Committee Report

Committee Name: **Laboratory Methods Review and Quality Assurance Committee**
Chairperson: **Stacey DeGrasse**
Date of Meeting: **Approved By:**_____
Recorder: **Printed Name:**

Committee Members:

Degrasse, Stacey X	Burrow, Richard X	Carey, David X
Couture, Darcie X	Dorsey, Carol X	Forester, Matthew X
Haines, Andy X	Hiatt, Brian	Howell, Tom X[1]
Knue, Jackie	Langlois, Gregg	Lankford, Shelley X
McFarland, Linda X	Porter, Leonora X	Schwarz, John
Sirois, Alison X	Smith, James	Rourke, Wade X
Vargas, Arturo	Wallace, Dave	Wickman, Kathleen X
Hansel, Joel	Chandler, Linda X	Lassitter, Cheryl X
Evans, Melissa X	Plakas, Steve X	Jessica, Jones X
Dortch, Quay X		

Refer to monthly LMRC meeting reports, available on the ISSC webpage, for more detail.

Charges

Charge 1: Proposal 05-111 Rapid Extraction Method for PSP and ASP

Findings & Conclusions: In 2011 the Conference took no action on the requested language change to the Model Ordinance that would allow the rapid extraction to be used with the kit due to insufficient data. The company has undergone a change and is now referred to as Scotia Rapid Testing. The kit, previously known as the JRT, is now referred to as the SRT; however, no changes were made to the kit. Proposal 05-111 was reintroduced for consideration during the 2015 Biennial Meeting. New data for the rapid extraction for the PSP kit were submitted for consideration and the LMRC has reviewed the data.

Recommendation 1: The LMRC recommends to Task Force I to change the name of the Jellett Rapid Test to Scotia Rapid Test and the Jellett Rapid Extraction to Scotia Rapid Extraction in the next revision of the NSSP Guide for the Control of Molluscan Shellfish (Section IV. Guidance Documents Chapter II Growing Areas 4. Approved Limited Use Methods for Marine Biotoxin Testing). (**See attached letter**)

Recommendation 2: The LMRC recommends to Task Force I to refer Proposal 05-111 for PSP to an appropriate committee as determined by the Conference Chair and further recommends to Task Force I to direct the Executive Office to send a letter to the method submitter requesting additional information as detailed by the LMRC.

[1] Tom Howell was present for the AM session, but absent for the PM session

Recommendation 3: The LMRC recommends that Task Force I take no action on the Scotia Rapid Extraction Method for ASP.

Charge 2: Proposal 11-109 Update PSP Laboratory Evaluation Checklist

Findings & Conclusions: The LMRC has thoroughly reviewed, discussed, and revised the PSP checklist that was originally submitted as proposal 11-109. Track changes were made to the original proposal to indicate the revisions that have been made by the LMRC.

Recommendation: The LMRC recommends to Task Force I that Proposal 11-109 be adopted as amended. (**See attached revision**)

Charge 3: Proposal 13-109 Expanding the Use of the Abraxis Shipboard ELISA for the Determination of Paralytic Shellfish Poisoning (PSP) Toxins

Findings & Conclusions: Proposal 13-109 seeks to expand the use of the Abraxis Shipboard ELISA to allow (1) for the rapid extraction or the AOAC extraction method and (2) for the kit to be used as a screening method beyond the onboard screening protocol. No data were submitted with this proposal.

Recommendation: The LMRC recommends to Task Force I that Proposal 13-109 be referred to an appropriate committee as determined by the Conference Chair until data that supports the use of the Abraxis ELISA beyond the use of the onboard procedure is made available.

Charge 4: Proposal 13-110 Immunoassay Method for Detection of Saxitoxin (PSP) from Shellfish

Findings & Conclusions: The recommendation on Proposal 13-110 during the 2013 Conference was that the submission was incomplete. The LMRC identified data gaps and requested that the submitter provide additional data. The LMRC has reviewed subsequent experimental designs by the submitter that they planned to conduct to address the data gaps. No further data have been submitted.

Recommendation: The LMRC recommends to Task Force I that Proposal 13-110 be referred to the appropriate committee as determined by the Conference Chair until additional data are received.

Charge 5: Proposal 13-111 DSP PPIA Kit for Determination of Okadaic Acid Toxins Group (OA, DTX1, DTX2) in Molluscan Shellfish

Findings & Conclusions: The recommendation on Proposal 13-111 during the 2013 Conference was that the submission was incomplete. The LMRC identified data gaps and requested that the submitter provide additional data. No further data have been submitted.

Recommendation: The LMRC recommends to Task Force I that Proposal 13-111 be referred to an appropriate committee as determined by the Conference Chair until additional data are received.

Charge 6: Proposal 13-112 Reveal 2.0 ASP

Findings & Conclusions: The recommendation on Proposal 13-112 during the 2013 Conference was to adopt this method as an Approved Limited Use Method. At that time, the submitters requested that the Conference be made aware that they are looking for naturally contaminated samples that could be used in validation studies for the method to be further considered. No new data have been submitted.

Recommendation: The LMRC recommends to Task Force I that no action be taken on Proposal 13-112.

Charge 7: Proposal 13-113 Reveal 2.0 DSP

Findings & Conclusions: The recommendation on Proposal 13-113 during the 2013 Conference was that the submission was incomplete. The LMRC identified data gaps and requested that the submitter provide additional data. The LMRC has reviewed subsequent experimental designs by the submitter that they planned to conduct to address the data gaps. No further data have been submitted.

Recommendation: The LMRC recommends to Task Force I that Proposal 13-113 be referred to an appropriate committee as determined by the Conference Chair until additional data are received.

Charge 8: Proposal 13-114 Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination

Findings & Conclusions: The recommendation on proposal 13-114 during the 2013 Conference was to adopt this method as Approved for mussels and Approved Limited Use for clams and scallops. The proposal was referred back to the LMRC for consideration for use with oysters. The LMRC has been informed that the validation study for oysters is underway, yet no new data have been submitted.

Recommendation: The LMRC recommends to Task Force I that Proposal 13-114 be referred to an appropriate committee as determined by the Conference Chair until additional data for oyster matrix are received.

Charge 9: Proposal 13-115 PSP HPLC-PCOX Method Evaluation Checklist

Findings & Conclusions: Proposal 13-115 was referred back to Committee at the 2013 Biennial Meeting. The LMRC has reviewed and revised the language of the checklist for consistency with other checklists and taking into consideration the need for the checklist to be applied broadly to all laboratories using the method. Track changes were made to the original proposal to indicate the revisions made by the LMRC. Additionally, it was noted that the Proposal Subject line was incorrect in the 2015 Biennial Meeting Proposals for Consideration booklet. The correct Proposal Subject line should read PSP HPLC-PCOX Method Evaluation Checklist.

Recommendation: The LMRC recommends to Task Force 1 that Proposal 13-115 be adopted, as amended. (**See attached revision**)

Charge 10: Proposal 13-117 Certification of State Shellfish Laboratory Evaluation Officers

Findings & Conclusions: Proposal 13-117 was referred to Committee during the 2013 Biennial Meeting. The LMRC has reviewed and revised the proposal to offer clarity. Track changes were made to the original proposal to indicate the revisions made by the LMRC. Recommendations were made on this revised proposal during a previous conference call. The proposal was reconsidered and amended during the conference.

Recommendation: The LMRC recommends to Task Force 1 that Proposal 13-117 be adopted, as amended. (**See attached revision**)

Charge 11: Proposal 15-101 Monthly Laboratory Grade Water Testing

Findings & Conclusions: Proposal 15-101 was discussed during the LMRC calls and it was agreed that this proposal was not needed and that no action would be the best course since the test is for internal laboratory use. As such, the test can be at the discretion of the laboratory as long as it is recognized as fit for purpose.

Recommendation: The LMRC recommends to Task Force I that no action be taken on Proposal 15-101 as this test is for internal laboratory use so the method of analysis used is at the discretion of the laboratory. The only requirement is that the test method chosen be recognized as fit for purpose.

Charge 12: Proposal 15-108 PCOX Method Status

Findings & Conclusions: Proposal 15-108 requests that the PSP PCOX method be classified as Approved as opposed to Approved Limited Use. The LMRC reviewed the proposal and found that sufficient data and usage of the method supported it being Approved for the species that were included in the original SLV package.

Recommendations: The LMRC recommends to Task Force 1 that Proposal 15-108 be adopted.

Charge 13: Proposal 15-109 PSP HPLC-PCOX Species Expansion

Findings & Conclusions: Proposal 15-109 seeks to expand the PSP PCOX method to additional shellfish species. Data for some species were provided by submitters from AK; however, the LMRC has not had adequate time to review. The LMRC requested that the submitters from ME provide the supporting data on the other species.

Recommendation: Recommendation: The LMRC recommends to Task Force I that Proposal 15-109 be referred to an appropriate committee as determined by the Conference Chair for evaluation of data and until additional data are received.

Charge 14: Proposal 15-110 V.p. Enumeration and Detection through MPN & Real-Time PCR

Findings & Conclusions: The method described in Proposal 15-110 was initially reviewed by the LMRC as an interim method. Based on initial review, the LMRC recommended on April 21, 2015 that the method be adopted as an interim method and that a full proposal be submitted. The LMRC is awaiting submission of the full proposal.

Recommendation: The LMRC recommends to Task Force I that Proposal 15-110 be referred to an appropriate committee as determined by the Conference Chair to await completed SLV data.

