ISSC 2017
Committee Report

Committee Name : Laboratory
Chairperson: Stacey DeGrasse
Date of Meeting: 10/15/17 **Approved By:**_____
Recorder: S. DeGrasse/A. Haines/J. Jones **Printed Name:**

Committee Members Present:

☒Stacey DeGrasse (Chairperson)
☒Richard Burrow
☒Darcie Couture
☐ Joseph Decrescenzo
☒ Matthew Forester
☒ Andrew Haines
☒ Tom Howell
☒ Shelly Lankford
☐ Jill McLeod
☒ Linda McFarland
☒ Leonora Porter
☒ Diane Regan
☐ Wade Rourke
☐ Kathleen Wickman
☒Joel Hansel (EPA Delegate)
☒Lizzie Evans (FDA Delegate)
☒Cheryl Lassiter (NOAA Delegate)
☐ Linda Chandler (FDA Advisor)
☒ Jessica Jones (FDA Advisor)
☒ Johnna Fay (FDA Advisor)
☒ Quay Dortch (NOAA Advisor)
☒ Maggie Broadwater (NOAA Advisor)

Charges

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

Findings/Conclusions: The Laboratory Committee reviews methods, not extraction procedures alone. After extensive communications between the Laboratory Committee and the method submitter, the submitter responded that they do not intend to pursue this proposal further at this time.

Recommendations: The Laboratory Committee recommends that Task Force I take no action on Proposal 05-111.

Charge 2: Proposal 13-109 Expanding the Use of the Abraxis Shipboard ELISA for the Determination of Paralytic Shellfish Poisoning (PSP) Toxins

Findings/Conclusions: Data were not submitted with the proposal. After numerous discussions, the Laboratory Committee concluded that there was no need or interest in expanding the Abraxis Shipboard ELISA for PSP at this time.

Recommendations: The Laboratory Committee recommends that Task Force I take no action on Proposal 13-109.

Charge 3: Proposal 13-110 Immunoassay Methods for Detection of Saxitoxin (PSP) from Shellfish

Findings/Conclusions: The Laboratory Committee received notification from the method submitter that they no longer intended to pursue this proposal due to loss of staff designated to work on the validation.

Recommendations: The Laboratory Committee recommends that Task Force I take no action on Proposal 13-110.

Charge 4: Proposal 13-111 DSP PPIA Kit for Determination of Okadaic Acid Toxins Group (OA, DTX1, DTX2) in Molluscan Shellfish

Findings/Conclusions: While additional information was provided to the Laboratory Committee in support of this proposal, the Laboratory Committee identified performance characteristics that still were not supported by datasets. The Laboratory Committee has drafted a response to submit to the method submitter explaining the outstanding data gaps and concerns.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 13-111 to an appropriate committee as determined by the Conference Chair.

Charge 5: Proposal 13-113 Reveal 2.0 DSP

Findings/Conclusions: The Laboratory Committee has provided feedback on the initial dataset submitted and has provided suggestions on experimental design to obtain additional data. Since that time the proposal has been inactive. Due to this lack of activity the Laboratory Committee would like to remove this proposal from the list of charges. The method submitter is encouraged to resubmit a proposal when the data have been obtained.

Recommendations: The Laboratory Committee recommends that Task Force I take no action on Proposal 13-113.

Charge 6: Proposal 13-114 Receptor Binding Assay (RBA) for Paralytic Shellfish Poisoning (PSP) Toxicity Determination

Findings/Conclusions: Action was taken on this proposal to adopt as an Approved Method for mussels and as an Approved Limited Use Method for scallops and clams. While there was interest in this method for oysters, data were lacking to support any action and the proposal was referred to committee for that matrix. The Laboratory Committee has reviewed an initial dataset, provided feedback to the method submitter, and is awaiting the submitter's additional data.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 13-114 to an appropriate committee as determined by the Conference Chair.

Charge 7: Proposal 15-109 PSP HPLC-PCOX Species Expansion

Findings/Conclusions: Data for some species were provided in the initial proposal; however, the Laboratory Committee awaits the data for other species. Additionally, the Laboratory Committee is awaiting a revised data package based on the feedback provided on the initial dataset.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 15-109 to an appropriate committee as determined by the Conference Chair.

Charge 8: Proposal 15-110 V.p. Enumeration and Detection through MPN & Real-Time PCR

Findings/Conclusions: This proposal was submitted by the ISSC Executive Board because the method was granted interim approval. However, no additional data have

been submitted since the interim approval was given and the original method submitter indicated that they would not be submitting anything further at this time. Data may be submitted at a later time under a new proposal.

Recommendations: The Laboratory Committee recommends that Task Force I take no action on Proposal 15-110.

Charge 9: Proposal 15-112 Direct Plating Method for trh

Findings/Conclusions: The Laboratory Committee has reviewed data from the original submission and provided feedback to the submitter. The Laboratory Committee awaits the submission of the revised dataset.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 15-112 to an appropriate committee as determined by the Conference Chair.

