PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
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SHELLFISH LABORATORY EVALUATION CHECKLIST

LABORATORY:		
ADDRESS:		
TELEPHONE:	FAX:	EMAIL:
DATE OF EVALUATION:	DATE OF REPORT:	LAST EVALUATION:

LABORATORY REPRESENTED BY:	TITLE:
LABORATORY EVALUATION OFFICER:	SHELLFISH SPECIALIST:
	REGION:
OTHER OFFICIALS PRESENT:	TITLE:

Items which do not conform are noted by:

C – Critical K - Key O - Other NA - Not Applicable Conformity is noted by a "√"

PART I – QUALITY ASSURANCE			
Code	**REF**		**Item Description**
			1.1 Quality Assurance (QA) Plan
K	5, 8		1.1.1 Written Plan adequately covers all the following: (check √ those that apply) a. Organization of the laboratory. b. Staff training requirements. c. Standard operating procedures. d. Internal quality control measures for equipment, their calibration, maintenance, repair, performance and rejection criteria established. e. Laboratory safety. f. Internal performance assessment. g. External performance assessment.
C	**5**		**1.1.2 QA Plan is implemented.**
			1.2 Educational/Experience Requirements
C	**State's Human Resources Department**		**1.2.1 In state/county laboratories, the supervisor meets the state/county educational and experience requirements for managing a public health laboratory.**
K	**State's Human Resources Department**		1.2.2 In state/county laboratories, the analyst(s) meets the state/county educational and experience requirements for processing samples in a public health laboratory.
C	**USDA Microbiology & EELAP**		**1.2.3 In commercial/private laboratories, the supervisor must have at least a bachelor's degree or equivalent in microbiology, biology, chemistry, or another appropriate discipline with at least two years of laboratory experience.**
K	**USDA Microbiology & EELAP**		1.2.4 In commercial/private laboratories, the analyst must have at least a high school diploma and shall have at least three months of experience in laboratory sciences.
C	**5**		**1.2.5 LC-Operator must be competent in the operation and maintenance of a basic liquid chromatography system.**
			1.3 Work Area
O	5, 8		1.3.1 Adequate for workload and storage.
O	8		1.3.2 Clean and well lighted.
O	8		1.3.3 Adequate temperature control.
O	8		1.3.4 All work surfaces are nonporous and easily cleaned.
			1.4 Laboratory Equipment.
O	6		1.4.1 The pH meter has a standard accuracy of 0.1 unit.
K	6		1.4.2 pH paper in the appropriate range (i.e. 1-4), if used, is used with minimum accuracy of 0.5 pH units.
K	10		1.4.3 pH electrodes consist of pH half-cell and reference half-cell or equivalent combination electrode/triode (free from Ag/AgCl or contains an ion exchange barrier to prevent passage of Ag ions into the medium that may result in inaccurate pH readings).