Charge 15: Proposal 15-111 MPN Real-Time PCR for Pathogenic

Findings & Conclusions: The method described in Proposal 15-111 was initially reviewed by the LMRC as an interim method. Based on initial review, the LMRC recommended on April 21, 2015 that the method be adopted as an interim method and that a full proposal be submitted. The LMRC received and reviewed the full SLV data.

Recommendation: The LMRC recommends to Task Force I that Proposal 15-111 be adopted and direct the Executive Office to request the submitter revise the SOP so that the BAM MPN calculator be used for determination of MPN values.

Charge 16: Proposal 15-112 Direct Plating Method for trh

Findings & Conclusions: The method described in Proposal 15-112 was initially reviewed by the LMRC as an interim method. Based on initial review, the LMRC recommended on April 21, 2015 that the method be adopted as an interim method and that a full proposal be submitted. The LMRC received and has reviewed the full SLV data.

Recommendation: The LMRC recommends to Task Force I that Proposal 15-112 be referred to an appropriate committee as determined by the Conference Chair to further review the data submitted.

Charge 17: Proposal 15-113 MPN Real-Time PCR for Total *Vibrio parahaemolyticus* (V.p.)

Findings & Conclusions: The method described in Proposal 15-113 was initially reviewed by the LMRC as an interim method. Based on initial review, the LMRC recommended on April 21, 2015 that the method be adopted as an interim method and that a full proposal be submitted. The LMRC was received and reviewed the full SLV data.

Recommendations: The LMRC recommends to Task Force I that Proposal 15-113 be adopted and direct the Executive Office to request the submitter revise the SOP so that the BAM MPN calculator be used for determination of MPN values.

Charge 18: Proposal 15-114 MSC Enumeration in Wastewater by Direct Double-Agar Overlay

Findings & Conclusions: Proposal 15-114 was a pre-proposal request asking for permission to submit a full proposal for the Conference to consider a method for enumerating male specific coliphage. The LMRC agreed that the pre-proposal request was sufficient and that

there is a need for the method. The method submitter was notified by the Executive Office and invited to submit a full SLV and an LMRC liaison was assigned.

Recommendations: The LMRC recommends to Task Force I that Proposal 15-114 be referred to an appropriate committee as determined by the Conference Chair to await SLV data.

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

PUBLIC HEALTH SERVICE **U.S. FOOD AND DRUG ADMINISTRATION** OFFICE OF FOOD SAFETY SHELLFISH AND AQUACULTURE POLICY BRANCH **~~SHELLFISH PROGRAM IMPLEMENTATION BRANCH~~** **~~SHELLFISH SAFETY TEAM~~** **5100 PAINT BRANCH PARKWAY** **COLLEGE PARK, MD 20740-3835** **TEL. 240-402-2151/2055~~301-436-2151/2147~~ 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~~UALITY~~ Assurance~~SSURANCE~~

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~~that~~ apply) ~~1. a. Organization of the laboratory.~~ ~~2. b. Staff training requirements.~~ ~~3. c. Standard operating procedures.~~ ~~4. d. Internal quality control measures for equipment, calibration, maintenance, repair and performance.~~ ~~5. e. Laboratory safety.~~ ~~6. f. Quality assessment.~~ ~~g. Proper animal care.~~
		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~~2~~ Work Area

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O ~~O~~O ~~O~~O ~~O~~O	5, 6 5		1.3.1 Adequate for the workload and storage. 1.3.2~~.~~ Clean and well lighted. 1.3.3~~.~~ Adequate temperature control. 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~~3~~ 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~~14), if ~~is~~ used, measures accurately to a ~~with~~ minimum ~~accuracy~~ 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. ~~or with each use.~~ Results are recorded and ~~R~~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 ~~(2 & 7)~~ 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~~efficiency~~ is determined daily or with each use by the ~~following either slope or~~ millivolt procedure or through determination of slope. (Circle method used).
K	2		1.4.8~~.~~ The balances being used provide~~s~~ an appropriate sensitivity at the weights of use.~~of at least 0.1g at a load of 150 grams.~~ 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, ~~or~~ ASTM Class 1or 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~~O~~	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~~at -20°C or below~~.
K~~O~~	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~~14.~~ All glassware is clean.
C~~O~~	**5**		**1.4.16~~15.~~ With each load of labware/glassware washed, the contact surface of**

			~~Once during each day of washing,~~ several dry pieces ~~of glassware~~ from each load~~batch washed~~ 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~~4~~ Reagents and Reference Solution Preparation and Storage
C	9		**1.5.1 Any residual (unused) STX diHCl~~l~~ standard solution is never stored after the ampule has been opened. ~~Opened PSP reference stand solution (100 µg/ml) is not stored.~~**
K	15		1.5.2~~.~~ PSP reference~~working standard~~ solution (1 µg/mL~~l~~) ~~and all dilutions are~~ is prepared gravimetrically and ~~prepared with~~ diluted with 0.001 M HCl~~,~~ solution. ~~pH 3 water, using 'Class A' volumetric glassware (flasks and pipettes) or prepared gravimetrically.~~
K	9		1.5.3~~.~~Prepared ~~Refrigerated storage of~~ 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~~working standard solution (1µg/ml) does not exceed 6 months and is checked gravimetrically for~~ 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~~l~~.**
K	9		1.5.5~~4~~. All~~PSP~~ working dilutions prepared from the PSP reference solution -are discarded appropriately after use.
C~~K~~	**5**		**1.5.6~~5.~~ Reagent~~Make up~~ water is distilled or deionized (circle appropriate choice~~circle one~~), tested monthly and exceeds 0.5 megohm – cm resistance ~~(2 megohms-cm in-line)~~ or is less than 2.0 µ~~-~~Siemens/cm conductivity at 25 °C. (Circle the appropriate water quality descriptor determined). ~~to be tested and~~Results are recorded and records are maintained.~~monthly for resistance or conductivity (circle the appropriate).~~**
~~O~~K	5		1.5.7~~6.~~ Reagent~~Make up~~ water is analyzed for residual chlorine monthly and is at a non-detectable level (≤ 0.1 mg/L~~ppm~~). Results are recorded and records are maintained. Specify method of determination _____.
~~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.~~
K~~O~~	5		1.5.8~~.~~ Reagent~~Makeup~~ water contains < 100~~0~~ CFU/mL~~l~~ as determined monthly using the heterotrophic plate count method. Results are recorded and records are maintained.
			1.6~~5~~ 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 ~~appropriately~~ labeled with ~~the~~ collector's name, type of shellstock, the source or harvest area, sampling station, ~~and~~ time, ~~and~~ date and place (if applicable) of collection.
C~~K~~	**2**		**1.6.3~~.~~ Immediately after collection, shellstock samples are placed in dry storage ~~for transport~~ (~~e.g. cooler~~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 lab, samples are placed under refrigeration.~~
K	15, 9		1.6.4~~.~~ T~~he t~~ime from collection to initiation~~completion~~ of the extraction~~bioassay~~ should not exceed 24 hours. However, if ~~there are~~ significant ~~transportation~~ delays are anticipated or if they occur, the laboratory has an appropriate contingency plan in place to handle these samples.~~, then shellstock samples are processed immediately~~ 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 ~~*(circle the appropriate choice)*~~: a. refrigerated or frozen until extracted; b. homogenized and frozen until extracted; or; c. extracted, the supernatant decanted, and refrigerated or frozen until assayed. ~~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~~.
C~~K~~	**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~~EXAMINATION OF SHELLFISH FOR PSP TOXIN~~

			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~~is~~ 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~~K~~	**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.

