IN-HOUSE METHOD VALIDATION FOR DETECTION AND IDENTIFICATION OF VIBRIO SPP. FROM VARIOUS FOOD MATRICES BY CULTURAL METHODS AND REAL-TIME PCR


Background:
Vibrio species are the most dominant bacterial pathogens in farm-reared shrimps and other seafoods such as oysters, fishes, crabs, and mussels. These are known to cause “vibriosis”, which is one of the most serious bacterial diseases in the aquaculture and food industries posing major threat on the health and economic issues globally. Standardized methods with high sensitivity and specificity are needed for accurate detection of Vibrio spp. in different seafood products to control or eradicate food pathogens.

Objectives:
This study aimed to conduct in-house method validation of Vibrio parahaemolyticus, following ISO 21872-1:2017, which includes both conventional microbiological procedures and Real-time Polymerase Chain Reaction (qPCR) method. The study also aimed to determine the prevalence of V. parahaemolyticus in seafood products by collecting and analyzing raw and ready-to-eat (RTE) seafood samples from Metro Manila.

Methods:
The verification of both conventional and qPCR methods were conducted following the performance requirements of National Association of Testing Authorities, Australia (NATA) Technical Note 17. In addition, analysts participated in a proficiency testing (PT) program organized by IFM Quality Services, Inc. to assess their performance of the method. A total of 65 seafood samples: 33 RTE (cooked, undercooked, sashimi) and 32 raw were collected and analyzed for the presence of V. parahaemolyticus.

Results and Findings:
Validation results showed that both the conventional and qPCR methods were precise and possess 100% sensitivity, selectivity, and trueness. Both conventional and qPCR analyses revealed that recovering V. parahaemolyticus in seafood samples decreased when target pathogen is in low numbers. In addition, the laboratory showed competency in the method performance through satisfactory PT results.

From the incidence study, 50% (16/32) of the raw seafood samples was positive for V. parahaemolyticus while none of the ready-to-eat samples were contaminated with the pathogen.

Conclusion and Recommendation:
Both the cultural and real-time PCR methods were fit for their intended use in the laboratory. Since the pathogen has been shown to be abundant in raw seafood, care must be done in the consumption of undercooked seafood products to avoid acquiring foodborne illness brought about by the pathogen.