Performance Summary

iQ-Check *Vibrio* Method

Introduction

The iQ-Check *Vibrio* PCR Detection Kit is a test based on gene amplification and detection by real-time PCR after seafood samples are enriched in *Vibrio* Enrichment Broth (VEB) or alkaline peptone water (APW). The method can detect and differentiate *Vibrio cholerae, Vibrio parahaemolyticus,* and *Vibrio vulnificus* in all seafood matrices, and can be used to confirm typical colonies isolated from trypto-casein-soy agar (TSA) + 2% NaCl, thiosulfate citrate bile saccharose (TCBS), and *Vibrio* Chromogenic Agar. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) highly specific for *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. A synthetic DNA internal control is included in the reaction mix. An internal control is critical in any reaction to monitor for inhibitors and allow for the validation of any negative result. The iQ-Check *Vibrio* method has been rigorously tested and validated by AOAC International (Table 1).

Table 1. Validation for the iQ-Check *Vibrio* method.

Validation	Certificate Number
AOAC	PTM 032002

Inclusivity/Exclusivity Testing

Inclusivity testing is performed to verify that the method can detect *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* while exclusivity studies test nontarget strains, including other species of *Vibrio*, to ensure there is no cross-reactivity. Exclusivity strains were enriched in nonselective broth for 20–24 hr at 35 ± 1°C and were tested at high levels. A target of 10–100 colony forming units (CFU) of each *Vibrio* inclusivity strain was cultured in VEB for 7 hr and APW for 6 hr at 35 ± 1°C and diluted to a low level (~10^3) before testing. Results are shown in Table 2.

Table 2. Results of inclusivity/exclusivity testing.

Strains Tested	Positives Detected/Identified	Results
150 *Vibrio* strains (50 of each strain)	150	100% inclusivity
116 non-*Vibrio* and non–*Vibrio cholerae*, *parahaemolyticus*, and *vulnificus* strains	0	100% exclusivity

Limit of Detection

Limit of detection (LOD_{50}) is an estimate of the contamination level required to achieve positive detection in 50% of cases. This is measured by inoculating food matrices with *Vibrio* strains and carrying out the validated enrichment, extraction, and detection protocols (Table 3).

The average LOD_{50} of the iQ-Check *Vibrio* method was determined to be 0.666 (range: 0.356–1.249) with VEB and 0.693 (range: 0.370–1.297) with APW.

Table 3. LOD_{50} for the iQ-Check *Vibrio* method.

Matrix/Strain Pair	Conditions	LOD_{50}, CFU/sample size (range)
Cooked shrimp/*V. cholerae*	VEB, 125 g	0.594 (0.331–1.068)
Cooked shrimp/*V. cholerae*	APW, 125 g	0.594 (0.331–1.068)
Cooked shrimp/*V. cholerae*	APW, 25 g	0.761 (0.411–1.410)
Raw mussels/*V. cholerae*	VEB, 25 g	0.623 (0.340–1.142)
Raw mussels/*V. cholerae*	APW, 25 g	0.807 (0.428–1.519)
Raw shrimp/*V. parahaemolyticus*	VEB, 25 g	0.617 (0.328–1.161)
Raw shrimp/*V. parahaemolyticus*	APW, 25 g	0.706 (0.368–1.354)
Raw oysters/*V. parahaemolyticus*	VEB, 25 g	0.640 (0.341–1.200)
Raw oysters/*V. parahaemolyticus*	APW, 25 g	0.640 (0.341–1.200)
Raw tuna/*V. vulnificus*	VEB, 25 g	0.857 (0.439–1.674)
Raw tuna/*V. vulnificus*	APW, 25 g	0.647 (0.339–1.232)

Method Comparison/Matrix Studies

Matrix testing is critical to demonstrating the performance of a method compared to the reference method with real-world food samples. The iQ-Check *Vibrio* method has been verified with external and internal testing on a wide variety of foods. No significant difference was found between the reference method and alternative method for all matrices tested (Table 4).

Table 4. Matrices tested with the iQ-Check *Vibrio* method.

Category	Matrices
Fishery products Raw, processed	**Raw:** Mussels, oysters, shrimp, tuna **Processed:** Shrimp

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