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

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~~1.~~ 100 grams of homogenized sample is weighed into a beaker.
K	2		2.2.3~~2.~~ The sample homogenate is extracted in a 1:1 weight/volume ratio by adding ~~An equal amount of~~ 0.1 M~~N~~ HCl~~1~~ or~~/~~ 0.18 M~~N~~ HCl ~~is added to the homogenate and thoroughly mixed~~ (*circle the appropriate choice ~~normality~~*).
K	2		**2.2.4 Homogenate/acid mixture is stirred thoroughly before boiling to completely mix the contents.**
~~C~~			~~3. pH is checked and, if necessary adjusted to between pH 2.0 and 4.0.~~
C	**2**		**2.2.5~~4.~~ To prevent toxin transformation, the pH of the homogenate/acid mixture before boiling is 3.0 ± 1.0, adjusted if necessary with ~~Adjustment of pH is made by~~ the dropwise addition of either ~~the acid (~~5 M~~N~~ HCl~~)~~ to lower the pH or ~~base (~~0.1 M~~N~~ NaOH~~)~~ to raise the pH, as appropriate, while constantly stirring the mixture.**
C	**2**		**2.2.6~~5.~~ The homogenate/acid mixture is promptly brought to its boiling point, ~~a boil, 100 ± 1°C,~~ then gently boiled at 100 ± 1 °C for 5 minutes.**
O	9		2.2.7~~6.~~ The homogenate/acid mixture is boiled under adequate ventilation (e.g.~~i.e.~~ fume hood).
O	9		2.2.8~~7.~~ The homogenate/acid mixture~~extract~~ is allowed to cool~~ed~~ to room temperature.
C	**2**		**2.2.9~~8.~~ The pH of the cooled mixture after boiling is 3.0 ± 1.0, ~~extract is determined and~~ adjusted~~,~~ if necessary, ~~to between pH 2 and 4, preferably to pH 3~~ with the ~~stirred~~ dropwise addition of 5 M ~~N~~ HCl to lower the pH or 0.1 M~~N~~ 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~~			~~9. The extract volume (or mass) is adjusted to 200 mls (or grams) with dilute HCl, pH 3 water.~~
K	2		2.2.11~~10.~~ The homogenate/acid mixture ~~extract is returned to the beaker, stirred to homogeneity and~~ is allowed to separate by gravity or by centrifugation ~~settle to remove particulates; or, if necessary, an aliquot of the stirred supernatant is~~ (e.g. centrifuged at 3,000 RPM for 5 minutes)~~ before injection~~.
K	9		2.2.12~~11.~~ If the extracted sample ~~mice~~ cannot be assayed~~injected~~ immediately, then the supernatant is decanted and stored in a sealed container under~~should be removed from the centrifuge tubes and~~ refrigeration~~ed~~ for up to 24 hours or frozen for longer storage.
K	9		2.2.13~~12.~~ 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~~O~~	2		2.3.1~~.~~ A 26-gauge hypodermic needle is used for intraperitoneal injections.
C~~K~~	**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 ~~M~~mice are never~~ot~~ re-used for a bioassay. Stock strain: Source: ~~Stock strain used _____ Source of mice_____~~
C	9		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.
C	9		2.3.-~~4.~~ A conversion factor (CF) for the lab has been appropriately determined. ~~as _____. Month and year when current CF 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*) ~~Date of most recent CF check _____~~ ~~CF verified/CF not verified *(Circle appropriate 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~~			~~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	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~~3~~ times in a~~per~~ year, the lab CF~~bioassay~~ is recalculated through a restandardization~~ed~~ procedure.
K~~O~~	9		2.3.8~~.~~ Mice are weighed to the nearest 0.15~~5~~ g~~ram~~.
C	2		2.3.9~~.~~ Mice are injected intraperi~~ti~~oneally with 1 mL~~l~~ of ~~the acid~~ extracted sample.
K	2		2.3.10~~.~~ For ~~the~~ CF checks, ~~at least 5~~five mice are injected~~used~~.
K~~C~~	9		2.3.11~~.~~ For routine assays, three~~At least 3~~ mice (two when both survive) are injected~~used~~ per sample ~~in routine assays~~.
C	2		2.3.-12~~.~~ Elapsed time post-injection is accurately determined and recorded.
C~~K~~	2		2.3.13~~.~~ When~~If~~ death occurs, the time of death to the nearest second is noted at~~by~~ 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~~14.~~ If the median corrected mouse unit is greater than 1.92 ~~death time(~~

			~~2 out of 3 mice injected die) is <~ (5 minutes), then the sample is ~~a~~ dilut~~ed~~ion is made with ~~dilute~~0.001 M HCl as appropriate~~, pH 3 water,~~ to achieve~~obtain~~ a median corrected mouse unit, MCMU of 1.39-1.92 (a death time ~~in the range~~ of 5~~ to~~ 7 minutes).
	2.4 Calculation of t~~T~~oxicity for MBA		
C	2		2.4.-1~~.~~ The death time for~~of~~ each mouse is converted to mouse units (MU) using Sommer's Table ~~(Table 6 *Recommended Procedures*, 4th edition)~~ and recorded. Any~~The death time of~~ mice surviving beyond 60 minutes are~~is considered to be~~ recorded as < 0.875 MU.
C~~K~~	2		2.4.2~~.~~ The~~A~~ weight for each mouse is corrected~~ion~~ to mouse units ~~in MU~~ using the table of weights in ~~is made for each mouse injected using Table 7 in~~ Recommended Procedures (Table 7) and interpolated for weights not listed.~~, 4th edition.~~
C	2		2.4.-3~~.~~ The ~~death time of each mouse in MU is multiplied by a weight correction in MU to give the C~~corrected M~~m~~ouse U~~u~~nit (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 ~~m~~Median Corrected Mouse Unit (MCMU) for each sample is calculated and used in the final toxicity calculation for that sample. ~~value of the array of corrected mouse units (CMU) is determined to give the median corrected mouse unit (MCMU).~~
C	2		2.4.-5~~. The concentration of toxin is determined by the formula, MCMU x CF X Dilution Factor X 200.~~ 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~~6.~~ Any value equal to or greater than 80 µg STX eq/l00 g~~rams~~ of sample~~meat~~ 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.

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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.**

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

LABORATORY: **DATE OF EVALUATION:**

SHELLFISH LABORATORY EVALUATION CHECKLIST

SUMMARY OF NONCONFORMITIES

Page	Item	Observation	Documentation Required

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

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

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 L~~l~~aboratory is not in conformity with NSSP requirements when any of the following apply. ~~if:~~

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.

~~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 ≥ 1 but < 3~~

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

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:_____

~~NSSP Form Lab-100 Rev. 2005~~

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH ~~PROGRAM IMPLEMENTATION~~AND AQUACULTURE POLICY BRANCH
~~SHELLFISH SAFETY TEAM~~
5100 PAINT BRANCH PARKWAY
COLLEGE PARK, MD 20740-3835
TEL. ~~301~~240-4~~36~~02-2151/2~~147~~055 FAX 301-436-26~~72~~01

SHELLFISH LABORATORY EVALUATION CHECKLIST

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

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

Items which do not conform are noted by:

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

PART I – QUALITY ASSURANCE			
Code	REF		Item Description
			1.1 Quality Assurance (QA) Plan
K	5, 8		1.1.1 Written Plan adequately covers all the following: (check √ those that apply) a. Organization of the laboratory. b. Staff training requirements. c. Standard operating procedures. d. Internal quality control measures for equipment, their calibration, maintenance, repair, ~~and~~ performance and rejection criteria established. e. Laboratory safety. f. Internal performance assessment. g. External performance assessment.
C	5		**1.1.2 QA Plan is implemented.**
			1.2 Educational/Experience Requirements
C	State's ~~Human~~ Resources Department		**1.2.1 In state/county laboratories, the supervisor meets the state/county educational and experience requirements for managing a public health laboratory.**
K	State's Human Resources Department		1.2.2 In state/county laboratories, the analyst(s) meets the state/county educational and experience requirements for processing samples in a public ~~health laboratory.~~
C	USDA ~~Microbiology~~ & EELAP		**1.2.3 In commercial/private laboratories, the supervisor must have at least a bachelor's degree or equivalent in microbiology, biology, chemistry, or another appropriate discipline with at least two years of laboratory experience.**
K	USDA~~Z~~ Microbiology & EELAP		1.2.4 In commercial/private laboratories, the analyst must have at least a high school diploma and shall have at least three months of experience in laboratory sciences.
C	5		**1.2.5 LC-Operator must be competent in the operation and maintenance of a basic liquid chromatography system.**
			1.3~~2~~ Work Area
O	5, 8		1.3.1 Adequate for workload and storage.
O	8		1.3.2 Clean and well lighted.
O	8		1.3.3 Adequate temperature control.
O	8		1.3.4 All work surfaces are nonporous and easily cleaned.
			1.4~~3~~ Laboratory Equipment.
O	6		1.4.1 The pH meter has a standard accuracy of 0.1 unit.
K	6		1.4.2 pH paper in the appropriate range (i.e. 1-4), if used, is used with minimum accuracy of 0.5 pH units.
K	10		1.4.3 pH electrodes consist of pH half-~~-~~cell and reference half-~~-~~cell or equivalent combination electrode/triode (free from Ag/AgCl or contains an ion exchange barrier to prevent passage of Ag ions into the medium that may result in inaccurate pH readings).

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K	5		1.4.4 pH meter is calibrated daily when in ~~or with each~~ use. Results are recorded and r~~R~~ecords are maintained.
K	8		1.4.5 Effect of temperature has been compensated for by an ATC probe, use of a triode or by manual adjustment.
K	8		1.4.6 A minimum of two standard buffer solutions is ~~(2 & 7) are~~ used to calibrate the pH meter. The first must be near the electrode isopotential point (pH 7). The second must be near the expected sample pH (i.e. pH 2, 4 or 11) as appropriate. Standard buffer solutions are used once and discarded.
K	5, 11		1.4.7 Electrode acceptability~~efficiency~~ is determined daily or with each use following either slope or millivolt procedure.
K	6		1.4.8 The balances being used provide~~s~~ an appropriate sensitivity at the weights of use, ~~of~~ at least ~~0.000~~0.1 g for laboratory precision balances and 0.1 mg for analytical balances. ~~at a load of 5 grams.~~
K	8, 9		1.4.9 The balance calibration is checked monthly using NIST class S, ASTM class 1 or 2 weights or equivalent. Results are recorded and r~~R~~ecords are maintained.
K	1		1.4.10 Refrigerator temperature is maintained between 0 and 4 ºC.
K	8		1.4.11 Refrigerator temperature is monitored at least once daily. Results are recorded and r~~R~~ecords maintained.
K	1		1.4.12 Freezer temperature is maintained at -20 ºC or below.
~~O~~K	8		1.4.13 Freezer temperature is monitored at least once daily. Results are recorded and r~~R~~ecords maintained.
C	13		**1.4.14 All in-service thermometers are properly calibrated and immersed.**
~~O~~K	5		~~1.4.14~~1.4.15 All glassware is clean.
K	3		~~1.4.15~~1.4.16 A ~~H~~high performance liquid chromatography system (HPLC) equipped with the following is used: ~~a. Low dead volume,~~ ~~b.~~a binary mobile phase ~~solvent~~ system delivering a pulse-free flow of 0.5-2.0 mL/min, ~~c.~~b solvent degasser, ~~d.~~c autosampler (refrigerated preferred) with loop suitable for 5-30 µL injections, ~~e.~~d temperature controlled column compartment capable of controlling temperature between 10 – 50 ºC, and ~~f.~~e. fluorescence detector able to achieve the required sensitivity at an excitation wavelength (λ) of ~~=~~ 330 nm and emission of ~~λ =~~390 nm.
K	3, 4		~~1.4.16~~1.4.17 The ~~Post~~post-column reaction system used is equipped with the following: a. ~~Reactor~~ reactor module capable of maintaining 85 ºC, b. dual reagent pumps capable of delivering accurate flows of 0.4 mL/min, and c. if applicable, a~~knitted~~ reaction coil (knitted or equivalent) having a total volume of ~~,~~ 1 mL ~~volume,~~and a length of 5 m x 0.5 mm.
K	6		~~1.4.17~~1.4.18 Autopipettors are calibrated for the appropriate volumes used and checked annually for accuracy. Results are recorded and r~~R~~ecords are maintained.
K	3		~~1.4.18~~1.4.19 A b~~B~~oiling water bath with sufficient volume to cover the sample/acid mixture is used for extraction.
~~K~~O	3		~~1.4.19~~1.4.20 Centrifuge capable of holding 50 mL polypropylene tubes. ~~and generating ~ 3000 RCF.~~

