Proposal for Task Force Consideration at the ISSC 2015 Biennial Meeting	☒ Growing Area ☐ Harvesting/Handling/Distribution ☐ Administrative

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Proposal Subject	Update PSP Laboratory Evaluation Checklist
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas .12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists-Laboratory Evaluation Checklist - PSP
Text of Proposal/ Requested Action	Update PSP Laboratory Evaluation Checklist. Please find the updated PSP Laboratory Checklist attached - word document titled "Revised PSP Checklist 11-08-2010.doc". A summary of the changes is: • Added the checklist items for Jellett Rapid Test for PSP • Renumbered checklist items to accommodate proposed additions and deletions and to better identify each checklist item. • Added, deleted or changed language for checklist items to be consistent with the microbiology laboratory evaluation checklist including added laboratory education and experience requirements • Deleted the requirement for metals testing on reagent water • Clarified and defined requirements for laboratory equipment, reagents and the mouse bioassay method.
Public Health Significance	The current PSP laboratory checklist was last revised in 2005. Since that time the Jellett Rapid Test has received approval and is not in the checklist. Deficiencies have been identified while using the PSP checklist in evaluation of laboratories and the PSP checklist is inconsistent with some requirements in the microbiology checklist which has more recently been revised. It is important that the checklist items and quality assurance requirements are clear and understandable. It is important that quality assurance requirements among the different laboratory evaluation checklists remain as consistent as possible since many monitoring laboratories perform multiple types of tests and are evaluated using multiple checklists; inconsistencies among the checklist cause confusion, extra expense and work for the laboratories.
Cost Information	None
Action by 2011 Laboratory Methods Review & Quality Assurance Committee	Recommend Proposal 11-109 be referred to the appropriate committee as determined by the Conference Chairman.
Action by 2011 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 11-109.
Action by 2011 General Assembly	Adopted recommendation of 2011 Task Force I on Proposal 11-109.
Action by FDA February 26, 2012	Concurred with Conference action on Proposal 11-109.
Action by 2013	Recommended Proposal 11-109 be referred to the appropriate committee as

Laboratory Methods Review & Quality Assurance Committee	determined by the Conference Chairman.
Action by 2013 Task Force I	Recommended adoption of Laboratory Methods Review and Quality Assurance Committee recommendation on Proposal 11-109.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 11-109.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 11-109.

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
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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 "√"

Check the applicable assays performed:

	Mouse Bioassay (MBA)
	Jellett Rapid Test (JRT)

PART I – QUALITY ASSURANCE

ITEM		
CODE		
		1.1 Quality Assurance (QA) Plan
K	☐	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 (SOPs).
		d. Internal quality control measures for equipment, calibration, maintenance repair ~~and~~, performance and rejection criteria established.

		e. Laboratory safety.
		f. ~~Quality assessment.~~ Internal performance assessment.
		g. ~~Proper animal care.~~ External performance assessment.
		h. Animal care.
C	☐	**2. 1.1.2 QA plan implemented.**
		1.2 Educational/Experience Requirements
C	☐	**1.2.1 In state/county laboratories, the supervisor meets the state/county educational and experience requirements for managing a public health laboratory.**
K	☐	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	☐	**1.2.3 In commercial laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology or an equivalent discipline with at least two years of laboratory experience.**
K	☐	1.2.4 In commercial laboratories, the analysts must have at least a high school diploma and shall have at least three months of experience in laboratory science.
		1.~~2~~3 Work Area
O	☐	1. 1.3.1 Adequate for workload and storage.
O	☐	2. 1.3.2 Clean and well lighted.
O	☐	3. 1.3.3 Adequate temperature control.
O	☐	4. 1.3.4 All work surfaces are nonporous and easily cleaned.
C	☐	**5. 1.3.5 A separate, quiet area with adequate temperature control for mice acclimation and injection is maintained.**
		1.~~3~~4 Laboratory Equipment
O	☐	1.1.4.1 The pH meter has a standard accuracy of 0.1 pH unit.
K	☐	~~pH paper in the appropriate range (i.e. 1-4) is used with minimum accuracy of 0.5 pH units.~~ 2. 1.4.2 pH paper in the appropriate range (i.e., pH <2 to >4.5) having a minimum accuracy of 0.5 units is used.
K	☐	3.1.4.3 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 ~~medium that may result in inaccurate pH readings~~ substance being measured.
K	☐	4.1.4.4 pH meter is calibrated daily or with each use. Results are recorded and records 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	☐	6.1.4.6 A minimum of two standard buffer solutions (pH 2 & pH 7) is used to calibrate the pH meter. Standard buffer solutions are used once and discarded.
K	☐	7.1.4.7 Electrode ~~efficiency~~acceptability is determined daily or with each use ~~following either slope or~~ by the millivolt procedure or through determination of the slope. (circle the method used.)
K	☐	~~8. The balance provides a sensitivity of at least 0.1g at a load of 150 grams.~~ 1.4.8 The differing sensitivities in weight measurements required by the various steps in the assay are met by the balance/balances being used. a. To prepare the reference solution, the balance used must have a sensitivity of at least 0.1 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 gravimetric extract volume adjustment, the balance used must have a

