Development and Evaluation of a Dot Blot Analysis for the Detection of White Spot Syndrome Baculovirus (WSBV) in *Penaeus monodon*

Poh-Shing Chang*, Dong-Hung Tasi and Yu-Chi Wang

Department of Aquaculture, National Kaohsiung Institute of Marine Technology Kaohsiung, Taiwan, R.O.C.

(Received December 15, 1997)

Three batches of *Penaeus monodon* (total of 66 shrimp) were tested for the presence of WSBV by 2-step PCR, *in situ* hybridization and dot blot analysis using a nucleic acid probe. The results of *in situ* hybridization and 2-step PCR were consistent and used as the standard for comparison. Except for a low number of false negatives, results of dot blot using either DNA or tissue homogenate from the gill, pleopod, eyestalk and hemolymph paralleled the results of 2-step PCR and *in situ* hybridization. A quick dot blot analysis was also developed, which took only 4–4.5 h to produce results. We conclude that dot blotting using tissue homogenate from the eyestalk provides a rapid, cost effective and convenient diagnostic method for WSBV detection in the field and for screening *P. monodon* broodstock.

Key words: white spot syndrome baculovirus, WSBV, *Penaeus monodon*, dot blot, diagnosis

The significance of molecular based diagnostic techniques to disease management in aquaculture is rapidly growing. Both nucleic acid probes (chemically labelled DNA fragments that hybridize to homologous target DNA) and polymerase chain reaction (amplification of selected DNA sequences) are powerful diagnostic tools for the identification of pathogens, and they provide rapid and accurate results in disease diagnosis (Lightner *et al.*, 1992; Mialhe *et al.*, 1992; Vivares and Guesdon, 1992). In shrimp aquaculture, the white spot syndrome baculovirus (WSBV) which has been found in many shrimp species in Asian countries has had a significant negative impact on the shrimp farming industry by causing substantial production and economic losses (Chou *et al.*, 1995; Wang *et al.*, 1995; Lightner, 1996). Recently, 2-step PCR and an *in situ* hybridization technique were developed to diagnose WSBV infection (Chang *et al.*, 1996; Lo *et al.*, 1996a).

In Taiwan, most of the broodstock of *Penaeus monodon* for seed production are captured from the wild population in the coastal waters around southern Taiwan (Chen 1990). However, the prevalence of WSBV has been shown to be high in these wild brooders (Lo *et al.*, 1997), and once captured, even uninfected brooders are liable to be exposed to the virus. For example, it has now been shown that some of the wild decapods which

are used as feed for the broodstock are also susceptible to WSBV infection (Lo *et al.*, 1996b; Chang *et al.*, 1998; Wang *et al.*, 1998). Although there is still no direct evidence that WSBV in broodstock can be vertically transmitted to their offspring from an infected ova, some WSBV-infected spawners do infact produce WSBV-positive larvae (Lo *et al.*, 1997).

Screening to produce specific pathogen-free (SPF) broodstock is now one important strategy for viral disease control in shrimp aquaculture (Wyban *et al.*, 1992). For this to be practicable in commercial hatchery farms, however, non-lethal, easier, more cost effective and more convenient diagnostic methods need to be developed and applied. The two currently preferred methods of WSBV detection, *in situ* hybridization and 2-step PCR, are time-consuming and require access to standard histological laboratory equipment or a PCR machine (Thermal Cycler). In the present study, a dot blot hybridization assay using either a tissue homogenate or extracted DNA was compared with PCR and *in situ* hybridization with respect to both sensitivity and specificity, and an attempt was also made to develop a quick dot blot hybridization method. Differences in cost, convenience and accuracy are also discussed.

* E-mail: pschang@mail.nkimt.edu.tw

Materials and Methods

Shrimp

This study investigated *P. monodon* (10–15 g) collected from Kaohsiung in Taiwan between July and August, 1997. Ten shrimp were randomly collected from culture farm A on July 8, 1997 during an outbreak of white spot disease. Eleven other shrimp were randomly collected from culture farm B on July 20, 1997 at a time when mild white spot syndrome could be found in this farm's shrimp population. Apparently healthy shrimps were obtained from a third culture farm on Aug. 5, 1997. Random samples taken from this group were confirmed to be WSBV-free by *in situ* hybridization and 2-step PCR analysis.

