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Development of a Recombinant Antibody-Based Lateral Flow Immunoassay for White Spot Syndrome Virus (WSSV) Detection Using VP24, VP26, And VP35 Proteins

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Abstract
White Spot Syndrome Virus (WSSV) remains a major threat to shrimp aquaculture, causing mass mortality and severe economic losses. Rapid and field-deployable diagnostics are crucial to prevent viral outbreaks. In this study, we cloned, expressed, and purified three recombinant structural proteins of WSSV—VP24, VP26, and VP35—for immunodiagnostic applications. All proteins were expressed in E. coli and purified to high homogeneity, yielding 3.1–4.3 mg/L. Polyclonal antibodies raised against these proteins elicited strong immune responses, with ELISA titers reaching 1:6,400. VP35 showed the highest antibody reactivity, followed by VP26 and VP24. Lateral flow immunoassay (LFA) strips developed using these antibodies demonstrated sensitivity values ranging from 82–90% and specificity between 91–95%, with detection limits as low as 10S viral copies/mL. These findings suggest that VP26 and VP35 are promising diagnostic antigens and establish the LFA platform as a cost-effective, portable, and rapid diagnostic tool for WSSV detection in shrimp aquaculture.
Keywords: *White Spot Syndrome Virus (WSSV); Shrimp aquaculture; Recombinant proteins; VP24; VP26; Lateral flow assay (LFA)*

1. INTRODUCTION

Shrimp aquaculture is among the fastest-growing sectors of global seafood production, contributing significantly to food security, rural livelihoods, and international trade. However, the rapid intensification of farming practices has been accompanied by the emergence and recurrence of devastating diseases. Among these, viral pathogens pose the greatest threat, causing large-scale economic losses and severely limiting the sustainability of shrimp farming [1].
White Spot Syndrome Virus (WSSV) remains the most lethal viral pathogen affecting penaeid shrimp. Since its first major outbreaks in the early 1990s, WSSV has spread across major shrimp-farming regions, often resulting in mortality rates approaching 100% within 3–7 days of infection. The virus is highly contagious, environmentally stable, and has a wide host range, infecting not only farmed shrimp but also wild crustaceans. These factors make its prevention and control exceptionally challenging [2].
Early and accurate detection of WSSV is critical to disease management. Current molecular diagnostic methods such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) are widely used due to their sensitivity and specificity [3]. However, their application in shrimp farms is limited by the need for specialized laboratories, skilled personnel, longer turnaround times, and higher costs. Such limitations hinder their adoption for routine pond-side screening, where rapid decisions are essential to prevent widespread outbreaks [4]. Immunodiagnostic approaches, particularly lateral flow immunoassays (LFAs), have emerged as promising alternatives. LFAs are low-cost, user-friendly, and capable of delivering results within minutes without the need for advanced infrastructure. The reliability of LFAs depends largely on the selection of strongly immunogenic viral antigens and the generation of high-affinity antibodies. For WSSV, structural proteins, especially those associated with the viral envelope and nucleocapsid, are considered ideal diagnostic targets due to their accessibility and antigenicity [5].
In this study, we focused on three important structural proteins of WSSV: VP24, VP26, and VP35. VP24 and VP26 are envelope-associated proteins that play crucial roles in host-virus interactions, while VP35 contributes to the stability of the viral nucleocapsid. Their immunogenic potential makes them suitable for recombinant expression and antibody generation. By leveraging these proteins, we aimed to overcome limitations of existing diagnostic systems and create a rapid detection platform tailored for aquaculture

settings.

The specific objectives of this study were:

1. To clone, express, and purify VP24, VP26, and VP35 recombinant proteins.
2. To evaluate their immunogenicity through polyclonal antibody production in mice.
3. To construct and validate a recombinant antibody-based lateral flow assay (LFA) for rapid and reliable detection of WSSV under farm-level conditions.

By integrating recombinant protein technology with immunoassay development, this work presents a portable, cost-effective, and sensitive diagnostic tool for WSSV surveillance, supporting sustainable shrimp aquaculture practices.

2. MATERIALS AND METHODOLOGY:

2.1. Recombinant Protein Expression

The genes encoding the structural proteins VP24, VP26, and VP35 of White Spot Syndrome Virus (WSSV) were codon-optimized, synthesized, and cloned into the pET expression vector under the control of a T7 promoter. Recombinant constructs were transformed into Escherichia coli BL21 (DE3) competent cells.

