PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
SHELLFISH PROGRAM IMPLEMENTATION BRANCH
SHELLFISH SAFETY TEAM
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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		Item Description
		1.1 Quality Assurance (QA) Plan
K		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, calibration, maintenance, repair and performance. e. Laboratory safety. f. Internal performance assessment g. External performance assessment
C		**2. QA Plan is implemented.**
		1.2 Work Area
O		1. Adequate for workload and storage.
O		2. Clean and well lighted.
O		3. Adequate temperature control.
O		4. All work surfaces are nonporous and easily cleaned.
		1.3 Laboratory Equipment.
O		1. The pH meter has a standard accuracy of 0.1 unit.
K		2. pH paper in the appropriate range (i.e. 1-4) is used with minimum accuracy of 0.5 pH units.
K		3. pH electrodes consist of pH half cell and reference half cell or equivalent combination electrode (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		4. pH meter is calibrated daily or with each use. Records maintained.
K		5. Effect of temperature has been compensated for by an ATC probe or by manual adjustment.
K		6. A minimum of two standard buffer solutions (2 & 7) are used to calibrate the pH meter. Standard buffer solutions are used once and discarded.
K		7. Electrode efficiency is determined daily or with each use following either slope or millivolt procedure.
K		8. The balance provides a sensitivity of at least 0.0001 g at a load of 5 grams.
K		9. The balance calibration is checked monthly using NIST class S, ASTM class 1 or 2 weights or equivalent. Records maintained.
K		10. Refrigerator temperature is maintained between 0 and 4ºC.
K		11. Refrigerator temperature is monitored at least once daily. Records maintained.
K		12. Freezer temperature is maintained at -20ºC or below.
O		13. Freezer temperature is monitored at least once daily. Records maintained.
O		14. All glassware is clean.
K		15. High performance liquid chromatography system equipped with the following: a. Low dead-volume, b. binary solvent system delivering a pulse-free flow of 0.5-2.0 mL/min, c. solvent degasser, d. autosampler with loop suitable for 5-30 µL injections, e. temperature controlled column compartment capable of controlling temperature between 10 – 50ºC, and f. fluorescence detector able to achieve the required sensitivity at excitation λ=330nm and emission λ=390nm.
K		16. Post-column reaction system equipped with the following:Reactor module

		capable of maintaining 85ºC, b. dual reagent pumps capable of delivering accurate flows of 0.4 mL/min, and c. knitted reaction coil, 1 mL volume, 5 m x 0.5 mm.
K		17. Autopipettors are calibrated annually. Records maintained.
K		18. Boiling water bath with sufficient volume to cover sample/acid mixture.
K		19. Centrifuge capable of holding 50 mL polypropylene tubes and generating ~ 3000 RCF.
K		20. Microcentrifuge capable of generating ~16000 RCF.
		1.4 Reagents and Reference Solution Preparation and Storage
O		1. All solvents and reagents used are analytical or LC grade materials.
K		2. Water is distilled or deionized and exceeds 0.5 megaohm resistance or is less than 2 µSiemens/cm conductivity at 25ºC to be tested and recorded monthly for resistance or conductivity.
O		3. Water is analyzed for residual chlorine monthly and is at a nondetectable level (≤0.1 ppm) Records maintained.
K		4. Water is free from trace (< 0.5 mg/l) dissolved metals specifically, Cd, Cr, Cu, Ni, Pb, and Zn as determined annually with total heavy metal content ≤ 1.0 mg/l. Records maintained.
O		5. Water contains < 1000 CFU/ml as determined monthly using the heterotrophic plate count method. Records maintained.
O		6. Reagents are properly stored and labeled with the date of receipt and date opened.
C		**7. 0.5 M 1-heptane sulphonate is prepared the day of use or refrigerated.**
C		**8. pH of mobile phases and oxidant are as follows and records maintained:** **a. GTX/STX toxins mobile phase A&B is 7.1,** **b. C toxins mobile phase A is 5.8, and** c. **Oxidant is 7.8.**
K		9. Mobile phases and post-column reagents are filtered through 0.2 µm nylon filter membrane before use.
C		**10. Only certified reference materials are used for standard solutions. Source of the reference standard: _____**
K		11. All primary standards are stored appropriately as per supplier recommendations.
K		12. Standards are prepared gravimetrically using "Class A" glassware.
K		13. Intermediate mixes of primary standards are made up in 0.003 M HCl (GTX/STX toxins) or Milli-Q water (C toxins), and stored appropriately.
K		14. Working standards are made up from primary standard mixes by dilution with toxin-free, deproteinated mussel or oyster extract (GTX/STX toxins) or Milli-Q water (C toxins).
K		15. Working standards are stored in the refrigerator at 4°C.
		1.5 Collection and Transportation of Samples
O		1. Shellstock are collected in clean, waterproof, puncture resistant containers.
K		2. Samples are appropriately labeled with the collector's name, type of shellstock, the source, the harvest area, time, date and place (if market sample) of collection.
K		3. Immediately after collection, shellstock samples are placed in dry storage between 0 and 10ºC until analyzed.
K		4. The time from collection to completion of the assay should not exceed 24 hours. However, if there are significant transportation delays, then shellstock

