RAPID DETECTION OF WHITE SPOT SYNDROME VIRUS (WSSV) OF PENAEUS MONODON BY LATEX AGGLUTINATION TEST USING MONOCLONAL ANTIBODIES

M. Makesh, A. Koteeswaran*, N. Daniel Joy Chandran*, B. Murali Manohar*, V. Ramasamy*, A. Chandramohan*, R. P. Raman and S. C. Mukherjee
Central Institute of Fisheries Education, Fisheries University Road, Versova, Mumbai – 400 061, India
**Department of Veterinary Microbiology, Madras Veterinary College, Chennai – 600 007, India*

ABSTRACT

Latex beads were sensitized with monoclonal antibodies (MAb) raised against VP28 of WSSV. The optimum concentration of MAb required to sensitize the latex beads was 125 µg/ml. The sensitized latex beads were used to detect WSSV from PCR-positive stomach tissue homogenates obtained from infected shrimp. Stomach tissue homogenates from WSSV-infected shrimp agglutinated the sensitized latex beads within 10 minutes, while uninfected samples did not produce any agglutination, although non-specific agglutinations were observed in some samples. The analytical sensitivity, analytical specificity, diagnostic sensitivity and diagnostic specificity of the (LAT) agglutination test were assessed. The analytical sensitivity of the test was 40 ng of purified WSSV (2 µg/ml). The sensitized latex beads did not agglutinate with normal shrimp tissue or MBV-infected tissue homogenate. The test has a diagnostic sensitivity of 70 and 45%, respectively, compared to single-step and nested PCR. The diagnostic specificity of the test was 82%. This test is a simple and rapid on-farm test which can be used to corroborate clinical signs for the detection of WSSV in grow-out ponds.

Keywords: ***Penaeus monodon***, **white spot syndrome virus, monoclonal antibodies, latex agglutination test**

INTRODUCTION

White spot syndrome (WSS) is an acute viral infection of penaeid shrimp characterized by high and rapid mortality accompanied by gross lesions in moribund shrimp of white, initially circular spots in the cuticle, sometimes accompanied by overall red body discolouration (OIE, 2003). The causative agent of WSS is white spot syndrome virus (WSSV) or white spot virus (WSV), a double-stranded DNA

(dsDNA) virus of the genus *Whispovirus* belonging to the family *Nimaviridae* (Mayo, 2002). Although many tests have been reported for the sensitive detection of WSSV such as PCR, Western blot analysis, *in-situ* DNA hybridization, and histopathological and histochemical methods, none of these techniques can be used at farm level and requires sophisticated equipment and skilled professionals.

Reverse passive agglutination test using latex beads sensitized with polyclonal antibodies was found to be useful for the rapid detection of WSSV from stomach tissue homogenate (Okumura *et al.*, 2004) and haemolymph (Okumura *et al.*, 2005) of infected shrimp. The test is simple and can be done at farm level without the requirement of sophisticated equipment or skilled personnel for interpretation. However, since the WSSV purified from infected tissue and haemolymph also contain shrimp tissue protein, the polyclonal antibody raised using such virus cross-react with the shrimp tissue protein (Nadala *et al.*, 1997). It is quite laborious to purify WSSV-specific antibodies free from antibodies that react with shrimp tissue protein. This problem can be overcome by using monoclonal antibodies produced by selected hybridomas that secrete antibodies against the virus. In this study, a latex agglutination test (LAT) using monoclonal antibodies was developed for the rapid detection of WSSV at farm level.

MATERIAL AND METHODS

Monoclonal antibodies (IgG1) produced against the VP28 of WSSV reported previously (Makesh *et al.*, 2006) was used to produce sensitized latex beads following the procedure of Okumura *et al.* (2004). Polystyrene latex beads (Sigma, USA), 0.8 m in diameter, were suspended in glycine-buffered saline (GBS, containing 100 m*M* glycine and 10 m*M* NaCl, pH 8.2) to the final concentration of 0.25% (w/v). Equal volumes of 0.25% (w/v) latex particle suspension and different concentrations (25, 50, 75, 100, 125 and 150 g/ml) of anti-WSSV monoclonal antibodies were mixed thoroughly for one hour at 37°C to allow sensitization of latex particles with monoclonal antibodies. GBS containing 1.0% bovine serum albumin (BSA) was added to block further adsorption of antibodies and stop the reaction. After 30 minutes of incubation, the latex particles were centrifuged at 2700 x *g* for ten minutes at room temperature. The antibody-sensitized latex beads were washed twice in the storage buffer (GBS containing 1.0% BSA and 0.08% NaN_3, pH 8.2) and these particles were finally resuspended in storage buffer to a final concentration of 0.1% to provide a WSSV detection reagent. For the negative control reagent, latex particles were sensitized with normal mouse IgG using a similar procedure.