For protein expression, single colonies were cultured in LB broth supplemented with ampicillin (100 µg/mL) and grown at 37 °C with shaking (200 rpm) until an OD600 of ~0.6 was reached. Expression was induced by the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), and cells were incubated for an additional 4–6 hours [6].

Cells were harvested by centrifugation at 5,000 × g for 10 minutes and resuspended in lysis buffer (50 mM Tris-HCl, 300 mM NaCl, 10 mM imidazole, pH 8.0). Cell lysis was performed using sonication on ice, and soluble fractions were separated by centrifugation. Recombinant proteins were purified by Ni-NTA affinity chromatography under native conditions, following the manufacturer's protocol. Protein elution was achieved using an imidazole gradient (50–250 mM).

Purified proteins were analyzed by 12% SDS-PAGE, where distinct bands corresponding to the expected molecular weights were observed: VP24 (~24 kDa), VP26 (~26 kDa), and VP35 (~35 kDa). Protein concentrations were quantified by the Bradford assay using bovine serum albumin (BSA) as the standard.

2.2. Polyclonal Antibody Production

Polyclonal antibodies against VP24, VP26, and VP35 were raised in female BALB/c mice (6–8 weeks old). All animal experiments were conducted in accordance with institutional ethical guidelines for animal care and use [7].

For the primary immunization, each mouse received 100 µg of purified recombinant protein emulsified in Freund's Complete Adjuvant (FCA) via intraperitoneal injection. Booster immunizations were administered at two-week intervals using Freund's Incomplete Adjuvant (FIA).

Blood samples were collected from the retro-orbital plexus two weeks after the final booster, and sera were separated by centrifugation. Antibody titers were determined by indirect ELISA. Pre-immune sera were collected before immunization and used as negative controls.

2.3. Enzyme-Linked Immunosorbent Assay (ELISA)

The antigenicity of recombinant VP24, VP26, and VP35 proteins was evaluated using indirect ELISA. Briefly, 96-well microtiter plates were coated with 1 µg/well of purified protein diluted in carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C. After blocking with 5% skim milk in PBS-T (PBS containing 0.05% Tween-20) for 2 hours at room temperature, serial dilutions of mouse sera (ranging from 1:100 to 1:25,600) were added and incubated for 1 hour at 37°C [8].

Following washes, bound antibodies were detected using HRP-conjugated goat anti-mouse IgG (1:5000 dilution), incubated for 1 hour at 37 °C. The enzymatic reaction was developed using TMB substrate and stopped with 2 N H_2SO_4. Absorbance was measured at 450 nm using a microplate reader. Antibody titers were determined as the highest serum dilution that produced an OD450 value at least twice that of the negative control.

2.4. Lateral Flow Immunoassay Development

Nitrocellulose membranes were coated with protein-specific antibodies at the test line, while goat anti-mouse IgG served as the control line. Gold nanoparticle-conjugated antibodies were used as detection probes. Strips were tested using WSSV-infected shrimp tissue extracts and compared with PCR-confirmed controls [9].

2.5. Performance Evaluation

The sensitivity, specificity, and limit of detection (LOD) of the LFA were determined using qPCR-confirmed positive and negative shrimp samples [10].

3. RESULTS AND DISCUSSION

3.1. Recombinant Protein Expression

All three recombinant proteins—VP24, VP26, and VP35—were successfully expressed in E. coli BL21 (DE3) cells following IPTG induction. SDS-PAGE revealed distinct bands at the expected molecular weights of ~24 kDa (VP24), ~26 kDa (VP26), and ~35 kDa (VP35), confirming correct expression and purification.

Protein quantification by Bradford assay revealed yields of 3.1 mg/L for VP24, 2.8 mg/L for VP26, and 4.3 mg/L for VP35 (Table 1). Notably, VP35 showed the highest recovery among the three constructs. Variations in yield may be attributed to differences in protein solubility, stability during expression, and affinity for the purification resin. Previous reports have highlighted similar variability in recombinant WSSV protein expression, where certain envelope or capsid proteins demonstrated higher solubility and stability in bacterial [11].