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K	3		~~1.4.20~~1.4.21 Microcentrifuge capable of holding 1.5 mL microcentrifuge tubes and ~~of~~ generating a minimum of ~~–~~16000 g or equivalent is used ~~RCF~~.
			1.54 Reagents and Reference Solution Preparation and Storage
~~OC~~	3		**1.5.1 All solvents and reagents used are analytical or LC grade materials.**
~~KC~~	8		**1.5.2 Water is glass distilled or deionized and exceeds 0.5 megaohm ~~resistance or is less than 2 µSiemens/cm conductivity at 25 °C to be~~ tested and recorded monthly for resistance or conductivity and the results are recorded.**
~~O~~K	8		1.5.3 Water is analyzed for residual chlorine monthly and is at a nondetectable level (≤0.1 ppm) Results are recorded and records are maintained.
~~K~~			~~1.5.4 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~~K	8		~~1.5.5~~1.5.4 Water contains < 100~~0~~ CFU/ml as determined monthly using the heterotrophic plate count method. Results are recorded and records are maintained.
~~O~~K	8		~~1.5.6~~1.5.5 Reagents are properly stored and labeled with the date of receipt, ~~and~~ date opened or date prepared and expiration date.
C	3		**1.5.6 The binary mobile phase system used to analyze the GTX and STX toxins consists of:** **1.5.6.1 Mobile Phase A, which contains 11 mM heptane sulfonate and 5.5 mM phosphoric acid (H_3PO_4), pH 7.1,** **1.5.6.2 Mobile Phase B, which contains 11 mM heptane sulfonate, 16.5 mM H_3PO_4 and11.5% acetonitrile (MeCN), pH 7.1,**
C	3		**1.5.7 The binary mobile phase system used to analyze the C toxins consists of** **1.5.7.1 Mobile Phase A, which contains 2 mM tetrabutyl ammonium phosphate, pH 5.8,** **1.5.7.2 Mobile Phase B, which contains 2 mM tetrabutyl ammonium phosphate in 4% acetonitrile, pH 5.8,**
C	3		**1.5.8 The post-column oxidant consists of 100 mM H_3PO_4 and 5 mM periodic acid (H_5IO_6), pH 7.8,**
C	3		**1.5.9 The post-column acid used is ~~1~~0.75 M nitric acid (HNO_3),**
C	3		~~1.5.7~~**1.5.10 The~~0.5 M 1-~~ heptane sulphonate used in mobile phase A and mobile phase B to analyze for GTX and STX toxins is prepared the day of use or refrigerated for up to one week,**
C	3		~~1.5.8~~**1.5.11 The pH of mobile phases and the post-column oxidant are adjusted as follows ~~and records maintained~~:** a. **Mobile phase A and mobile phase B for the GTX and/ STX toxins are adjusted to ~~mobile phase A&B is~~ 7.1 with ammonium hydroxide (NH_4OH),**

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			b. **Mobile phase A and mobile phase B for the C toxins are adjusted to ~~mobile phase A is~~ 5.8 in one direction only with 10% acetic acid (HOAc) ~~if~~ if too basic or 1% NH_4OH if too acidic, and**
			c. **The post-column o~~O~~xidant is adjusted to 7.8 with 5 M sodium hydroxide (NaOH).**
~~K~~O	3		d. Mobile phases and post-column reagents are filtered ~~through 0.2 µm nylon filter membrane~~ before use if the HPLC does not have a degreaser.
C	3,7		~~1.5.9~~**1.5.12 Only certified reference materials are used for standard solutions. Source of the reference standard: _____**
C	7		**1.5.13 NRC Zero-Mus or a negative control matched matrix is used as a matrix blank as appropriate. Source of the negative matrix: _____**
~~K~~C	7		~~1.5.10~~**1.5.14 All primary standards are stored appropriately as per supplier recommendations.**
C	7		**1.5.15 All standards used are within expiration date.**
~~K~~C	~~7~~3		~~*1.5.11*~~**1.5.16 All s~~S~~tandards are prepared gravimetrically. ~~using "Class A" glassware.~~**
K	3		~~1.5.12~~1.5.17 Intermediate mixes of primary standards are made up in 0.003 M HCl for the ~~(~~GTX/STX toxins~~)~~ or pH 5 glass distilled/deionized water for the ~~Milli-Q water (~~C toxins~~)~~ labeled with the date of preparation and the expiration date ~~,~~ and stored appropriately. The pH of the glass distilled/deionized water is adjusted when necessary by the dropwise addition of 10% acetic acid (HOAc).
~~K~~C	3		~~1.5.13~~**1.5.18 Working standards are made up from primary standard or intermediate mixes by dilution with toxin-free, deproteinated, matrix matched ~~mussel or oyster~~ extracts ~~(GTX/STX toxins) or Milli-Q water (C toxins)~~.**
C	7		**1.5.19 Zero-Mus is stored according to manufacturer's instructions.**
C	2		**1.5.20 Quality Control shellfish tissues are stored frozen.**
~~K~~C	7		~~1.5.14~~**1.5.21 Working standards are labeled with the date of preparation, stored ~~in the refrigerator at 4°C~~ appropriately and used within 3 months of preparation.**
			1.~~6~~5 Collection and Transportation of Samples
O	6		1.6.1 Shellstock are collected in clean, waterproof, puncture resistant containers.
K	6		1.6.2 Samples are appropriately labeled with the collector's name, type of shellstock, ~~the source,~~ the harvest area, and time and~~,~~ date ~~and place (if market sample)~~ of collection.

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~~KC~~	6		**1.6.3 Immediately after collection, shellstock samples are placed in dry storage (ice chest or equivalent) which is maintained between 0 and 10 °C with ice or cold packs for transport to the laboratory.~~Immediately after collection, shellstock samples are placed in dry storage between 0 and 10 °C during transport and held under refrigeration in the laboratory until processedanalyzed.~~**
K	~~9~~14		1.6.4 T~~he t~~ime from collection to initiation~~completion~~ of the extraction~~analysisassay~~ should not exceed 24 hours. However, if~~ there are~~ significant ~~transportation~~ delays are anticipated or if they occur, the laboratory has an appropriate contingency plan in place to handle the~~n shellstock~~ 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~~are processed immediately~~ as follows ~~(circle the appropriate choice)~~: a. refrigerated or frozen until extracted~~Washed, shucked, drained, frozen until extracted~~; b. homogenized and frozen until extracted; or~~Washed, shucked, drained, homogenized and frozen; or~~ c. extracted, the supernatant decanted, and refrigerated or frozen until assayed.~~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.~~
C	6		~~1.6.4~~**1.6.5 Frozen shucked product or homogenates are allowed to thaw completely and all liquid is included as part of the sample before being processed further.**
PART II – EXAMINATION OF SHELLFISH FOR PSP TOXINS			
			2.1 Preparation of Sample
C	6		**2.1.1 At least 12 animals are used per sample or the laboratory has an appropriate contingency plan for dealing with non-typical species of shellfish.**
O	6		2.1.2 The outside of the shell is thoroughly cleaned with fresh water.
O	6		2.1.3 Shellstock are opened by cutting the adductor muscles.
O	6		2.1.4 The inside surfaces of the shells are rinsed with fresh water to remove sand and other foreign materials.
O	6		2.1.5 Shellfish meats are removed from the shell by separating the adductor muscles and tissue connecting at the hinge.
~~KC~~	6		**2.1.6 Damage to the body of the mollusk is minimized in the process of opening.**
O	6		2.1.7 Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.

