

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

NATSUKO TANAKA¹, YOSHITO IWAE², WATARU YAMAZAKI³, FUMIO GONDAIRA⁴, VARAPORN
VUDDHAKUL⁵, YOSHITSUGU NAKAGUCHI⁶ AND MITSUAKI NISHIBUCHI^{6*}

¹Graduate School of Medicine and ⁶Center for Southeast Asian Studies, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan; ²Mie Prefecture Health and Environment Research Institute, Sakura-cho, Yokkaichi-shi, Mie 512-1211, Japan; ³Department of Veterinary Science, Faculty of Agriculture, University of Miyazaki, Gakuenkibanadainishi, Miyazaki-shi, Miyazaki 889 2192, Japan; ⁴Denka Seiken Co., Ltd., Nihonbashi-Muromachi, Chuo-ku, Tokyo 103-8338, Japan; and ⁵Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai 90110, Thailand

Although thermostable direct hemolysin-producing (*tdh*⁺) *Vibrio parahaemolyticus* is the leading cause of seafood-borne gastroenteritis, the enumeration of *tdh*⁺ *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-IS¹-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 (*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 *tdh*⁺ *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 *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 *tdh*⁺ *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 *tdh*⁺ *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 *tdh*⁺ *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-IS¹-LAMP procedure can be used by any health authority in the world to measure the *tdh*⁺ *V. parahaemolyticus* levels in shellfish products.