		sensitivity of at least 0.1 gram at a load of 200 grams. d. To determine the weight of the mice, the balance must have a sensitivity of at least 0.1 gram at a load of 20 grams.
K	☐	~~9. The balance calibration is checked monthly using NIST Class S or ASTM Class 1or 2 weights or equivalent. Records maintained.~~ 1.4.9 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. Results are recorded and records maintained.
K	☐	10.1.4.10 Refrigerator temperatures ~~is~~are maintained between 0 and 4°C.
O	☐	111.4.11 Refrigerator temperatures ~~is~~are monitored at least once daily on workdays. Results are recorded and records maintained.
K	☐	12.1.4.12 Freezer temperatures ~~is~~ are maintained at ~~20°C~~or below -15°C.
O	☐	13.1.4.13 Freezer temperatures ~~is~~ are monitored at least once daily on workdays. Results are recorded and records maintained.
O	☐	14.1.4.14 All glassware is clean.
O C	☐	~~15. Once during each day of washing, several pieces of glassware from each batch washed are tested for residual detergent with aqueous 0.04% bromthymol blue solution. Records are maintained.~~ **1.4.15 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) with aqueous 0.04% bromthymol blue (BTB) solution. Results are recorded and records maintained.**
C	☐	**1.4.16 An alkaline or acid based detergent is used for washing glassware/labware**
		1.41.5 Reagent and Reference Solution Preparation and Storage
C	☐	**1.5.1 Opened PSP ~~reference~~ standard solution (100µg/mL) is not stored.**
K	☐	~~2. PSP working standard solution (1 µg/ml) and all dilutions are prepared with dilute HCl, pH 3 water, using 'Class A' volumetric glassware (flasks and pipettes) or prepared gravimetrically.~~ 1.5.2 PSP reference solution (1µg/mL) is prepared by weight (grayimetrically) with dilute HCl, pH 3 water.
K	☐	~~3. Refrigerated storage of PSP working standard solution (1µg/ml) does not exceed 6 months and is checked gravimetrically for evaporation loss.~~ 1.5.3 Refrigerated storage of PSP reference solution (1µg/mL) in a sealed container is stored indefinitely as long as there is no evaporation loss as checked by weight. If evaporation is detected, the solution is discarded appropriately. Records are maintained.
C	☐	**1.5.4 Dilutions of the 1µg/mL reference solution are prepared by weight or** **volume using dilute HCl, pH 3 water.**
K	☐	4.1.5.5 PSP working dilutions(dilutions of the 1µg/mL reference solution) are discarded after use.
K	☐	5. ~~Make up water is distilled or deionized (*circle one*) and exceeds 0.5 megohm resistance or is less than 2 µ Siemens/cm conductivity at 25°C to be tested and recorded monthly for resistance or conductivity *(circle the appropriate).*~~ 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 the records maintained.
O	☐	6. 1.5.7 ~~Make up~~ Reagent water is analyzed for residual chlorine monthly and is at a nondetectable

