Proposal No. 19-101

Proposal for Task Force Consideration at the ISSC 2019 Biennial Meeting	1. a. ☒ Growing Area b. ☐ Harvesting/Handling/Distribution c. ☐ Administrative
2. Submitter	Michael Hickey, Jeff Kennedy, Diane Regan
3. Affiliation	Massachusetts Division of Marine Fisheries
4. Address Line 1	836 S Rodney French Blvd
5. Address Line 2	
6. City, State, Zip	New Bedford, MA 02744
7. Phone	(508) 990-2860
8. Fax	(508) 990-0449
9. Email	Michael.hickey@mass.gov
10. Proposal Subject	Conditionally Conforming Laboratory Status
11. Specific NSSP Guide Reference	Section II. Model Ordinance Chapter I. Shellfish Sanitation Program Requirements for the Authority @.03 B. 1. b. Section II. Model Ordinance Chapter III. Laboratory @.01 Section II. Model Ordinance Chapter XV. Depuration .03 J. (4)
12. Text of Proposal/ Requested Action	The requested action is to create a NSSP laboratory status of conditionally conforming. This status is based on a demonstrated proficiency of laboratory method performance. Laboratories that are found to conditionally conform for a laboratory analysis may support the NSSP. **MO Chapter 1.@.03 B. 1. b.** v. Performance Evaluation: Conditionally Conforms. Tto be deemed conditionally conforming under the NSSP, a laboratory must meet one of the following laboratory performance criteria: (a) Complete an appropriate ISSC Accepted SLV; or (b) Complete a Method Verification Study, Section IV. Chapter II. .20 that successfully transfers; or (c). Successfully complete a proficiency and/or inter-laboratory study approved by the FDA Shellfish LEO or State certified Shellfish LEO. (d) This laboratory status will remain in effect until an technical FDA Shellfish LEO or FDA certified State Shellfish LEO Evaluation occurs as in @.03 B. **MO Chapter III. @.01 Quality Assurance** A. NSSP Conformance Required for all laboratories supporting the NSSP. All laboratory analyses shall be performed by a laboratory found to conform, conditionally conform or provisionally conform by the FDA Shellfish LEO or FDA certified State Shellfish LEO in accordance with the requirements established under the NSSP. **MO Chapter XV. .03 J. (4)** (a) Are analyzed by a laboratory which has been evaluated and found to conform or conditionally conform to the NSSP pursuant to the requirements in Chapter III, using an NSSP-Approved Method;
13. Public Health Significance	A technical Laboratory evaluation, as outlined in MO Chapter 1.@.03B.1.b.ii, is conducted to verify that conditions are present *in the laboratory* which **should**

	result in the accurate outcome of method data. A performance evaluation **verifies** that the method data produced *by the laboratory and for all analysts* is accurate. A technical evaluation does not examine the quality of a laboratory's method data for validity, standardization or for individual analysts. If a laboratory has successfully passed a proficiency study, SLV or MV, and statistically confirmed method data results, the laboratory can be assumed to have technically performed the method correctly. Under current interpretation a laboratory may have completed and had accepted by the conference a method SLV with accompanying checklist yet not be able to support the NSSP with data until a FDA Shellfish LEO or FDA certified State Shellfish LEO conducts a technical inspection at their laboratory using the laboratory's own checklist. If a laboratory has proven its ability to perform a method, then the laboratory should be able to conditionally support the NSSP with data. A cooperative goal of the NSSP, FDA and the SSCA is to assure that a laboratory's data is accurate, verified and standardized. Method based performance evaluations confirm data which results in standardization across laboratories. Method based performance evaluations statistically verify data accuracy. Performance Evaluations therefore support the legal defensibility of the laboratory's Laboratory Quality Management System.
14. Cost Information	Cost of conducting SLV, MV or Proficiency Participation
Action by 2019 Laboratory Committee	Recommended no action on Proposal 19-101. Rationale: This issue is addressed by Proposal 19-301.
Action by 2019 Task Force I	Recommended adoption of Proposal 19-101 as submitted.
Action by 2019 General Assembly	Recommended referral of Proposal 19-101 to an appropriate committee as determined by the Conference Chair.
Action by FDA February 21, 2020	Concurred with Conference action on Proposal 19-101.
Action by 2023 Laboratory Committee	Recommended referral of Proposal 19-101 to an appropriate committee as determined by the Conference Chairperson
Action by 2023 Task Force I	Recommended referral of Proposal 19-101 as amended to the Laboratory Committee with the provision that a recommendation for interim approval be provided at the 2023 Fall Executive Board Meeting. **MO Chapter 1.@.03 B. 1. b.** v. Performance Evaluation: Conditionally Conforms. Tto be deemed conditionally conforming under the NSSP, a laboratory must meet one of the following laboratory performance criteria: (a) Complete an appropriate ISSC Accepted SLV; or (b) Complete a Method Verification Study, Section IV. Chapter II. .20 that successfully transfers; or ~~(c). Successfully complete a proficiency and/or inter-laboratory study approved by the FDA Shellfish LEO or State certified Shellfish LEO.~~ (c~~d~~)

