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Efficiency and sensitivity determination of Shrimple®, an immunochromatographic assay for white spot syndrome virus (WSSV), using quantitative real-time PCR

James W.B. Powell [a,c,*], Erin J. Burge [b,c], Craig L. Browdy [a], Eleanor F. Shepard [a,c]

[a] South Carolina Department of Natural Resources, Marine Resources Research Institute, 217 Fort Johnson Road, Charleston, SC 29412, USA
[b] College of Charleston, Grice Marine Laboratory, 205 Fort Johnson Road, Charleston, SC 29412, USA
[c] Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, USA

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Abstract

White spot syndrome virus (WSSV) is a prevalent and virulent pathogen affecting both wild and cultured penaeid shrimp worldwide. Molecular diagnostic tools have made detection of the virus increasingly accurate. However, these techniques are often not readily available for rapid diagnosis in the field or in shrimp production facilities. Shrimple®, an immunochromatographic detection assay for WSSV, was designed specifically for use by shrimp producers. In this study, WSSV-infected shrimp were tested with both real-time PCR and Shrimple®, in order to determine the range of sensitivity in which the diagnostic test kit is capable of detecting viral infection and the efficiency of the test kit when compared to the real-time PCR. *Litopenaeus vannamei* were injected with a WSSV inoculum and sampled from 1 to 32 h post injection (p.i.), prior to developing gross anatomical signs of disease. By analyzing the corresponding samples from each specimen, the Shrimple® test results were correlated with estimated viral copy numbers from quantitative PCR. Real-time PCR detected infections in 100% of the inoculated shrimp, while the Shrimple® test kits detected infection in only 34.7% of the specimens. The findings of this study indicate that the Shrimple® test kits fail to detect WSSV infection prior to 12 h post infection and demonstrate a significant reduction in detection efficiency during early onset of infection—failing to detect any viral infection from 1 to 8 h p.i. compared to 100% with real-time PCR. False negative results were observed for specimens containing 4-1061 viral copies/ng genomic DNA. Faint positives were observed for specimens containing 36-1784 viral copies/ng genomic DNA. Although considerably less sensitive than real-time PCR, the Shrimple® test kits provide a useful tool for the detection of WSSV infections prior to development of gross signs of acute disease.

Keywords: White spot syndrome virus; WSSV; Penaeid shrimp; Real-time PCR; Shrimple® diagnostic test kit

1. Introduction

White spot syndrome virus (WSSV) was identified by following the mortality events on east Asian shrimp farms during 1992–1993 (Huang et al., 1994; Nakano et al., 1994; Zhan et al., 1998) and it has since become one of the most serious causes of disease in cultivated shrimp. Cumulative mortalities from white spot disease (WSD) can reach 100% within 5 to 7 days (Chou et al., 1995) and economic losses have been estimated at nearly US$1 billion per year since 1994 (Lightner et al., 1998). In recent years, numerous studies have reported

* Corresponding author. Tel.: +1 843 762 8831; fax: +1 843 762 8737.
E-mail address: powellj@mrd.dnr.state.sc.us (J.W.B. Powell).

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the presence of WSSV in wild crustaceans (Lo et al., 1996b; Chakraborty et al., 2002; Chapman et al., 2004) suggesting potentially significant implications for wild penaeid shrimp populations.

There are currently no treatments available for WSD, so preventative practices are needed for its control. Proposed strategies include strict biosecurity protocols and use of specific-pathogen free (SPF) shrimp stocks. Implementation depends heavily upon the availability of economically feasible, rapid assessment tools (Lightner, 1999).

Current diagnostic methods for the detection of WSSV range from clinical observations (Chou et al., 1995) to antibody-based diagnostic assays (Takahashi et al., 2003), and molecular diagnostic techniques, including real-time polymerase chain reaction (real-time PCR) (Lo et al., 1996a; Kim et al., 1998; Tang and Lightner, 2000; Durand and Lightner, 2002). Of these techniques, only real-time PCR allows simultaneous detection and quantification of WSSV infection. The cost of equipment and technical expertise necessary for real-time PCR is often beyond the means of typical commercial shrimp farms. Despite this, the virulence of WSSV makes rapid detection critical to prevent complete loss of shrimp stocks.