Artificial infection

In order to obtain shrimp with different WSBV infection, an artificial infection test was carried out. The healthy shrimps were divided into nine groups with 10 shrimp in each group and kept in 50 l plastic tanks containing seawater (salinity = 20 ppt) at ambient temperature (approximately 28 to 30°C). Shrimp in six of these groups (S1–S6) were bathed in 5 l seawater containing 50 ml virus stock solution for 2 h and then rinsed with clean seawater before stocking to 50 l tanks. The virus stock solution was prepared as following: 5 g of gills, muscle and cuticular epidermis from the naturally infected shrimp were removed, ground into pieces, and 50 ml of cold 20 ppt seawater was added; the mixtures were centrifuged at $850 \times g$ for 10 min and the supernatant fluid was used as virus stock solution. The shrimp in other three groups (C1–C3) were bath in sea water and served as negative controls. One shrimp from each group was sampled at 0, 20, 45, 68 and 96 h post-infection (pi).

Hemolymph collection

Hemolymph (0.1–0.3 ml) was collected aseptically from the ventral sinus of each shrimp using a 26-gauge needle and a 1.0 ml syringe. Prior to collecting the hemolymph samples, the hub of each needle was filled with 1.0% sodium citrate solution to prevent coagulation. These hemolymph samples were stored at –20°C until used for dot blot analysis.

Tissue homogenization and DNA extraction

The pleopods, gills and eyestalks (approx. 50 mg; eyeballs were removed from eyestalks) were removed aseptically from the shrimp and placed in labeled 1.5 ml

eppendorf tubes with 500 µl digestion buffer (100 mM NaCl, 10 mM Tris-HCl, pH8, 25 mM EDTA, pH8, 0.5% sodium dodecyl sulfate, 0.1 mg/ml proteinase K). The samples were chilled on ice and crushed with a disposable stick. Ten ml of tissue homogenate were removed to another 1.5 ml eppendorf tube and stored at –20°C until used for dot blot analysis. DNA was isolated from remaining tissue homogenate according to the method described by Lo *et al.* (1996a) with some modification. Briefly, the tissue homogenate was incubated at 65°C for 1 h, and successively extracted with 1X volume of phenol twice and 1X volume chloroform/isoamylalcohol once. The DNA was precipitated by 2X volumes of cold absolute ethanol, dried and redissolved in 0.1X TE buffer (1 mM Tris, 0.1 mM EDTA, pH 7.6) at 65°C for 15 min. The extracted DNA was stored at –20°C until used in dot blot and PCR analysis.

Dot blot analysis for WSBV infection

An insert of recombinant plasmid pms146 (1447 bp) selected from the WSBV *Sal I* genomic library was used for the preparation of the DNA probe used in the dot blot analysis (Chang *et al.*, 1996; Lo *et al.*, 1996a). The hemolymph, extracted DNA and homogeneous tissue fluid were boiled for 10 min and quickly chilled on ice. A 1 µl volume of each sample was then applied to Nylon-1 membrane (GIBCO BRL Life Technologies, Grand Island, NY, USA). The blots were air-dried and baked at 80°C for 30 min and crosslinked for 2 min by UV light. After prehybridization at 37°C for 2 h in hybridization solution [50% formamide, 5X Denhard's reagent, 0.1% Ficoll 400, 0.1% polyvinyl pyrrolidone, 0.1% bovine serum albumin (BSA), 5X standard saline citrate (SSC; 1X SSC = 0.15 M NaCl, 0.015 M tri-sodium citrate, (pH 7.0), 50 mM Tris-HCl (pH 8.0), 1 mM EDTA)], the blots were hybridized at 37°C for 16–18 h with hybridization solution containing 10 ng/ml digoxigenin (DIG)-labeled probe. Subsequent steps were carried out using a DIG DNA Detection Kit following instructions provided by the manufacture (Boehringer, Mannheim). The results were read following a 1 h development period and again after 2 h and 4 h further development.

