Development of primers for loop-mediated isothermal amplification of Philippine white spot syndrome virus isolates

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Abstract. Because there are currently no effective chemicals or drugs to completely treat bacterial and viral diseases, effective means of prevention such as early detection methods are continuously being explored and fully exhausted. Over the past few years, we have seen the development of cutting edge technologies to specifically and efficiently detect White Spot Syndrome Virus (WSSV), one of the major viruses that devastated global shrimp aquaculture. The recent discovery of loop mediated isothermal amplification (LAMP) brought about opportunities for fast diagnosis of a lot of animal diseases including humans. Here, the development of alternative LAMP primers specifically for Philippine WSSV isolates was discussed. The sensitivity of the established detection protocol was found to be at 0.3954 pg of shrimp DNA template while exhibiting high specificity to the viral target. In addition, alternative visualization techniques and comparison with other detection protocols are also discussed.

Key Words: WSSV, Philippines, shrimp, Litopenaeus vannamei, LAMP.

Introduction. The global shrimp aquaculture industry is worth in excess of 10 billion US dollars annually, but continues to be plagued by endemic viral diseases (Johnson et al 2008) mainly because there are currently no effective chemicals or drugs to completely treat these diseases. Thus, the ability to vaccinate shrimp and other crustaceans against specific viral diseases is of global economic and biosecurity significance. Immune-stimulation and vaccine-based approaches are likely to prove difficult in shrimp because these organisms primarily rely on innate immunity and therefore lack the ability to produce antibodies. This is the main reason why quarantining and environmental management are the most commonly used practices in combating shrimp diseases (Xiang 2001). These strategies, however, are non-specific in combating infectious diseases and cannot boost the shrimp’s ability to cope with future infection even with the same pathogen (Hoffmann et al 1999). In this regard, effective means of prevention such as early detection methods are continuously being explored and fully exhausted. Various methods have been developed for the detection of White Spot Disease (WSD). These include polymerase chain reaction (PCR) (Lo et al 1996; Kim et al 1998; Tapay et al 1999), in situ hybridization using DNA probes (Durand et al 1996), transmission electron microscopy (TEM), histological analysis using hematoxylin and eosin–stained tissues from moribund shrimp (Wang et al 1997), and immunological methods (Lu et al 1996) and recently, loop-mediated isothermal amplification (Kono et al 2004). Distinct advantages and disadvantages in terms of sensitivity, specificity, cost and convenience vary in each