A commercially available immunochromatographic diagnostic test kit (Shrimple®) has been developed for the detection of the white spot syndrome virus from fresh samples (EnBioTec Laboratories; Tokyo, Japan). The advertised benefits of this method include diagnosis within approximately 20 min, a low cost per sample, and ease of use by untrained personnel. The aim of this investigation was to quantitatively evaluate the sensitivity range of Shrimple® test kits as well as to compare the efficiency of the test kit with that of the real-time PCR.

2. Materials and methods

2.1. Maintenance of animals

Specific-pathogen free (SPF) Pacific white shrimp (*Litopenaeus vannamei*) were obtained from the Oceanic Institute (U.S. Marine Shrimp Farming Program; Kailua-Kona, HI) as post-larvae and reared in an indoor, environmentally-controlled, biosecure husbandry facility. Water quality parameters were monitored regularly and adjusted to maintain optimal conditions.

Experimental infections were carried out in a biosecure, environmentally-controlled challenge laboratory (27 °C, 12L:12D photoperiod) in 19 L polypropylene aquaria filled with 3 L of artificial seawater. Shrimp (5.0–7.0 g) were stocked at a density of 10 animals per aquarium and allowed to acclimate for three days prior to the injection of viral inoculum. Daily maintenance of laboratory animals in the challenge system included a 50% water exchange prior to feeding 1 pellet of a commercial shrimp grower diet per shrimp per aquarium.

2.2. Timecourse bioassay experimental design

WSSV inoculum was prepared by homogenizing previously infected, frozen whole shrimp heads in 1% TN-buffered saline (20 mM Tris-Cl, 400 mM NaCl, pH = 7.4) (1 g infected tissue/10 mL saline), centrifuging the homogenate at 1800 g for 20 min, and filtering the supernatant through a 0.45 μm polyethersulfone (PES) filter (Prior et al., 2003). Shrimp ($n = 125$) were injected with 5 μL of a 1:100 dilution of the stock viral inoculum on the lateral side between the second and third abdominal segment. The negative control group included 10 shrimp injected with 1% saline and 10 shrimp injected with an inoculum prepared from specific pathogen free (SPF) shrimp.

Shrimp were sacrificed at 1, 2, 4, 8, 12, 16, 20, 24, and 32 h post injection (p.i.) and pleopod samples were collected. Sample size varied from 10 to 30 shrimp per timepoint, based on previously determined estimates of the progression of WSSV infection. Paired pleopods were removed from segments 1 to 3. Three pleopods (one from each segment) were preserved for Shrimple® and real-time PCR analysis, respectively.

2.3. Shrimple® diagnostic test kit

All Shrimple® tests were performed according to the manufacturer's protocol (EnBioTec Laboratories; Tokyo, Japan). Kit components consisted of Shrimple® test strip, disposable eye dropper, tissue grinder, and a 1.5 mL microcentrifuge tube filled with grinding buffer. Pleopods were removed from the shrimp, placed in the microcentrifuge tube, and ground using the tissue grinder. Three drops, or approximately 75 μL, of the supernatant were applied to the test strip.

The immunochromatographic assay (Shrimple®) developed by EnBioTec Laboratories utilizes a sandwich immunoassay. A monoclonal rat anti-WSSV antibody-colloid gold conjugate pad is positioned next to the sample pad region on the membrane test strip. The membrane test strip is pre-coated with anti-rat IgG on the control (C) zone, where a pink band will appear if the test kit is valid and has performed properly, and monoclonal rat anti-WSSV on the test (T) zone, where a pink band will appear if the animal being tested is positive for WSSV. A test that results in pink bands both at the C-zone and at the T-zone is positive for white spot syndrome virus (EnBioTec Laboratories) (Fig. 1).