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K	6		2.1.8 Pieces of shell and drainage are discarded.
C	6		**2.1.9 Drained meats or previously cooled/refrigerated shucked meats and their drip loss liquid or thawed homogentates with their freeze-thaw liquid are blended at high speed until homogenous (60-120 seconds).**
			2.2 Digestion of Sample
K	6		2.2.1 Sample homogenates are extracted as soon as possible (preferably the same day) or stored in the freezer.
C	3		**2.2.2 Five (5) grams of homogenized sample is weighed into a 50 mL polypropylene centrifuge tube and subsequently extracted.**
K	3		~~2.2.2~~2.2.3 The s~~S~~ample homogenate is extracted in a 1:1 w/v ratio with 0.1 M HCl~~, preferably 5g tissue in 5mL acid~~
K	3		~~2.2.3~~2.2.4 Homogenate/acid mixture is vortexed thoroughly before boiling to completely mix the contents.
C	3		~~2.2.4~~**2.2.5 To prevent toxin transformation, the pH of the homogentate/acid mixture before boiling is 3.0 ± 1.0, adjusted if necessary ~~with~~with the dropwise addition of either 5 M HCl to lower the pH or 0.1 M NaOH to raise the pH.**
C	3		~~2.2.5~~**2.2.6 Samples ~~are extracted in a boiling water bath for 5 minutes,~~ in capped 50 mL polypropylene centrifuge tubes are extracted in a boiling water bath for 5 minutes.**
K	3		~~2.2.6~~2.2.7 The pH of the cooled mixture after boiling is 3.0 ± 1.0, adjusted if necessary with the dropwise addition of 5 M HCl. Any sample with a pH of less than 2.0 is discarded and extracted again.
K	3		~~2.2.7~~2.2.8 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	3		**2.3.1 500 µL of sample e~~E~~xtract is deproteinated with 25 µL of 30% trichloroacetic acid ~~(50 µL TCA per 1000 µL aliquot of supernatant)~~, vortexed thoroughly and centrifuged at ~ 16,000 *g* for 5 minutes.**
C	3		**2.3.2 The pH of the deproteinated extract is adjusted with 35 µL of~~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		2.3.3 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 Analysis~~say~~
C	2		2.4.1 **A standard calibration curve (of at least six concentrations) is performed upon initial instrument set up, following any major hardware maintenance activity, or when the continuing calibration verification (CCV) indicates significant drift (> 30% for individual**

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			toxin) from the calibration. Results are recorded and rRecords are maintained.
~~K~~			~~2.4.2 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		~~2.4.3~~2.4.2 10 µL is injected for GTX/STX toxins and 5 µL is injected~~analyzed~~ for C-toxins.
K	3		~~2.4.4~~2.4.3 Samples are stored in the sample compartment of the autosampler at 4 °C during analysis. Otherwise samples must be analyzed within 20 hours if the autosampler is held at room temperature.
~~O~~K	3		~~2.4.5~~2.4.4 A column heater that is capable of maintaining 30-40 °C for the GTX/STX toxins and 10-20 °C for the C toxins is used in the analysis.
~~OC~~	3		~~2.4.6~~2.4.5 **The appropriate analytical column is used.** **a. GTX/STX Toxins: Agilaent Zorbax Bonus-RP column, 4.6 mm x 150 mm, 3.5 µm~~, Agilent catalog number 863668-901~~ or equivalent.** b. **C Toxins: Thermo BetaBasic 8, 4.6 mm x 250 mm, 5 µm~~, Fisher catalog number 71405-254630~~ or equivalent.**
			2.5 System Suitability
K	2		2.5.1 The correlation coefficient for the linear regression of the calibration standards ~~(r^2)~~ must be ≥ 0.990 for each individual toxin.
~~K~~C	3		**2.5.2 The rResolution and rRetention tTime cCriteria that must be met are:** ~~GTX/STX Toxins.~~ **a. For GTX and STX toxins, the mMatrix peak must be at least 70% baseline resolved between GTX3 and GTX2.** **b. For GTX and STX toxins, GTX5 must be at least 40% baseline resolved between dcGTX3 and dcGTX2.** **c. For GTX and STX toxins, dcSTX and STX must be at least 70% baseline resolved.** ~~d.~~ **For GTX and STX toxins, the retention time of GTX4 ~~retention time should~~must be between 5 and 7 minutes.** **d. ~~C Toxins.~~** **e. For the C toxins, ~~C1 and~~ C2 must be at least 70% baseline resolved between C1 and C2.** **f. For the C toxins, the retention time of C1 ~~retention time should~~must be between ~~4~~5 and ~~7~~8 minutes.**
C	2		**2.5.3 Daily injection schedules must include the adequate frequency of injection standards based on an assessment of individual standard toxin variability. Variability in peak response must be less than 10% for calculation of toxicity in samples.**
			2.6 Calculation of Toxicity
C	4		2.6.1 **The toxicity of the individual toxins is calculated as follows:**

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$$\mu gSTXdiHCleq/100g = \mu M \times \frac{372.2}{1000mL} \times \frac{Fvol}{Ext.vol} \times \left(\frac{Wt + Vol}{Wt}\right) \times ReTx \times 100$$

~~7.~~15.

Where:

µM = Concentration of toxin in the extract, in µM;

Fvol = Final volume of the deproteinized extract (e.g. ~~112~~560 µL);

Ext.vol = Volume of crude extract used (e.g. ~~10~~500 µL);

Wt = Weight of sample used;

Vol = Volume of acid extractant used (e.g. 5 mL); and

ReTx = Relative toxicity of toxin vs. Saxitoxin.

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

~~8.~~16.

C | 3 | 2.6.2 **The individual toxicities for each toxin are summed to obtain the overall sample toxicity in µg STX equivalents/100 g (µg/100 g).**

C | 12 | 2.6.3 **Any value at or above~~greater than~~ 80 µg STX equivalents /100 g of meat is actionable.**

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7. Consult reference standard product literature.
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~~9. Personal communication with USFDA Washington Seafood Laboratory Branch, Office of Seafood, CFSAN, 1998-1999.~~

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LABORATORY:			**DATE OF EVALUATION:**
SHELLFISH LABORATORY EVALUATION CHECKLIST~~H~~ **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)** Nonconforma~~a~~ities Total # of Key (K) Nonconforma~~a~~ities Total # of Critical, Key, and Other (O) Nonconfom~~a~~ities	_____ _____ _____

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

1. Conforms Status: The PSP, PCOX component of this Laboratory is in conformity with NSSP requirements if all of the following apply.

a. No Critical nonconformities,

b. and <6 Key nonconformities,

c. and <12 Total nonconformities.

~~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, PCOX 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~~y~~ **≤ 4.** ~~3~~

b. and **≤ 6** Key nonconformities.

c. and **≤ 12** Total ~~N~~nonconformities.~~-~~

3. **Does Not Conform Status** : The PSP, PCOX component of this laboratory is not in conformity with NSSP requirements when any of the following apply.

a. The total # of Critical nonconformities is **≥ 4.**

b. or the total # of Key nonconformities is **≥ 6.**

~~d.~~c. or the total # of Critical, Key, or Other is **≥ 12.**

C. Laboratory Status (*circle appropriate)*

Does Not Conform – Provisionally Conforms – Conforms

Acknowledgement by Laboratory Director/Supervisior:

All corrective Action will be implemented and verifying substantiating documentation received by the

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Laboratory Evaluation Officer on or before _____.

Laboratory Signature: _____ Date:_____

LEO Signature:_____ Date: _____

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Scotia
Rapid Testing

October 8, 2015

Interstate Shellfish Sanitation Conference
209-2 Dawson Road
Columbia, SC 29223

Attention: Ken Moore

Dear Mr. Moore.

A legal agreement dated November 14, 2014 between the former owners of Jellett Rapid Testing Ltd, established that W. Hywel Morgan would become the sole owner of that company, and that the name "Jellett" be no longer associated with the company or its activities.

Accordingly, on December 12, 2014 the company name was changed to Scotia Rapid Testing Ltd, and the name of its products, from Jellett Rapid Tests to Scotia Rapid Tests. This second provision of course applies to the Scotia Rapid Test for PSP. Scotia Rapid Testing Ltd. requests that the ISSC acknowledge this change in the next revision of the NSSP guide for the control of Molluscan Shellfish.

We also have to request that in future the ISSC refer to the Jellett Rapid Extraction Method as the Scotia Rapid Extraction Method.

We hope that these requests cause you no problems, but if you should need any further details, we would be happy to comply.

Yours sincerely,

Andrea Swinamer

Andrea Swinamer

Proposal No. 13-117

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

Submitter	US Food & Drug Administration (FDA)
Affiliation	US Food & Drug Administration (FDA)
Address Line 1	Center for Food Safety & Applied Nutrition (CFSAN)
Address Line 2	5800 Paint Branch Parkway
City, State, Zip	College Park, Maryland 20740
Phone	240-402-1410
Fax	301-436-2601
Email	linda.chandler@fda.hhs.gov
Proposal Subject	Certification of State Shellfish Laboratory Evaluation Officers
Specific NSSP Guide Reference	Section IV. Guidance Documents Chapter II. Growing Areas
Text of Proposal/ Requested Action	.12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists Laboratory results from the ~~bacteriological~~ microbiological and marine Biotoxin testing of shellfish and shellfish growing waters ~~and meats~~ are widely used in the National Shellfish Sanitation Program (NSSP) to aid in determining the safety of shellfish for human consumption. Experience with the ~~bacteriological~~ microbiological and marine Biotoxin analyses of shellfish and shellfish growing waters have indicated that minor differences in laboratory procedures or techniques might cause wide variations in the results. ~~Improper handling of the sample may also cause variations in results during collection or transportation to the laboratory.~~ To ensure uniformity ~~nationwide~~ NSSP wide in the application of standards for shellfish and shellfish growing waters, a comprehensive, effective laboratory quality assurance (QA) program is necessary to ~~substantiate~~demonstrate the validity of analytical results. ~~A~~ Thee laboratory ~~quality assurance~~QA program is the systematic application of the practices essential to remove or minimize errors that may occur in any laboratory operation caused by personnel, ~~apparatus,~~ equipment, media, reagents, ~~sampling procedures,~~ and analytical methodology. ~~(APHA, 1985).~~ Integral to laboratory quality assurance is a strong program for the external assessment or evaluation of laboratory performance. The laboratory evaluation process has evolved over the years to accommodate changes in microbiology and marine Biotoxin procedures brought about by NSSP Workshops and more recently by the Interstate Shellfish Sanitation Conference (ISSC). In 1985, FDA issued an interpretation entitled "Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers" (SS#35). This Interpretation allowed NSSP laboratories which had been previously evaluated by FDA Shellfish Laboratory Evaluation Officers to be subsequently evaluated by qualified state personnel as certified State Shellfish Laboratory Evaluation Officers. This guidance describes the procedure for the certification of these individuals as State Shellfish Laboratory Evaluation Officers. ~~Requirements for evaluating laboratories that analyze samples under the NSSP have increased significantly since the 1970's. The number of laboratories participating in the shellfish program has also increased. Several states now have multiple laboratories that provide these analyses. Some states have officially designated city, county or private laboratories to conduct analyses supporting their shellfish sanitation~~

