Laboratory Evaluation Checklist – Mouse Bioassay and Scotia Rapid Test for Paralytic Shellfish Poisoning (PSP)

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. 240-402-2151/2055/4960 FAX 301-436-2672

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 "√"

Mouse Bioassay Assay (MBA) and Scotia Rapid Test (SRT) for Paralytic Shellfish Poisoning (PSP)			
PART I - Quality Assurance			
Code	**REF**	**Item Description**	
		1.1 Quality Assurance (QA) Plan	
K	5, 6, 8	1.1.1 Written Plan adequately covers all of the following: (check √ those items which apply)	
			a. Organization of the laboratory.
			b. Staff training requirements.
			c. Standard operating procedures (SOPs).
			d. Internal quality control measures for equipment, calibration, maintenance, repair, performance and rejection criteria established.
			e. Laboratory safety.
			f. Internal performance assessment.
			g. External performance assessment.
			h. Animal care.
C	**6**	**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 analyst(s) 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/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(s) meets the state/county educational and experience requirements for processing samples in a public health laboratory.
		1.3 Work Area	
O	5, 6		1.3.1 Adequate for the workload and storage.
O	5		1.3.2 Clean and well lighted.
O	5		1.3.3 Adequate temperature control.
O	5		1.3.4 All work surfaces are nonporous and easily cleaned.
C	**8**		**1.3.5 A separate, quiet area with adequate temperature control for mice acclimation and injection is maintained.**
		1.4 Laboratory Equipment	
O	2		1.4.1 The pH meter has a standard accuracy of 0.1 pH units.
K	9		1.4.2 pH paper in the appropriate range (i.e. 1-5), if used, measures accurately to a minimum of 0.5 pH units over the covered pH range.
K	7		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	6		1.4.4 pH meter is calibrated daily when in use. Results are recorded and records are maintained.
K	5		1.4.5 Effect of temperature has been compensated for by an ATC probe; use of a triode or by manual adjustment.
K	5		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	6, 12		1.4.7 Electrode acceptability is determined daily or with each use by the millivolt procedure or through determination of slope. (Circle method used).
K	2		1.4.8 The balances being used provide an appropriate sensitivity at the weights of use. a. To prepare reference solution, the balance must have a sensitivity of at least 0.1 g at a load of 1 g. b. For sample extraction, the balance must have a sensitivity of at least 0.1 g at a load of 100 g. c. For gravimetric extract volume adjustment, the balance must have a sensitivity of at least 0.1 g at a load of 200 g. d. To weigh mice for assay, the balance must have a sensitivity of at least 0.1 g at a load of 20 g.
K	4,5		1.4.9 The balance calibration is checked monthly according to the manufacturer's specifications 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˚C.
K	5		1.4.11 Refrigerator temperature is monitored at least once daily on workdays. Results are recorded and records are maintained.
K	4		1.4.12 Freezer temperature is maintained within manufacturer's tolerance.
K	5		1.4.13 Freezer temperature is monitored at least once daily on workdays. Results are recorded and records are maintained.
C	**10**		**1.4.14 All in-service thermometers are properly calibrated and immersed. Results are recorded and records are maintained.**
O	6		1.4.15 All glassware is clean.
C	**5**		**1.4.16 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% bromthymol blue (BTB) solution. Results are recorded and records are maintained.**
C	**9**		**1.4.17 An alkaline or acid based detergent is used for washing glassware/labware.**
			1.5 Reagents and Reference Solution Preparation and Storage
C	**9**		**1.5.1 Any residual (unused) STX diHCl standard solution is never stored after the ampule has been opened.**
K	15		1.5.2 PSP reference solution (1 µg/mL) is prepared gravimetrically and diluted with 0.001 M HCl solution.
K	9		1.5.3 Prepared PSP reference solution is stored under refrigeration in a sealed non-reactive container. Solution may be stored indefinitely as long as there is no detectable evaporation loss as determined by weight. If evaporation is detected, the solution is discarded appropriately. Records are maintained.
C	**14**		**1.5.4 All working dilutions from the PSP reference solution are prepared gravimetrically using 0.001 M HCl.**
K	9		1.5.5 All working dilutions prepared from the PSP reference solution are discarded appropriately after use.
C	**5**		**1.5.6 Reagent water is distilled or deionized (circle appropriate choice), tested monthly and exceeds 0.5 megohm – cm resistance (2 megohms-cm in-line) or is less than 2.0 µSiemens/cm conductivity at 25 °C. (Circle the appropriate water quality descriptor determined). Results are recorded and records are maintained.**
K	5		1.5.7 Reagent water is analyzed for residual chlorine monthly and is at a non-detectable level (≤ 0.1 mg/L). Results are recorded and records are maintained. Specify method of determination_____.
K	5		1.5.8 Reagent water contains < 100 CFU/mL as determined monthly using the