		samples are processed immediately as follows (circle the appropriate choice):Washed, shucked, drained, frozen until extracted; b. Washed shucked, drained, homogenized and frozen; c. Washed, shucked drained, extracted, the supernatant decanted and refrigerated (**best choice**); or d. The laboratory has an appropriate contingency plan in place to handle samples which can't be analyzed within 24 hours due to transportation issues.
		5. Frozen shucked product or homogenates are allowed to thaw completely and all liquid is included as part of the sample before being processed futher.
PART II – EXAMINATION OF SHELLFISH FOR PSP TOXINS		
		2.1 Preparation of Sample
C		**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		2. The outside of the shell is thoroughly cleaned with fresh water.
O		3. Shellstock are opened by cutting the adductor muscles.
O		4. The inside surfaces of the shells are rinsed with fresh water to remove sand and other foreign materials.
O		5. Shellfish meats are removed from the shell by separating the adductor muscles and tissue connecting at the hinge.
K		6. Damage to the body of the mollusk is minimized in the process of opening.
O		7. Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.
K		8. Pieces of shell and drainage are discarded.
C		**9. Drained meats or thawed homogentates are blended at high speed until homogenous (60-120 seconds).**
		2.2 Digestion of Sample
K		1. Sample homogenates are extracted as soon as possible (same day) or stored in the freezer.
K		2. Sample homogenate is extracted in a 1:1 w/v ratio with 0.1 M HCl, preferably 5g tissue in 5mL acid
K		3. Homogenate/acid mixture is vortexed thoroughly before boiling to completely mix the contents.
C		**4. To prevent toxin transformation, the pH of the homogentate/acid mixture before boiling is 3.0 ± 1.0, adjusted if necessary with 5M HCl or 0.1 M NaOH.**
C		**5. Samples are extracted in a boiling water bath for 5 minutes, in capped 50mL polypropylene centrifuge tubes.**
K		6. The pH of the cooled mixture after boiling is 3.0 ± 1.0, adjusted if necessary with 5M HCl. Any sample with a pH of less than 2.0 is discarded and extracted again.
K		7. The homogenate/acid mixture is allowed to separate by gravity or by centrifugation at 2500 *g* for 10 minutes. Supernatant is then decanted into a scintillation vial.
		2.3 Deproteination
C		**1. Extract is deproteinated with 30% trichloroacetic acid (50 µL TCA per 1000 µL aliquot of supernatant), vortexed thoroughly and centrifuged at 16,000 *g* for 5 minutes.**
C		**2. The pH of the deproteinated extract is adjusted to 3.0 ± 1.0 with 1.0 M**

