Development of QuickEZ Kit and a Non-stop, Single-tube, Semi-nested PCR Technique for Diagnosing WSSV in *Penaeus Monodon* (Shrimps)

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Abstract: White spot syndrome is a viral infection of *Penaeid* shrimp. The disease is highly lethal, contagious and killing shrimps quickly. Since this disease is caused by a virus, there is no anti-viral treatment for the disease. Sri Lanka is a country involving in large scale intensive shrimp farming. Most of shrimp products are export oriented. WSSV cause severe damage to shrimps and prawns while leading to economic lost. DNA extraction kits are available in the country to find out the positive samples for white spot disease. But these kits are expensive. Therefore this study aimed to optimize a molecular detection kit totally made locally for detecting WSSV in shrimps for a lower cost. A viral DNA extraction kit and single-tube, non-stop, semi-nested polymerase chain reaction (PCR) technique was developed for simultaneous detection and severity grading of white spot syndrome virus (WSSV) infections in the black tiger shrimp. The test uses 1 sense primer and 3 antisense primers that produce up to 3 PCR products (1100, 526 and 250 base pairs) depending upon the severity of infection. Specifically, heavy infections (≥2×104 viral particles) of WSSV produce all 3 fragments, while moderate infections (around 2×103 viral particles) produce 2 (526 and 250 bp) and light infections (20 to 200 viral particles) produce 1 (250bp). The non-stop, single-tube, semi-nested PCR technique is simple and convenient and can detect as little as 5fg WSSV DNA (20 viral particles) in crude extracts of post larval samples or extracts of gills, tail and pleopods from larger shrimp.

Keywords: Semi-nested PCR, WSSV