			heterotrophic plate count method. Results are recorded and records are maintained.
		1.6 Collection and Transportation of Samples	
O	2		1.6.1 Shellstock are collected in clean, waterproof, puncture resistant containers, loosely sealed.
K	2		1.6.2 Shellstock samples are labeled with collector's name, type of shellstock, the source or harvest area, sampling station, time, date and place (if applicable) of collection.
C	**2**		**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	15, 9		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 these 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	**14**		**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 – Analysis of Shellfish for PSP Toxins - MBA			
		2.1 Preparation of Samples for Analysis – Homogenization	
C	**15, 9**		**2.1.1 At least 12 animals (or more to provide 100 g of shellfish meat) are used per sample or the laboratory has an appropriate contingency plan for dealing with non-typical species of shellfish.**
O	2		2.1.2 The outside of the shell is thoroughly cleaned with fresh water.
O	2		2.1.3 Shellstock are opened by cutting the adductor muscles.
O	2		2.1.4 The inside surfaces of the shells and meats are rinsed with fresh water to remove sand or other foreign material.
O	2		2.1.5 Shellfish meats are removed from the shell by separating the adductor muscles and tissue connecting at the hinge.
C	**2**		**2.1.6 Damage to the body of the mollusk is minimized in the process of opening.**
O	2		2.1.7 Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.
K	2		2.1.8 Pieces of shell and drainage are discarded.
C	**2**		**2.1.9 Drained meats or previously cooled/refrigerated shucked meats and their drip loss liquid or thawed homogenates with their freeze-thaw liquid are blended at high speed until homogenous** (60 - 120 seconds).
		2.2 Preparation of Samples for Analysis – APHA/AOAC Digestion & Extraction	
K	15, 9		2.2.1 Sample homogenates are extracted as soon as possible (preferably the same day) or stored in the freezer.
K	2		2.2.2 100 grams of homogenized sample is weighed into a beaker.
K	2		2.2.3 The sample homogenate is extracted in a 1:1 weight/volume ratio by adding 0.1 M HCl or 0.18 M HCl (*circle the appropriate choice*).
K	2		2.2.4 Homogenate/acid mixture is stirred thoroughly before boiling to completely mix the contents.
C	**2**		**2.2.5 To prevent toxin transformation, the pH of the homogenate/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, as appropriate, while constantly stirring the mixture.**
C	**2**		**2.2.6 The homogenate/acid mixture is promptly brought to its boiling point, then**