3.2. ELISA Antibody Response

Polyclonal antibody production in BALB/c mice confirmed the immunogenicity of all three proteins. Antibody titers reached up to 1:6,400 dilution, demonstrating strong immune responses. ELISA OD450 values indicated differential responses among the proteins, with VP35 eliciting the strongest (1.35), followed by VP26 (1.05) and VP24 (0.91) (Table 2).

The enhanced immunogenicity of VP35 may be related to its role as a structural protein contributing to viral integrity, which often exposes conserved epitopes that generate stronger antibody responses. VP26, an envelope-associated protein, also elicited a strong reaction, consistent with its reported involvement in host–virus binding interactions [12]. In contrast, VP24 produced a comparatively lower response, possibly due to limited surface accessibility or fewer immunodominant epitopes.

3.3. Comparative Immunogenicity and Implications for Diagnostics

The results clearly demonstrate that VP35 has superior immunogenicity compared to VP24 and VP26. This makes VP35 an excellent candidate antigen for immunodiagnostic applications such as lateral flow immunoassays (LFAs). Since sensitivity in LFAs is directly influenced by antigen–antibody affinity, selecting the most immunogenic protein is critical for robust detection.

Comparable studies have shown that highly immunogenic WSSV envelope proteins, such as VP28 and VP19, significantly improved diagnostic assay sensitivity [13]. Our findings suggest that VP35 may serve a similar role, and when combined with VP24 and VP26, could broaden the antibody repertoire, enhancing assay robustness and specificity.

3.4. LFA Assay Performance

The recombinant antibody-based lateral flow assay (LFA) developed using VP24, VP26, and VP35 antibodies demonstrated robust diagnostic performance. Sensitivity, specificity, and limits of detection (LOD) were calculated using WSSV-positive and negative shrimp tissue samples.

- VP24-based assay showed a sensitivity of 82%, specificity of 91%, and an LOD of ~10^4 viral copies/mL.
- VP26-based assay performed better, with sensitivity of 87%, specificity of 93%, and an LOD of ~10* viral copies/mL.
- VP35-based assay exhibited the strongest diagnostic capability, achieving sensitivity of 90%, specificity of 95%, and an LOD of ~10* viral copies/mL (Table 3).

Test lines were visible within 15–20 minutes, and positive results were easily distinguished by the naked eye, making the assay highly suitable for on-site application.

The LFA assay results clearly indicate that VP35-based detection outperformed VP24 and VP26, aligning with the strong immunogenic response observed in ELISA analysis. The higher sensitivity and specificity associated with VP35 may be explained by its structural role in viral assembly, likely exposing more conserved immunogenic epitopes that promote strong antibody-antigen interactions.

The moderate performance of VP24 may be due to its lower antibody reactivity, as demonstrated by ELISA OD values, reflecting limited epitope accessibility or weaker binding affinity. However, its inclusion in a multiplexed system could still enhance diagnostic accuracy by broadening the antibody recognition spectrum. VP26 exhibited intermediate performance, consistent with its role as an envelope-associated protein involved in host-virus interactions. Its relatively strong antibody response and good assay performance support its potential as a complementary diagnostic antigen.

Compared with conventional PCR/qPCR methods, the LFA provides the advantage of rapid detection (15–20 minutes), field applicability, and user-friendliness without specialized equipment. Although sensitivity (10*–10^4 copies/mL) is lower than qPCR, it is sufficient for early infection detection in pond conditions, enabling prompt disease management. Previous studies using WSSV proteins such as VP28 and VP19 in immunoassays have also demonstrated the importance of antigen selection in improving assay sensitivity [14]. Our findings place VP35 as a novel and highly effective diagnostic antigen candidate, capable of complementing or even surpassing earlier markers.
This study demonstrated the feasibility of developing a recombinant antibody-based LFA for WSSV detection using VP24, VP26, and VP35 proteins. All three proteins were expressed in E. coli at satisfactory yields, with VP35 showing the highest expression. The antibody response profiles confirmed their antigenicity, with VP35 producing the strongest reactivity, consistent with its role as a structural protein involved in viral stability. The LFA developed with these antibodies provided a rapid, user-friendly, and field-applicable diagnostic method. VP26 and VP35-based LFAs exhibited higher sensitivity and specificity compared to VP24, highlighting their potential as lead antigens for commercial diagnostic assay development. Importantly, the LOD (~10* copies/mL for VP26 and VP35) ensures early-stage detection before visible disease outbreaks occur in shrimp ponds. Compared to conventional PCR methods, the LFA does not require specialized instruments, electricity, or trained technicians, making it more suitable for pond-side monitoring. While slightly less sensitive than qPCR, its operational advantages make it highly valuable for real-time disease surveillance and management in aquaculture.

