Proposal No. 15-111

Proposal for Task Force Consideration at the ISSC 2015 Biennial Meeting	☒ Growing Area ☐ Harvesting/Handling/Distribution ☐ Administrative

Submitter	Executive Board
Affiliation	Interstate Shellfish Sanitation Conference (ISSC)
Address Line 1	209 Dawson Road
Address Line 2	Suite 1
City, State, Zip	Columbia, SC 29223-1740
Phone	803-788-7559
Fax	803-788-7576
Email	issc@issc.org
Proposal Subject	MPN-Real-Time PCR for Pathogenic *V.p.*
Specific NSSP Guide Reference	Section IV. Guidance , Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests
Requested Action	This method was developed by Jessica Jones (FDA Gulf Coast Seafood Laboratory) 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.
Text of Proposal	Submitted by method developer Jessica Jones (FDA Gulf Coast Seafood Laboratory)

5. Approved Methods for Vibrio Enumeration

	Vibrio Indicator Type:	Application: PHP Sample Type: Shucked	Application: Reopening
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-Real Time PCR[6]	*tdh+ and trh+ Vibrio parahaemolyticus (V.p.)*	X	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] MPN-real time PCR method for the *tdh* and *trh* genes for total *V. parahaemolyticus*

	as described in Kinsey et al., 2015.
Public Health Significance	The current NSSP method for enumeration of *tdh+ Vp* requires a minimum of four days from receipt of sample to results reporting. Currently, there is no NSSP-approved method for enumeration of *trh+ Vp*. At the 2013 conference, proposal 13-202 was adopted which requires testing for the presence of *tdh* and *trh* prior to reopening of growing areas closed as a result of *Vp* illnesses [Chapter II @.01.F(5)]. This proposed MPN-real-time PCR method provides results in as little as 24h from receipt of sample. Availability of this more rapid method will facilitate reopening decision making.
Cost Information	This method costs ~$120 per sample for laboratory consumables, supplies, and reagents. Most equipment needed for testing is standard microbiology equipment, but purchase of a heat block (~$400) and/or centrifuge (~$2,500) may be necessary. Purchase of a real-time PCR instrument will be required ($30,000-$45,000). Additional costs for a laboratory would vary based on their operational overhead and labor.