	This laboratory status will remain in effect until an technical FDAShellfish LEO or FDA certified State Shellfish LEO Evaluation occurs as in @.03 B. **MO Chapter III. @.01 Quality Assurance** A. NSSP Conformance Required for all laboratories supporting the NSSP. All laboratory analyses shall be performed by a laboratory found to conform, conditionally conform or provisionally conform by the FDA Shellfish LEO or FDA certified State Shellfish LEO in accordance with the requirements established under the NSSP. **MO Chapter XV. .03 J. (4)** (a) Are analyzed by a laboratory which has been evaluated and found to conform or conditionally conform to the NSSP pursuant to the requirements in Chapter III, using an NSSP-Approved Method;
Action by 2023 General Assembly	Adopted the recommendation of Task Force I on Proposal 19-101.
Action by FDA July 7, 2023	Concurred with Conference action on Proposal 19-101.
Action by 2024 Laboratory Committee	Recommended Proposal 19-101 as substituted and submit to the 2024 Executive Board for interim approval. **Chapter III** **@.01 Quality Assurance** C. FDA Responsibilities. The FDA will ensure that all laboratories generating data in support of the NSSP will be evaluated at a minimum frequency of once every three (3) years. (1) Evaluations will be conducted by either an FDA Shellfish LEO or an FDA certified State Shellfish LEO as appropriate. Normally the initial evaluation of a laboratory will be conducted by FDA. (2) Evaluations are ~~generally~~ conducted onsite; however, evaluations may be conducted ~~but can under certain circumstances be~~ by desk/virtual audit wen scheduling or extenuating circumstances will not permit a timely onsite evaluation. ~~(evaluation follow up, action plan monitoring, nonconformity corrections, major changes in personnel, workload or facilities, etc.).~~ (3) When there is an immediate and critical need, and: (a) no NSSP conforming or provisionally conforming laboratory exists to perform a specific Approved NSSP Method, or (b) no NSSP conforming or provisionally conforming laboratory is accessible (i.e. due to distance, etc.) to perform specific Approved NSSP method, or (c) following a request for emergency consideration as defined in Section II, Ch. 1 @.01 I. The following alternative laboratories may implement that Approved ISSC Method in support of the NSSP:

	(a) A laboratory that has not been evaluated or assigned an NSSP operational status, provided that: i. the ISSC Executive Board is notified within a reasonable period of time regarding the laboratory used; and, ii. the appropriate FDA Office is notified within a reasonable period of time regarding the laboratory used; and, iii. the laboratory meets the following laboratory performance criteria: 1. completed the SLV study which resulted in the Approved NSSP method; or 2. maintains a recognized laboratory accreditation status (e.g., ISO 17025:2017, LAAF, etc.), with documentation of accreditation provided to the FDA prior to method implementation; and 3. successfully completed a Method Verification Study, as outlined in Section IV, Chapter II, .20, and the method verification data package has been approved by an FDA Shellfish LEO. If an FDA Shellfish LEO is unavailable to review the method verification data package in a timely manner, an FDA certified State Shellfish LEO may review and approve the data package instead. State Shellfish LEOs will only review method verification data packages pertaining to methods for which they have documented proficiency and evaluation standardization. (b) An existing NSSP conforming or provisionally conforming laboratory that has not been evaluated for the specific Approved NSSP method to be used, provided that: i. the ISSC Executive Board is notified within a reasonable period of time regarding the laboratory used; and, ii. the appropriate FDA Office is notified within a reasonable period of time regarding the laboratory used; and, iii. the laboratory has successfully completed a Method Verification Study, as outlined in Section IV, Chapter II, .20, and the method verification data package has been approved by an FDA Shellfish LEO. If an FDA Shellfish LEO is unavailable to review the method verification data package in a timely manner, an FDA certified State Shellfish LEO may review and approve the data package instead. State Shellfish LEOs will only review method verification data packages pertaining to methods for which they have documented proficiency and evaluation standardization. If the immediate and critical need for analysis becomes an ongoing or recurring need, the laboratory implementing the method will work with the FDA

	Shellfish LEOs to promptly initiate the evaluation process. D. Wet Storage and Post-Harvest Processors. For any laboratory providing analytical testing services for depuration, wet storage or PHP, initial and subsequent triennial evaluations will be required and conducted in accordance with @.01 and @.02 of this Chapter by an FDA Shellfish LEO or an FDA certified State Shellfish LEO ~~as appropriate~~. It is understood that academic laboratories involved in PHP Validation or Verification have special circumstances such as extended periods of inactivity resulting from university schedules or funding constraints; however, written documentation of Quality Control practices will be required for time periods in which they are preparing for or actively participating in a PHP Validation or Verification. Times in which the lab is inactive can be explained with a not applicable notation.
Action by 2023 Executive Board	Adopted the recommendation of 2024 Laboratory Committee. Interim Approval on October 30, 2024.

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
5001 CAMPUS DRIVE
COLLEGE PARK, MD 20740-3835
TEL. 240- 402-4960/9258/7629, 301-796-0788
CFSANDSSLEOS@FDA.HHS.GOV

SHELLFISH LABORATORY EVALUATION CHECKLIST
Receptor Binding Assay for Paralytic Shellfish Poisoning (RBA PSP)

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

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

Items which do not conform are noted by: **Conformity is noted by a "√"**

C- Critical K - Key O - Other N/A- Not Applicable

PART I – QUALITY ASSURANCE			
CODE	**REF**		**ITEM**
		1.1 Quality Assurance (QA) Plan	
K	1, 2, 3		1.1.1 Written Plan (Check √ those items which apply).
			a. Organization of the Laboratory.
			b. Staff training requirements. Training must include radiation lab safety.
			c. Standard operating procedures (SOPs).
			d. Internal quality control measures for equipment, their calibration, maintenance, repair, performance and rejection criteria established.
			e. Laboratory safety. Radiation safety practices (e.g., handling and disposal) must be included.
			f. Internal performance assessment.
			g. External performance assessment.
C	**2**		**1.1.2 The 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 analysts meet the state/county educational and experience requirements for processing samples in a public health laboratory.
C	**USDA Microbiology & EELAP**		**1.2.3 In commercial laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology or other appropriate discipline with at least two years of laboratory experience.**
K	USDA Microbiology & EELAP		1.2.4 In commercial laboratories, the analysts must have at least a high school diploma and at least three months of experience in laboratory sciences.
C	**6**		**1.2.5 Training regarding radiation laboratory safety, handling and disposal practices is documented and records are maintained.**
C	**15**		**1.2.6 Laboratory has a Nuclear Regulatory Commission (NRC) or equivalent state license for the use of tritiated saxitoxin in this assay. Alternatively, the laboratory uses less than 50 µCi per year and adheres to the American Radiolabeled Chemical (ARC) exemption status.**
		1.3 Work Area	
O	2		1.3.1 The work area is adequate for the workload and storage.
K	2		1.3.2 The work area is clean and well lighted.
K	2		1.3.3 The work area has adequate temperature control.
O	3		1.3.4 All work surfaces are nonporous, easily cleaned and disinfected.
C	**3,4**		**1.3.5 The work area is located in an appropriate space designated for low-level radiation work. Radioactive materials are only handled and manipulated in designated areas which are clearly identified and labeled accordingly.**