			gently boiled at 100 ± 1 ºC for 5 minutes.
O	9		2.2.7 The homogenate/acid mixture is boiled under adequate ventilation (e.g. fume hood).
O	9		2.2.8 The homogenate/acid mixture is allowed to cool to room temperature.
C	**2**		**2.2.9 The pH of the cooled mixture after boiling is 3.0 ± 1.0, 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	2		2.2.10 The homogenate/acid mixture is adjusted gravimetrically to the pre-boiling weight using 0.001 M HC1.
K	2		2.2.11 The homogenate/acid mixture is allowed to separate by gravity or by centrifugation (e.g. centrifuged at 3,000 RPM for 5 minutes).
K	9		2.2.12 If the extracted sample cannot be assayed immediately, then the supernatant is decanted and stored in a sealed container under refrigeration for up to 24 hours or frozen for longer storage.
K	9		2.2.13 Refrigerated extracts are allowed to reach ambient temperature before being bioassayed or tested by the SRT for PSP.
		2.3 Mouse Bioassay (MBA) for PSP	
K	2		2.3.1 A 26-gauge hypodermic needle is used for intraperitoneal injections.
C	**2**		**2.3.2 Healthy mice in the weight range of 17.0 -23.0 grams (19 - 21 grams is preferable) from a stock colony are used for routine assays. Previously injected mice are never re-used for a bioassay.** **Stock strain: Source:**
C	**9**		**2.3.3 Mice are allowed to acclimate at least 24 hours prior to injection. In some cases, 48 hours may be required.**
C	**9**		**2.3.4 A conversion factor (CF) for the lab has been appropriately determined.** **Lab CF: Date CF established:**
C	**2**		**2.3.5 The CF value is checked weekly if assays are done on one or several days during the week or once each day that assays are performed if they are performed less than once per week.** **Date of current CF check: CF verified: yes/no (*circle choice*)**
C	**2**		**2.3.6 If the lab CF is not verified during a check, the lab follows the appropriate procedure for establishing a temporary CF to use for the day/week.**
C	**2, 9**		**2.3.7 If the lab CF fails to be verified, the cause is investigated and the situation is corrected. If the cause cannot be determined with reasonable certainty and the lab CF fails to be verified > three times in a year, the lab CF is recalculated through a restandardization procedure.**
K	9		2.3.8 Mice are weighed to the nearest 0.1 g.
C	**2**		**2.3.9 Mice are injected intraperitoneally with 1 mL of extracted sample.**
K	2		2.3.10 For CF checks, five mice are injected.
K	9		2.3.11 For routine assays, three mice (two when both survive) are injected per sample.
C	**2**		**2.3.12 Elapsed time post-injection is accurately determined and recorded.**
C	**2**		**2.3.13 When death occurs, the time of death to the nearest second is noted at the last gasping breath and recorded.**
C	**9, 2**		**2.3.14 Mice are continually observed for up to 20 minutes after injection, then periodically observed for a total time of up to 60 minutes after injection.**
C	**2**		**2.3.15 If the median corrected mouse unit is greater than 1.92 (5 minutes), then the sample is diluted with 0.001 M HCl as appropriate to achieve a median corrected mouse unit, MCMU of 1.39-1.92 (a death time of 5-7 minutes).**
		2.4 Calculation of toxicity for MBA	
C	**2**		**2.4.1 The death time for each mouse is converted to mouse units (MU) using Sommer's Table and recorded. Any mice surviving beyond 60 minutes are recorded as < 0.875 MU.**
C	**2**		**2.4.2 The weight for each mouse is corrected to mouse units using the table of weights in Recommended Procedures (Table 7) and interpolated for weights not listed.**