~~programs. Some states are also authorizing the use of private laboratories to monitor depuration operations. More states are maintaining a marine biotoxin analytical capability in their laboratories; and more foreign laboratories are involved in the NSSP. Historically, FDA has evaluated all these laboratories. Reduction in FDA staffing has made it difficult to evaluate the many state, county, municipal, and foreign shellfish laboratories operating in support of the NSSP. If states with multiple laboratory support would exercise their option to accept responsibility for evaluating their laboratories by employing a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO), FDA would be able to better meet its NSSP responsibilities.~~

General Provisions

1. If the State Shellfish Control Authority (Authority) uses the analytical services of private/commercial/fee for services laboratories to support the NSSP, then he/she should select a qualified individual to become certified as a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO).
2. If the Authority uses the analytical services of multiple public laboratories (state, county, parish town, etc.) to support the NSSP, then he/she may select a qualified individual to become a State Shellfish LEO.
3. ~~If the Authority chooses not to participate in the certification process,~~ FDA can evaluate the state's public laboratories. FDA, however, does not normally evaluate private/commercial/fee for service laboratories once they (FDA) have established that the laboratory meets NSSP requirements. FDA may under certain circumstances and as resources permit evaluate these laboratories after their initial successful evaluation if their services continue to be necessary to support the NSSP. The Authority may accompany FDA during such evaluations if the lab being evaluated performs regulatory testing for that State. ~~If the Authority chooses not to participate in the certification process, FDA can evaluate the state's public laboratories. FDA, however, does not normally evaluate private/ commercial/fee for services laboratories. FDA may, under certain circumstances as resources permit, evaluate these laboratories on a case- by case basis at the request of the Authority. This request must be in writing and made through the FDA Regional Shellfish Specialist.~~
4. State Shellfish LEOs may perform official NSSP evaluations of laboratories (public and private/commercial/fee for service) which have been previously evaluated by FDA and found to fully conform to NSSP requirements. ~~State Shellfish LEOs will perform official NSSP evaluations of laboratories which have been previously evaluated by FDA and been found to fully conform to NSSP laboratory requirements.~~
5. State Shellfish LEOs may evaluate laboratories in a different state under a memorandum of understanding between the states involved and FDA consistent with NSSP requirements.
6. State Shellfish LEOs may not evaluate laboratories in which they are employed or which they supervise or laboratories within the same supervisory chain of command to ensure complete objectivity in the evaluation process and avoid the appearance of a conflict of interest.
7. To qualify for certification, the prospective State Shellfish LEO should be:
   a. A state employee;

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b. Have shellfish laboratory experience or a laboratory background;
c. Preferably have laboratory evaluation experience; and,
d. Be free from any commercial, financial or other pressures or conflicts of interest that might cause or appear to cause the prospective State Shellfish LEO to act in other than an impartial or non-discriminatory manner.

8. If the prospective or current State Shellfish LEO is employed by the laboratory supporting the NSSP, that laboratory must be fully conforming to NSSP requirements or the individual will not be certified and if currently certified, certification will be revoked.

Responsibilities of the State Shellfish Control Authority

1. The Authority must ensure that appropriate written documentation is provided to FDA to demonstrate that a prospective State Shellfish LEO is adequately qualified to assume the responsibilities of a State Shellfish LEO as described above.
2. The Authority must provide or ensure that adequate time, resources and support are made available to the State Shellfish LEO to fully participate in the certification process and to fulfill his/her obligation as a State Shellfish LEO.

FDA's Responsibilities

1. FDA is responsible for the certification/recertification of State Shellfish LEOs.
2. As a result FDA must:
   a. Select qualified individuals to receive training based upon the documentation supplied by the Authority;
   b. Develop and provide training that will enable prospective and current State Shellfish LEOs to consistently and uniformly apply evaluation criteria in determining the competence of laboratories to support or continue to support the NSSP;
   c. Certify prospective State Shellfish LEOs that successfully complete the certification process;
   d. Maintain communication with State Shellfish LEOs as needed to provide guidance and updates relevant to the NSSP laboratory evaluation program;
   e. Recertify current State Shellfish LEOs pursuant to the criteria established for satisfactory performance below;
   f. Monitor the performance of State Shellfish LEOs to ensure that the evaluation process is being performed consistent with NSSP requirements as described in the current NSSP Guide for the Control of Molluscan Shellfish and this guidance;
   g. Maintain communication as needed with the Authority and other pertinent state officials, prospective and current State Shellfish LEOs and FDA Regional Shellfish Specialists relevant to the certification/recertification process;
   h. Revoke certification of State Shellfish LEOs for cause; and,
   i. Void certification when the need for a State Shellfish LEO no longer exists within the state shellfish sanitation program or when the State Shellfish LEO is no longer employed by the state.

~~Selection of State Shellfish LEOs should be based on the following criteria:~~

~~1.The individual must be administratively attached to a state central shellfish sanitation laboratory that has been found by the FDA to be in full conformance with NSSP requirements. To avoid the appearance of impropriety and maintain objectivity in the evaluation process, individuals certified as State Shellfish LEOs will not be allowed to evaluate their own laboratories. FDA will maintain the responsibility for evaluating these laboratories.~~

~~2.The individual must be an experienced analyst and should have laboratory supervision experience. To maintain the integrity of the evaluation process, this individual should not, however, have overall supervisory responsibilities for the laboratory or laboratories to be evaluated. If deemed necessary by an FDA Laboratory Evaluation Officer, the individual must conduct several laboratory evaluations jointly with the FDA Laboratory Evaluation Officer.~~

~~3.During the joint on site laboratory evaluation with an FDA Laboratory Evaluation Officer, the individual must demonstrate competence in evaluating the laboratory's capability to support the NSSP. The evaluation will be performed and documented using the most current version of the applicable FDA Shellfish Laboratory Evaluation Checklist.~~

~~4 The individual must submit a written narrative report of the joint on site evaluation to the FDA co evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this evaluation write up; and, where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations must be included in this write up.~~

~~The FDA will issue a letter certifying each individual who successfully completes the certification process and will clear the evaluation report(s) for distribution to the laboratories evaluated with copies to the appropriate Shellfish Specialist.~~

~~Certification is normally effective for a period of three (3) years. Once certified, the individual is then expected to assume the following responsibilities:~~

State Shellfish Laboratory Evaluation Officer's Responsibilities

1. Conduct onsite laboratory evaluations at least every three (3) years. However, more frequent evaluations are strongly encouraged and may be ~~required~~necessary with marginally performing laboratories, or when major changes in workloads or priorities have occurred or when there has been a substantial turnover of personnel, or, at the specific request of the Authority. ~~State Shellfish Control Authorities:~~
2. Provide appropriate post-evaluation follow-up for each laboratory evaluated;
3. Prepare timely narrative evaluation reports for all laboratories evaluated. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist for the component(s) evaluated and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this narrative; and, where relevant, an explanation

provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should also be included in the narrative report. ~~Incorporating the requirements specified in 4 above;~~

4. Distribute completed evaluation reports with checklists with checklists to FDA and to FDA and to the appropriate FDA Regional Shellfish Specialist.~~:~~
5. Inform ~~the appropriate~~ FDA Shellfish Laboratory Evaluation Officers when a laboratory has been found to be in nonconforming status.~~;~~
6. Coordinate proficiency testing at least yearly for all laboratories in the state supporting the microbiology component of the NSSP.
7. Prepare ~~at least~~ annually (in December) a summary list of ~~qualified analysts for each~~ all laboratories and qualified analysts within each laboratory by NSSP laboratory component supported ~~laboratory supporting the NSSP in the state~~ and transmit it to the ~~appropriate~~ FDA Shellfish Laboratory Evaluation Officers.

Certification Process

Certification is designed to be accomplished through individualized training and field standardization. Individuals are certified for evaluating either the microbiological and/or post-harvest processing (PHP) and/or marine Biotoxin components of the NSSP depending on their qualifications and the needs of the state shellfish sanitation program and at the discretion of FDA.