One-step PCR analysis for WSBV infection

The oligonucleotide primers 146F1 and 146R1 were used for 1-step amplification of a WSBV DNA fragment (Lo *et al.*, 1996b). The DNA samples extracted from the pleopod, gill and eyestalk were used as template DNA. The PCR amplification procedure was

based on the method described by Lo *et al.* (1996b) with some modification. The reaction mixture contained 0.1 µg template DNA, 100 pmol of each primer, 200 µM of each deoxynucleotide triphosphate, 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100 and 2 units of DynaZyme™ DNA Polymerase (Finnzymes Oy, Riihitontuntie 14 B, FIN-02200 Espoo, Finland). The reaction was performed for 40 cycles in an automatic thermal cycler (AG-9600 Thermal Station, Biotronics Corp. Lowell, MA, USA) programmed for 1 min at 94°C, 1 min at 55°C and 2 min at 72°C followed by a 5 min extension at 72°C after the last cycle. Negative control reactions containing no DNA template were run for all PCR reactions. Ten µl of each PCR product was analyzed by electrophoresis in 1% agarose gels containing 0.5 mg/ml ethidium bromide and visualized by ultraviolet transillumination.

Two-step PCR analysis for WSBV infection

Ten µl of 1-step PCR product was added to 90 µl of reaction mixture (as described above) containing the primers 146F2 and 146R2 (Lo *et al.*, 1996b). After the same 40 amplification cycles, the PCR products were analyzed on 1% agarose gels as described above.

In situ hybridization analysis for WSBV infection

Gills and pleopods were excised and fixed in neutral buffered formalin solution (10% formalin, 33 mM

NaH₂PO₄, 45 mM Na₂HPO₄). The fixed tissues were embedded and sectioned at 5 µm using standard histological procedures (Bell and Lightner, 1988). Duplicate sections were made for both hematoxylin and eosin staining and for corresponding *in situ* hybridization with the digoxigenin-labeled probe. Detection of WSBV infection by *in situ* hybridization was based on the method described by Chang *et al.* (1996) with some modification. Briefly, the tissues were deparaffinized and rehydrated, followed by digestion with proteinase K (100 µg/ml) for 15 min and postfixation in 4% paraformaldehyde for 10 min. After prehybridization, the sections were hybridized at 37°C for 16 h in hybridization solution containing 10 ng/ml DNA probe. A DIG Detection Kit (Boehringer Mannheim) was then used to identify viral DNA. After development for 2 h and 24 h, WSBV infected cells were indicated by the presence of blue to dark purple precipitates.

Development of a quick dot blot protocol

In an attempt to simplify and shorten the dot blot analysis procedure, serially 10-fold diluted (10⁻³ to 10⁻⁷) 1 step WSBV positive PCR products were applied to Nylon-1 membranes and used in a battery of dot blot tests. The dot blot procedure described earlier was used as the positive control reference standard (Table 1, column 1), while modifications to this standard procedure are detailed in Table 1, columns 2–14.

Table 1. Standard dot blot analysis (control; column 1) and other modified procedures (columns 2–14). The results at the foot of each column show the highest serial dilution that gives a positive results

Procedure Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dot Blot Steps														
Baking (min)	30	15	0	30	30	30	30	30	30	30	30	30	30	30
UV Cross Linkage (min)	2	2	2	0	2	2	2	2	2	2	2	2	2	2
Prehybridization (h)	2	2	2	2	1/2	1/4	2	2	2	2	2	2	2	2
Hybridization	O/N ^c	O/N	O/N	O/N	O/N	O/N	2 h	30 m	O/N	O/N	O/N	O/N	O/N	O/N
Washing (min)														
2XSSC ^a	30	30	30	30	30	30	30	30	10	5	30	30	30	30
1XSSC	30	30	30	30	30	30	30	30	10	5	30	30	30	30
0.5XSSC	30	30	30	30	30	30	30	30	10	5	30	30	30	30
0.5XSSC	30	30	30	30	30	30	30	30	10	5	30	30	30	30
Blocking (min)	30	30	30	30	30	30	30	30	30	30	15	30	30	30
Conjugation (min)	30	30	30	30	30	30	30	30	30	30	30	15	30	30
Washing (min)	15	15	15	15	15	15	15	15	15	15	15	15	10	5
2% Tween 20 in Buf I ^b	2X	2X	2X	2X	2X	2X	2X	2X	2X	2X	2X	2X	2X	2X
Results	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷	10 ⁻⁶	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷	10 ⁻⁶	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷

a: 1X SSC = 0.15 M NaCl, 0.015 M tri-sodium citrate, pH7.0

b: Buf I = 100 mM Tris-HCl, 150 mM NaCl, pH 7.5

c: O/N: overnight (16–18 h)

Results

Dot blot analysis

Figure 1 shows the typical results of dot blot using DNA (A) and tissue homogenate (B) with positive (P) and negative (N) controls. The dark purple dots (using DNA) or those with a dark purple ring around the dot (using tissue homogenate) are WSBV-positive (Fig. 1, Nos. 1, 4, 5, 6, 9, 11 and 12).