K	5		1.4.4 pH meter is calibrated daily when in use. Results are recorded and records are maintained.
K	8		1.4.5 Effect of temperature has been compensated for by an ATC probe, use of a triode or by manual adjustment.
K	8		1.4.6 A minimum of two standard buffer solutions is used to calibrate the pH meter. The first must be near the electrode isopotential point (pH 7). The second must be near the expected sample pH (i.e. pH 2, 4 or 11) as appropriate. Standard buffer solutions are used once and discarded.
K	5, 11		1.4.7 Electrode acceptability is determined daily or with each use following either slope or millivolt procedure.
K	6		1.4.8 The balances being used provide an appropriate sensitivity at the weights of use, at least 0.1 g for laboratory precision balances and 0.1 mg for analytical balances.
K	8, 9		1.4.9 The balance calibration is checked monthly using NIST class S, ASTM class 1 or 2 weights or equivalent. Results are recorded and records are maintained.
K	1		1.4.10 Refrigerator temperature is maintained between 0 and 4 ºC.
K	8		1.4.11 Refrigerator temperature is monitored at least once daily. Results are recorded and records maintained.
K	1		1.4.12 Freezer temperature is maintained at -20 ºC or below.
K	8		1.4.13 Freezer temperature is monitored at least once daily. Results are recorded and records maintained.
C	**13**		**1.4.14 All in-service thermometers are properly calibrated and immersed.**
K	5		1.4.15 All glassware is clean.
K	3		1.4.16 A high performance liquid chromatography system (HPLC) equipped with the following is used: a. binary mobile phase system delivering a pulse-free flow of 0.5-2.0 mL/min, b. solvent degasser, c. autosampler (refrigerated preferred) with loop suitable for 5-30 µL injections, d. temperature controlled column compartment capable of controlling temperature between 10 – 50 ºC, and e. fluorescence detector able to achieve the required sensitivity at an excitation wavelength (λ) of 330 nm and emission of 390 nm.
K	3, 4		1.4.17 The post-column reaction system used is equipped with the following: a. reactor module capable of maintaining 85 ºC, b. dual reagent pumps capable of delivering accurate flows of 0.4 mL/min, and c. if applicable, a reaction coil (knitted or equivalent) having a total volume of 1 mL and a length of 5 m x 0.5 mm.
K	6		1.4.18 Autopipettors are calibrated for the appropriate volumes used and checked annually for accuracy. Results are recorded and records are maintained.
K	3		1.4.19 A boiling water bath with sufficient volume to cover the sample/acid mixture is used for extraction.
O	3		1.4.20 Centrifuge capable of holding 50 mL polypropylene tubes.
K	3		1.4.21 Microcentrifuge capable of holding 1.5 mL microcentrifuge tubes and generating a minimum of 16000 g or equivalent is used.
			1.5 Reagents and Reference Solution Preparation and Storage
C	**3**		**1.5.1 All solvents and reagents used are analytical or LC grade materials.**
C	**8**		**1.5.2 Water is glass distilled or deionized and exceeds 0.5 megaohm resistance or is less than 2 µSiemens/cm conductivity at 25 ºC to be tested and recorded monthly for resistance or conductivity and the results are recorded.**
K	8		1.5.3 Water is analyzed for residual chlorine monthly and is at a nondetectable level

			(≤0.1 ppm) Results are recorded and records are maintained.
K	8		1.5.4 Water contains < 100 CFU/ml as determined monthly using the heterotrophic plate count method. Results are recorded and records are maintained.
K	8		1.5.5 Reagents are properly stored and labeled with the date of receipt, date opened or date prepared and expiration date.
C	**3**		**1.5.6 The binary mobile phase system used to analyze the GTX and STX toxins consists of:** **1.5.6.1 Mobile Phase A, which contains 11 mM heptane sulfonate and 5.5 mM phosphoric acid (H_3PO_4), pH 7.1.** **1.5.6.2 Mobile Phase B, which contains 11 mM heptane sulfonate, 16.5 mM H_3PO_4 and11.5% acetonitrile (MeCN), pH 7.1.**
C	**3**		**1.5.7 The binary mobile phase system used to analyze the C toxins consists of** **1.5.7.1 Mobile Phase A, which contains 2 mM tetrabutyl ammonium phosphate, pH 5.8.** **1.5.7.2 Mobile Phase B, which contains 2 mM tetrabutyl ammonium phosphate in 4% acetonitrile, pH 5.8.**
C	**3**		**1.5.8 The post-column oxidant consists of 100 mM H_3PO_4 and 5 mM periodic acid (H_5IO_6), pH 7.8.**
C	**3**		**1.5.9 The post-column acid used is 0.75 M nitric acid (HNO_3).**
C	**3**		**1.5.10The heptane sulphonate used in mobile phase A and mobile phase B to analyze for GTX and STX toxins is prepared the day of use or refrigerated for up to one week.**
C	**3**		**1.5.11The pH of mobile phases and the post-column oxidant are adjusted as follows:** **a. Mobile phase A and mobile phase B for the GTX and STX toxins are adjusted to 7.1 with ammonium hydroxide (NH_4OH),** **b. Mobile phase A and mobile phase B for the C toxins are adjusted to 5.8 in one direction only with 10% acetic acid (HOAc) if too basic or 1% NH_4OH if too acidic, and** **c. The post-column oxidant is adjusted to 7.8 with 5 M sodium hydroxide (NaOH).**
O	3		d. Mobile phases and post-column reagents are filtered before use if the HPLC does not have a degreaser.
C	**3,7**		**1.5.12Only certified reference materials are used for standard solutions. Source of the reference standard:**
C	**7**		**1.5.13NRC Zero-Mus or a negative control matched matrix is used as a matrix blank as appropriate. Source of the negative matrix:**
C	**7**		**1.5.14All primary standards are stored appropriately as per supplier recommendations.**
C	**7**		**1.5.15All standards used are within expiration date.**
C	**3**		**1.5.16All standards are prepared gravimetrically.**
K	3		1.5.17Intermediate mixes of primary standards are made up in 0.003 M HCl for the GTX/STX toxins or pH 5 glass distilled/deionized water for the C toxins labeled with the date of preparation and the expiration date and stored appropriately. The pH of the glass distilled/deionized water is adjusted when necessary by the dropwise addition of 10% acetic acid (HOAc).
C	**3**		**1.5.18Working standards are made up from primary standard or intermediate mixes by dilution with toxin-free, deproteinated, matrix matched extracts.**
C	**7**		**1.5.19Zero-Mus is stored according to manufacturer's instructions.**

