggs, often lacks sensitivity, particularly during early or chronic stages of infection [8,9]. This diagnostic gap underlines the crucial need for novel methods that can be used to diagnose *F. gigantica* infection quickly, accurately, and easily, particularly in resource constrained contexts [1]. Recent progress in immunodiagnostics has facilitated the creation of point-of-care tests for fasciolosis. Several *Fasciola* antigens, including saposin-like protein 2 (SAP2) [10,11], cathepsin L-like proteases (CatL) [12-18], fatty acid-binding proteins (FABP) [19-21], cathepsin B3 (CatB3) [22], glutathione S-transferases (GST) [23,24] and 28.5 kDa tegumental antigens [25], have been investigated as potential targets for serological assays.

SAP2 in *Fasciola* spp. is a protein critical for parasite survival and digestion [20,21]. It is highly expressed in the caecal epithelium and has emerged as a promising candidate due to its strong immunogenicity and demonstrated diagnostic potential [11,28-30]. Earlier investigations employing ELISA tests centered on recombinant *F. gigantica* SAP2 (rFgSAP2) showed impressive sensitivity and precision, particularly in the context of early-stage infections, which exhibited specificity for *F. gigantica* in serum samples from infected animals, with no cross-reactivity observed with ruminant parasite infections [10]. However, despite these promising results, ELISA-based methods still require laboratory infrastructure, trained personnel, and longer processing times, which limit their applicability in field settings and resource-limited environments. This study presents the development and evaluation of an immunochromatographic (IC) strip test utilizing rFgSAP2 for the rapid and accurate detection of *F. gigantica* infection. IC strip tests are known for their cost-effectiveness, simple use, and low equipment requirements, aligning well with the World Health Organization's ASSURED standards for point-of-care diagnostics [31-33].

Materials and methods

Expression and purification of rFgSAP2

The rFgSAP2 was prepared as described in a previous study [27], with some modifications. Briefly, The *Escherichia coli* BL21(DE) contains the recombinant FgSAP2/pET-30b plasmid. The rFgSAP2 was expressed in LB medium containing 100 µg/ml kanamycin and it was induced using 1 M isopropyl β-D-thiogalactoside (IPTG; 1 mM final concentration; Merck, Germany) with shaking at 250 rpm. The expression protein was purified under denatured conditions via Ni- NTA affinity chromatography (Qiagen, Germany). The purified rFgSAP2 was dialyzed and concentrated using SnakeSkin™ Pleated Dialysis Tubing, 3.5K MWCO (Thermo Scientific, USA) and a concentrated centrifugal filter tube (Ultracel-3K, Millipore, Germany). The rFgSAP2 was stored at –20 °C.

Production and purification of rabbit IgG1 antibody against rFgSAP2

Rabbit anti-rFgSAP2 serum was purified using an Affi-Gel Protein A MAPS II Kit (Bio-Rad, USA). Afterward, the purified rabbit PoAb against rFgSAP2 was dialyzed and concentrated using SnakeSkin™ Dialysis Tubing, 3.5K MWCO (Thermo Scientific, USA) in 0.01 M phosphate-buffered saline (PBS) with a pH of 7.4 and a centrifugal filter tube (Ultracel-3K, Millipore, Germany), respectively. The concentrated rabbit PoAb against rFgSAP2 was analyzed using SDS-PAGE and stored at 4 °C.

Determination of IgG against FgSAP2 using indirect ELISA

A 96-well maxisorb microtiter plate was coated with 100 µL of 0.1 µg rFgSAP2 in a coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6) at 4 °C for overnight. The plate was washed three times using 0.05% PBS-Tween 20 (PBS-T), and non-specific binding was blocked using 1% bovine serum albumin (BSA) in 0.01 M PBS at room temperature (RT). Subsequently, all samples including non-infected (n = 30) mice serum and infected mice (n = 30) serum that infected with ten *F. gigantica* metacercariae were obtained from the Research Unit of Vaccine and Diagnosis of Parasitic Diseases, Burapha University. The samples were diluted at a ratio of 1:8

with 0.01 M PBS and incubated for 1 h with shaking. After washing, HRP-conjugated goat anti-mouse IgG (H+L) (Invitrogen, USA) diluted with 0.01M PBS at a ratio of 1 to 5,000 µL was added at 100 µL per well, shook for 1 h, and washed three times. Afterward, the reaction was developed via the addition of 100 µL of TMB substrate per well (SureBlue™, Germany) and incubation in the dark for 15 min at RT. The reactions were stopped by using 1 N HCl, and the optical density was measured at 450 nm with a microplate reader machine (VERSAMAX, Molecular Devices, USA). The cut-off OD value was calculated as mean ± 3SD. OD values were higher than the cut-off was considered positive results, whereas OD values lower than the cut-off were considered negative results.