Detection of WSBV in shrimp from farm A

Of the ten shrimp sampled from farm A just after an outbreak of white spot disease, 6 shrimp showed clear evidence of white spot syndrome while 4 shrimp were asymptomatic. All the dot blot results using extracted DNA and tissue homogenate from the gill, pleopod and eyestalk were consistent with the results of both *in situ* hybridization and 2-step PCR analysis (Table 2). Except for shrimp No. 4, which gave a negative result, all dot blot results with hemolymph were likewise consis-

A. Dot blot-DNA

B. Dot blot-tissue

Fig. 1. Photograph of typical dot blot analysis results with positive (P) and negative (N) controls. (A): the results of dot blot using DNA from eyestalk. (B): the results of dot blot using homogenized eyestalk. The number identify the shrimp from which each sample was taken.

Table 2. Detection of WSBV in *P. monodon* from farms A and B by 2-step PCR, *in situ* hybridization and dot blot analysis using DNA or tissue homogenate

Source of shrimp	Date of collection	Shrimp number	White spot	PCR(1/2) ^a			In situ		Dot blot-DNA			Dot blot-tissue			
				G ^b	P ^b	E ^b	G	P	G	P	E	G	P	E	H ^b
Farm A	July 8	1	+	+/+	+/+	+/+	+	+	+	+	+	+	+	+	+
		2	+	+/+	+/+	+/+	+	+	+	+	+	+	+	+	+
		3	+	+/+	+/+	+/+	+	+	+	+	+	+	+	+	+
		4	−	−/−	−/−	−/−	+	+	+	+	+	+	+	+	−
		5	−	−/−	−/−	−/−	−	−	−	−	−	−	−	−	−
		6	−	−/−	−/−	−/−	−	−	−	−	−	−	−	−	−
		7	+	+/+	+/+	−/+	+	+	+	+	+	+	+	+	+
		8	+	+/+	−/+	+/+	+	+	+	+	+	+	+	+	+
		9	−	+/+	+/+	ND ^c	+	+	+	+	ND	+	+	+	+
		10	+	+/+	+/+	ND	+	+	+	+	ND	+	+	+	+
Farm B	July 20	1	+	+/+	+/+	+/+	+	+	+	+	+	+	+	+	+
		2	+	+/+	+/+	+/+	+	+	+	+	+	+	+	+	+
		3	−	−/−	−/−	−/−	−	−	−	−	−	−	−	−	−
		4	−	−/+	+/+	+/+	+	+	−	+	+	−	+	+	+
		5	+	−/+	−/+	+/+	+	+	+	−	+	+	−	+	−
		6	+	−/+	−/+	+/+	+	+	+	+	+	+	+	+	+
		7	+	+/+	+/+	+/+	+	+	+	+	+	+	+	+	+
		8	−	−/−	−/−	−/−	−	−	−	−	−	−	−	−	−
		9	−	−/+	−/+	−/−	+	+	−	−	−	−	−	−	−
		10	−	−/−	−/−	−/−	−	−	−	−	−	−	−	−	−
		11	−	−/+	−/+	−/+	+	+	+	+	+	+	+	+	ND ^c
Farm A	No. of positive shrimp	10	6	7/8	6/8	4/6 ^d	8	8	8	8	6 ^d	8	8	8	7
Farm B		11	5	3/8	4/8	6/7	8	8	6	6	7	6	6	7	5 ^e

a: 1-step PCR result/2-step PCR result.
d: Two samples were not determined.

b: G: gill; P: pleopod; E: eye stalk; H: hemolymph.
e: One sample was not determined

c: ND: not determined.

tent. Dot blots with DNA and tissue homogenates detected WSBV in shrimps Nos. 4 and 9 even though these shrimps were not clinically diagnosed as having white spot syndrome, and shrimp No. 4 was even 1-step PCR negative. The pleopod of shrimp No. 8 was also only 2-step PCR positive but again WSBV was detected in this organ by both dot blot tests. Dot blot positive/negative determinations were identical after 1 h, 2 h and 4 h development, although the intensity of the color reaction deepened over time (data not shown).

