

<b>Proposal for Task Force Consideration at the ISSC 2015 Biennial Meeting</b>		<input checked="" type="checkbox"/> Growing Area <input type="checkbox"/> Harvesting/Handling/Distribution <input type="checkbox"/> Administrative																													
Submitter	Executive Board																														
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Proposal Subject	MPN-Real-Time PCR for Total Vp																														
Specific NSSP Guide Reference	Section IV. Guidance Documents 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)  <b>5. Approved Methods for Vibrio Enumeration</b> <table border="1" data-bbox="467 1008 1442 1381"> <thead> <tr> <th></th> <th>Vibrio Indicator Type:</th> <th>Application: PHP Sample Type: Shucked</th> <th><u>Application: Reopening</u></th> </tr> </thead> <tbody> <tr> <td>EIA<sup>1</sup></td> <td><i>Vibrio vulnificus (V.v.)</i></td> <td>X</td> <td></td> </tr> <tr> <td>MPN<sup>2</sup></td> <td><i>Vibrio vulnificus (V.v.)</i></td> <td>X</td> <td></td> </tr> <tr> <td>SYBR Green 1 QPCR-MPN<sup>5</sup></td> <td><i>Vibrio vulnificus (V.v.)</i></td> <td>X</td> <td></td> </tr> <tr> <td>MPN<sup>3</sup></td> <td><i>Vibrio parahaemolyticus (V.p.)</i></td> <td>X</td> <td></td> </tr> <tr> <td>PCR<sup>4</sup></td> <td><i>Vibrio parahaemolyticus (V.p.)</i></td> <td>X</td> <td></td> </tr> <tr> <td><u>MPN-Real Time PCR<sup>6</sup></u></td> <td><u><i>Vibrio parahaemolyticus (V.p.)</i></u></td> <td><u>X</u></td> <td><u>X</u></td> </tr> </tbody> </table> Footnotes: <sup>1</sup> EIA procedure of Tamplin, et al, as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992. <sup>2</sup> 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). <sup>3</sup> 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. <sup>4</sup> 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. <sup>5</sup> <i>Vibrio vulnificus</i> , ISSC Summary of Actions 2009. Proposal 09-113, Page 123. <sup>6</sup> <u>MPN-real time PCR method for the <i>tlh</i> gene for total <i>V. parahaemolyticus</i> as described in Kinsey et al., 2015.</u>				Vibrio Indicator Type:	Application: PHP Sample Type: Shucked	<u>Application: Reopening</u>	EIA <sup>1</sup>	<i>Vibrio vulnificus (V.v.)</i>	X		MPN <sup>2</sup>	<i>Vibrio vulnificus (V.v.)</i>	X		SYBR Green 1 QPCR-MPN <sup>5</sup>	<i>Vibrio vulnificus (V.v.)</i>	X		MPN <sup>3</sup>	<i>Vibrio parahaemolyticus (V.p.)</i>	X		PCR <sup>4</sup>	<i>Vibrio parahaemolyticus (V.p.)</i>	X		<u>MPN-Real Time PCR<sup>6</sup></u>	<u><i>Vibrio parahaemolyticus (V.p.)</i></u>	<u>X</u>	<u>X</u>
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<p>Public Health Significance</p>	<p>The current NSSP method for enumeration of <i>Vp</i> requires a minimum of four days from receipt of sample to results reporting. The MPN-real-time PCR method provides results in as little as 24h from receipt of sample. At the 2013 conference, proposal 13-202 was adopted which requires testing prior to reopening of growing areas closed as a result of <i>Vp</i> illnesses [Chapter II @.01.F(5)]. Availability of this more rapid method will facilitate reopening decision making.</p>
<p>Cost Information</p>	<p>This method costs ~\$100 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.</p>