Preparation of colloidal gold solution of IC strip tests

For conjugation with rFgSAP2, 25 nm of colloidal gold (DCN Diagnostic Inc, USA) was used. The pH of colloidal gold was adjusted to 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 with 0.1 M NaOH in order to obtain the optimal value. Then, one milliliter of colloidal gold was gently mixed with 100 µL of 0.5 mg/mL of rFgSAP2 and rotary shaken for 30 min at RT. The solution was investigated by adding 50 µL of 10% NaCl. For optimal concentration of rFgSAP2 conjugated with colloidal gold, one milliliter of colloidal gold was gently mixed with a serial dilution of rFgSAP2 with 0.1 M phosphate buffer (PB), pH 7.0. The mixed solutions were rotary shaken for 30 min at RT, and then 50 µL of 10% NaCl was added. The solutions were mixed using a rotary shaker for 30 min at RT and observed. Colloidal gold with 0.1 M PB was used as a negative control. A semi-quantitative analysis was conducted using a microplate reader machine (VERSAMAX, Molecular Devices, USA), with an OD of 525 nm. In this study, the working concentration utilized in an assay exceeded the optimum concentration by 20%. Subsequently, one milliliter of colloidal gold was mixed with 100 µL of rFgSAP2. The mixed solution was rotary shaken for 30 min at RT. A blocking solution was added to the tube. The colloidal gold conjugated with rFgSAP2 was centrifuged at 10,000 g for 30 min at 4 °C and the supernatant was discarded. The pellet was resuspended using passive gold diluent (2% sucrose, 0.05% NaN3 in 0.1 M PB) and stored at 4 °C until use.

IC strip tests production

IC strip tests were prepared by assembling several components, including colloidal gold, conjugate pad, absorption pad, sample pad and coated nitrocellulose membrane. For conjugate pad preparation, Glass fibers (STD17, Cytiva, USA) were cut into approximately 4x8 mm pieces and saturated with 4 µL of the colloidal gold solution. The saturated conjugate pads were dried at 37 °C for 15 min and then stored in a desiccator at RT.

The nitrocellulose membrane (AE99, Cytiva, USA) was coated with 0.4 µL of 2 µg of recombinant protein A (test line) and 0.4 µL of 2 µg of purified rabbit IgG PoAb against rFgSAP2 (control line). After coating, the membrane was incubated at RT for 30 min, followed by drying in a humidity-controlled cabinet. The coated nitrocellulose membrane was then blocked by incubation with a blocking solution (2.5% BSA in 0.1 M PB) for 30 min. After that, the membrane was soaked in a 5% sucrose solution at RT for 15 min, and then dried in a cabinet at 37 °C for 40 min. The membrane was stored under dry conditions in a desiccator at RT. Sample and absorption pad preparation, the sample pad (Fusion 5, Cytiva, USA) was soaked with 2% triton X-100 in 0.1 M PB. Both the soaked sample pad and the absorption pad (CF 7, Cytiva, USA) were dried at 80 °C for 1 h. Once dried, the pads were cut into approximately 4×18 mm pieces and stored in a desiccator at RT.

Final step, the IC strips were assembled by manually overlapping and attaching the sample pad, conjugate pad, coated nitrocellulose membrane (with test line and control line), and absorption pad onto a plastic backing plate (**Figure 1**). The completed IC strips were stored in plastic bags with desiccating gel at RT.

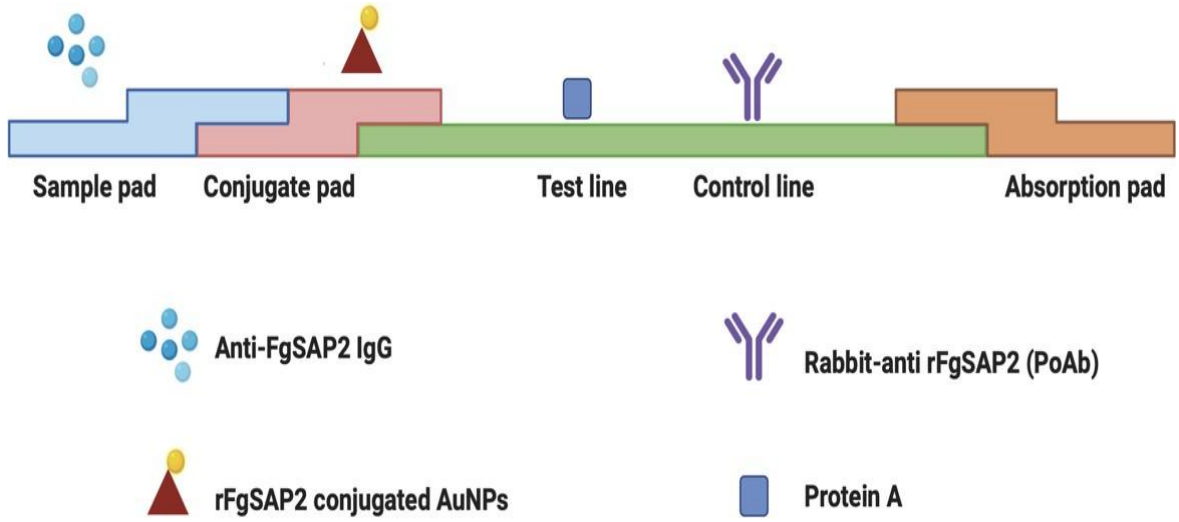

Figure 1 Schematic diagram of IC strip test for antibody detection model.

Parasite antigen and serum samples

All samples, including non-infected mice serum (n = 30), mice serum experimentally infected with ten *F. gigantica* metacercariae (n = 30), non-infected cattle serum (n = 14) that parasitologically confirmed no *F. gigantica* infection but were obtained from ruminant parasites including *Setaria labiato-papillosa* (*S. labiato-papillosa*), *Paramphistomum cervi* (*P. cervi*), *Gastrothylax crumenifer* (*G. crumenifer*), *Calicophoron cotylophorum* (*C. cotylophorum*), and *Gigantocotyle explanatum* (*G. explanatum*), and naturally infected cattle (n = 12) that parasitologically confirmed *F. gigantica*, *S. labiato-papillosa*, *P. cervi*, *G. crumenifer*, *C. cotylophorum*, and *G. explanatum* infection, were obtained from the Research Unit of Vaccine and Diagnosis of Parasitic Diseases, Burapha University. The serum was diluted at a ratio of 1:8 with a running buffer (1% Triton X-100 in 0.1 M Phosphate buffer, pH 7.0) and added to a well of a microplate. The IC strips were then dipped into the sample solution and removed after 15 min to observe the results. If both the test and control lines exhibited positive dots, the samples were evaluated as positive result, indicating the presence of IgG against FgSAP2. Conversely, if the control line showed positive dots on the control line but no positive dots appeared on the test line, the samples were evaluated as negative result. The test was considered invalid if no positive dots appeared on the control line. This animal protocol was established following the Ethical Principles and Guidelines and was approved by the Animal Care and Use Committee of Thammasat University (protocol number 013/2024), and the Animal Care and Use Committee of Burapha University, Thailand (protocol code: IACUC 020/2024).