C	**2**		**1.5.20 Quality Control shellfish tissues are stored frozen.**
C	**7**		**1.5.21 Working standards are labeled with the date of preparation, stored appropriately and used within 3 months of preparation.**
			1.6 Collection and Transportation of Samples
O	6		1.6.1 Shellstock are collected in clean, waterproof, puncture resistant containers.
K	6		1.6.2 Samples are appropriately labeled with the collector's name, type of shellstock, the harvest area, and time and date of collection.
C	**6**		**1.6.3 Immediately after collection, shellstock samples are placed in dry storage (ice chest or equivalent) which is maintained between 0 and 10 °C with ice or cold packs for transport to the laboratory.**
K	14		1.6.4 Time from collection to initiation of the extraction should not exceed 24 hours. However, if significant delays are anticipated or if they occur, the laboratory has an appropriate contingency plan in place to handle the samples. For samples shipped live in accordance with 1.6.3, the contingency plan ensures samples remain within allowable temperature tolerances and animals are alive upon receipt. The contingency plan also addresses field and/or laboratory processing that ensures the integrity of the sample or extract until initiation of the assay. For example, samples are washed, shucked, drained and processed as follows: a. refrigerated or frozen until extracted; b. homogenized and frozen until extracted; or c. extracted, the supernatant decanted, and refrigerated or frozen until assayed.
C	**6**		**1.6.5 Frozen shucked product or homogenates are allowed to thaw completely and all liquid is included as part of the sample before being processed further.**
PART II – EXAMINATION OF SHELLFISH FOR PSP TOXINS			
			2.1 Preparation of Sample
C	**6**		**2.1.1 At least 12 animals are used per sample or the laboratory has an appropriate contingency plan for dealing with non-typical species of shellfish.**
O	6		2.1.2 The outside of the shell is thoroughly cleaned with fresh water.
O	6		2.1.3 Shellstock are opened by cutting the adductor muscles.
O	6		2.1.4 The inside surfaces of the shells are rinsed with fresh water to remove sand and other foreign materials.
O	6		2.1.5 Shellfish meats are removed from the shell by separating the adductor muscles and tissue connecting at the hinge.
C	**6**		**2.1.6 Damage to the body of the mollusk is minimized in the process of opening.**
O	6		2.1.7 Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.
K	6		2.1.8 Pieces of shell and drainage are discarded.
C	**6**		**2.1.9 Drained meats or previously cooled/refrigerated shucked meats and their drip loss liquid or thawed homogentates with their freeze-thaw liquid are blended at high speed until homogenous (60-120 seconds).**
			2.2 Digestion of Sample
K	6		2.2.1 Sample homogenates are extracted as soon as possible (preferably the same day) or stored in the freezer.
C	**3**		**2.2.2 Five (5) grams of homogenized sample is weighed into a 50 mL polypropylene centrifuge tube and subsequently extracted.**
K	3		2.2.3 The sample homogenate is extracted in a 1:1 w/v ratio with 0.1 M HCl.