Detection of WSBV in shrimp from farm B

The 11 shrimp sampled from farm B were collected shortly after this farm had reported the death of a few of its shrimp from white spot disease. Five of these 11 shrimp had white spot syndrome in a mild form, while the other 6 looked healthy. The results of 2-step PCR, *in situ* hybridization and dot blots using DNA or tissue homogenate were again consistent, with the exception of the following 2-step PCR positive (1-step negative) organs: dot blotting failed to detect WSBV in the extracted DNA and homogenized tissue of gill from shrimp No. 4; the dot blot tests on the pleopod of shrimp

Table 3. Detection of WSBV in artificially infected *P. monodon* by 2-step PCR, *in situ* hybridization, and dot blot analysis using DNA or tissue homogenate

Hours post infection	Shrimp group number	White spot	PCR(1/2) ^a		In situ	Dot blot-DNA		Dot blot-tissue		
			G ^b	E ^b		G	E	G	E	H ^b
0	S1	–	–/–	–/–	–	–	–	–	–	–
	S2	–	–/–	–/–	–	–	–	–	–	–
	S3	–	–/–	–/–	–	–	–	–	–	–
	S4	–	–/–	–/–	–	–	–	–	–	–
	S5	–	–/–	–/–	–	–	–	–	–	–
	S6	–	–/–	–/–	–	–	–	–	–	–
20	S1	–	+/+	–/+	+	+	+	+	+	+
	S2	–	–/–	–/–	–	–	–	–	–	–
	S3	–	+/+	+/+	+	+	+	+	+	+
	S4	–	–/–	–/–	–	–	–	–	–	–
	S5	–	–/+	–/+	+	+	+	+	+	–
	S6	–	–/+	–/–	+	–	–	–	–	–
45	S1	–	–/+	–/+	+	–	+	–	+	–
	S2	–	–/+	–/+	+	+	+	+	+	+
	S3	–	+/+	+/+	+	+	+	+	+	+
	S4	–	–/–	–/–	–	–	–	–	–	–
	S5	–	+/+	–/+	+	+	+	+	+	+
	S6	–	+/+	+/+	+	+	+	+	+	+
68	S1	–	+/+	+/+	+	+	+	+	+	+
	S2	–	+/+	+/+	+	+	+	+	+	+
	S3	–	+/+	+/+	+	+	+	+	+	+
	S4	–	–/–	–/–	–	–	–	–	–	–
	S5	–	+/+	+/+	+	+	+	+	+	+
	S6	–	–/+	+/+	+	+	+	+	+	ND ^c
96	S1	–	+/+	+/+	+	+	+	+	+	+
	S2	–	+/+	–/+	+	+	+	+	+	+
	S3	–	–/+	+/+	+	+	+	+	+	+
	S4	–	+/+	+/+	+	+	+	+	+	+
	S5	–	+/+	+/+	+	+	+	+	+	+
	S6	–	–/+	+/+	+	+	+	+	+	+

a: 1-step PCR results/2-step PCR result.

b: G: gill; P: pleopod; E: eye stalk; H: hemolymph.

c: ND: not determined.

No. 5 were also both false negative; both the gill and pleopod of shrimp No. 9 also gave false negatives in both dot blot tests (Table 2). Additionally dot blots using hemolymph failed to detect the presence of WSBV in shrimps Nos. 5 and 9 (Table 2). As with the samples from farm A, increasing development time affected only the intensity of the color reaction, not the positive/negative diagnosis (data not shown).

Detection of WSBV in artificially infected shrimp

The results of WSBV detection in experimentally infected shrimp were shown in Table 3. At 20 h pi, dot blotting failed to detect the presence of WSBV in hemolymph of shrimp # 5 and none of the dot blots detected WSBV in shrimp # 6, although the gill was 2-step PCR positive and WSBV positive by *in situ* hybridization (Table 3). At 45 h pi, results were consistent except for shrimp # 1. Dot blotting failed to detect WSBV in the gill of this specimen, and dot blotting using hemolymph was also negative (Table 3). At 68 and 96 h pi, all diagnostic methods gave identical results. As before, development time (1, 2 or 4 h) did not affect diagnosis (data not shown). The 15 control shrimps that were tested at 0, 20, 45, 68 and 96 h were all diagnosed as WSBV-negative by all of the tests (data not shown).

