

National Shellfish Sanitation Program
Guide for the Control of Molluscan Shellfish
2007

Section IV. Guidance Documents
Chapter II. Growing Areas

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.10 Approved National Shellfish Sanitation Program Laboratory Tests: Microbiological and Biotxin Analytical Methods

1. Microbiological Methods

Application	Sample Type	Total Coliform ¹			Fecal Coliform ²					Standard Plate Count ³	
		APHA Decimal Dilution MPN	12 tube single dilution MPN	Other	A-1M		APHA				ETCP
					Decimal dilution MPN	12 tube single dilution MPN	Decimal dilution MPN	12 tube single dilution MPN	mTEC		
Growing Area Survey & Classification	Seawater	X	X		X ⁴	X ⁴	X	X	X		
	Shellfish						X				
Controlled Relaying	Seawater	X	X		X ⁴	X ⁴	X	X	X		
	Shellfish						X				
Wet Storage	Seawater			X					X		
	Shellfish						X				
Controlled Purification	UV Effluent			X					X		
	Shellfish						X	X		X	
Market Shellfish	Shellstock						X				X
	Shucked						X				X
PHP	Shucked										
	In Shell										

References

1. Total Coliform Methods
 - o American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition, APHA, New York, N. Y. [Decimal Dilution MPN test]
 - o American Public Health Association, American Water Works Association, and Water Environmental Federation. 1992. Section 9221. Examination of a 100 ml aliquot by the Multiple Tube Fermentation

- Method (MTF). *Standards Methods for the Examination of Water and Wastewater*, 18th Edition, APHA/AWWA/WEF. Washington, D.C. [Decimal Dilution MPN test]
 - o Redman, J. H. 1974. A simpler multiple fermentation tube test for monitoring the bacteriological quality of shellfish harvest waters; the examination of twelve 1.0 ml sample portions, p.123-124. *In* Wilt, D. S. (ed.), *Proceedings 8th National Shellfish Sanitation Workshop*, U.S. Food and Drug Administration, Washington, D.C. [12-tube, Single Dilution MPN test]
 - o Springer, J. A. 1974. Statistical considerations in using the twelve-tube MPN test for routine monitoring of shellfish waters, p.125-126. *In* Wilt, D. S. (ed.), *Proceedings 8th National Shellfish Sanitation Workshop*. U.S. Food and Drug Administration, Washington, D.C. [12-tube, Single Dilution MPN test]
2. Fecal Coliform Methods
- o 40 CFR 136.3 Table I-A. List of Approved Microbiological Methods
 - o A-1M, 1990 AOAC International - *Official Methods of Analysis*, 15th Edition. Association of Official Analytical Chemists. Washington, D.C. [A-1 Modified MPN test]
 - o APHA. 1998. Standard Methods for the Examination of Water and Wastewater APHA, 20th Edition, APHA, Washington, DC
 - o American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition, APHA, New York, N.Y.
 - o Rippy, Scott, et. al, Enumeration of Fecal Coliforms and E. coli in marine and estuarine waters: an alternative to the APHA-MPN approach. *Journal Water Pollution Control Federation*. August 1987, pg. 795-798.
 - o U.S. Food and Drug Administration. 2001. (Revised 12-8-02). NSSP Interpretation, 03-III-@.02-100, Options for the use of the 12-tube, single dilution MPN test. [12-tube, Single Dilution MPN test for seawater]
 - o U.S. Food and Drug Administration. 2001 (Revised 12-8-02). NSSP Interpretation 03-XV-.03-100, Method for determining fecal coliform levels in end product depurated shellfish. [12-tube, single dilution MPN test for the controlled purification of shellfish]
 - o Cabelli, V. J. and W. P. Heffernan. 1970. Accumulation of *Escherichia coli* by the northern quahog. *Appl. Microbiol.* 19:239-244. [ETCP for the controlled purification of hard- and soft-shelled clams]
3. Standard Plate Count Method
- o American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition, APHA, New York, N. Y.

Footnote:

⁴ The use of A-1 Medium Minus Salicin is optional with the following condition. Any State that eliminates Salicin must show equivalency with a minimum of 30 samples seasonally (4 sets of 30 samples). The samples must generate results and the results must not be significantly different as shown by using a T test. The data generated to support the elimination of Salicin must be submitted to FDA for review and concurrence prior to State action to eliminate the use of Salicin.

2. Marine Biotxin Method

Application	Sample Type	Paralytic Shellfish Poison (PSP)	Neurotoxic Shellfish Poison (NSP)
Growing Area Survey & Classification	Shellfish	X	X
Controlled Relaying	Shellfish	X	X

References:

1. Paralytic Shellfish Poison (PSP) and Neurotoxic Shellfish Poison (NSP) Methods
American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition, APHA, New York, N.Y.

3. Type III and Type IV Microbiological Test Methods

Application	Sample Type	Total Coliform	Fecal Coliform	Other
Growing Area Survey & Classification	Seawater			
Controlled Relaying	Seawater			
	Shellfish			
Wet Storage	Seawater			
	Shellfish			
Controlled Purification	UV Effluent	Type III ¹		
	Shellfish			
Market Shellfish	Shellstock			
	Shucked			

Footnotes:

1. Single step direct mEndo-LES Membrane Filter Technique used in the Newburyport Depuration Plant, Massachusetts.

4. Type III and Type IV Marine Biotoxin Test Method

Application	Sample Type	NSP	PSP	DSP	AS	Other
Growing Area Survey & Classification	Shellfish		Type III ²		Type III ¹	
Controlled Relaying	Shellfish				Type III ¹	

Footnotes:

1. Peer recognized HPLC Methods with or without clean up.
2. Jellett Rapid Test for PSP
 - i. Method can be used to determine when to perform a mouse bioassay in a previously closed area.
 - ii. A negative result can be substituted for a mouse bioassay to maintain an area in the open status.
 - iii. A positive result shall be used for a precautionary closure.

5. Interim Approval by ISSC Executive Board August 2007

Application	Sample Type	V _v	V _v	V _p	V _p
PHP	Shucked	EIA ¹	MPN ²	MPN ³	PCR ⁴
	In-Shell	EIA ¹	MPN ²	MPN ³	PCR ⁴

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.

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.