		1.4 Laboratory Equipment	
C	**4**		**1.4.1 Any lab equipment that may come into contact with [^{3}H]-STX at any point in the preparation or assay procedures must be specially labelled and must remain in the work area designated for low-level radiation work.**
O	5		1.4.2 The pH meter has a standard accuracy of 0.1 pH units.
K	7		1.4.3 The pH electrodes being used consist of a pH half cell and reference half cell or equivalent combination electrode/triode free from silver/silver chloride (Ag/AgCl) or contains an ion exchange barrier to prevent the passage of silver (Ag) ions into the substance being measured.
K	3, 8		1.4.4 The pH meter is calibrated daily when in use. Results are recorded and records maintained.
K	1		1.4.5 The effect of temperature on the pH has been compensated for by an ATC probe, use of a triode, or by manual adjustment.
K	1		1.4.6 The pH meter manufacturer instructions are followed for calibration, or a minimum of two (2) standard buffer solutions is used to calibrate the pH meter. If the calibration sequence of standard buffer solutions is not stipulated by the manufacturer, the first must be near the isopotential point (pH 7) and the second near the expected sample (i.e., pH 4 or pH 10). Standard buffer solutions are used once and discarded.
O	9		1.4.7 Electrode acceptability is determined daily or with each use by the millivolt procedure or through determination of the slope.
K	6		1.4.8 pH paper in the appropriate pH range (i.e., 1-5), if used, measures accurately to a minimum of 0.5 pH units over the covered pH range.
K	6		1.4.9 The differing sensitivities in weight measurements required by the various steps in the assay are met by the balance(s) being used. a. To prepare Phenyl methylsulfonyl fluoride solution (PMSF), the balance used must have a sensitivity of at least 0.001 gram at a load of 1 gram. b. For sample extraction, the balance used must have a sensitivity of at least 0.1 gram at a load of 100 grams. c. For MOPS buffer preparation, the balance used must have a sensitivity of at least 0.01 gram at a load of 100 grams.
K	1, 3		1.4.10 Balance calibrations are checked monthly according to manufacturer's specifications using NIST Class S or ASTM Class 1 or 2 weights or equivalent. The accuracy of the balance is verified at the weight range of use.
K	2		1.4.11 Refrigerator temperatures are maintained between 0 and 4 °C. Freezer security for ^{3}HSTX and cold STX must meet state and federal requirements for these materials.
K	1		1.4.12 Refrigerator temperatures are monitored at least once daily on workdays. Results are recorded and records maintained.
C	**4, 6, 10**		**1.4.13 Freezer temperature used to store [^{3}H] STX standard, rat brain membrane tissue preparation, interassay calibration standard (QC check) and archived shellfish tissue homogenate is maintained at -80 °C or below. Freezer security for ^{3}HSTX and cold STX must meet state and federal requirements for these materials.**

