1. Type I and Type II Microbiological Methods

		Application										
		Growing Area Survey and Classification	Controlled Relaying		Wet Storage			Controlled Purification			Market Shellfish	
Sample Type												
Method		Seawater	Seawater	Shellfish	UV Treated Seawater	Untreated Seawater	Shellfish	UV Treated Seawater	Untreated Seawater	Shellfish	In Shell	Shucked
Total Coliform	APHA Decimal Dilution[1]	X	X									
	12 tube single dilution[2,12]	X	X									
	Other APHA[3]				X			X				
Fecal Coliform	A-1M Decimal dilution MPN[4]	X	X									
	A-1M 12 tube single dilution MPN[5,12]	X	X									
	APHA Decimal dilution MPN[6]	X	X	X			X			X	X	X
	12 tube single dilution MPN[7,12]	X	X							X		
	mTEC[8]	X	X									
	ETCP[9]									X		
Standard Plate Count[10]											X	X

References:

1. Total Coliform Decimal Dilution MPN Methods (seawater)
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]

2. Total Coliform Single Dilution MPN Method (seawater)
   A. 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]
   B. 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]
3. Total Coliform Other Multiple Tube Fermentation Methods
   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.
4. Fecal Coliform A-1M Decimal Dilution MPN Methods (seawater)
   A-1M, 1990 AOAC International - Official Methods of Analysis, 15th Edition. Association of Official Analytical Chemists. Washington, D.C.
5. Fecal Coliform A-1 Single Dilution MPN Method (seawater)
   U.S. Food and Drug Administration. 2001. (Revised 12-8-02). NSSP Interpretation 09-III-@.02-100, Options for use of the 12-tube, single dilution MPN test.
6. Fecal Coliform APHA Decimal Dilution MPN Methods (seawater and shellfish)
   American Public Health Association. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish, 4th Edition, APHA, New York, N.Y.
7. Fecal Coliform APHA Single Dilution Methods (seawater and shellfish)
   A. U.S. Food and Drug Administration. 2001. (Revised 12-8-02). NSSP Interpretation 09-III-@.02-100, Options for use of the 12-tube, single dilution MPN test (for seawater).
   B. U.S. Food and Drug Administration. 2001. (Revised 12-8-02). NSSP Interpretation 09-XV-.03-100, Method for determining fecal coliform levels in end product depurated shellfish (for controlled purification of shellfish).
8. Fecal Coliform Methods (mTEC)
   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.
9. Fecal Coliform Methods (ETCP)
   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).
10. Standard Plate Count Method
    American Public Health Association. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish, 4th Edition, APHA, New York, N.Y.

Footnotes:

[11]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.

[12]The 12-tube single dilution MPN is not acceptable for use in conjunction with the analysis of growing waters under Systematic Random Sampling. U.S. Food and Drug Administration. 2003. NSSP Interpretation 09-IV-@.02-102.

2. Type I and Type II Marine Biotoxin Methods			
		Application	
		Growing Area Survey & Classification	**Controlled Relaying**
Sample Type		**Shellfish**	**Shellfish**
Method			
Paralytic Shellfish Poison (PSP)			
APHA	**Mouse Bioassay**[1]	X	X
Neurotoxic Shellfish Poison (NSP)			
APHA	**Mouse Bioassay**[1]	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								
	Growing Area Survey & Classification			Controlled Relaying		Wet Storage		Controlled Purification	
Sample Type	**Seawater**	**Shellfish**	**Seawater**	**Shellfish**	**Seawater**	**Shellfish**	**UV Treated Seawater**	**Shellfish**	**UV Treated Seawater**
Method									
mEndo-LES MF[1]									X
Other									
MSC[2]		X							

Footnotes:

[1]American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1989. Section 9222 B. Standard Fecal Coliform Membrane Filter Procedure. 5d. Alternative single-step direct technique. Standard Methods for the Examination of Water and Wastewater, 17th Edition, APHA/AWWA/WPCF. Washington DC. (Type III).

[2]Modified Double Agar Overlay Method for Determining Male-specific Coliphage In Soft Shelled Clams and American Oysters. ISSC Summary of Actions 2009. Proposal 05-114, Page 50. (Type IV).

4. Type III and Type IV Marine Biotoxin Test Methods		
	Application	
	Growing Area Survey & Classification	**Controlled Relaying**
Sample Type	**Shellfish**	**Shellfish**
Method		
Neurotoxic Shellfish Poison (NSP)		
Paralytic Shellfish Poison (PSP)		
JRT[2]	X	X
PCOX[3]	X	X
Diarrhetic Shellfish Poison (DSP)		
Amnesic Shellfish Poison (ASP)		
HPLC[1]	X	X

Footnotes:

[1]M.A. Quilliam, M.Xie and W.R. Hardstaff. 1991. Rapid Extraction and Cleanup Procedure for the Determination of Domoic Acid in Tissue Samples. NRC Institute for Marine Biosciences, Technical Report #64, National Research Council Canada #33001. This method may also be used direct without cleanup. (Type III).

[2]Jellett Rapid Test for PSP (Type III), Jellett Rapid Testing Ltd.

a. Method can be used to determine when to perform a mouse bioassay in a previously closed area.
b. A negative result can be substituted for a mouse bioassay to maintain an area in the open status.
c. A positive result shall be used for a precautionary closure.
d.

[3]Rourke et al. 2008. Rapid Postcolumn Methodology for Determination of Paralytic Shellfish Toxins in Shellfish Tissue. Journal of AOAC International. Vol.91, No 3: 589-597 (Type IV).

5. Methods Approved For Vibrio Enumeration		
Method	**Application**	
	PHP	
Sample Type:	**Shucked**	**In-Shell**
Vibrio vulnificus (Vv)		
EIA[1]	X	X
MPN[2]	X	X
SYBR Green 1 QPCR-MPN[5]	X	X
Vibrio parahaemolyticus (Vp)		
MPN[3]	X	X
PCR[4]	X	X

Footnotes:

[1] EIA procedure of Tamplin, et al, as described in Chapter 9 of the FDA Bacteriological Analytical Manual, 7th Edition, 1992 (Type III).
[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) (Type III).
[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 (Type III).
[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 (Type III).
[5]Vibrio vulnificus, ISSC Summery of Actions 2009. Proposal 09-113, Page 123 (Type IV).
[6]For additional information - ISSC Constitution, Bylaws & Procedures (Updated March 1, 2011) - Procedure XVI, Page 30.

6. Other	
	Application
	Controlled Purification
Sample Type	**Seawater**
Method	
Nephelometer[1] Turbidity	X

Footnotes:

[1]Nephelometer [seawater]
American Public Health Association. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish, 4th Edition, APHA, New York, N. Y.