		level (<0.1ppm). Results are recorded and records maintained.
K	☐	7. Make up 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	☐	8.1.5.8 ~~Makeup~~ Reagent water contains ~~<1000~~ <100 CFU/mL as determined monthly using the heterotrophic plate count method. Results are recorded and records maintained.
		1.~~5~~6 Collection and Transportation of Samples
O	☐	1. ~~Shellstock are collected in clean, waterproof, puncture resistant containers.~~ 1.6.1 Shellfish are collected in clean, waterproof , loosely sealed, puncture resistant containers.
K	☐	2.1.6.2 Samples are appropriately labeled with the collector's name, harvest area, sampling station and time and date of collection.
K	☐	3. ~~Immediately after collection, shellstock samples are placed in dry storage for transport (e.g. cooler) which is maintained between 0 and 10°C. Upon receipt at the lab, samples are placed under refrigeration.~~ 1.6.3 Immediately after collection, shellfish 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. Upon receipt at the laboratory, samples are placed under refrigeration.
K	☐	4.1.6.4 The time from collection to completion of the bioassay should not exceed 24 hours. However, if there are significant transportation delays, then shellstock samples are processed immediately as follows (*circle the appropriate choice*): a. 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.
K**C**	☐	**5.1.6.5 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~~ ANALYSIS OF SHELLFISH FOR PSP TOXINS		
		2.1 Preparation of the Sample
C	☐	**1. 2.1.1 At least 12 animals (equivalent to at least 100 g of shellfish meat) are used per sample or the laboratory has a~~n~~ ~~appropriate~~ proven effective 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	☐	3. 2.1.3 Shellstock are opened by cutting adductor muscles.
O	☐	4. 2.1.4 The inside of the shell is rinsed with fresh water to remove sand or other foreign material.
O	☐	5. 2.1.5 Shellfish meats are removed from the shell by separating adductor muscles and tissue connecting at the hinge.
K	☐	6. 2.1.6 Damage to the body of the mollusk is minimized in the process of opening.
O	☐	7.2.1.7 Shucked shellfish are drained on a #10 mesh sieve (or equivalent) without layering for 5 minutes.
K	☐	8. 2.1.8 Pieces of shell and drainage are discarded.
C	☐	**9. ~~Drained meats or thawed homogenates are blended at high speed until homogenous~~ ~~(60 – 120 seconds).~~** **2.1.9 Drained meats or previously cooled/refrigerated, shucked, drained meats and their drip-loss liquid or thawed, shucked meat with its freeze-thaw liquid or thawed homogenates with their freeze-thaw liquid are blended at high speed until homogenous (60 – 120 seconds).**
		2.2 Extraction
K	☐	1. 2.2.1 100 grams of homogenized sample is weighed into a beaker.