		NaOH (70 µL NaOH per 1000 µL aliquot of supernatant), vortexed thoroughly and centrifuged at 16,000 *g* for 5 minutes.
K		3. An aliquot of the deproteinated, pH-adjusted supernatant is filtered through a 0.2 µm filter into two 2 mL autosampler vials (one vial for GTX/STX analysis and one vial for C-Toxins analysis).
		2.4 Assay
C		**1. A calibration 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. Records maintained.**
K		2. For GTX/STX toxins, no more than ten samples should be made between standard analyses. For C toxins, no more than five samples injections should be made between standard analyses.
K		3. 10 µL is injected for GTX/STX toxins and 5 µL is analyzed for C-toxins.
K		4. Samples are stored in the sample compartment at 4°C during analysis.
O		5. A column heater is used in the analysis.
O		6. The appropriate analytical column is used. a. GTX/STX Toxins: Zorbax Bonus-RP column, 4.6 mm x 150 mm, 3.5 µm, Agilent catalog number 863668-901 or equivalent. b. C Toxins: BetaBasic 8, 4.6 mm x 250 mm, 5 µm, Fisher catalog number 71405-254630 or equivalent.
		2.5 System Suitability
K		1. The correlation coefficient for the linear regression (r^2) must be ≥ 0.990 for each individual toxin.
K		2. Resolution and Retention Time Criteria. a. GTX/STX Toxins. i. Matrix peak must be at least 70% baseline resolved between GTX3 and GTX2. ii. GTX5 must be at least 40% baseline resolved between dcGTX3 and dcGTX2. iii. dcSTX and STX must be at least 70% baseline resolved. iv. GTX4 retention time should be between 5 and 7 minutes. b. C Toxins. i. C1 and C2 must be at least 70% baseline resolved. ii. C1 retention time should be between 5 and 8 minutes.
		2.6 Calculation of Toxicity
C		**1. The toxicity of the individual toxins is calculated as follows:** $$\mu\text{gSTXdiHCle q/100g} = \mu\text{M} \times \frac{372.2}{1000\text{mL}} \times \frac{\text{Fvol}}{\text{Ext.vol}} \times \left(\frac{\text{Wt} + \text{Vol}}{\text{Wt}}\right) \times \text{ReTx} \times 100$$ **Where:** **µM = Concentration of toxin in the extract, in µM;** **Fvol = Final volume of the deproteinized extract (1120 µL);** **Ext.vol = Volume of crude extract used (1000 µL);** **Wt = Weight of sample used;** **Vol = Volume of acid extractant used (e.g. 5 mL); and** **ReTx = Relative toxicity of toxin vs. Saxitoxin.** **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**	
C		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		3. **Any value greater than 80 µg STX equivalents /100 g of meat is actionable.**					

REFERENCES

1. 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.
2. Adams, W.N. and S.A. Furfari. 1984. Evaluation of laboratory performance of the AOAC method for PSP toxin in shellfish. *J. Assoc. Off. Anal. Chem.* Vol 67, 6:1147-1148.
3. American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition. APHA, Washington, D.C.
4. American Public Health Association. 192. *Standard Methods for the Examination of Dairy Products*, 16th Edition. APHA, Washington, D.C.
5. Assocation of Official Analytical Chemists International. 1990. *Methods of Analysis*, 15th Edition. AOAC, Arlington, VA.
6. APHA/WEF/AWWA. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition. APHA, Washington, D.C.
7. Title 21, Code of Federal Regulations, Part 58, *Good Laboratory Practice for Nonclinical Laboratory Study*. U.S. Government Printing, Washington, D.C.
8. National Research Council. 1996. *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, D.C.
9. Personal communication with USFDA Washington Seafood Laboratory Branch, Office of Seafood, CFSAN, 1998-1999.

LABORATORY:	**DATE OF EVALUATION:**

SHELLFISH LABORATORY EVALUATION CHECKLISH

SUMMARY OF NONCONFORMITIES

Page	**Item**	**Observation**	**Documentation Required**

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	
PARALYTIC SHELLFISH POISON COMPONENT: PARTS I AND II	
A. Results Total # of **Critical (C)** Nonconformaties Total # of Key (K) Nonconformaties Total # of Critical, Key, and Other (O) Nonconfomaties	_____ _____ _____

B. Criteria for Determining Laboratory Status of the PSP Component

1. **Does Not Conform Status** The PSP component of this laboratory is not in conformity with NSSP requirements if:
   a. The total # of Critial nonconformities is ≥ **3 or**
   b. The total # of Key nonconformities is ≥ **6 or**
   c. The total # of Critical, Key, or Other is ≥ **10**

2. **Provisionally Conforms Status** The PSP component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is ≥ 1 by <3.

C. Laboratory Status (*circle appropriate)*

Does Not Conform – Provisionally Conforms – Conforms

Acknowledgement by Laboratory Director/Supervisior:

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: _____