K	3		2.2.4 Homogenate/acid mixture is vortexed thoroughly before boiling to completely mix the contents.
C	**3**		**2.2.5 To prevent toxin transformation, the pH of the homogentate/acid mixture before boiling is 3.0 ± 1.0, adjusted if necessary with the dropwise addition of either 5 M HCl to lower the pH or 0.1 M NaOH to raise the pH.**
C	**3**		**2.2.6 Samples in capped 50 mL polypropylene centrifuge tubes are extracted in a boiling water bath for 5 minutes.**
K	3		2.2.7 The pH of the cooled mixture after boiling is 3.0 ± 1.0, adjusted if necessary with the dropwise addition of 5 M HCl. Any sample with a pH of less than 2.0 is discarded and extracted again.
K	3		2.2.8 The homogenate/acid mixture is allowed to separate by gravity or by centrifugation.
			2.3 Deproteination
C	**3**		**2.3.1 ~~500~~ 1000 µL of sample extract is deproteinated with ~~25~~ 50 µL of 30% trichloroacetic acid, vortexed thoroughly and centrifuged at ~ 16,000 *g* for 5 minutes.**
C	**3**		**2.3.2 The pH of the deproteinated extract is adjusted with 35 µL of ~~1~~2.0 M NaOH vortexed thoroughly and centrifuged at ~ 16,000 *g* for 5 minutes.**
K	3		2.3.3 An aliquot of the deproteinated supernatant is filtered through a 0.2 µm filter.
			2.4 Analysis
C	2		2.4.1 **A standard calibration curve (of at least six concentrations) is performed upon initial instrument set up, following any major hardware maintenance activity, or when the continuing calibration verification (CCV) indicates significant drift (> 30% for individual toxin) from the calibration. Results are recorded and records are maintained.**
K	3		2.4.2 10 µL is injected for GTX/STX toxins and 5 µL is injected for C-toxins.
K	3		2.4.3 Samples are stored in the sample compartment of the autosampler at 4 °C during analysis. Otherwise samples must be analyzed within 20 hours if the autosampler is held at room temperature.
K	3		2.4.4 A column heater that is capable of maintaining 30-40 °C for the GTX/STX toxins and 10-20 °C for the C toxins is used in the analysis.
C	**3**		**2.4.5 The appropriate analytical column is used.** **a. GTX/STX Toxins: Agilent Zorbax Bonus-RP column, 4.6 mm x 150 mm, 3.5 µm or equivalent.** **b. C Toxins: Thermo BetaBasic 8, 4.6 mm x 250 mm, 5 µm or equivalent.**
			2.5 System Suitability
K	2		2.5.1 The correlation coefficient for the linear regression of the calibration standards must be ≥ 0.990 for each individual toxin.

C	**3**		**2.5.2 The resolution and retention time criteria that must be met are:** **a. For GTX and STX toxins, the matrix peak must be at least 70% baseline resolved between GTX3 and GTX2.** **b. For GTX and STX toxins, GTX5 must be at least 40% baseline resolved between dcGTX3 and dcGTX2.** **c. For GTX and STX toxins, dcSTX and STX must be at least 70% baseline resolved.** **d. For GTX and STX toxins, the retention time of GTX4 must be between 5 and 7 minutes.** **e. For the C toxins, C2 must be at least 70% baseline resolved between C1 and C2.** **f. For the C toxins, the retention time of C1 must be between 4 and 7 minutes.**
C	**2**		**2.5.3 Daily injection schedules must include the adequate frequency of injection standards based on an assessment of individual standard toxin variability. Variability in peak response must be less than 10% for calculation of toxicity in samples.**
			2.6 Calculation of Toxicity
C	**4**		**2.6.1 The toxicity of the individual toxins is calculated as follows:** $\mu\text{gSTXdiHCleq}/100\text{g} = \mu\text{M} \times \frac{372.2}{1000\text{mL}} \times \frac{\text{Fvol}}{\text{Ext.vol}} \times \left(\frac{\text{Wt} + \text{Vol}}{\text{Wt}}\right) \times \text{ReTx} \times 100$ **14.** **Where:** **µM = Concentration of toxin in the extract, in µM;** **Fvol = Final volume of the deproteinized extract (e.g. 560 µL);** **Ext.vol = Volume of crude extract used (e.g. 500 µL);** **Wt = Weight of sample used;** **Vol = Volume of acid extractant used (e.g. 5 mL); and** **ReTx = Relative toxicity of toxin vs. Saxitoxin.**
C	**3**		**2.6.2 The individual toxicities for each toxin are summed to obtain the overall sample toxicity in µg STX equivalents/100 g (µg/100 g).**
C	**12**		**2.6.3 Any value at or above 80 µg STX equivalents /100 g of meat is actionable.**