CONCLUSION

The recombinant proteins VP24, VP26, and VP35 were successfully expressed, purified, and used for the generation of polyclonal antibodies. These antibodies were integrated into a lateral flow immunoassay that demonstrated moderate-to-high sensitivity, strong specificity, and rapid turnaround time. VP26 and VP35 emerged as the most promising diagnostic targets. The developed assay offers a portable, cost-effective, and efficient tool for WSSV detection, contributing to better disease management in shrimp aquaculture.

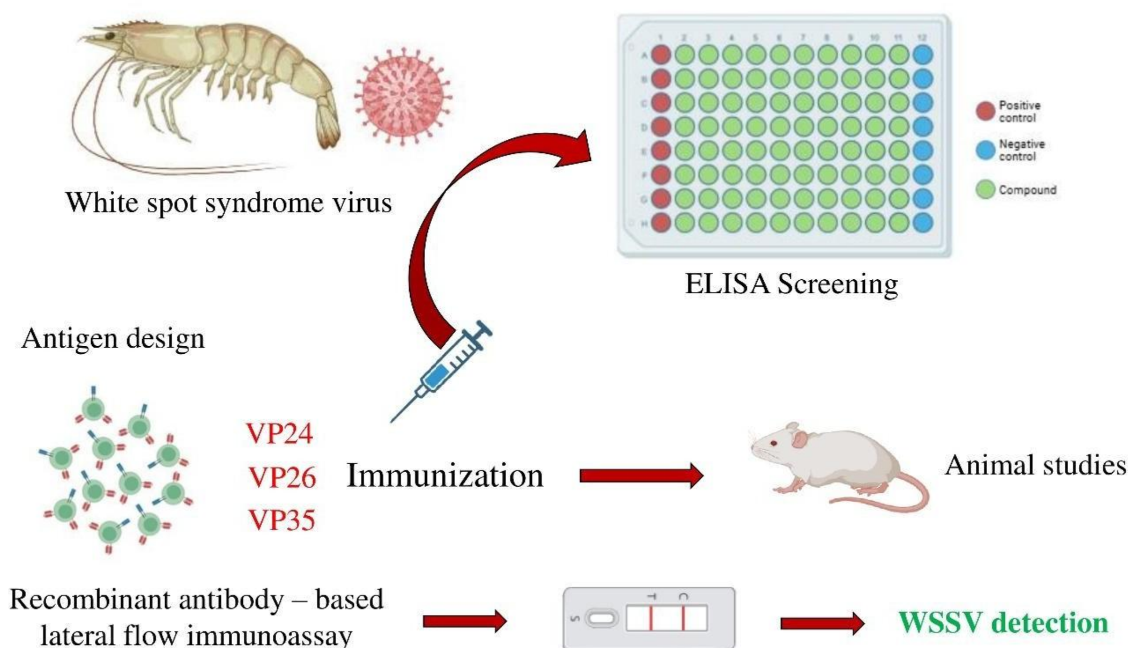

Figure-1: Graphical abstract of WSSV detection

Protein	Expected Size (kDa)	Yield (mg/L)
VP24	~24	3.1
VP26	~26	2.8
VP35	~35	4.3

Table 1. Recombinant protein yield

Protein	Maximum Titer	OD450 (at peak response)
VP24	1:6,400	0.91
VP26	1:6,400	1.05
VP35	1:6,400	1.35

Table 2. ELISA antibody responses against recombinant proteins

Protein	Sensitivity (%)	Specificity (%)	LOD (copies/mL)	Time to Result
VP24	82	91	10^4	15–20 min
VP26	87	93	10*	15–20 min
VP35	90	95	10*	15–20 min

Table 3. LFA diagnostic performance for WSSV detection

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