K	☐	2. 2.2.2 An equal amount of 0.1 N/0.18 N HCl is added to the homogenate and thoroughly mixed. (*circle the appropriate normality*).
C	☐	**3. 2.2.3 The pH is checked and, if necessary adjusted to between pH 2.0 and 4.0.**
C	☐	**4. 2.2.4 Adjustment of the pH is made by the dropwise addition of either (5 N HCl) or base (0.1 N NaOH) as appropriate while constantly stirring the mixture.**
C	☐	**5. 2.2.5 The homogenate/acid mixture is promptly brought to a boil, 100 +1°C then gently boiled for 5 minutes.**
O	☐	6. 2.2.6 The homogenate/ acid mixture is boiled under adequate ventilation (i.e., fume hood).
O		7. 2.2.7 The extract is cooled to room temperature.
C	☐	**8. 2.2.8 The pH of the extract is determined and adjusted if necessary to between pH 2 and 4 preferably to pH 3 with the stirred dropwise addition of 5 N HCl to lower the pH or 0.1 N NaOH to raise the pH.**
K		9. 2.2.9 The extract volume ~~(or mass)~~ is adjusted to 200 mL (or grams) with dilute HCl, pH 3.0 water.
K	☐	10.2.2.10 The extract is returned to the beaker, stirred to homogeneity and allowed to settle to remove particulates; or, if necessary, an aliquot of the stirred supernatant is centrifuged at 3,000 RPM for 5 minutes before ~~injection~~ being bioassayed.
K	☐	11. ~~If mice cannot be injected immediately then the supernatant should be removed from the centrifuge tubes and refrigerated for up to 24 hours.~~ 2.2.11 If the extract cannot be bioassayed or the Jellett Rapid Test (JRT) for PSP cannot be performed immediately, then the supernatant is removed from the centrifuge tubes and sealed and refrigerated for up to 24 hours.
K	☐	12. 2.2.12 Refrigerated extracts are allowed to reach ambient temperature before being bioassayed or tested by the JRT for PSP.
		2.3 Bioassay
O	☐	1. 2.3.1 A 26-gauge hypodermic needle is used for injection.
KC	☐	~~2. Healthy mice in the weight range of 17 – 23 grams (19 – 21 grams is preferable) from a stock colony are used for routine assays. Mice are not reused for the bioassay.~~ ~~Stock strain used_____ Source of the mice _____~~ **2.3.2 Healthy mice in the weight range of 17 – 23 grams (19 – 21 grams is preferable) from a stock colony are used for routine assays. Mice are not reused for the bioassay.** **Stock strain used_____ Source of the mice _____**
C	☐	**1. 2.3.3 Mice are allowed to acclimate for at least 24 hours prior to injection. In some cases up to 48 hours may be required.** **2.**
C	☐	**4. 2.3.4 A conversion factor (CF) has been determined as _____. Month and year when current CF determined _____.**
C	☐	**5. 2.3.5 CF value is checked weekly if assays are done on several days during the week, or, once each day that assays are performed if they are performed less than once per week.** **Date of most recent CF check_____** **CF verified ~~/CF not verified~~: *yes / no*: (*circle the appropriate choice*).**
C	☐	**6. 2.3.6 If the CF is not verified, 5 additional mice are injected with the dilution used in the CF check to complete a group of 10 mice. Ten additional mice are also injected with this dilution to produce a second group of 10 mice. The CF is calculated for each group of 10 mice and averaged to give the CF to be used in sample toxicity calculations for the day's or week's work only. All subsequent work must make use of the original laboratory CF value unless this value continues to fail to be verified by routine CF checks.**