Relative Toxicity Values

Toxin	ReTx	Toxin	ReTx
GTX1	0.9940	NEO	0.9243
GTX2	0.3592	STX	1.0000
GTX3	0.6379	dcSTX	0.5131
GTX4	0.7261	C1	0.0060
GTX5	0.0644	C2	0.0963
dcGTX2	0.1538	C3	0.0133
dcGTX3	0.3766	C4	0.0576

15.

REFERENCES:

1. American Public Health Association. 1984. *Compendium for the Microbiological Examination of foods,* 2nd Edition. APHA. Washington D.C.
2. Good Laboratory Practice.
3. AOAC Official Methods of Analysis (2011). AOAC Official Method 2011.02 Paralytic Shellfish Toxins in Mussels, Clams, Oysters, and Scallops Post-Column Oxidation (PCOX) Method.
4. Oshima, Y. 1995. J. AOAC Int. 78: 528-532.
5. Association of Official Analytical Chemists (AOAC). 1991. *Quality Assurance Principles for Analytical Laboratories.* AOAC, Arlington, VA.
6. American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition. APHA, Washington, D.C.
7. Consult reference standard product literature.
8. APHA/WEF/AWWA. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition. APHA, Washington, D.C.
9. American Public Health Association. 1992. *Standard Methods for the Examination of Dairy Products*, 16th Edition. APHA, Washington, D.C.
10. Fisher, J. 1985. Measurement of pH. *American Laboratory* 16: 54-60.
11. Consult pH electrode product literature.
12. U.S. Food and Drug Administration (FDA) and Interstate Shellfish Sanitation Conference (ISSC). 2011. *NSSP Guide to the Control of Molluscan Shellfish.* FDA/ISSC, Washington, D.C. and Columbia, S.C.
13. U.S. Department of Commerce. 1976. NBS Monograph 150. U.S. Department of Commerce, Washington, D.C.
14. Compendium of Methods for the Microbiological Examination of Foods, 3rd Edition, pg. 901.

LABORATORY:			**DATE OF EVALUATION:**
SHELLFISH LABORATORY EVALUATION CHECKLIST **SUMMARY OF NONCONFORMITIES**			
Page	**Item**	**Observation**	**Documentation Required**
LABORATORY STATUS			

LABORATORY	DATE
LABORATORY REPRESENTATIVE:	

PARALYTIC SHELLFISHPOISON COMPONENT: PARTS I AND II

A. Results

Total # of **Critical (C)** Nonconformities	_____
Total # of Key (K) Nonconformities	_____
Total # of Critical, Key, and Other (O) Nonconfomities	_____

B. Criteria for Determining Laboratory Status of the PSP, PCOX Component

1. **Conforms Status: The PSP, PCOX component of this Laboratory is in conformity with** NSSP requirements if a ll of the following apply.
   a. No Critical nonconformities.
   b. and <6 Key nonconformities.
   c. and <12 Total nonconformities.

2. **Provisionally Conforms Status:** The PSP, PCOX component of this laboratory is determined to be provisionally conforming to NSSP requirements if a ll of the following apply.
   a. the number of critical nonconformities is ≥ 1 but **< 4**.
   b. and **< 6** Key nonconformities.
   c. and **< 12** Total nonconformities.

3. **Does Not Conform Status** : The PSP, PCOX component of this laboratory is not in conformity with NSSP requirements when any of the following apply.
   a. The total # of Critical nonconformities is **≥ 4**.
   b. or the total# of Key nonconformities is **≥ 6.**
   c. or the total # of Critical, Key, or Other is **≥ 12.**

C. Laboratory Status (*circle appropriate)*

Does Not Conform **Provisionally Conforms** **Conforms**

Acknowledgement by Laboratory Director/Supervisor:

All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____.

Laboratory Signature: _____ Date:_____

LEO Signature: _____ Date: _____