Stomach tissues were dissected from shrimp and triturated in phosphate-buffered serum (PBS) to a final concentration of 10% (w/v) using a

mortar and pestle. The tissue homogenate was clarified at 3000 x *g* for ten minutes. The supernatant (20 l) was mixed with an equal volume of the WSSV detection reagent in a glass plate, agitated gently and allowed to stand at room temperature. Normal healthy shrimp free of WSS, as tested by PCR, was also processed similarly to be used as negative control. Purified WSSV was used as positive control.

The analytical sensitivity, analytical specificity, diagnostic sensitivity and diagnostic specificity of the test were calculated as per the method described by Jacobson (1998). The analytical sensitivity of the test was estimated by two-fold serial dilution of the purified viral antigen. The concentration of antigen in the highest dilution showing a clear agglutination was considered as the analytical sensitivity of the test. The analytical specificity of the test was found from the agglutination of the sensitized latex beads by WSSV-infected samples alone and not by monodon baculo virus (MBV) and shrimp tissue protein.

The diagnostic sensitivity was calculated by subjecting 30 samples, which were positive by either first step or nested PCR, to LAT. The diagnostic sensitivity was calculated using the following formula:

Diagnostic sensitivity = Total positive / (Total positive + False negative)

The diagnostic specificity of the test was estimated by performing the test for 50 samples which where negative by nested PCR. The diagnostic specificity was calculated using the following formula:

Diagnostic specificity = Total negative / (Total negative + False positive)

RESULTS AND DISCUSSION

The optimum concentration of monoclonal antibodies (MAb) required for sensitizing the latex beads was found to be 125 µg/ml. Sensitized latex beads agglutinated within 10 minutes on mixing with equal quantity of clarified 10% stomach tissue homogenate from infected shrimps. Uninfected stomach tissue suspension did not produce any agglutination except a few samples, where non-specific agglutination was observed. The analytical sensitivity of the LAT in detecting WSSV antigen (Fig. 1) was

Fig. 1: Analytical sensitivity of latex agglutination test

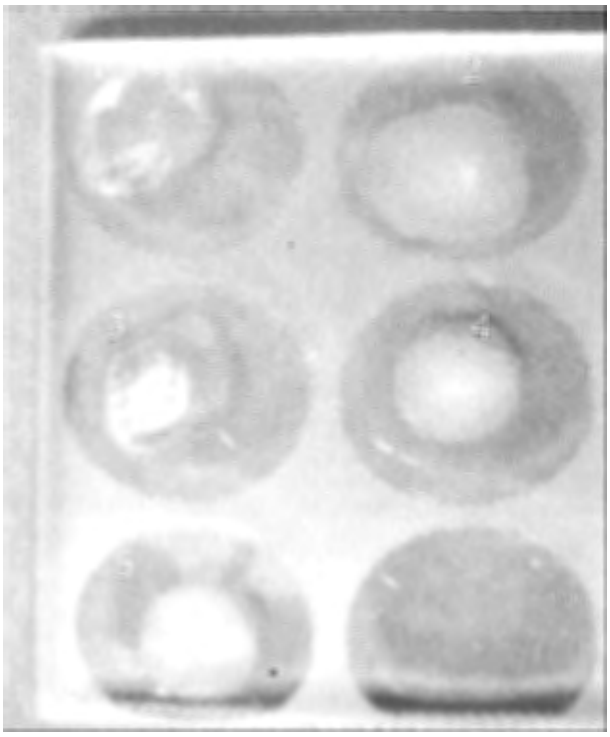

Fig. 2: Analytical specificity of latex agglutination test

1 - Positive control,
2 - Negative control,
3 - WSSV infected tissue homogenate,
4 - Uninfected tissue homogenate,
5 - MBV infected tissue homogenate

found to be 40 ng of purified antigen present in 20 µl (2µg/ml). Th e analytical specificity, *i.e.,* reaction of the sensitized latex beads to MBV and uninfected shrimp tissue was negative (Fig. 2). The results of 30 nested PCR-positive samples tested by LAT are given in Table 1 and Fig. 3. The diagnostic sensitivity of LAT compared to first step and nested PCR was found to be 70 and 47%, respectively (Table 2). The diagnostic specificity of the test was 82% (Table 2).