IC strip test

All serum samples of non-infected mice, experimentally infected mice, non-infected cattle, and naturally infected cattle serum were investigated in duplicate. The results of non-infected mice and experimentally infected mice were used to calculate diagnostic indices, while the results of non-infected cattle and naturally infected cattle were represented as definitive host that was investigated using the IC strip test. Diagnostic indices for the IC strip test including specificity, sensitivity, positive predictive value, negative predictive value, false positive rate, false negative rate, and accuracy, were calculated using the method described by Galen [34]. These values were then calculated and expressed as follows: Sensitivity = [number of true positives/(number of true positives + number of false negatives)]×100; Specificity = [number of true negatives/(number of true negatives + number of false positives)]×100; positive predictive value = [number of true positives/(number of true positives + number of false positives)]×100; negative predictive value = [number of true negatives/(number of true negatives + number of false negatives)]×100; False positive rate = [number of false positives/(number of false positives + number of true negatives)]×100; False negative rate = [number of false negatives/(number of false negatives + number of true positives)]×100; Accuracy = [all number of true

positives and negatives/all tests done]×100.

Results and discussion

Expression and purification of rFgSAP2

The recombinant *F. gigantica* SAP2 was expressed in a prokaryotic expression system, which was induced using 1 mM IPTG. The rFgSAP2 was purified under denature conditions via Ni-NTA affinity chromatography and analyzed using SDS-PAGE. SDS-PAGE showed mature rFgSAP2 at approximately 10 kDa (**Figure 2**).

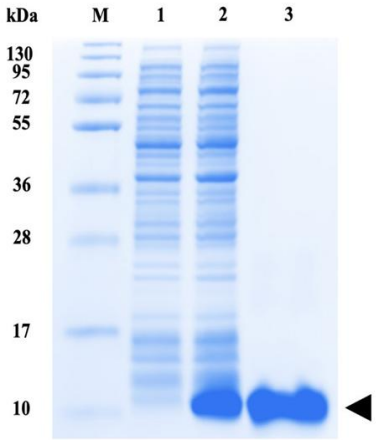

Figure 2 Coomassie® Brilliant blue R250 stained SDS-PAGE analysis of rFgSAP2 was purified using QIAexpress Ni-NTA Protein Purification System. PageRuler™ Plus Prestained Protein Ladder (lane M), non-induced conditions (lane 1), induce conditions (lane 2), and purified rFgSAP2. (lane 3). Black triangles indicate rFgSAP2 at 10 kDa.

Purification and SDS-PAGE analysis of rabbit PoAb against rFgSAP2

Rabbit anti-rFgSAP2 serum was purified using an Affi-Gel Protein A MAPS II Kit. The purified rabbit PoAb against rFgSAP2 was dialyzed and concentrated using SnakeSkin™ Pleated Dialysis Tubing and a centrifugal filter tube. The concentrated rabbit PoAb against rFgSAP2 was analyzed using SDS-PAGE. SDS-PAGE showed that the rabbit PoAb against rFgSAP2 presented heavy and light chains of immunoglobulin G at 50 and 25 kDa, respectively (**Figure 3**).

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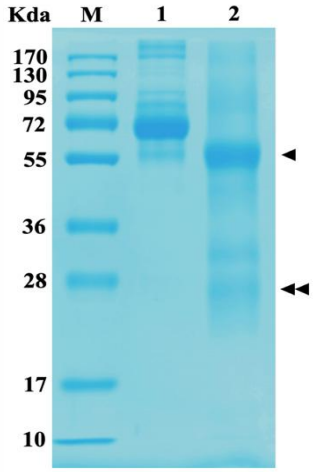

Figure 3 Coomassie® Brilliant blue R250 stained SDS-PAGE analysis of rabbit PoAb against rFgSAP2 was purified using Affi-Gel® protein A MAPS II Kit. PageRuler™ Plus Prestained Protein Ladder (lane M), rabbit serum (lane 1), and purified rabbit PoAb against rFgSAP2 (lane 2). Black triangles and double black triangles indicated that heavy and light chains of IgG, respectively.

Optimal pH of colloidal gold and concentration of rFgSAP2 for conjugation with colloidal gold probe

In this experiment, we utilized rFgSAP2 conjugated with 25 nm colloidal gold at pH 7.0. The concentration was increased by 20% beyond the stability point to enhance particle saturation [33]. Recombinant protein A was employed as the test line, while polyclonal antibodies served as the control line. An examination of the pH of colloidal gold revealed that a pH of 7.0 was optimal for conjugation with rFgSAP2, as shown in **Figures 4(A) - 4(B)**. rFgSAP2 was subjected to two-fold serial dilution for conjugation with the colloidal gold probe, with the concentration ranging from 25 to 200 µg/mL. After adding 10% NaCl, it was found that the optimal amount and lowest concentration of rFgSAP2 for conjugation with the colloidal gold probe was 125 µg/mL (**Figures 4(C) - 4(D)**). The working concentration utilized in the assay exceeded the optimum concentration by 20%.