Field Standardization

1. Field standardization is designed to evaluate the prospective State Shellfish LEO's ability to determine the competence of the laboratory to meet NSSP laboratory requirements; recognize laboratory practices inconsistent with NSSP requirements when they occur; make appropriate recommendations for corrective action; and, provide the necessary follow-up activity to bring the laboratory into conformity with the NSSP.
2. Field standardization consists of one or several joint but independent onsite evaluations with an FDA Shellfish Laboratory Evaluation Officer and preparation of the corresponding narrative evaluation reports. The report(s) should consist of the completed FDA Shellfish Laboratory Evaluation Checklist(s) and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in the narrative; and where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should be included in this narrative report(s).
3. Field standardization should be performed in NSSP laboratories within the prospective State Shellfish LEO's home state to provide realistic evaluation scenarios. The narrative evaluation report detailing the evaluation findings must be prepared. The draft narrative report(s) with accompanying checklist(s) must be submitted to the certifying FDA Shellfish Laboratory Evaluation Officer within 60 days of the evaluation(s). All documents submitted will be

reviewed for appropriate content, accuracy and uniformity of approach by the certifying FDA Shellfish Laboratory Evaluation Officer.

4. Field standardization is based on a pass fail system.

Certification

1. Certification is dependent upon the perspective State Shellfish LEO satisfying all the following performance criteria.
   a. Demonstration of good familiarity with evaluation requirements.
   b. Demonstration of a thorough knowledge of the evaluation methods and documents.
   c. Demonstration of the technical knowledge/familiarity with the analytical procedures being used.
   d. Ability to communicate effectively both orally and in writing.
   e. Successful completion of both training and field standardization.
2. Upon successful completion of the certification process, a letter of certification will be issued by the FDA Shellfish Laboratory Evaluation Officer and a copy will be sent to both the requesting Authority and the FDA Regional Shellfish Specialist.
3. Certification is normally valid for up to five (5) years unless revoked or voided.

Failure to be Certified

1. If a prospective State Shellfish LEO fails to satisfy any of the performance criteria listed above, he/she will not be certified.
2. As resources permit and at the discretion of FDA, the prospective State Shellfish LEO may receive additional training to better prepare him/her to be certified.
3. The requesting Authority may withdraw the prospective State Shellfish LEO from consideration.

Recertification

1. Recertification normally occurs every five (5) years and is contingent upon the continuing need in the state shellfish sanitation program for the services of a State Shellfish LEO.
2. Recertification is based on the State Shellfish LEO satisfactorily meeting the following employment and performance criteria.
   a. The individual must continue to be employed by the state and be free of any commercial, financial or other pressures or conflicts of interest real or perceived that may cause the State Shellfish LEO to act in other than an impartial and non-discriminatory manner.
   b. The individual must demonstrate continued competence in the evaluation of NSSP laboratories by performing one to several joint evaluations with an FDA Shellfish Laboratory Evaluation Officer and providing an appropriate narrative evaluation report to the FDA co-evaluator for review and comment for each of the laboratories jointly evaluated.
   c. The individual must have performed laboratory evaluations at the minimum frequency prescribed in the current edition of

	the Guide for the Control of Molluscan Shellfish and have all Narrative evaluation reports up to date. 3. State Shellfish LEOs who successfully complete recertification will be issued a letter of recertification by FDA and be cleared to distribute the completed report(s) to the appropriate Regional Shellfish Specialist. A copy of this letter will be sent to the State Shellfish Control Authority and appropriate Regional Shellfish Specialist. 4. If FDA is unable to conduct a recertification visit by the expiration of the individual's certification, his/her certification may be extended until such time as recertification can be completed. If requested, a letter extending the certification can be provided as appropriate. Revocation of Certification 1. State Shellfish LEO's who fail to meet any of the certification/recertification, employment or performance criteria listed above will have their certification revoked. 2. Certification may be voided when state shellfish sanitation programs no longer have a need for the services of a State Shellfish LEO. 3. Voided certifications may be reactivated at the discretion of FDA if the need for the analytical services of additional laboratories by the state shellfish sanitation program recurs. 4. Revoked certifications will not normally be restored. ~~Recertification of State Shellfish LEOs will normally occur triennially and will be based on satisfactorily meeting the following criteria:~~ ~~1. The individual must continue to be administratively attached to a central state shellfish laboratory which is in full conformance with NSSP requirements;~~ ~~2. The individual is not the supervisor of any of the laboratories to be evaluated;~~ ~~3. The individual must demonstrate continued competence in evaluating the capability of laboratories to support the NSSP. If considered necessary, the individual will be required to performance to several joint evaluations with FDA Laboratory Evaluation Officer.~~ ~~4. The individual must submit a written narrative report of the joint evaluation(s) to the FDA co-evaluator for review and comment. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and the narrative portion should be prepared as above;~~ ~~5. The individual must have all state laboratory evaluations, split sample(proficiency) test examinations, and reports current;~~ ~~6. The individual should receive training as necessary, in laboratory evaluations and analytical procedures to remain proficient.~~ ~~State Shellfish LEOs who successfully complete this process will be issued a Letter of recertification by FDA and be cleared to distribute the evaluation reports to the laboratories evaluated with a copy to the appropriate Regional Shellfish Specialist. Normally recertification is effective for a period of three (3) years. Individuals who fail to meet the requirements for recertification will lose their certification until it is demonstrated that all requirements including adequate training are met.~~
Public Health Significance	This guidance document is virtually unchanged since the inception of the program for utilizing State Shellfish Laboratory Evaluation Officers (State Shellfish LEOS) in the NSSP. This revised guidance updates and clarifies the process for selection,

	certification and recertification of State Shellfish LEOs
Cost Information	NA
Action by 2013 Task Force I	Recommended referral of Proposal 13-117 to an appropriate committee as determined by the Conference Chairman.
Action by 2013 General Assembly	Adopted recommendation of 2013 Task Force I on Proposal 13-117.
Action by FDA May 5, 2014	Concurred with Conference action on Proposal 13-117.
Action by 2015 Laboratory Committee	.12 Evaluation of Laboratories By State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists Laboratory results from the ~~bacteriological~~ microbiological and marine Biotoxin testing of shellfish and shellfish growing waters ~~and meats~~ are widely used in the National Shellfish Sanitation Program (NSSP) to aid in determining the safety of shellfish for human consumption. Experience with the ~~bacteriological~~ microbiological and marine Biotoxin analyses of shellfish and shellfish growing waters have indicated that minor differences in laboratory procedures or techniques might cause wide variations in the results. ~~Improper handling of the sample may also cause variations in results during collection or transportation to the laboratory.~~ To ensure uniformity ~~nationwide~~ NSSP wide in the application of standards for shellfish and shellfish growing waters, a comprehensive, effective laboratory quality assurance (QA) program is necessary to ~~substantiate~~demonstrate the validity of analytical results. ~~A~~ Thee laboratory ~~quality assurance~~QA program is the systematic application of the practices essential to remove or minimize errors that may occur in any laboratory operation caused by personnel, ~~apparatus,~~ equipment, media, reagents, ~~sampling procedures,~~ and analytical methodology. ~~(APHA, 1985).~~ Integral to laboratory quality assurance is a strong program for the external assessment or evaluation of laboratory performance. The laboratory evaluation process has evolved over the years to accommodate changes in microbiology and marine Biotoxin procedures brought about by NSSP Workshops and more recently by the Interstate Shellfish Sanitation Conference (ISSC). In 1985, FDA issued an interpretation entitled "Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers" (SS#35). This Interpretation allowed NSSP laboratories which had been previously evaluated by FDA Shellfish Laboratory Evaluation Officers to be subsequently evaluated by qualified state personnel as certified State Shellfish Laboratory Evaluation Officers. This guidance describes the procedure for the certification of these individuals as State Shellfish Laboratory Evaluation Officers. ~~Requirements for evaluating laboratories that analyze samples under the NSSP have increased significantly since the 1970's. The number of laboratories participating in the shellfish program has also increased. Several states now have multiple laboratories that provide these analyses. Some states have officially designated city, county or private laboratories to conduct analyses supporting their shellfish sanitation programs. Some states are also authorizing the use of private laboratories to monitor depuration operations. More states are maintaining a marine biotoxin analytical capability in their laboratories; and more foreign laboratories are involved in the NSSP. Historically, FDA has evaluated all these laboratories. Reduction in FDA staffing has made it difficult to evaluate the many state, county, municipal, and foreign shellfish laboratories operating in support of the NSSP. If states with multiple laboratory support would exercise their option to accept responsibility for evaluating their laboratories by employing a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO), FDA would be able to better meet its NSSP responsibilities.~~

General Provisions

1. If the State Shellfish Control Authority (Authority) uses the analytical services of private/commercial/fee for services laboratories to support the NSSP, then he/she should select a qualified individual to become certified as a State Shellfish Laboratory Evaluation Officer (State Shellfish LEO).
2. If the Authority uses the analytical services of multiple public laboratories (state, county, parish town, etc.) to support the NSSP, then he/she may select a qualified individual to become a State Shellfish LEO.
3. If the Authority chooses not to participate in the certification process, FDA can evaluate the state's public laboratories. FDA, however, does not normally evaluate private/commercial/fee for services laboratories. FDA may, under certain circumstances as resources permit, evaluate these laboratories on a case-by-case basis at the request of the Authority. This request must be in writing and made through the FDA Regional Shellfish Specialist.
4. State Shellfish LEOs will perform official NSSP evaluations of laboratories which have been previously evaluated by FDA and been found to fully conform to NSSP laboratory requirements.
5. State Shellfish LEOs may evaluate laboratories in a different state under a memorandum of understanding between the states involved and FDA consistent with NSSP requirements.
6. State Shellfish LEOs may not evaluate laboratories in which they are employed or which they supervise or laboratories within the same supervisory chain of command to ensure complete objectivity in the evaluation process and avoid the appearance of a conflict of interest.
7. To qualify for certification, the prospective State Shellfish LEO should be:
   a. A state employee;
   b. Have shellfish laboratory experience or a laboratory background;
   c. Preferably have laboratory evaluation experience; and,
   d. Be free from any commercial, financial or other pressures or conflicts of interest that might cause or appear to cause the prospective State Shellfish LEO to act in other than an impartial or non-discriminatory manner.
8. If the prospective or current State Shellfish LEO is employed by the laboratory supporting the NSSP, that laboratory must be fully conforming to NSSP requirements or the individual will not be certified and if currently certified, certification will be revoked.