C	☐	**7. 2.3.7 If the CF fails to be verified, the cause is investigated and the situation corrected. If the cause cannot be determined with reasonable certainty and fails >3 times per year, the bioassay is restandardized.**
O	☐	8. 2.3.8 Mice are weighed to the nearest ~~0.5 gram~~ 0.1 gram .
C	☐	**9. 2.3.9 Mice are injected intrapertioneally with 1 mL of the acid extract.**
K	☐	10.2.3.10 For the CF check at least 5 mice are used.
C	☐	**11. 2.3.11 At least 3 mice are used per sample in routine assays.**
C	☐	**12.2.3.12 Elapsed time is accurately determined and recorded.**
K	☐	13. 2.3.13 If death occurs, the time of death to the nearest second is noted by the last gasping breath.
C	☐	**2.3.14 Mice are continually observed for up to 20 minutes after injection with periodic checks for a total of 60 minutes as appropriate.**
C	☐	**14. 2.3.15 If the median death time ~~(2 out of 3 mice injected die)~~ is <5 minutes, a dilution is made with dilute HCl, pH 3 water, to obtain a median death time in the range of 5 to 7minutes.**
		2.4 Calculation of Toxicity
C	☐	**1. 2.4.1 The death time of each mouse is converted to mouse units (MU) using Sommer's Table (Table 6, *Recommended Procedures for the examination of Sea Water and Shellfish, Fourth* ~~4th~~ Fourth Edition). The death time of mice surviving beyond 60 minutes is considered to be <0.875 MU.**
K	☐	2. 2.4.2 A weight correction in MU is made for each mouse injected using Table 7 in *Recommended Procedures for the Examination of Sea Water and Shellfish*, Fourth 4th Edition.
C	☐	**3. 2.4.3 The death time of each mouse in MU is multiplied by a weight correction in MU to give the corrected mouse unit (CMU), the true death time for each mouse.**
C	☐	**4. 2.4.4 The median value of the array of corrected mouse units (CMU) is determined to give the median corrected mouse unit (MCMU), median death time.**
C	☐	**5. 2.4.5 The concentration of toxin is determined by the formula, MCMU x CF x Dilution Factor (DF) x 200.**
C	☐	**1. 2.4.6 Any value greater than 80 µg/100 grams of meat is actionable.**
PART III – JELLETT RAPID TEST (JRT) FOR PSP		
		3.1 Procedure
K	☐	3.1.1 The batch/lot numbers of the test strips and buffers, their expiration dates, date received and date used are recorded.
K	☐	3.1.2 When placed into service, test strips and buffers (PSP & Matrix) are within their respective expiration dates.
C	☐	**3.1.3 When opened, the test strip desiccant pouch is blue in color indicating its suitability for use. Test strips emerging from desiccant pouches which are pink in color are never used.**
K	☐	3.1. 4 Test strips and buffer are stored according to the manufacturer's instructions.
C	☐	**3.1.5 Negative extracts are spiked at a low level concentration (40 – 60 µg/100 grams of sample) or equivalent (a bioassayed extract) and used as a positive control for testing both new batches/lots of kits and buffers. Results are recorded and records maintained.**
C	☐	**3.1.6 Micropippettors capable of accurately delivering volumes of 100 and 400 µL are used to transfer buffer and sample extracts and to inoculate test strips with diluted extract.**
K	☐	3.1.7 Volumes delivered by the micropippettor are checked for accuracy at 100 and 400 µL monthly while in service. Results are recorded and records maintained.
C	☐	**3.1.8 400 µL of the buffer supplied with the test kits is accurately transferred to a small tube.**
C	☐	**3.1.9 100 µL of the sample extract is added to the buffer.**
K	☐	3.1.10 The sample/extract is thoroughly mixed with buffer by inserting the tip of the micropippettor into the buffer/sample extract mixture and pipetting up and down at least three (3) times.
C	☐	**3.1.11 100 µL of the thoroughly mixed diluted sample extract is inoculated into the test strip sample well.**
K	☐	3.1.12 Micropippettor tips are not reused.

K	☐	3.1.13 Inoculated test strips are allowed to react with the sample extract for the period of time specified by the manufacturer.
C	☐	**3.1.14 The test is interpreted according to the manufacturer's instruction card which is specific to each batch/lot of test strips.**
K	☐	3.1.15 When invalid tests are repeated, the pH of the sample extract is checked and adjusted as necessary to between pH 2.0 and pH 4.0. An aliquot of Matrix buffer and a fresh test strip is used to reassay the sample.
C	☐	**3.1.16 When a repeated JRT test for PSP gives identical invalid results, the sample contains interfering substances which require the use of the mouse bioassay for testing.**
C	☐	**3.1.17 A positive JRT for PSP is actionable.**

LABORATORY	DATE OF EVALUATION:

SHELLFISH LABORATORY EVALUATION CHECKLIST
SUMMARY OF NONCONFORMITIES

Page	Item	Observation	Documentation Required

LABORATORY STATUS

LABORATORY:	**DATE:**
LABORATORY REPRESENTATIVE:	

PARALYTIC SHELLFISH TOXIN COMPONENT: PARTS I and II and III

A. Results:

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

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 Critical Nonconformities is **>3 or**
   B. The total # of Key Nonconformities is **>6 or**
   C. The total # of Critical, Key and 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 **< 3 and** the number of **Key** Nonconformities is **<6 and** the number of **Other** Nonconformities is **<4.**

3. **Conforming Status.** The PSP component of this Laboratory is determined to be conforming when it has **no Critical** Nonconformities **and < 6 Key** Nonconformities **and < 4 Other** Nonconformities.

C. Laboratory Status *(circle appropriate choice)*:

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