DEVELOPMENT OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) METHODS USING A SIMPLE TURBIDIMETER FOR DETECTION OF INFECTIOUS MYONECROSIS VIRUS (IMNV) AND MACROBRACHIUM ROSENBERGI/NODAVIRUS (MrNV)

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ABSTRACT

LAMP is a novel method for DNA amplification that is rapid, specific, and highly sensitive under isothermal conditions. The aim of the present study was to develop LAMP detection methods combined with a simple turbidity measurement using a designed portable machine for diagnosis of IMNV and MrNV in field applications. Turbidity in LAMP reactions results from accumulation of white precipitates of magnesium pyrophosphate. The optimal conditions of RT-LAMP for IMNV and MrNV were 63°C at 30 min. and 65°C at 40 min., respectively. Using these conditions, LAMP-turbidity measurement revealed that RT-LAMP-GEL and RT-LAMP-LFD had comparable sensitivity that was higher than that of traditional RT-PCR and nested RT-PCR. Cross reactions with other shrimp viral pathogens were not detected, indicating that our LAMP methods were highly specific for both IMNV and MrNV. With the use of a rapid RNA extraction method, the entire LAMP-turbidity assay process took less than 1 hr. compared to 4-8 hrs. for a nested RT-PCR method. In addition, the devised end-point detection occurred immediately at the end of the LAMP reaction without the need to add further reagents or to open the reaction tube (risk of contamination is also minimized). This system could be easily adapted to produce a hand-held detection device.

KEY WORDS: IMNV/MrNV/LAMP-TURBIDITY MEASUREMENT/ LAMP-LFD

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