Responsibilities of the State Shellfish Control Authority

1. The Authority must ensure that appropriate written documentation is provided to FDA to demonstrate that a prospective State Shellfish LEO is adequately qualified to assume the responsibilities of a State Shellfish LEO as described above.
2. The Authority must provide or ensure that adequate time, resources and support are made available to the State Shellfish LEO to fully participate in the certification process and to fulfill his/her obligation as a State Shellfish LEO.

FDA's Responsibilities

1. FDA is responsible for the certification/recertification of State Shellfish LEOs.
2. As a result FDA must:
   a. Select qualified individuals to receive training based upon the documentation supplied by the Authority;
   b. Develop and provide training that will enable prospective and current State Shellfish LEOs to consistently and uniformly apply evaluation criteria in determining the competence of laboratories to support or continue to support the NSSP;
   c. Certify prospective State Shellfish LEOs that successfully complete the certification process;
   d. Maintain communication with State Shellfish LEOs as needed to provide guidance and updates relevant to the NSSP laboratory evaluation program;
   e. Recertify current State Shellfish LEOs pursuant to the criteria established for satisfactory performance below;
   f. Monitor the performance of State Shellfish LEOs to ensure that the evaluation process is being performed consistent with NSSP requirements as described in the current NSSP Guide for the Control of Molluscan Shellfish and this guidance;
   g. Maintain communication as needed with the Authority and other pertinent state officials, prospective and current State Shellfish LEOs and FDA Regional Shellfish Specialists relevant to the certification/recertification process;
   h. Revoke certification of State Shellfish LEOs for cause; and,
   i. Void certification when the need for a State Shellfish LEO no longer exists within the state shellfish sanitation program or when the State Shellfish LEO is no longer employed by the state.

~~Selection of State Shellfish LEOs should be based on the following criteria:~~

~~1.The individual must be administratively attached to a state central shellfish sanitation laboratory that has been found by the FDA to be in full conformance with NSSP requirements. To avoid the appearance of impropriety and maintain objectivity in the evaluation process, individuals certified as State Shellfish LEOs will not be allowed to evaluate their own laboratories. FDA will maintain the responsibility for evaluating these laboratories.~~

~~2.The individual must be an experienced analyst and should have laboratory supervision experience. To maintain the integrity of the evaluation process, this individual should not, however, have overall supervisory responsibilities for the laboratory or laboratories to be evaluated. If deemed necessary by an FDA Laboratory Evaluation Officer, the individual must conduct several laboratory evaluations jointly with the FDA Laboratory Evaluation Officer.~~

~~3.During the joint on-site laboratory evaluation with an FDA Laboratory Evaluation Officer, the individual must demonstrate competence in evaluating the laboratory's capability to support the NSSP. The evaluation will be performed and documented using the most current version of the applicable FDA Shellfish Laboratory Evaluation Checklist.~~

~~4. The individual must submit a written narrative report of the joint on-site evaluation to the FDA co-evaluator for review and comment. The report~~

~~should consist of the completed FDA Shellfish Laboratory Evaluation Checklist and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this evaluation write up; and, where relevant an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations must be included in this write up.~~

~~The FDA will issue a letter certifying each individual who successfully completes the certification process and will clear the evaluation report(s) for distribution to the laboratories evaluated with copies to the appropriate Shellfish Specialist.~~

~~Certification is normally effective for a period of three (3) years. Once certified, the individual is then expected to assume the following responsibilities:~~

State Shellfish Laboratory Evaluation Officer's Responsibilities

1. Conduct onsite laboratory evaluations at least every three (3) years. However, more frequent evaluations are strongly encouraged and may be ~~required~~necessary with marginally performing laboratories, or when major changes in workloads or priorities have occurred or when there has been a substantial turnover of personnel, or, at the specific request of the Authority. ~~State Shellfish Control Authorities:~~
2. Provide appropriate post-evaluation follow-up for each laboratory evaluated;
3. Prepare timely narrative evaluation reports for all laboratories evaluated. The report should consist of the completed FDA Shellfish Laboratory Evaluation Checklist for the component(s) evaluated and a narrative discussion that accurately and concisely describes the overall operation of the laboratory. All nonconformities noted should be described in this narrative; and, where relevant, an explanation provided relating the potential impact of the deficiency on the analytical results. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should also be included in the narrative report. ~~Incorporating the requirements specified in 4 above;~~
4. Distribute completed evaluation reports with checklists with checklists to FDA and to FDA and to the appropriate FDA Regional Shellfish Specialist.~~:~~
5. Inform ~~the appropriate~~ FDA Shellfish Laboratory Evaluation Officers when a laboratory has been found to be in nonconforming status.~~;~~
6. Coordinate proficiency testing at least yearly for all laboratories in the state supporting the microbiology component of the NSSP.
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Certification Process

Certification is designed to be accomplished through individualized training and field standardization. Individuals are certified for evaluating either the microbiological

and/or post-harvest processing (PHP) and/or marine Biotoxin components of the NSSP depending on their qualifications and the needs of the state shellfish sanitation program and at the discretion of FDA.

Field Standardization

1. Field standardization is designed to evaluate the prospective State Shellfish LEO's ability to determine the competence of the laboratory to meet NSSP laboratory requirements; recognize laboratory practices inconsistent with NSSP requirements when they occur; make appropriate recommendations for corrective action; and, provide the necessary follow-up activity to bring the laboratory into conformity with the NSSP.
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3. Field standardization should be performed in NSSP laboratories within the prospective State Shellfish LEO's home state to provide realistic evaluation scenarios. The narrative evaluation report detailing the evaluation findings must be prepared. The draft narrative report(s) with accompanying checklist(s) must be submitted to the certifying FDA Shellfish Laboratory Evaluation Officer within 60 days of the evaluation(s). All documents submitted will be reviewed for appropriate content, accuracy and uniformity of approach by the certifying FDA Shellfish Laboratory Evaluation Officer.
4. Field standardization is based on a pass fail system.

Certification

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   b. Demonstration of a thorough knowledge of the evaluation methods and documents.
   c. Demonstration of the technical knowledge/familiarity with the analytical procedures being used.
   d. Ability to communicate effectively both orally and in writing.
   e. Successful completion of both training and field standardization.
2. Upon successful completion of the certification process, a letter of certification will be issued by the FDA Shellfish Laboratory Evaluation Officer and a copy will be sent to both the requesting Authority and the FDA Regional Shellfish Specialist.
3. Certification is normally valid for up to five (5) years unless revoked

	or voided. Failure to be Certified 1. If a prospective State Shellfish LEO fails to satisfy any of the performance criteria listed above, he/she will not be certified. 2. As resources permit and at the discretion of FDA, the prospective State Shellfish LEO may receive additional training to better prepare him/her to be certified. 3. The requesting Authority may withdraw the prospective State Shellfish LEO from consideration. Recertification 1. Recertification normally occurs every five (5) years and is contingent upon the continuing need in the state shellfish sanitation program for the services of a State Shellfish LEO. 2. Recertification is based on the State Shellfish LEO satisfactorily meeting the following employment and performance criteria. a. The individual must continue to be employed by the state and be free of any commercial, financial or other pressures or conflicts of interest real or perceived that may cause the State Shellfish LEO to act in other than an impartial and non-discriminatory manner. b. The individual must demonstrate continued competence in the evaluation of NSSP laboratories by performing one to several joint evaluations with an FDA Shellfish Laboratory Evaluation Officer and providing an appropriate narrative evaluation report to the FDA co-evaluator for review and comment for each of the laboratories jointly evaluated. c. The individual must have performed laboratory evaluations at the minimum frequency prescribed in the current edition of the Guide for the Control of Molluscan Shellfish and have all Narrative evaluation reports up to date. 3. State Shellfish LEOs who successfully complete recertification will be issued a letter of recertification by FDA and be cleared to distribute the completed report(s) to the appropriate Regional Shellfish Specialist. A copy of this letter will be sent to the State Shellfish Control Authority and appropriate Regional Shellfish Specialist. 4. If FDA is unable to conduct a recertification visit by the expiration of the individual's certification, his/her certification may be extended until such time as recertification can be completed. If requested, a letter extending the certification can be provided as appropriate. Revocation of Certification 1. State Shellfish LEO's who fail to meet any of the certification/recertification, employment or performance criteria listed above will have their certification revoked. 2. Certification may be voided when state shellfish sanitation programs no longer have a need for the services of a State Shellfish LEO. 3. Voided certifications may be reactivated at the discretion of FDA if the need for the analytical services of additional laboratories by the state shellfish sanitation program recurs.

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