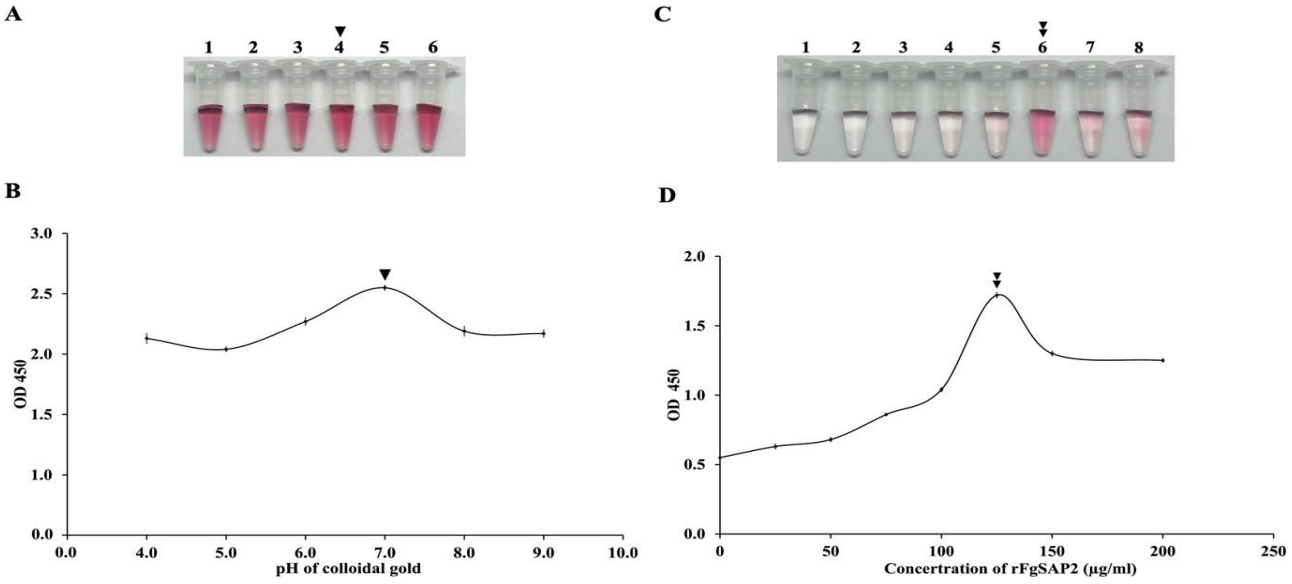

Figure 4 The optimal concentration of pH colloidal gold for conjugation with rFgSAP2 (A): (1) pH 4.0, (2) pH 5.0, (3) pH 6.0, (4) pH 7.0, (5) pH 8.0, (6) pH 9.0 The optimal concentration of rFgSAP2 for conjugation with colloidal gold probe (C): (1) negative control, (2) 25 µg/mL, (3) 50 µg/ mL, (4) 75 µg/mL, (5) 100 µg/mL, (6) 125 µg/mL, (7) 150 µg/mL, (8) 200 µg/mL. The semi-quantitative analysis of pH for colloidal gold conjugation with rFgSAP2 and the optimal concentration of rFgSAP2 for conjugation with the colloidal gold solution are shown in panels B and D, respectively. The single-headed and double-headed arrows indicate the optimal pH and concentration of rFgSAP2 for the experiment.

Detection of IgG against FgSAP2 using Indirect ELISA

The non-infected mice serum (n = 30) and infected mice serum (n = 30) samples were confirmed for IgG against FgSAP2 using an indirect ELISA. All the non-infected mice serum samples presented OD values of 0.04 - 0.05. In contrast, all infected mice serum samples showed high OD values, ranging from 0.16 to 0.65. A cut-off OD value of 0.06 was established, with values below this threshold considered negative and values above considered positive (**Figure 5**).

At present, methods for detecting liver fluke infections have low efficacy and sensitivity. This is primarily because the liver fluke takes approximately two to four weeks to mature and reside in the host's liver or bile ducts [1]. During this period, the parasite sustains itself by secreting protease enzymes to obtain nutrients and protect itself from the host's immune system. Consequently, it is challenging to detect the infection in its early stages; detection is usually possible only when the parasite reaches adulthood [1,7,8]. Immunological methods, which detect antibodies produced by the host in response to antigens

secreted by the parasite, are widely used to diagnose such infections. These methods can accurately and efficiently identify the infection once the host has mounted an immune response, reflecting the presence of the mature parasite [6,11,23]. In our study, we developed an immunochromatographic strip test as an antibody detection model targeting the IgG against FgSAP2, and we used rFgSAP2 in the model to detect IgG against SAP2. SAP2 circulates in the host's bloodstream to facilitate survival. A recent study indicated that SAP2 is a highly immunogenic, thus leading to a robust host immune response, with high levels of antibody production detectable approximately two weeks post-infection [10,26]. Previous studies demonstrated the feasibility of adapting assays for the diagnosis of human fascioliasis by using rSAP2 in ELISA, Western blot and Luminex assays. These diagnostic assays exhibited sensitivity ranging from 87% to 100%, specificity between 95.6% and 98%, and accuracy from 96% to 98.5% [14,29,30,35].

