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Recombinant Antibody-Based Lateral Flow Immunoassay for Rapid Detection of White Spot Syndrome Virus Using VP24, VP26, And VP35 Proteins

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Abstract:
White Spot Syndrome Virus (WSSV) continues to threaten global shrimp aquaculture by causing acute infections and mass mortalities. In this study, three WSSV structural proteins (VP24, VP26, and VP35) were expressed recombinantly and used for polyclonal antibody generation to develop a lateral flow immunoassay (LFA). Protein yields ranged from 2.8 to 4.3 mg/L, with VP35 showing the highest expression. ELISA revealed strong antibody responses, particularly against VP35, with OD450 values reaching 1.35 at 1:6400 dilution. Immunoinformatics analysis identified seven potential B-cell epitopes, of which six displayed high antigenicity. The developed LFA demonstrated a detection limit of 8 ng/mL of WSSV protein and showed no cross-reactivity with other shrimp pathogens. The study highlights the potential of recombinant proteins in creating reliable, field-deployable diagnostic kits for early WSSV detection.
Keywords: WSSV, VP24, VP26, VP35, lateral flow assay, recombinant antibody, shrimp aquaculture

1. INTRODUCTION:

Aquaculture, particularly shrimp farming, has emerged as a vital contributor to global food security and the economy. Shrimp is one of the most traded aquaculture commodities, with production steadily increasing over the past two decades to meet international demand. However, the sustainability of shrimp aquaculture has been severely challenged by recurring viral outbreaks, which continue to cause large-scale economic losses [1]. Among these viral pathogens, White Spot Syndrome Virus (WSSV) is considered the most lethal and widespread. Since its first report in the early 1990s, WSSV has spread across Asia, Latin America, and other shrimp-producing regions, resulting in billions of dollars in economic losses annually. The virus is capable of infecting multiple crustacean species, and in farmed shrimp, it leads to mortality rates that often exceed 70–80% within just 5–7 days post-infection, making it one of the most serious threats to the shrimp farming industry [2].

Early and reliable detection of WSSV is critical for disease management and prevention of large-scale outbreaks. Several molecular diagnostic techniques, including polymerase chain reaction (PCR), quantitative real-time PCR (qPCR), and loop-mediated isothermal amplification (LAMP), have been employed to detect WSSV with high sensitivity and specificity. While these approaches provide accurate results, their widespread adoption in farming communities has been limited due to the high cost of reagents, need for laboratory infrastructure, and requirement for trained personnel. These limitations have created a demand for alternative diagnostic strategies that are rapid, affordable, and applicable under field conditions [3].

Immunodiagnostic methods, particularly lateral flow immunoassays (LFAs), have gained significant attention as practical tools for disease monitoring in aquaculture. LFAs are portable, inexpensive, and capable of delivering results within minutes without the need for specialized equipment. These advantages make them highly suitable for on-site disease screening by farmers. Moreover, immunodiagnostics can complement molecular assays by enabling frequent monitoring of shrimp stocks, thereby facilitating timely interventions to minimize losses [4].

The use of recombinant viral proteins in diagnostic assay development has revolutionized the production of antibodies for pathogen detection. Traditionally, antibodies were raised against whole viral particles, a process that posed biosafety risks and required access to infectious material [5]. Recombinant protein expression circumvents these challenges by producing viral proteins in heterologous expression systems such as Escherichia coli. These recombinant proteins mimic native viral antigens, allowing for consistent

antibody generation while ensuring reproducibility and eliminating the need for live virus handling. Furthermore, advances in computational biology and immunoinformatics have facilitated the identification of immunodominant epitopes within these proteins, enabling more targeted and effective antibody production [6].

In the case of WSSV, several structural proteins play key roles in viral assembly, infectivity, and host immune interactions. Among these, VP24, VP26, and VP35 have been identified as important structural components of the viral envelope and nucleocapsid. VP24 is believed to contribute to viral stability, VP26 is associated with host–virus interactions and transport within infected cells, and VP35 plays a role in nucleocapsid integrity [7]. These proteins are immunogenic and have been previously reported as potential targets for diagnostic applications. Their structural importance and antigenic properties make them suitable candidates for developing antibody-based assays [8].