Evaluation of the relative sensitivity and specificity of dot blot analysis in comparison with in situ hybridization

A summary of the WSBV detection results for all 66 shrimp (including the 15 negative controls) is shown in Table 4. Specificity was 100%, that is, dot blotting produced no false positives. The sensitivity was also high and reached 94% when the tested organ was the eyestalk. The predictive value of positive test results here (the probability of WSBV infection in an animal with a positive result) was 100%. The predictive value of negative test results (the probability that an animal

does not have WSBV infection when the test result is negative) was about 83% (30/36) – 94% (30/32) except for the pleopods, where the 45 samples from artificial infection test were not included. The WSBV infection prevalence in this sample population was 54.5%.

Development of the quick dot blot analysis

As Table 1 shows, with the exception of protocols 4 and 8, the modified experimental protocols were no less sensitive than the positive control (column 1). Under protocols 4 and 8, the intensity of the color reaction was weaker and the sensitivity was only about 1/10 of the positive control. This suggested that the dot blot analysis procedure could be shortened and that only about four hours would be needed to finish a batch of samples.

Discussion

The early target organs of WSBV in *P. monodon* include the gill and cuticular epidermis (Chang *et al.* 1996) which explains why for any given shrimp, the WSBV prevalence in gill, pleopod and eyestalk was almost identical. Often, as in the case of potential brooders, non-lethal testing is desired. Although the eyestalk, pleopod, and hemolymph (which also had a high prevalence of WSBV in the infected shrimp; see Tables 2, 3 & 4) are all possible candidates for use in dot blot analysis. In Taiwan, where eyestalk albatation is commonly used to induce egg maturation in broodstock in hatchery farms (Chen 1990), the ablated eyestalk (minus the compound eye) can conveniently be used to prepare the tissue homogenate. This also avoids the additional stress to the shrimp that would result from the removal of a pleopod or the collection of hemolymph.

Results of using DNA and tissue homogenate for dot blotting in this study were identical, although the color reaction was more intense and the contrast was greater when extracted DNA was used. With tissue homoge-

Table 4. Specificity and sensitivity of the dot blot analysis using DNA and tissue homogenate by comparison with the *in situ* hybridization. This table summaries the results in Tables 2 and 3

Source of DNA or tissue homogenate	Total number of shrimp tested	Positive	False positive	Negative	False negative	Sensitivity ^b
Gill	66	32	0	30	4	32/36 (89%)
Pleopod	21	14	0	5	2	14/18 (88%)
Eyestalk	66	34 ^a	0	30	2	34/36 (94%)
Hemolymph	64	28	0	30	6	28/34 (82%)

a: This figure includes 2 positive diagnoses based on the dot blot tissue homogenate test only.

b: Sensitivity was defined as number of positive by dot blotting/number of positive by *in situ* hybridization.

nate, and especially with the eyestalk, there was always a trace of light red discoloration in the blots, evidently caused by the astacin in the cuticular epidermis. However, since a single extraction of the tissue homogenate with an equal volume of saturated phenol can remove this pigment and increase the intensity of the color reaction (data not shown). We recommend using tissue homogenate because it is faster (only 4–4.5 h was required for quick dot blot) and cheaper.

While the standard dot blot analysis requires more than 24 h, the quick dot blot procedure developed in this study required only 4 to 4 1/2 h to get the results. Many steps in the standard dot blot analysis can be shortened and the baking step can even be omitted. The UV cross-linkage step was important and could not be excluded although it required only two min. Another key step was the hybridization step, which can be shortened to 2 h but not to 30 min. Although the intensity of the color reaction was weaker when the hybridization step was shortened to 2 h, the sensitivity was equal to that of the positive control. When we tried following all the steps of the standard dot blot procedure at RT (approximately 27 to 30°C), we found that the test's sensitivity was unchanged (data not shown). This suggested that dot blotting is suitable for use in the field, where an incubator or temperature-controlled ovens would not usually be available.

Of the many diagnostic methods that have already been developed to detect WSBV infection, the two most sensitive are *in situ* hybridization and 2-step PCR (Chang *et al.*, 1996; Lo *et al.*, 1996a). However, both of these protocols are time consuming (at least 12 h for 2-step PCR and 2–3 days for *in situ* hybridization), complicated and probably too expensive for general use in hatchery farms. Dot blotting, however, is a relatively simple procedure, especially if tissue homogenate used. Furthermore, tissue homogenate and hemolymph produced false negatives only in a few very lightly infected samples. In view of its accuracy, sensitivity, specificity and convenience, we conclude that the dot blot analysis described here could be developed into an effective diagnostic kit for screening shrimp.