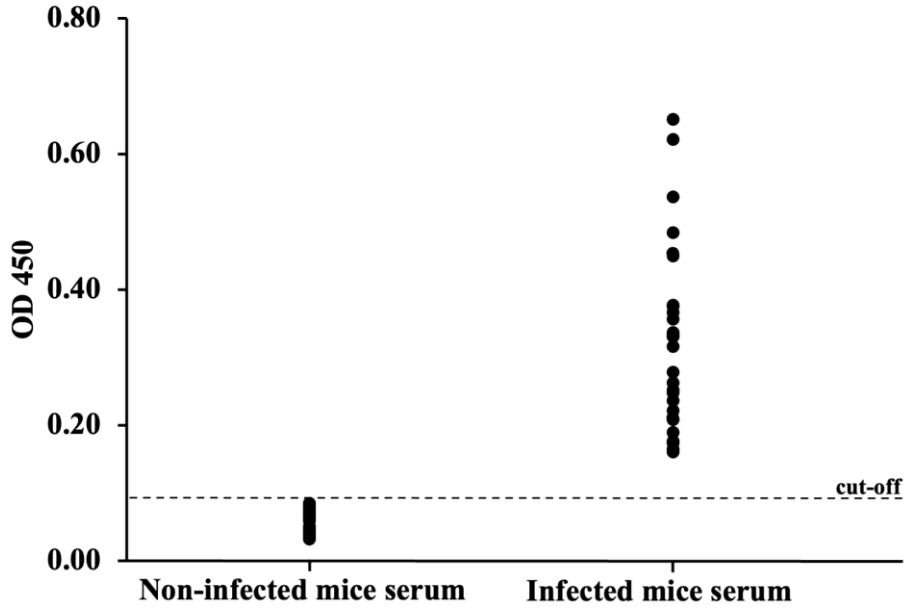

Figure 5 The graph of antibody detection was determined by indirect ELISA. Mice were infected with *F. gigantica* (n = 30), and non-infected mouse serum (n = 30) was classified as positive and negative by indirect ELISA assay.

Antibody detection of IC strip test

Serum samples from non-infected mice (n = 30), infected mice (n = 30), non-infected cattle (n = 14), and infected cattle (n = 12) were diluted with a running buffer at a 1:8 ratio and added to the wells of a microplate. The IC strips were then dipped into the sample solution and removed after 15 min to observe the results. A positive result was indicated by two red dots on both the test and control lines, while a negative result was indicated by a single red dot on the control line. The strips were considered invalid if no dots appeared on the control line. The results were as follows: Twenty-one non-infected mice samples and four non-infected cattle serum samples showed negative results, while two non-infected mice serum samples and one non-infected cattle serum samples showed false-positive results. Moreover, all infected mice and infected cattle serum samples showed positive results (**Figure 6**, **Tables 2** and **3**).

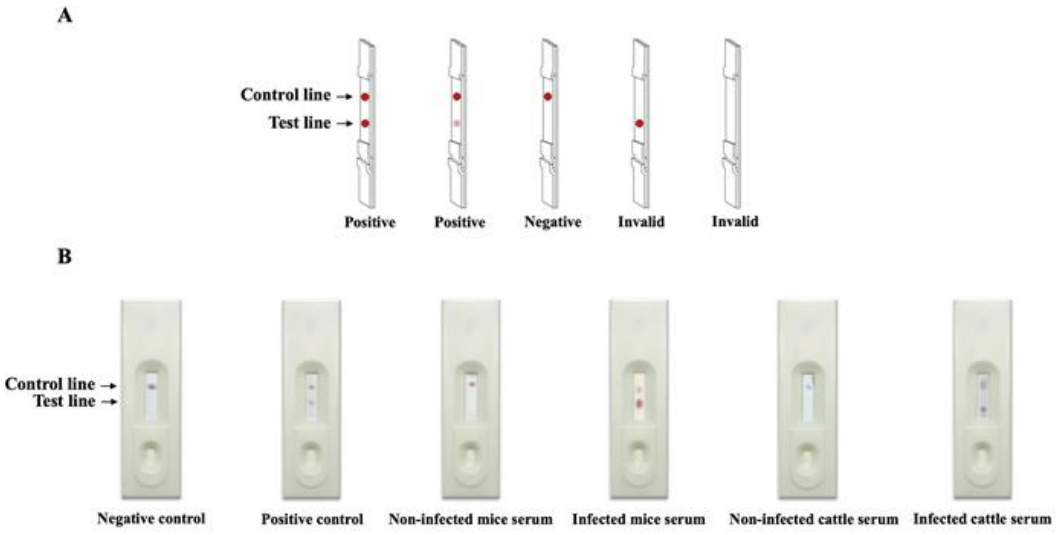

Figure 6 Schematic illustration of IC strip test results. Illustration of IC strip test result interpretation (A). Represented IC strip test results of negative control (0.1 M PB), positive control (rabbit PoAb against rFgSAP2), non- infected mice serum, infected mice serum, non-infected cattle serum, and infected cattle serum (B).

Table 2 Antibody detection of non-infected and infected mice serum using indirect ELISA and IC strip test.

Mice	Indirect ELISA		IC strip test	
	Positive	Negative	Positive	Negative
Non-infected	0	30	3	27
Infected	30	0	29	1
Total	30	30	32	28

Table 3 Antibody detection of non-infected and naturally infected cattle serum using IC strip test.