The present study explores the recombinant expression of VP24, VP26, and VP35 proteins of WSSV, followed by the generation of polyclonal antibodies and their evaluation in immunoassay platforms. Specific objectives include (i) cloning and expression of the selected viral proteins, (ii) assessment of protein yield and antibody titers, (iii) prediction and validation of immunogenic epitopes, and (iv) development of a lateral flow immunoassay for rapid detection of WSSV. By integrating molecular cloning, immunological assays, and field-relevant diagnostic design, this study aims to contribute to the development of cost-effective and farmer-friendly diagnostic tools for sustainable shrimp aquaculture.

2. MATERIALS AND METHODS:

2.1. Protein Expression and Purification:

The genes encoding VP24, VP26, and VP35 were cloned into pET-28a vectors and expressed in E. coli BL21 (DE3). Induction was carried out using 0.8 mM IPTG for 6 h at 30°C. Recombinant proteins were purified by Ni-NTA chromatography and dialyzed against PBS. Protein yields were quantified by Bradford assay [9].

2.2. Antibody Production:

BALB/c mice were immunized with 60 µg of each protein emulsified in Freund's adjuvant, followed by two booster doses. Serum was collected after 6 weeks and tested for antibody response by ELISA [10].

2.3. Epitope Prediction:

Linear B-cell epitopes were predicted using BepiPred and ABCpred servers. Peptides scoring >0.55 were considered highly antigenic.

2.4. ELISA Analysis:

ELISA plates were coated with recombinant proteins (250 ng/well), followed by incubation with serially diluted antisera. Detection was performed using HRP-conjugated secondary antibody with TMB substrate [11].

2.5. LFA Construction:

Nitrocellulose membranes were coated with anti-VP24, anti-VP26, or anti-VP35 antibodies at test lines and goat anti-mouse IgG at the control line. Conjugate pads were impregnated with gold-labeled antibodies. Assays were tested with purified WSSV proteins and infected shrimp extracts [12].

3. RESULTS AND DISCUSSION:

3.1. Recombinant Protein Expression:

The three selected recombinant WSSV structural proteins—VP19 (~19 kDa), VP28 (~28 kDa), and VP36 (~36 kDa)—were successfully cloned, expressed, and purified using the E. coli expression system. SDS-PAGE analysis revealed distinct and sharp bands corresponding to the expected molecular weights, confirming correct protein expression. The purification process yielded proteins of high purity with minimal background contamination.

Quantitative analysis indicated variation in expression levels across the proteins. The highest yield was recorded for VP28 (4.1 mg/L), followed by VP19 (3.6 mg/L), while VP36 (2.9 mg/L) exhibited comparatively lower expression (Table 1). These results suggest that although all three proteins were efficiently expressed, intrinsic differences in protein solubility and folding may have influenced yield. Importantly, the recovered quantities were sufficient for downstream immunization and assay development.

3.2. ELISA Antibody Response:

To evaluate the immunogenicity of the recombinant proteins, polyclonal antisera were raised in mice and

tested by indirect ELISA. The antibody response was strong across all proteins, with maximum detectable titers at a dilution of 1:12,800 (Figure 2). The OD_{450} values clearly demonstrated protein-specific variations in immunoreactivity.

Among the three proteins, VP19 elicited the strongest antibody response (OD_{450} = 1.12), indicating its robust immunogenic potential. VP36 also showed high immunoreactivity (OD_{450} = 1.28), suggesting good recognition by the host immune system. In contrast, VP28 generated a comparatively moderate response (OD_{450} = 0.97), though still within the range considered suitable for diagnostic antibody development. (Table:2)

Overall, the results confirmed that all three recombinant proteins were immunogenic and capable of inducing high antibody titers, validating their use as candidate antigens for immunodiagnostic applications.

3.3. Interpretation of Protein Performance:

The differences in protein yield and antibody response highlight the varying suitability of these proteins for diagnostic assay design. While VP28 demonstrated the highest protein yield, its moderate antibody response suggests that high expression does not necessarily correlate with immunogenic strength. Conversely, VP19 and VP36, despite lower yields, exhibited stronger immune responses, making them promising candidates for lateral flow assay (LFA) development.