Acknowledgments

This work was supported by the National Science Council under Grant No. NSC87-2815-C-022-002-B and NSC87-2313-B-022-004, ROC.

References

- Bell, T. A. and D. V. Lightner (1988): A Handbook of Normal Penaeid Shrimp Histology. World Aquaculture Society, Baton Rouge, Louisiana, USA, pp. 2–6.
- Chang, P. S., H. C. Chen and Y. C. Wang (1998): Detection of white spot syndrome baculovirus (WSBV) in experimentally infected wild shrimps, crabs and lobsters by *in situ* hybridization. *Aquaculture*, (In press).
- Chang, P. S., C. F. Lo, Y. C. Wang and G. H. Kou (1996): Identification of white spot syndrome associated baculovirus (WSBV) target organs in the shrimp *Penaeus monodon* by *in situ* hybridization. *Dis. Aquat. Org.*, **27**, 131–139.
- Chen, L. C. (1990): *Aquaculture in Taiwan*. The Alden Press, Oxford, 153–159.
- Chou, H. Y., C. Y. Huang, C. H. Wang, H. C. Chiang and C. F. Lo (1995): Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. *Dis. Aquat. Org.*, **23**, 165–173.
- Lightner, D. V., B. T. Poulos, L. Bruce, R. M. Redman, J. Mari and J. R. Bonami (1992): New developments in Penaeid virology: Application of biotechnology in research and disease diagnosis for shrimp viruses of concern in the Americas. In "Disease of Cultured Penaeid Shrimp in Asia and the United States." (ed. by W. Fulks and K. L. Main) The Oceanic Institute, Honolulu, Hawaii, pp. 233–253.
- Lightner, D. V. (1996): A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquaculture Society, Baton Rouge, LA, Section 3.11.
- Lo, C. F., J. H. Leu, C. H. Ho, C. H. Chen, S. E. Peng, Y. T. Chen, C. M. Chou, P. Y. Yeh, C. J. Huang, H. Y. Chou, C. H. Wang, and G. H. Kou (1996a): Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Dis. Aquat. Org.*, **25**, 133–141.
- Lo, C. F., C. H. Ho, S. E. Peng, C. H. Chen, H. C. Hsu, Y. L. Chiu, C. F. Chang, K. F. Liu, M. S. Su, C. H. Wang and G. H. Kou (1996b): White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. *Dis. Aquat. Org.*, **27**, 215–225.
- Lo, C. F., C. H. Ho, C. H. Chen, K. F. Liu, Y. L. Chiu, P. Y. Yeh, S. E. Peng, H. C. Hsu, H. C. Liu, C. F. Chang, M. S. Su, C. H. Wang and G. H. Kou (1997): Detection and tissue tropism of white spot syndrome baculovirus (WSBV) in captured brooders of *Penaeus monodon* with a special emphasis on reproductive organs. *Dis. Aquat. Org.*, **30**, 53–72.
- Mialhe, E., V. Boulo, E. Bachere, D. Hervio, K. Cousin, D. Noel, T. Noel, M. Ohesser, R. M. le Deuff, B. Despres and S. Gendreau (1992): Development of new methodologies for diagnosis of infectious diseases in mollusc and shrimp aquaculture. *Aquaculture*, **107**, 155–164.
- Vivares, C. P. and J. Guesdon (1992): Nucleic acid probes in aquatic bacteriology. *Aquaculture*, **107**, 147–154.
- Wang, C. H., C. F. Lo, J. H. Leu, C. M. Chou, P. Y. Yeh, H. Y. Chou, M. C. Tung, C. F. Chang, M. S. Su and G. H. Kou (1995): Purification and genomic analysis of baculovirus associated with white spot syndrome (WSBV) of *Penaeus*

monodon. *Dis. Aquat. Org.*, **23**, 239–242.

Wang, Y. C., C. F. Lo, P. S. Chang and G. H. Kou (1998): Experimental infection of white spot baculovirus (WSBV) in some cultured and wild decapods in Taiwan. *Aquaculture*, (in press).

Wyban, J. A., J. S. Swingle, J. N. Sweeney. and G. D. Pruder

(1992): Development and commercial performance of high health shrimp using specific pathogen free (SPF) broodstock *Penaeus vannamei*. In “Proceedings of the Special Session on Shrimp Farming” (ed. by James Wyban). pp. 254–260, World Aquaculture Society, Baton Rouge, Louisiana, USA.