Submitter

US Food & Drug Administration (FDA)
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Proposal Subject

MPN-Real-Time PCR for Enumeration of Vibrio vulnificus in Oysters

Specific NSSP Guide Reference

Section IV. Guidance Documents, Chapter II. Growing Areas .14 Approved NSSP Laboratory Tests.

Text of Proposal/ Requested Action

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**
MPN-Real Time PCR[7]	***Vibrio parahaemolyticus (V.p.)***	**X**	**X**
Direct Plating Method[8]	***Vibrio parahaemolyticus (V.p.)***		**X**
MPN-Real Time PCR[9]	***Vibrio vulnificus (V.v.)***	**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 gene probe for vvhA as described by Wright et al., or a method that a State can demonstrate is equivalent.

[3] MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, followed by confirmation using biochemical analyses or the DNA-alkaline phosphatase gene probe for tlh as described by McCarthy et al., or a method that a State can demonstrate is equivalent.

[4] MPN method in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, and as described in the "Direct Plating Procedure for the Enumeration of Total and Pathogenic *Vibrio parahaemolyticus* in Oyster Meats" developed by FDA, Gulf Coast Seafood Laboratory, 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. ISSC 2015 Summary of Actions Proposal 15-111, Page 397.

[7] MPN-Real Time PCR Method for the *tlh* gene for total *V. parahaemolyticus* as described in Kinsey et al., 2015. ISSC 2015 Summary of Actions Proposal 15-113, Page 418

[8] Direct Plating Procedure in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, May 2004 revision, and as described in the 'Direct Plating Procedure for the Enumeration of Total and Pathogenic *Vibrio parahaemolyticus* in Oyster Meats' developed by FDA, Gulf Coast Seafood Laboratory.

[9] MPN-Real Time PCR Method for the vvh gene for total *V. vulnificus* as described in Kinsey et al., 2015.

Public Health Significance

This MPN-real-time PCR method provides results in as little as 24 h from receipt of sample. The current NSSP methods for enumeration of *Vv* have limitations: the

	traditional MPN requires a minimum of 3 days and the SYBR Green PCR is only validated on an instrument platform which is no longer supported by the manufacturer. This method provides an additional option for laboratories to maintain the same level of testing as has been maintained in the program.
Cost Information	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.
Action by 2019 Laboratory Committee	Recommended adoption of Proposal 19-126 as submitted.
Action by 2019 Task Force I	Recommended the adoption of Laboratory Committee recommendation on Proposal 19-126.
Action by 2019 General Assembly	Adopted recommendation of Task Force I on Proposal 19-126.
Action by FDA February 21, 2020	Concurred with Conference action on Proposal 19-126.