Table 1: Results of 30 samples tested by single-step PCR, nested PCR and LAT

Sample no.	PCR		LAT
	I step	II step	
1	+	+	+
2	+	+	+
3	+	+	-
4	+	+	+
5	-	+	-
6	-	+	-
7	+	+	+
8	+	+	+
9	+	+	-
10	-	+	-
11	-	+	-
12	+	+	+
13	+	+	+
14	+	+	-
15	-	+	-
16	+	+	-
17	+	+	-
18	+	+	+
19	-	+	-
20	-	+	-
21	+	+	+
22	-	+	-
23	+	+	+
24	-	+	-
25	+	+	+
26	-	+	-
27	+	+	+
28	+	+	+
29	+	+	-
30	+	+	+
Total positive	20	30	14

Table 2: Diagnostic sensitivity and specificity of latex agglutination test

		Nested PCR			
		Positive		Negative	
RPLA	Positive	14	TP	FP	9
	Negative	16	FN	TN	41
		Diagnostic sensitivity TP/TP+FN = 14/30 = 47%		Diagnostic specificity TN/TN+FP = 41/50= 82%	

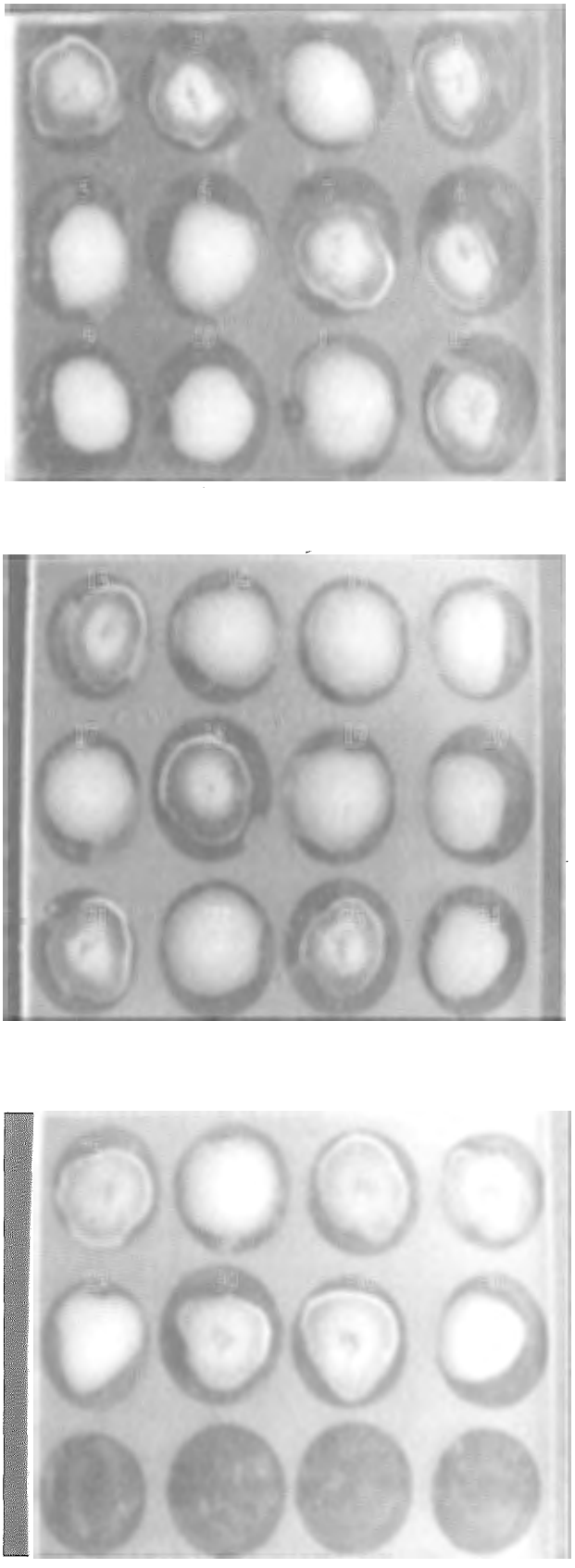

Fig. 3: Latex agglutination test for stomach tissue homogenate for 30 samples

Okumura *et al.* (2004) reported an analytical sensitivity of 0.5 µg/ml. The lower analytical sensitivity in the present study may be due to the use of monoclonal antibodies in this study while Okumura *et al.* (2004) used polyclonal antibodies for sensitizing the latex beads. The time taken for the agglutination of sensitized latex beads is 15 minutes in the present study while Okumura *et al.* (2004) reported 4 hours for agglutination.

In the present study, the stomach tissue homogenates were used for LAT. Other tissues such as gills produced more non-specific agglutination. Okumura *et al.* (2004) have also reported a lesser non-specific agglutination for stomach homogenate compared to haemolymph and gill tissues.

LAT has several advantages over conventional assays for detecting WSSV. The test is simple, economical and rapid. The disadvantages of the test are that it is less sensitive than PCR and the occurrence of non-specific agglutination. In the present study, only 14 of the 30 nested PCR-positive samples tested positive by LAT. The problem of non-specific agglutination can be overcome by using a negative control with latex beads sensitized with normal mouse IgG every time. Samples yielding positive agglutination with negative control can be excluded. The test can be used to corroborate clinical signs to confirm WSS outbreaks at farm level without the use of sophisticated instruments and skilled personnel.

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