Cattle	IC strip test	
	Positive	Negative
Non-infected (n = 14)	2	12
Infected (n = 12)	11	1
Total	13	13

Evaluation of IC strip test

The diagnostic values of the IC strips were evaluated using serum from experimental mice, with twenty-three non- infected and infected mice in each group. The IC strip test for antibody detection demonstrated a sensitivity of 96.67%, specificity of 90.00%, positive predictive value of 90.63%, negative predictive value of 96.43%, false-positive rate of 10.00%, false-negative rate of 3.33%, and accuracy of 93.33% (**Table 4**). The sensitivity is better than the previous assay using 28.5 kDa TA antigen at 94.55% [25], and FgCatB3 at 95% [22].

Table 4 Diagnostic values of the IC strip test for fasciolosis detection.

Diagnostic Value	Diagnostic values (%)
Accuracy	93.33
Specificity	90.00
Sensitivity	96.67
False negative rate	3.33
False positive rate	10.00
Positive predictive value	90.63
Negative predictive value	96.43

The IC strip test was investigated using serum samples from healthy and infected experimental mice, as well as healthy and naturally infected cattle. The results showed a sensitivity, specificity, accuracy, and false-positive rate 99.99%, 91.30%, 95.65%, and 8.7%, respectively (**Table 4**). False positives arise because animal serum contains various antibodies, not only those specific to SAP2. Moreover, the recombinant protein A has a high affinity for binding IgG antibodies; consequently, the results may reflect nonspecific binding due to the presence of other antibodies in the serum samples, leading to false positives [36]. The application of recombinant protein A at the test line of the IC strip for the detection of *F. gigantica* in animal serum offers several advantages. The strong binding capacity for mouse IgG guarantees the effective capture and concentration of *F. gigantica*-specific antibodies, which may improve the clarity and interpretation of the test line [31,32]. Furthermore, recombinant protein A's commercial availability and relatively low cost compared to recombinant protein production make it a cost-effective option, particularly for large-scale production or resource-limited settings [37,38]. The development of assays is further simplified when using recombinant protein A, because it eliminates the necessity of recombinant protein expression, and purification. Recombinant protein A, being a well-established technology with clearly defined properties, minimizes the necessity of extensive optimization and validation when compared to novel recombinant proteins. Additionally, its ability to bind to a variety of mouse IgG subclasses could provide a more thorough understanding of the immune response to *F. gigantica* [39]. Nevertheless, careful optimization and validation are crucial to minimizing the risk of potential non-specific binding and to ensure the accuracy and reliability of the assay [40]. Future modifications to the test line may be required to enhance specificity and decrease the occurrence of false positives. The IC strip test presents a compelling choice for diagnosing fascioliasis, owing to its straightforward nature, quick results, and user-friendly interpretation that does not require specialized staff. The antibody detection model exhibited high efficacy in *detecting F. gigantica* infection in blood samples from both infected mice and cattle.

Conclusions

The IC strip test, which uses a rabbit polyclonal antibody against rFgSAP2 as an antibody detection model for fasciolosis, demonstrated sensitivity, specificity, false positive rate, false negative rate, positive predictive value, negative predictive value, and accuracy of 96.67%, 90.00%, 10.00%, 3.33%, 90.63%, 96.43% and 93.33%, respectively. This antibody detection model is highly applicable for field detection of fasciolosis and offers significant clinical value for both prevention and early detection. In the future, the test kits will undergo large-scale validation in cattle, sheep and human, cross-reactivity studies and stability testing, further supporting their reliability for use in endemic areas.

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Declaration of Generative AI in Scientific Writing

The authors acknowledge that generative AI tools (e.g., ChatGPT by OpenAI) were used solely for language editing and grammar correction during the preparation of this manuscript. No content creation or data interpretation was performed by AI.

CRediT author statement

Komsil Rattanasroi: Conceptualization; Methodology; Validation; Investigation; Writing - Original Draft; Visualization; Funding acquisition. **Pornanan Kueakhai**: Methodology; Investigation; Resources; Writing - Review & Editing. **Narin Changklungmoa**: Conceptualization; Investigation; Writing - Review & Editing; Supervision; Project administration; Funding acquisition

References

[1] A Aftab, OK Raina, A Maxton and SA Masih. Advances in diagnostic approaches to *Fasciola* infection in animals and humans: An overviews. *Journal of Helminthology* 2024; **98**, e12.

[2] O Sezgin, E Altintas, S Disibeyaz, U Saritas and B Sahin. Hepatobiliary fascioliasis: Clinical and radiologic features and endoscopic management. *Journal of Clinical Gastroenterology* 2004; **38(3)**, 285-291.

[3] LR Rosas-Hostos Infantes, GA Paredes Yataco, Y Ortiz-Martínez, T Mayer, A Terashima, C Franco-Paredes, C Franco-Paredes, E Gonzalez-Diaz, AJ Rodriguez-Morales, DK Bonilla-Aldana, LV Barahona, AA Grimshaw, DB Chastain, S Sillau, LA Marcos and AF Henao-Martínez. The global prevalence of human fascioliasis: A systematic review and meta-analysis. *Therapeutic Advances in Infectious Disease* 2023; **10**, 1-18.

[4] N Changklungmoa, P Kueakhai, S Apisawetakan, S Riengrojpitak, P Sobhon and K Chaithirayanon. Identification and expression of *Fasciola gigantica* thioredoxin. *Parasitology Research* 2014; **113**, 2335-2343.

[5] S Chansap, W Cheukamud, T Suthisintong, P Kueakhai and N Changklungmoa. Development of multi-epitope Cathepsin L driven short peptide vaccine against *Fasciola gigantica*. *Frontiers in Veterinary Science* 2025; **12**, 1547937.