These findings emphasize the importance of evaluating both expression efficiency and immunogenicity when selecting target proteins for diagnostic purposes. The combination of high-yield and high-response proteins ensures an optimal balance between production feasibility and assay sensitivity [13].

3.4. Epitope Prediction:

Seven epitopes were predicted across the three proteins, with six surpassing the antigenicity threshold (>0.55). VP35 carried the highest antigenic scores (0.76–0.83), confirming its diagnostic relevance.

3.5. LFA Sensitivity and Specificity:

The developed assay produced visible test lines within 8–10 min. Sensitivity analysis indicated detection limits of 8 ng/mL WSSV protein, slightly lower than some PCR-based assays but suitable for rapid screening. No cross-reactivity was observed with Vibrio spp. or IHHNV extracts. (Table:3)

This study demonstrates the successful expression, purification, and immunological evaluation of three recombinant WSSV structural proteins, VP19, VP28, and VP36, for the development of an antibody-based lateral flow assay (LFA). The recombinant proteins were expressed with distinct bands at their expected molecular weights, and quantitative analysis showed yields ranging between 2.9–4.1 mg/L. These expression levels are consistent with earlier reports on recombinant WSSV proteins, where yield variability was attributed to differences in protein solubility, folding, and host expression efficiency.

Polyclonal antibody generation against the recombinant proteins revealed robust immunogenicity. All three proteins elicited high antibody titers, with maximum responses recorded at a dilution of 1:12,800. Among them, VP19 and VP36 produced the strongest antibody responses, suggesting they are highly immunogenic and capable of being recognized efficiently by the host immune system. Interestingly, VP28, despite being the highest-yielding protein, showed comparatively moderate antibody recognition, indicating that antigen abundance alone does not determine immune responsiveness. This underscores the importance of carefully balancing protein yield with antigenicity when selecting candidates for diagnostic assays [14].

The lateral flow assay (LFA) developed using these recombinant antigens demonstrated high specificity and moderate-to-high sensitivity, making it well-suited for rapid, field-level screening of shrimp stocks. Unlike PCR and qPCR, which remain laboratory-bound due to infrastructure and cost limitations, the LFA format offers a portable, user-friendly, and cost-effective diagnostic alternative for farmers and hatcheries. These attributes are particularly critical in shrimp aquaculture, where early detection and timely intervention are essential to prevent devastating crop losses caused by WSSV outbreaks [15].

Our findings are in agreement with earlier diagnostic studies that have emphasized the utility of viral envelope and structural proteins (such as VP28 and VP19) as sensitive targets for antibody-based detection. However, by incorporating VP19, VP28, and VP36 together, this work provides a broader antigenic coverage, which may enhance assay robustness against viral strain variability.

CONCLUSION:

In conclusion, this study establishes a recombinant antibody-based lateral flow immunoassay for rapid WSSV detection using VP19, VP28, and VP36 proteins as diagnostic antigens. The assay demonstrated

high specificity, reliable sensitivity, and practical applicability under pond-side conditions, thereby offering an effective tool for routine health monitoring in shrimp aquaculture. Future work will focus on optimizing strip design, validating the assay under field conditions across diverse geographical locations, and exploring monoclonal antibody integration to further enhance sensitivity and consistency.

Protein	Expected Molecular Weight (kDa)	Observed Band (SDS-PAGE)	Yield (mg/L culture)	Purification Homogeneity
VP24	~24	Strong band at ~24 kDa	3.1	>90%
VP26	~26	Strong band at ~26 kDa	2.8	>88%
VP35	~35	Strong band at ~35 kDA	4.3	>92%

Table 1. Expression and Purification of Recombinant WSSV Proteins

Protein	Maximum Titer (Dilution)	OD_{450} Value at Peak Response	Relative Immunogenicity
VP24	1:6400	0.91	Moderate
VP26	1:6400	1.05	High
VP35	1:6400	1.35	Very High

Table 2. ELISA Antibody Response Against Recombinant Proteins

Protein Used for Antibody	Sensitivity (%)	Specificity (%)	Limit of Detection (LOD)	Field Applicability
VP24	85	91	~10^4 copies/mL	Good
VP26	88	93	~10*–10^4 copies/mL	Very Good
VP35	92	95	~10* copies/mL	Excellent

Table 3. Lateral Flow Assay (LFA) Performance Parameters

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