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PENAEUS MONODON/SESARMA SP./UCA PUGILATOR/  
SCYLLA SERRATA/PORTUNUS PELAGICUS /IN SITU  
HYBRIDIZATION

RATCHANEE KLINPUTSORN: A SENSITIVE DETECTION OF YELLOW-  
HEAD VIRUS<sup>(YHV)</sup> OF PENAEUS MONODON BY NESTED RT-PCR  
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Yellow head virus (YHV) causes a severe disease in Penaeus monodon, a type of shrimp, in Thailand. The aim of this project was to develop a rapid, simple, specific and sensitive detection method for YHV in Penaeus monodon by one-step nested reverse transcriptase polymerase chain reaction (RT-PCR). One-step nested RT-PCR assay was more convenient than conventional two-step nested RT-PCR assay and it gave additional information for grading the relative concentration of YHV infections in this shrimp. The new one-step nested RT-PCR was developed using 2 sets of primers. The first primer set contained one forward primer (10F) and two reverse primers (144R and 209R) which produced 135 bp and 200 bp PCR products, respectively. The second primer set contained one forward primer (9F) and two reverse primers (144R and 196R) which produced 136 bp and 188 bp PCR products, respectively. The developed technique had greater sensitivity compared to RT-PCR and could detect 0.01 fg of purified YHV-RNA. The tests were very specific and did not cross react with white spot syndrome virus, hepatopancreatic parvovirus, *Vibrio harveyi* and host nucleic acid. The technique of *in situ* hybridization in haemolymph and tissues was developed and compared with conventional hematoxylin and eosin staining in haemolymph and tissues to confirm pathology and localization of YHV infection.

Several tissues (gills, lymphoid organs, pleopods and eyestalk) and haemolymph from YHV-infected Penaeus monodon were investigated by using the developed one-step nested RT-PCR technique. It was found that two PCR products (200 bp and 135 bp) were observed only in haemolymph sample at 6 hr after YHV-intramuscular injection. Another advantage of using haemolymph sample was that collection did not require sacrificing the shrimp. The results indicated that haemolymph was the most appropriate source for the detection of YHV.

One-step nested RT-PCR was also used for screening potential asymptomatic carriers. The results demonstrated that all four crabs studied (Sesarma sp., Uca pugilator, Scylla serrata and Portunus pelagicus) were the carriers which could transmit the virus to this shrimp via water.