Proposal No. **15-110**

Proposal for Task Force Consideration at the ISSC 2017 Biennial Meeting	a. ☒ Growing Area b. ☐ Harvesting/Handling/Distribution c. ☐ Administrative
Submitter	Executive Board
Affiliation	Interstate Shellfish Sanitation Conference (ISSC)
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Proposal Subject	Laboratory Method for *Vibrio parahaemolyticus (V.p.)* Enumeration and Detection through MPN and Real-Time PCR
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Text of Proposal/ Requested Action	This method was developed by William A. Glover (Washington State Public Health Laboratories) and is being submitted by the ISSC Executive Board. The Executive Board granted interim approval to this method on March 13, 2015. The Executive Board is submitting this proposal to comply with Article V. Section 1. of the ISSC Constitution, Bylaws, and Procedures.

Submitted by method developer William A. Glover (Washington State Public Health Laboratories)

5. Approved Methods for Vibrio Enumeration

	Vibrio Indicator Type:	Application: PHP Sample Type: Shucked
EIA[1]	*Vibrio vulnificus (V.v.)*	X
MPN[2]	*Vibrio vulnificus (V.v.)*	X
SYBR Green 1 QPCR-MPN[5]	*Vibrio vulnificus (V.v.)*	X
MPN[3]	*Vibrio parahaemolyticus (V.p.)*	X
PCR[4]	*Vibrio parahaemolyticus (V.p.)*	X
MPN and PCR[6]	*Vibrio parahaemolyticus (V.p.)*	X

Footnotes:

[1] EIA procedure of Tamplin, et al, as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992.

[2] MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, followed by confirmation using biochemical analyses or by the DNA -alkaline phosphatase labeled gene probe (vvhA).

[3] MPN format with confirmation by biochemical analysis, gene probe methodology as listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.

[4] PCR methods as they are listed in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, or a method that a State can demonstrate is equivalent.

	[5]*Vibrio vulnificus*, ISSC Summary of Actions 2009. Proposal 09-113, Page 123. [6]William A. Glover, II, Ph.D. D9ABMM), MT(ASCP) Food and Shellfish Bacteriology Laboratory (FSBL) at the Washington State Public Health Laboratories (WAPHL)
Public Health Significance	The purpose of this method is to provide laboratories supporting the NSSP the ability to rapidly quantify *Vibrio parahaemolyticus (V.p.)* from oysters using a high throughput real-time PCR protocol. The Food and Shellfish Bacteriology Laboratory (FSBL) at the Washington State Public Health Laboratories (WAPHL) tests on average over 200 oyster samples per year for *Vibrio parahaemolyticus (V.p.)*Culture based assays for the enumeration of *V.p.* take four days or longer and require the Kanagawa test (media based) to detect pathogenicity. Due to the large number of samples and need for accurate and timely results, the FSBL at the WAPHL has tested Pacific oysters (Crassostrea gigas) for (*V.p.*) using a MPN based real-time PCR assay for over 10 years. The real-time PCR assay utilized by the FSBL at the WAPHL has gone through redesigns and improvements by various scientists at the WAPHL based on new published literature, clinical *V.p.* case data, experiences in WA State over the course of a season or seasons, and requests from the Office of Shellfish & Water Protection for enhanced detection of pathogenic *V.p.* strains and additional surveillance capabilities. The real-time PCR assay redesigned and implemented in 2009 and utilized through the 2013 *V.p.* monitoring season (June – September) was designed to detect *V.p.* using the species-specific thermolabile hemolysin gene (tlh) and virulent *V.p.* using the thermostable direct hemolysin gene (tdh). This assay was designed for high throughput in a 384-well based format. Additionally, the tlh and tdh targets were redesigned yielding amplicons between 50-150 base pairs. This is optimal for real-time PCR and is known to produce consistent results1. Validation of the assay and concept of a “molecular MPN” was conducted using FERN guidelines and was compared to the FDA BAM method. This assay served as the backbone for which further improvements and redesigns were made in 2013.
Cost Information	
Action by 2015 Laboratory Method Review Committee	Recommended referral of Proposal 15-110 to an appropriate committee as determined by the Conference Chair to await completed SLV data.
Action by 2015 Task Force I	Recommended adoption of 2015 Laboratory Methods Review Committee recommendation on Proposal 15-110.
Action by 2015 General Assembly	Adopted recommendation of Task Force I on Proposal 15-110.
Action by FDA January 11, 2016	Concurred with Conference action on Proposal 15-110.