K	6, 10		1.4.14 Freezer temperature used for all other purposes is maintained at -20 °C or below.
O	1		1.4.15 Freezer temperature is monitored at least once daily on workdays. Results are recorded and records maintained.
O	8		1.4.16 All glassware is clean.
C	**3**		**1.4.17 An alkaline or acid-based detergent is used for washing glassware/labware.**
C	**1**		**1.4.18 With each load of labware/glassware washed, the contact surface of several dry pieces from each load are tested for residual detergent (acid or alkali as appropriate) with aqueous 0.04% bromothymol blue (BTB) solution. Results are recorded and records maintained.**
C	**6**		**1.4.19 Micropipettors are calibrated for the appropriate volumes used and checked annually for accuracy. Results are recorded and records are maintained.**
C	**11**		**1.4.20 Scintillation counter is serviced according to manufacturer specifications and calibrated annually. Results are recorded and records maintained.**
C	**4**		**1.4.21 Minimum radiation safety equipment and protocols include the following: A wipe-test is conducted in the radiation work area as described in the QA plan. Results are recorded and records maintained.**
		1.5 Reference Solution Reagent Storage, Preparation and Security	
C	**12**		**1.5.1 [^{3}H] STX standard is stored in a freezer at -80 °C or below.**
C	**10**		**1.5.2 Concentration of [^{3}H] STX standard is calculated from the lot information provided by the supplier with each batch.**
K	6		1.5.3 Unopened diHCl STX standard may be stored at room temperature or refrigerated.
C	**10**		**1.5.4 Preparation of MOPS assay buffer includes the following:** **a. 100 mM MOPS/L.** **b. 100 mM choline chloride/L.** **c. pH adjustment to 7.4 with NaOH.** **e. refrigerated storage at 4 °C.** **d. Maintained ice cold while in use.**
C	**10**		**1.5.6 Bulk standard curve dilutions are stored at 4 °C for up to one (1) month.**
K	1		1.5.7 Reagent water is distilled or deionized *(circle appropriate choice)* and is analyzed monthly for the following criteria, with all results recorded and records maintained: a. Exceeds 0.5 megohm-cm resistivity (2 megohm-cm in-line) or less than 2.0 µSiemens/cm conductivity at 25 °C *(circle appropriate choice)*. b. Residual chlorine is at a non-detectable level (<0.1 ppm). Specify method of determination_____ c. Water contains <100 CFU/mL using the heterotrophic plate count method.

