# DEVELOPMENT OF RAPID DIAGNOSTIC METHODS USING NUCLEIC ACID BASED MOLECULAR TECHNIQUES FOR WHITE SPOT SYNDROME VIRUS (WSSV)

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## Introduction

The emergence of white-spot syndrome (WSSV) has caused the marine prawn culture industry to experience huge economic losses all across Asia in recent years. WSSV has been identified to be a major threat to world shrimp culture production due to its virulent and untreatable properties (Lotz, 1997). The rapid spread and lethal nature of the white-spot syndrome virus (WSSV) can result in total crop loss in a matter of days. As such, the best protection against the disease at the currently is to prevent the introduction of the virus into the culture system. To achieve this requires the development of rapid, reliable and highly sensitive diagnostic tests. For this purpose, we proceeded to develop DNA-based testing protocols for the detection, screening and quantification of WSSV. In our research, gene probes and novel Polymerase Chain Reaction (PCR) based techniques specific for WSSV were developed.

## Materials and Methods

Development of gene probes using dot-blot hybridization assay format in detecting WSSV was conducted using biotin labeled WSSV 816 bp fragment using random priming method. The probe was used to hybridize with DNA sample blotted and cross-linked on nylon membranes. Hybridized DNA was then detected using streptavidin alkaline phospahatase labeled reporter molecules. The whole diagnostic procedure from DNA extraction to visualisation required two hours. The result was visualised with BCIP/NBT substrate system, which stained purple on a positive sample while a negative sample remained clear. Development of novel PCR methods was focused on identifying suitable DNA amplification technology. Due to the high risk of PCR contamination in routine diagnosis of white-spot syndrome (WSS) in diagnostic laboratory using two-tube nested PCR (conventional two-step PCR), research was undertaken to develop a single-tube nested PCR (STN-PCR) system as the substitute for the former system. For this purpose, one outer and four inner primer pairs were selected using genetic analysis software from an 889-bp conserve region of white-spot syndrome virus (WSSV), for its workability under STN-PCR condition.

## Results and Discussion

Diagnostic assays using WSSV gene probes were sensitive to detect 2.5 ng of viral DNA from shrimp muscle and integument samples. Positive results were obtained also from abdominal flesh, gills, pleopods and post larvae extracted with DNAzol (containing guanidine thiocyanate and detergent), while no reaction was obtained with similar tissue from uninfected shrimp, hence confirming the specificity of the gene probe developed. In developing STN-PCR techniques as diagnostic assays for WSSV, our evaluation revealed that these primer pairs did not work as expected in the PCR condition, due to insufficient thermodynamic driving of primers' functionality by annealing temperature (Ta) during thermal cycling. As such, two new primer pairs (one outer and one inner) with more than 10°C-difference of melting temperature were selected to substitute the previous primers. The functionality of these primer pairs were designed to be driven thermodynamically by high-to-low Ta during PCR. These primer pairs proved to be suitable for STN-PCR, making the new system feasible as a substitute. An important discovery using this new system was the fact PCR result obtained could provide semi-quantitative data on the infection level of samples analyzed. This feature was confirmed using quantitative competitive PCR experiments. The assay was sensitive in detecting 1-10 copies of double stranded WSSV DNA target. Apart from its extreme sensitivity, the assay was also superior than any presently available system as its was more user friendly, had a lower risk of contamination and more economical to use, making the PCR approach more accessible and applicable in the effective control of the disease.

## Conclusions

Effective disease diagnostic techniques employing DNA-based testing protocols were successfully developed. These molecular tools can now be employed as rapid, reliable and highly sensitive diagnostic tests for WSSV. The potential use of such nucleic acid based assay is great as these tests are reproducible allowing standardization in diagnostic protocols in national and regional laboratories. The culturing of screened, uninfected animals can prevent or minimize viral infections that can eventually help, sustain and support a viable shrimp industry.

## References

Lotz, J.M. 1997. Special topic review: Viruses, biosecurity and specific pathogen-free stocks in shrimp aquaculture. *World J. Microbial. Biotechnology.* 13: 405-413.

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