[6] SC Olaogun, C Byaruhanga, SO Ochai, GT Fosgate and MC Marufu. Comparison of three diagnostic methods to detect the occurrence of *Fasciola* Species in communally grazed cattle in the Northwest province, South Africa. *Pathogens* 2022; **11(12)**, 1398.

[7] J Gonzalez-Miguel, D Becerro-Recio and M Siles-Lucas. Insights into *Fasciola hepatica* juveniles: Crossing the fasciolosis rubicon. *Trends in Parasitology* 2021; **37(1)**, 35-47.

[8] NED Calvani and J Slapeta. *Fasciola* Species introgression: Just a fluke or something more? *Trends in Parasitology* 2021; **37(1)**, 25-34.

[9] DA Zarate-Rendon, J Vlaminck, B Levecke, A Briones-Montero and P Geldhof. Comparison of Kato-Katz thick smear, mini-FLOTAC, and flukefinder for the detection and quantification of *Fasciola hepatica* eggs in artificially spiked human stool. *The American Journal of Tropical Medicine and Hygiene* 2019; **101(1)**, 59-61.

[10] P Kueakhai, N Changklungmoa, K Chaithirayanon, M Phatsara, N Preyavichyapugdee, S Riengrojpitak, K Sangpairoj, P Chusongsang and P Sobhon. Saposin-like protein 2 has an immunodiagnostic potential for detecting fasciolosis gigantica. *Experimental Parasitology* 2015; **151-152**, 8-13.

[11] A Mirzadeh, F Jafarihaghighi, E Kazemirad, SS Sabzevar, MH Tanipour and M Ardjmand. Recent developments in recombinant proteins for diagnosis of human fascioliasis. *Acta Parasitologica* 2021; **66(1)**, 13-25.

[12] P Anuracpreeda, R Chawengkirttikul and P Sobhon. Immunodiagnosis of *Fasciola gigantica* infection using monoclonal antibody-based sandwich ELISA and immunochromatographic

assay for detection of circulating cathepsin L1 protease. *PLoS One* 2016; **11(1)**, e0145650.

[13] P Anuracpreeda, T Srirakam, S Pandonlan, N Changklungmoa, C Chotwiwatthanakun, Y Tinikul, J Poljaroen, K Meemon and P Sobhon. Production and characterization of a monoclonal antibody against recombinant cathepsin L1 of *Fasciola gigantica*. *Acta Tropica* 2014; **136**, 1-9.

[14] B Gottstein, M Schneeberger, G Boubaker, B Merkle, C Huber, M Spiliotis, N Müller, T Garate and MG Doherr. Comparative assessment of ELISAs using recombinant saposin-like protein 2 and recombinant cathepsin L-1 from *Fasciola hepatica* for the serodiagnosis of human fasciolosis. *PLoS Neglected Tropical Diseases* 2014; **8(6)**, e2860.

[15] P Suksomboon, P Kueakhai and N Changklungmoa. *Fasciola gigantica* Cathepsin L1H: High sensitivity and specificity of immunochromatographic strip test for antibody detection. *Tropical Medicine and Infectious Disease* 2023; **8(3)**, 164.

[16] S Wongwairot, P Kueakhai, N Changklungmoa, W Jaikua, V Sansri, K Meemon, S Songkoomkrong, S Riengrojpitak and P Sobhon. Monoclonal antibody against recombinant *Fasciola gigantica* athepsin L1H could detect juvenile and adult cathepsin Ls of *Fasciola gigantica*. *Parasitology Research* 2015; **114(1)**, 133-140.

[17] J Wang, K He, Z Wu, W Jin, W Wu, Y Guo, W Zhang and W Di. Development of a colloidal gold immunochromatographic strip for the rapid detection of antibodies against *Fasciola gigantica* in buffalo. *Frontiers in Veterinary Science* 2022; **9**, 1004932.

[18] S Liu, N Zhang, Q Yu, J Li, X Wang, X Li, X Zhang, S Cheng, T Yue, H Zhang, P Gong and X Zhang. Immunol detection of cathepsin L from *Fasciola hepatica* infection in sheep by monoclonal antibody-based colloidal gold test strip assay. *Acta Biochimica et Biophysica Sinica* 2023; **55(10)**:1668-1671.

[19] G Allam, IR Bauomy, ZM Hemyeda, TM Diab and TF Sakran. Diagnostic potential of *Fasciola gigantica*-derived 14.5 kDa fatty acid binding protein in the immunodiagnosis of bubaline fascioliasis. *Journal Helminthology* 2013; **87(2)**, 147-153.

[20] G Allam, IR Bauomy, ZM Hemyeda and TF Sakran. Evaluation of a 14.5 kDa-*Fasciola gigantica* fatty acid binding protein as a diagnostic antigen for human fascioliasis. *Parasitology Research* 2012; **110(5)**, 1863-1871.

[21] P Anuracpreeda, R Chawengkirttikul and P Sobhon. Immunodiagnostic monoclonal antibody-based sandwich ELISA of fasciolosis by detection of *Fasciola gigantica* circulating fatty acid binding protein. *Parasitology* 2016; **143(11)**, 1369-1381.

[22] P Anuracpreeda, R Chawengkirtikul, Y Tinikul, J Poljaroen, C Chotwiwatthanakun and P Sobhon. Diagnosis of *Fasciola gigantica* infection using a monoclonal antibody-based sandwich ELISA for detection of circulating cathepsin B3 protease. *Acta Tropica* 2013; **127(1)**, 38-45.