		1.6 Rat Brain Membrane Tissue Preparation and Storage		
C	**10**		**1.6.1**	**MOPS/choline chloride/phenyl methylsulfonyl floride (PMSF), pH 7.4 is used in preparing rat brain membrane tissue. PMSF is added to MOPS/choline chloride fresh on the day of use.**
C	**10**		**1.6.2**	**The cerebral cortex of 6-week old Sprague-Dawley rats is used in membrane tissue preparations, placed in iced MOPS/choline chloride/PMSF buffer (pH 7.4; 1 brain/12.5 mL) and homogenized with no visible chunks remaining in the homogenate. This procedure is repeated until twenty (20) rat brains have been processed.**
C	**10**		**1.6.3**	**The homogenized cerebral cortex tissue from the twenty (20) rat brain cortices is pooled and centrifuged at 20000 x g for 15 minutes at 4 °C.**
K	10		1.6.4	The pellet of the centrifuged rat brain tissue preparation is fully resuspended in ice cold MOPS/choline chloride/PMSF buffer (up to 10 mL/brain).
K	10		1.6.5	The resuspended rat brain tissue preparations are pooled and the centrifuge tubes used for these preparations are rinsed with a small amount of MOPS/choline chloride/PMSF buffer to recover all the rat brain tissue**.**
K	10		1.6.6	The total volume of the pooled rat brain tissue is adjusted to 200 mL with MOPS/choline chloride/PMSF buffer while iced.
K	10		1.6.7	The iced contents of the pooled rat brain tissue are blended using a Polytron at 70% power or a small hand- held blender at low speed for 20 seconds to obtain a homogeneous membrane tissue preparation.
C	**10**		**1.6.8**	**Two (2) mL/tube of the pooled, homogeneous rat brain membrane tissue preparation is aliquoted into cryovials, frozen and stored at -80 °C for up to six (6) months.**
		1.7 Rat Brain Membrane Tissue Protein Receptor Determination		
C	**10**		**1.7.1**	**The protein/receptor concentration of the rat brain membrane tissue preparation is determined for each new batch using a Pierce Micro BCA Protein Assay Reagent Kit No. 23235 (micro plate method) or No. 23225 (tube method) or equivalent.**
C	**10**		**1.7.2**	**The dilution of the protein/receptor concentration of the rat brain membrane tissue preparation needed to obtain a working stock of 1 mg/mL is determined.**
K	10		1.7.3	Dilutions of the protein/receptor concentration of the rat brain membrane tissue preparation of less than 1:4 are not used as they may be too viscous.
PART II – ANALYSIS OF SHELLFISH SAMPLES FOR PSP TOXINS - RBA				
		2.1 Collection and Transportation of Samples		
C	**5**		**2.1.1**	**A representative sample of shellfish is collected.**
K	5		2.1.2	Shellfish samples are collected in clean, waterproof, puncture resistant containers loosely sealed.
K	5		2.1.3	Shellfish samples are labeled with the collector's name, type of shellstock, the source or harvest area, sampling station, time, date and place (if applicable) of collection.
C	**5**		**2.1.4**	**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	6, 13		2.1.5 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 these samples. For samples shipped live in accordance with 2.1.4, 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.
		2.2 Preparation of Samples for Analysis – Homogenization	
C	**5, 6**		**2.2.1 At least 12 animals are used per sample, or the laboratory has an appropriate contingency plan for dealing with non-typical species of shellfish or collection conditions.**
O	5		2.2.2 The outside of the shell is thoroughly cleaned with fresh water.
O	5		2.2.3 Shellstock are opened by cutting the adductor muscles.
O	5		2.2.4 The inside surfaces of the shells and meats are rinsed with fresh water to remove sand or other foreign material.
O	5		2.2.5 Shellfish meats are removed from the shell by separating the adductor muscles and tissue connecting at the hinge.
C	**5**		**2.2.6 Damage to the body of the mollusk is minimized in the process of opening.**
O	5		2.2.7 Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.
K	5		2.2.8 Pieces of shell and drainage are discarded.
C	**5, 6**		**2.2.4 Meats are blended at high speed until homogenous (60 – 120 seconds), using the following criteria:** **a. Freshly drained/air dried meats are placed into the blender for homogenization.** **b. Previously frozen shucked, rinsed, and drained meats are completely thawed, then placed in the blender <u>with all freeze-thaw liquid</u> for homogenization.** **c. Previously frozen homogenates are completely thawed then placed in the blender <u>with all freeze-thaw liquid</u> for homogenization.**
K	6, 13		2.2.5 Homogenates should be extracted immediately. If homogenates must be stored, they should be frozen.
		2.3 Preparation of Samples for Analysis – Extraction	
K	5, 10		2.3.1 0.1 M HCl is used for extractions.
K	5, 10		2.3.2 At least five (5) grams of tissue +/- 0.1g is extracted using a 1:1 mass to volume ratio of 0.1 M HCl.
C	**10**		**2.3.3 The pH of the sample is checked and adjusted as necessary to between 3.0– 4.0.**

