

Loop-mediated isothermal amplification of the *toxR* gene coupled with lateral flow dipstick (LAMP-LFD) for the novel, rapid and specific visual detection of *Vibrio harveyi*

ABSTRACT

Aims: *Vibrio harveyi* is a serious pathogen for marine organisms particularly in hatcheries and grow-out ponds that attack their immune system. The rapid detection of *V. harveyi* is urgently needed to prevent bacterial spread. Here we described a rapid and specific visual detection method based on the Loop-Mediated Isothermal Amplification in combination with Lateral Flow Dipstick (LAMP-LFD). Methodology and results: A set of six novel primers were designed to target the *toxR* gene. These include the biotinlabelled inner primer that complements specifically to the target sequences. The resulting biotinylated LAMP amplicons were hybridised to the FAM-labelled probe resulting in lateral flow detection on the dipstick. The addition of loop primers improved the reaction time of LAMP by more than half and rapid detection was observed within 10-15 min. In comparison, the sensitivity of PCR-UV analysis was only at 10⁴ copies while LAMP-LFD was able to detect lower amounts at 10³ copies. The LFD provided higher specificity and selectivity since hybridization with specific probes to the LAMP amplicons was employed. In addition, detection of *V. harveyi* infected grouper was successful using the LAMP-LFD method described here. Conclusion, significance and impact of study: LAMP-LFD is specific to *V. harveyi*. Our method provides a useful tool to rapidly detect and monitor the outbreaks of the pathogen.