[23] K Mokhtarian, AR Meamar, M Khoshmirsafa, E Razmjou, L Masoori, M Khanmohammadi, L Akhlaghi and R Falak. Comparative assessment of recombinant and native immunogenic forms of *Fasciola hepatica* proteins for serodiagnosis of sheep fasciolosis. *Parasitology Research* 2018; **117(1)**, 225-232.

[24] J Duan, N Zhang, S Liu, J Li, P Gong, X Wang, X Li, X Zhang, B Tang and X Zhang. The detection of circulating antigen glutathione S-transferase in sheep infected with *Fasciola hepatica* with double-antibody sandwich signal amplification enzyme-linked immunosorbent assay. *Animals* 2024; **14(3)**, 506.

[25] P Anuracpreeda, C Wanichanon, R Chawengkirtikul, K Chaithirayanon and P Sobhon. *Fasciola gigantica*: Immunodiagnosis of fasciolosis by detection of circulating 28.5 kDa tegumental antigen. *Experimental Parasitology* 2009; **123(4)**, 334-340.

[26] A Mirzadeh, Z Valadkhani, A Yoosefy, J Babaie, M Golkar, ARE Rastaghi, E Kazemi-Rad and K Ashrafi. Expression, purification and *in vitro* refolding of the recombinant truncated Saposin-like protein 2 antigen for development of diagnosis of human fascioliasis. *Acta Tropica* 2017; **171**, 163-171.

[27] P Kueakhai, K Meemon, N Changklungmoa, K

Chaithirayanon, S Riengrojpitak and P Sobhon. Characterization and localization of saposin-like protein-2 (SAP-2) in *Fasciola gigantica*. *Parasitology Research* 2011; **108(6)**, 1493-1500.

[28] P Kueakhai, N Changklungmoa, K Chaithirayanon, S Songkoomkrong, S Riengrojpitak and P Sobhon. Production and characterization of a monoclonal antibody against recombinant saposin-like protein 2 of *Fasciola gigantica*. *Acta Tropica* 2013; **125(2)**, 157-162.

[29] O Figueroa-Santiago, B Delgado and AM Espino. *Fasciola hepatica* saposin-like protein-2-based ELISA for the serodiagnosis of chronic human fascioliasis. *Diagnostic Microbiology and Infectious Disease* 2011; **70(3)**, 355-361.

[30] SH Shin, A Hsu, HM Chastain, LA Cruz, ES Elder, SGH Sapp, I McAuliffe, AM Espino and S Handali. Development of two FhSAP2 recombinant-based assays for immunodiagnosis of human chronic fascioliasis. *The American Journal of Tropical Medicine and Hygiene* 2016; **95(4)**, 852-855.

[31] ML Boisen, D Oottamasathien, AB Jones, MM Millett, DS Nelson, ZA Bornholdt, ML Fusco, DM Abelson, SI Oda, JN Hartnett, MM Rowland, ML Heinrich, M Akdag, A Goba, M Momoh, M Fullah, F Baimba, M Gbakie, S Safa, R Fonnie, L Kanneh, RW Cross, JB Geisbert, TW Geisbert, PC Kulakosky, DS Grant, JG Shaffer, JS Schieffelin, RB Wilson, EO Saphire, LM Branco, RF Garry, SH Khan and KR Pitts. Viral Hemorrhagic Fever Consortium. Development of prototype filovirus recombinant antigen immunoassays. *The Journal of Infectious Diseases* 2015; **212(S2)**, s359-s367.

[32] K Nielsen, WL Yu, L Kelly, R Bermudez, T Renteria, A Dajer, E Gutierrez, J Williams, J Algire and STD Eschaide. Development of a lateral flow assay for rapid detection of bovine antibody to *Anaplasma marginale*. *Journal of Immunoassay and Immunochemistry* 2008; **29(1)**, 10-18.

[33] X Yu, L Wei, H Chen, X Niu, Y Dou, J Yang, Z Wang, Y Tang and Y Diao. Development of colloidal gold-based immunochromatographic assay for rapid detection of goose parvovirus. *Frontiers in Microbiology* 2018; **9**, 953.

[34] RS Galen. Predictive value and efficiency of laboratory testing. *Pediatric Clinics of North America* 1980; **27(4)**, 861-869.

[35] A Mirzadeh, A Yoosefy, E Kazemirad, Z Barati, M Golkar, J Babaie, F Jafarihaghighi and Z Valadkhani. Evaluation of a set of refolded recombinant antigens for serodiagnosis of human fascioliasis. *PLoS One* 2018; **13(10)**, e0203490.

[36] G Drescher, TCBD Vasconcelos, VS Belo, MMG Pinto, JO Rosa, LG Morello and FB Figueiredo. Serological diagnosis of fasciolosis (*Fasciola hepatica*) in humans, cattle, and sheep: A meta-analysis. *Frontiers in Veterinary Science* 2023; **10**, 1252454.

[37] AV Abhyankar, PK Dash, P Saxena, R Bhargava, MM Parida, AM Jana, AK Sahni and PVL Rao. Comparison of a dipstick dot-ELISA with commercial assays for anti-dengue virus IgM antibodies. *Viral Immunology* 2006; **19(4)**, 630-636.

[38] D Zhong, J Zheng, Z Ma, Y Wang and J Wei. Rapid detection of getah virus antibodies in horses using a recombinant E2 Protein-Based immunochromatographic strip. *Animals* 2024; **14(16)**, 2309.

[39] G Vidarsson, G Dekkers and T Rispens. IgG subclasses and allotypes: From structure to effector functions. *Frontiers in Immunology* 2014; **5**, 520.

[40] JR Crowther. ELISA. Theory and practice. *Methods in Molecular Biology* 1995; **42**, 1-218.