C	**10**		**2.3.4**	**Adjustment of the pH is accomplished by dropwise addition of either 5 N HCl or 0.1 N NaOH, as appropriate, while constantly stirring the sample.**
C	**6**		**2.3.5**	**The sample is promptly brought to a boil at 99.0 +/- 1.0 °C and gently boiled for 5 minutes.**
O	6		2.3.6	The sample is boiled under adequate ventilation (e.g., fume hood).
O	10		2.3.7	The sample is allowed to cool to room temperature.
C	**10**		**2.3.8**	**The pH of the cooled mixture after boiling is between 3.0 - 4.0, adjusted if necessary, with the dropwise addition of 5 M HCl to lower the pH or 0.1 M NaOH to raise the pH, as appropriate, while constantly stirring the mixture.**
K	5, 10		2.3.9	The volume of the sample is adjusted to the original (pre-boiling) volume, by adding 0.001N HCl (pH 3 water).
K	10		2.3.10	The sample is stirred gently to homogeneity, then treated as follows: a. The sample is allowed to settle to remove particulates, then the supernatant is carefully decanted into a clean container; then b. an aliquot of the sample is centrifuged at 3000 x g for 10 minutes, then the supernatant is carefully decanted into a clean container.
K	6, 10		2.3.11	The sample extract is analyzed immediately, refrigerated at 4 °C in a sealed container for up to 24 hours, or frozen at -20 °C.
		2.4 Sample Assay		
K	6		2.4.1	One analyst performs the entire plate set-up for the assay.
K	6		2.4.2	Microtubes containing dilutions and samples are vortexed immediately before dispensing.
K	10		2.4.3	The standard curve consists of at least 7 concentrations (minimum 6 x 10^{-10} M and maximum 6 x 10^{-6} M).
C	**10**		**2.4.4**	**The rat brain membrane tissue preparation is kept on ice and mixed often during addition to the plate to maintain a homogenous suspension.**
K	10		2.4.5	Each day an assay is conducted, a standard curve is required. However, filter plates of the same lot must be used if the assay requires multiple plates to accommodate all samples. If the filter plate lot changes over the course of a day, a new standard curve must be performed for the new lot of filter plates. An inter-assay QC calibration and reference blank are required for each plate analyzed.
C	**10**		**2.4.6**	**The standard curve, reference blank, interassay QC calibration standard, and test samples are all run in triplicate.**
K	10		2.4.7	Assay buffer is added to the plate before any other components of the assay, in order to properly wet the filter membrane.
K	10		2.4.8	All wells of the plate (including any unused wells) are filled with MOPS/choline chloride buffer during vacuum filtration, in order to ensure even pressure and filtration across the plate.
C	**10**		**2.4.9**	**Appropriate scintillation cocktail is used, depending on the type of scintillation counter (traditional or microplate).**
K	10		2.4.10	[^{3}H] STX working solution is checked for counts per minute (CPM) and is consistent within 15% of the expected value.
C	10		**2.4.11**	**An appropriate dark adaptation interval is employed, based on type of scintillation counter (traditional or microplate).**
K	10		2.4.12	Standard curve fitting is calculated using appropriate software program.

C	**10**		**2.4.13 Slope of standard curve is between -0.8 and -1.2 (the theoretical slope is - 1.0). If the slope falls outside these criteria, the assay results are rejected and the assay must be repeated.**
C	**10**		**2.4.14 The relative standard deviation of triplicate CPM for standards and samples must be less than 30%. If greater than 30%, the assay results are rejected and the assay must be repeated.**
C	**10**		**2.4.15 The IC_{50} is in acceptable range (2.0 nM +/- 30%). If the IC_{50} is outside this range, the assay results are rejected and the assay must be repeated**
C	**10**		**2.4.16 The inter-assay QC calibration standard (QC check) sample is in the acceptable range (3 nM +/- 30%). If the QC check sample is outside this range, the assay results are rejected and the assay must be repeated.**
C	**10**		**2.4.17 Sample dilutions are quantified only if B/B_0 is between 0.2 – 0.7. If B/B_0 is greater than 0.7, then the sample is reported as below the limit of detection. If B/B_0 is less than 0.2, then the sample should be further diluted and repeated if a quantification is needed.**
K	4		2.4.18 Assay materials are cleaned and disposed of in accordance with federal, state, and local requirements.
		2.5 Calculation of Sample Toxicity	
C	**10**		**2.5.1 When more than one dilution falls within B/B_0 of 0.2 – 0.7, all wells corresponding to these dilutions are used to calculate sample toxicity.**
C	**10**		**2.5.2 Sample toxicity is calculated as follows:** **(nM STX equiv.) x (sample dilution) x (210 µL total volume/35 µL sample = mM STX equivalent in extract** **(nM STX diHCl equiv. in extract) x 1L/1000 mL x 372 ng/nmol x1 µg/1000 ng =µg STX diHCl equiv./mL** **µg STX diHCl equiv./mL x mL extract/g shellfish x 1000 g/kg =µg STX diHCl equiv./kg**
C	**14**		**2.5.3 Any value equal to or greater than 80 µg STX diHCl equiv./100 g) of sample is actionable.**
C	**14**		**2.5.4 Shellfish Program Management is made aware of positive result. Laboratory action to identify positive result is:**

