Fit for Purpose Molecular Methods for Vibrio Detection

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A series of national and international risk assessments on *Vibrio* spp. in various seafoods have been released and indicate that a relatively small proportion of the vibrio population accounts for the overwhelming majority of illnesses and that limiting exposure to these more virulent strains is the most effective approach for reducing risk. Available "official" or "reference" methods are based on traditional culture techniques and are generally incapable of reliably determining levels of virulent subpopulations of *Vibrio* spp. in seafood or the environments where they are produced. Molecular detection methods such as real-time PCR are much more reliable at detecting and characterizing virulent vibrio populations and are typically applied in an MPN format for enumeration at appropriate limit of detection (LOD). Numerous PCR assays targeting species specific and virulence associated genes have been developed and have been widely applied to determine vibrio levels in seafood and the environment. Formal validation of these molecular detection methods has been hindered by the lack of reference methods with corresponding capabilities for detection of virulent vibrio subpopulations. An approach that couples evaluation of method performance and analyst proficiency using molecular detection methods was developed. Coded samples of pure cultures (50 *Vibrio parahaemolyticus* isolates and 30 near neighbor vibrio isolates) and oyster enrichments inoculated with various levels of *V. parahaemolyticus* that were prepared at a FDA laboratory. These samples were boiled and frozen at -80°C to avoid dangerous goods issues with international shipment of pathogens and were subsequently distributed to 12 laboratories in 10 countries. Most of the laboratories were able to correctly identify samples of pure cultures regardless of the molecular detection method used. The LOD with inoculated oyster enrichments varied widely. Most of the laboratories using conventional PCR were unable to detect *V. parahaemolyticus* DNA targets in any of the samples. Real-time PCR and LAMP assays were able to detect between ~10^2 and 10^3 targets/PCR. This approach could provide a timely mechanism to simultaneously determine method performance and analyst proficiency using molecular detection methods for vibrios and other pathogens in the food supply.