C	**2**		**2.4.3 The Corrected Mouse Unit (CMU) for each mouse injected is calculated as follows:** **Death time in MU x Weight correction in MU=CMU**
C	**2**		**2.4.4 The Median Corrected Mouse Unit (MCMU) for each sample is calculated and used in the final toxicity calculation for that sample.**
C	**2**		**2.4.5 The toxicity of each sample is calculated as follows:** **µg STX eq/100 g of sample = MCMU x CF x DF-x 200 except when less than 100 grams of sample is used for analysis.** **In this case an adjustment for sample weight must be made such that the formula for calculating sample toxicity becomes:** **µg STX eq/100 grams of sample = MCMU x CF x DF x 200/Adjusted weight of the acidified sample x 200.** **Where:** **MCMU=Median Corrected Mouse Unit for the sample** **CF=Laboratory Conversion Factor** **DF=Dilution Factor (e.g. 1:1 dilution, DF=2)**
C	**11**		**2.4.6 Any value equal to or greater than 80 µg STX eq/l00 g of sample is actionable.**
PART III – Examination of Shellfish for PSP Toxins – SRT			
		3.1 Screening by Scotia Rapid Test (SRT)	
K	9		3.1.1 Before beginning any screening, the following items are recorded for the SRT kit in use. a. Date received. b. Batch/lot numbers for all kit components (test strip and PSP AOAC buffer). c. Expiration dates for all kit components. d. Date opened and/or used.
K	13		3.1.2 When placed into service, all kit components are within the accepted expiration dates.
C	**13**		**3.1.3 The desiccant pouch inside the test strip wrapping is blue in color, indicating suitability for use. Any test strip wrapping containing a pink desiccant pouch is discarded.**
K	13		3.1.4 All kit components are stored according to the manufacturer's recommendations.
C	**9**		**3.1.5 A positive control of 80 µg STX eq/100 g of sample is used to test new kit lots and buffers. Results are recorded and records maintained.**
C	**9**		**3.1.6 Micropipettes with appropriate ranges for the volumes being measured are used.**
K	9		3.1.7 All micropipettes are maintained and calibrated according to manufacturer's instructions. Results are recorded and records maintained.
C	**13**		**3.1.8 400 µL of buffer solution is accurately transferred to a small tube.**
C	**13**		**3.1.9 100 µL of sample extract is accurately added to the buffer.**
K	13		3.1.10 The buffer/sample mixture is carefully mixed by inserting the tip of the micropipette into the mixture and pipetting up and down at least three times.
C	**13**		**3.1.11 100 µL of the thoroughly mixed solution is added to the test strip sample well.**
K	9		3.1.12 Micropipette tips are not reused.
K	13		3.1.13 Inoculated test strips are allowed to react with the sample mixture for the period of time recommended by the manufacturer.
C	**13**		**3.1.14 The test strip result is interpreted according to the instruction card provided by the manufacturer, which is specific to each batch/lot of test strips. Results are recorded and records are maintained.**
K	13		3.1.15 If a test result is interpreted as invalid; the pH of the sample extract is checked and adjusted as needed to fall between pH 2.0 – 4.0. Fresh PSP AOAC buffer is used to re-test the sample on a new test strip.
C	**13**		**3.1.16 If the same sample is interpreted as invalid on two different test strips, then the sample is assumed to contain interfering substances, and an alternative test**

			method is used.
C	**11**		**3.1.17 Any positive result on a SRT is actionable.**

REFERENCES:

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2. American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition. APHA, Washington, D.C.
3. American Public Health Association. 1984. *Compendium for the Microbiological Examination of Foods*, 2nd Edition. APHA, Washington, D.C.
4. Consult freezer product literature.
5. APHA/WEF/AWWA. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition. APHA, Washington, D.C.
6. Association of Official Analytical Chemists (AOAC). 1991. *Quality Assurance Principles for Analytical Laboratories.* AOAC, Arlington, VA.
7. Fisher, J. 1985. Measurement of pH. *American Laboratory.* 16:54-60.
8. National Research Council. 1996. *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, D.C.
9. Good Laboratory Practice
10. U.S. Department of Commerce. 1976. NBS Monograph 150. U.S. Department of Commerce, Washington, D.C.
11. U.S. Food and Drug Administration (FDA) and Interstate Shellfish Sanitation Conference (ISSC). 2013. *NSSP Guide to the Control of Molluscan Shellfish.* FDA/ISSC, Washington, D.C. and Columbia, S.C.
12. Consult pH electrode product literature.
13. Consult SRT manufacturer instruction manual / literature
14. Personal Communication with Dr. Sherwood Hall, USFDA.
15. Wilt, D.S. (ed). 1974. Proceedings of the 8th National Shellfish Sanitation Workshop. U.S. Food and Drug Administration, Washington, D.C.

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

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

B. Criteria for Determining Laboratory Status of the PSP, MBA and/or SRT Component

1. **Conforms Status:** The PSP, MBA and/or SRT 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 PSP, MBA and/or SRT 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 PSP, MBA and/or SRT 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**

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:_____