REFERENCES

1. American Public Health Association (APHA). 1992. *Standard Methods for Examination of Water and Wastewater*, 18th Edition. APHA/AWWA/WEF, Washington, D.C.
2. American Public Health Association (APHA). 1984. *Compendium of Methods for the Microbiological Examination of Foods*, 2nd Edition. APHA, Washington, D.C.
3. American Public Health Association (APHA). 1992. *Standard Methods for the Examination of Diary Products*, 16th Edition. APHA, Washington, D.C.
4. Appendix C: Radiation Safety Requirements, ISSC Proposal 13-114 Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination.
5. American Public Health Association (APHA). 1970. Recommended Procedures for the Examination of Sea Water and Shellfish, Fourth Edition. APHA, Washington, D.C.
6. Good Laboratory Practice.
7. Fisher J. 1985. Measurement of pH. *American Laboratory* 16:54-60.
8. Association of Official Analytical Chemists (AOAC). 1991. *Quality Assurance Principles for Analytical Laboratories*. AOAC, Arlington, VA.
9. Consult pH electrode product literature.
10. Association of Official Analytical Chemists (AOAC). 2016. Official Method 2011.27 Paralytic Shellfish Toxins (PSTs) in Shellfish Receptor Binding Assay.
11. Consult instrument manufacturer instructions.
12. Technical Data Sheet, American Radiolabeled Chemicals, Inc. 101 Arc Drive, St. Louis, MO 63146.
13. Wilt, d. s. (ed). 1974. Proceedings of the 8th National Shellfish Sanitation Workshop. U. S. Food and Drug Administration, Washington, D.C.
14. U. S. Food and Drug Administration (FDA) and Interstate Shellfish Sanitation Conference (ISSC). 2017. NSSP *Guide for the Control of Molluscan Shellfish*. FDA/ISSC, Washington D.C. and Columbia, S.C.
15. U. S. Nuclear Regulatory Commission Materials, Section 30.18, 10 CFR Part 30, and American Radiolabeled Chemicals Licenses.

LABORATORY:	DATE OF EVALUATION:

SHELLFISH LABORATORY EVALUATION CHECKLIST

SUMMARY OF NONCONFORMITIES

Page	Item	Observation	Documentation Required

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	
RECEPTOR BINDING ASSAY FOR PSP COMPONENT: (Part I-II)	
A. Results Total # of Critical (C) Nonconformities Total # of Key (K) Nonconformities Total # of Critical (C), Key (K), and Other (O) Nonconformities	 _____ _____ _____

B. Criteria for Determining Laboratory Status of the RBA for PSP:

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

2. **Provisionally Conforms Status:** The RBA for PSP component of this laboratory is determined to be provisionally conforming to NSSP requirements if all 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 RBA for PSP 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 total # of Key nonconformities is ≥ 6;
   c. or the total # of Critical, Key and Others is ≥ 12.

C. Laboratory Status (*circle appropriate*)

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

Acknowledgment 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:_____

NSSP Form *11 – RBA PSP Checklist*, Rev. September 2024