Development of Quantitative Real-time Polymerase Chain Reaction for the Detection of *Vibrio vulnificus* Based on Hemolysin (vvhA) Coding System

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Objective To establish a TaqMan real-time fluorescent quantitative PCR to detect *Vibrio vulnificus* based on the hemolysin gene (vvhA) coding cytolysin. **Methods** Primers and probes in the conserved region of the vvhA gene sequence were designed for the TaqMan real-time PCR to detect 100 bp amplicon from *V. vulnificus* DNA. Recombinant plasmid pMD19-vvhA100 was constructed and used as a positive control during the detection. Minimal amplification cycles (*Ct* value) and fluorescence intensity enhancement (*ΔRn* value) were used as observing indexes to optimize the reaction conditions of TaqMan real-time PCR. The TaqMan assay for the detection of *Vbirio vulnificus* was evaluated in pure culture, mice tissue which artificially contaminated *Vibrio vulnificus* and clinical samples. **Results** The established TaqMan real-time PCR showed positive results only for *Vibrio vulnificus* DNA and pMD19-vvhA100. The standard curve was plotted and the minimum level of the vvhA target from the recombinant plasmid DNA was 10^3 copies with a *Ct* value of 37.94±0.19, as the equivalent of 0.01 ng purified genomic DNA of *Vibrio vulnificus*. The results detected by TaqMan PCR were positive for the 16 clinical samples and all the specimens of peripheral blood and subcutaneous tissue of mice which were infected with *Vibrio vulnificus*. **Conclusion** TaqMan real-time PCR is a rapid, effective, and quantitative tool to detect *Vibro vulnificus*, and can be used in clinical laboratory diagnosis of septicemia and wound infection caused by *Vibrio vulnificus.*

Key words: *Vibrio vulnificus*; vvhA gene; TaqMan probe; Real-time quantitative PCR; Detection

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