十足目虹彩病毒 MCP 基因即時定量 PCR 檢測方法的優化與驗證

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摘要

十足目虹彩病毒 1 (DIV1) 是一種新興的甲殼類病毒性疾病，可導致養殖蝦、對蝦和小龍蝦大量死亡。 2020 年亞太水產養殖中心網絡（NACA）在疾病卡中建議並文件化該疾病第 I 至 III 級診斷方法，並於 2021 年時被世界動物衛生組織（WOAH）納入表列疾病。本研究旨在優化和驗證 Qiu 等人於 2020 年已發表之 DIV1 MCP 基因的即時定量 PCR (QPCR)方法，以建立在地標準化流程及測定檢測性能範圍。在實驗中根據 WOAH 手冊傳染性疾病檢測方法之驗證程序，以健康白蝦的核酸萃取溶液作為標準陽性質體樣品連續稀釋之基質，來優化與評估此 QPCR 分析性能特徵。結果顯示最佳試驗條件之 DNA 模板體積為 4 µL，其標準曲線在 4.6×10^2–4.6×10^9 DNA copies/reaction 範圍內顯示出高度線性相關，相關係數 R^2 值為 0.996，回歸方程為 Ct = -3.474·log (DIV1 DNA copies) + 42.023。進一步分析此 QPCR 檢測方法的分析特異性（ASp）和分析靈敏度（ASe）分別為 100%（CI 95%、94.5%、100.0%）和 92%（CI 95%、82.6%、97.3%），而 100% 檢測極限值 (LOD) 為 4.6×10^2 拷貝/reaction。根據 WOAH 手冊傳染性疾病檢測方法之驗證程序，已證明此 QPCR 在檢測 DIVI 方面具有良好的第一階段分析性能，並有利於未來持續第二階段診斷測定驗證程序之進行。

Optimization and Validation of Real-time PCR for Detection of Decapod Iridescent Virus 1 MCP Gene

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Abstract

Decapod iridescent virus 1 (DIV1) is an emerging viral disease of crustaceans and infection of DIV1 causes mass mortality in farmed shrimp, prawns, and crayfish. In 2020, the Network of Asia-Pacific Aquaculture Centres (NACA) recommended and documented the diagnosis methods of levels I to III for this disease in the disease card. In 2021, the method were included in the World Organisation for Animal Health (WOAH) list of diseases. This study aimed to optimize and validate the real-time quantitative PCR (QPCR) method for the DIV1 major capsid protein (MCP) gene published by Qiu *et al*. in 2020 to establish a locally standardized process and assay performance range. According to the WOAH Manual about the validation procedures for infectious disease detection methods, the nucleic acid extraction solution of healthy white shrimp was used as the matrix for serial dilution of standard positive plasmid samples to optimize and evaluate the performance characteristics of this QPCR analysis. The results showed that the DNA template volume of the best test result was 4 μL. Its standard curve showed a high linear correlation within the range of 4.6×10^2–4.6×10^9 DNA copies/reaction, and the correlation coefficient R^2 value was 0.996; the regression equation was Ct = -3.474·log (DIV1 DNA copies) + 42.023. The analytical specificity (ASp) and analytical sensitivity (ASe) of this QPCR assay were further analyzed to be 100% (CI 95%, 94.5%, 100.0%) and 92% (CI 95%, 82.6%, 97.3%), respectively, while the 100% limit of detection (LOD) was 4.6×10^2 copies/reaction. This QPCR has demonstrated good phase 1 analytical performance for detecting DIV1 and will facilitate future ongoing phase 2 diagnostic assay validation procedures.