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Most-Probable-Number Loop-Mediated Isothermal Amplification-Based Procedure Enhanced with K Antigen-Specific Immunomagnetic Separation for Quantifying \textit{tdh}^+ \textit{Vibrio parahaemolyticus} in Molluscan Shellfish

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Although thermostable direct hemolysin-producing (\textit{tdh}^+) \textit{Vibrio parahaemolyticus} is the leading cause of seafood-borne gastroenteritis, the enumeration of \textit{tdh}^+ \textit{V. parahaemolyticus} remains challenging due to its low densities in the environment. In this study, we developed a most-probable-number (MPN)-based procedure designated A-IS\textsuperscript{1}-LAMP, in which an immunomagnetic separation (IMS) technique targeting as many as 69 established K antigens and a loop-mediated isothermal amplification (LAMP) assay targeting the thermostable direct hemolysin (\textit{tdh}) gene were applied in an MPN format. Our IMS employed PickPen, an eight-channel intrasolution magnetic particle separation device, which enabled a straightforward microtiter plate-based IMS procedure (designated as PickPen-IMS). The ability of the procedure to quantify a wide range of \textit{tdh}^+ \textit{V. parahaemolyticus} levels was evaluated by testing shellfish samples in Japan and southern Thailand, where shellfish products are known to contain relatively low and high levels of total \textit{V. parahaemolyticus}, respectively. The Japanese and Thai shellfish samples showed, respectively, relatively low (<3 to 11 MPN/10 g) and considerably higher (930 to 110,000 MPN/10 g) levels of \textit{tdh}^+ \textit{V. parahaemolyticus}, raising concern about the safety of Thai shellfish products sold to domestic consumers at local morning markets. LAMP showed similar or higher performance than conventional PCR in the detection and quantification of a wide range of \textit{tdh}^+ \textit{V. parahaemolyticus} levels in shellfish products. Whereas a positive effect of PickPen-IMS was not observed in MPN determination, PickPen-IMS was able to concentrate \textit{tdh}^+ \textit{V. parahaemolyticus} 32-fold on average from the Japanese shellfish samples at an individual tube level, suggesting a possibility of using PickPen-IMS as an optional tool for specific shellfish samples. The A-IS\textsuperscript{1}-LAMP procedure can be used by any health authority in the world to measure the \textit{tdh}^+ \textit{V. parahaemolyticus} levels in shellfish products.