Charge 10: Proposal 15-114 MSC Enumeration in Wastewater by Direct Double-Agar Overlay

Findings/Conclusions: The Laboratory Committee has reviewed data from the original submission and provided feedback to the submitter. The Laboratory Committee awaits the submission of the revised dataset.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 15-114 to an appropriate committee as determined by the Conference Chair.

Charge 11: Proposal 17-102 Update Definition of Replicate

Findings/Conclusions: The definition was updated to be more general. The Laboratory Committee had amendments to further revise the definition.

Recommendations: The Laboratory Committee recommends that Task Force I adopt Proposal 17-102 as amended by Committee.

Charge 12: Proposal 17-103 LC-MS/MS for Monitoring DSP Toxins

Findings/Conclusions: An extensive dataset was provided on a new method for DSP toxins. There is currently no Approved Method for DSP toxins, making this submission a challenge in terms of comparability but also demonstrating a need for such a method. After a few iterations of communication between the Laboratory Committee and the method submitter for additional information and clarity, the method was found to be acceptable for clams, as per the NSSP SLV guidelines. Limited data were provided for mussels and oysters. For those matrices the Laboratory Committee awaits additional data prior to making a recommendation as to adoption for matrices other than clams.

Recommendations: (1) The Laboratory Committee recommends that Task Force I adopt Proposal 17-103 as an Approved Method for clams. (2) The Laboratory Committee recommends that Task Force I refer Proposal 17-103 to an appropriate committee as determined by the Conference Chair for mussels and oysters.

Charge 13: Proposal 17-104 Growing Areas .20 Quantitative Analytical Method Verification

Findings/Conclusions: Guidance for verifying quantitative Approved or Approved Limited Use Methods is needed. The Laboratory Committee made amendments to clarify and to establish consistency in terminology.

Recommendations: The Laboratory Committee recommends that Task Force I adopt Proposal 17-104 as amended by Committee.

Charge 14: Proposal 17-106 RBA PSP Geoduck

Findings/Conclusions: The proposal seeks to expand the RBA to geoduck. While the data summaries look promising, the submission was lacking in detail such as sample preparation information and raw data. The Laboratory Committee has drafted a response to the method submitter describing the outstanding data gaps, and the submitter provided information to the Laboratory Committee at this Conference. The Laboratory Committee will review the newly received information at the earliest opportunity to continue deliberation of this proposal.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 17-106 to an appropriate committee as determined by the Conference Chair.

Charge 15: Proposal 17-107 ELISA NSP

Findings/Conclusions: The proposal represents an extensive dataset in support of an ELISA for NSP for hard clams, sunray venus clams, and oysters. The Laboratory Committee reviewed the data, provided feedback, and reviewed a revised submission. While comparability between the ELISA and mouse bioassay (current Approved Method) was not strong, other indicators of comparability were presented providing confidence in the method. In particular the method is submitted as an Approved Limited Use Method. Based on the data and proposed use, the Laboratory Committee is confident in the method as proposed.

Recommendations: The Laboratory Committee recommends that Task Force I adopt Proposal 17-107 as submitted.

Charge 16: Proposal 17-108 Detection of ASP Biotoxins in Mytilus edulis (Blue Mussel) Shellfish by ELISA for Domoic Acid

Findings/Conclusions: The proposal included validation data for the method. Upon review, the Laboratory Committee has identified outstanding data gaps to be addressed and has drafted a response to inform the method submitter of the additional information needed.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 17-108 to an appropriate committee as determined by the Conference Chair.

Charge 17: Proposal 17-109 Domoic Acid ASP HPLC Checklist

Findings/Conclusions: The Laboratory Committee reviewed the HPLC ASP Checklist and made amendments for clarification and consistency with other checklists.

Recommendations: The Laboratory Committee recommends that Task Force I adopt Proposal 17-109 as amended by Committee.

Charge 18: Proposal 17-110 Vibrio Probe Checklist

Findings/Conclusions: The Laboratory Committee received the Vibrio Probe Checklist. However, given the extensive charges for this Conference and the time needed to amend the checklist for clarification and consistency with other checklists, the Laboratory Committee was unable to complete the review at this time. The Laboratory Committee will resume review and deliberation at the earliest opportunity.

Recommendations: The Laboratory Committee recommends that Task Force I refer Proposal 17-110 to an appropriate committee as determined by the Conference Chair.

Charge 19: Proposal 17-111 Vibrio PCR Checklist

Findings/Conclusions: The Laboratory Committee reviewed the Vibrio PCR Checklist and made amendments for clarification and consistency with other checklists.

Recommendations: The Laboratory Committee recommends that Task Force I adopt Proposal 17-111 as amended by Committee.

Charge 20: Proposal 17-112 State Shellfish LEO Guidance

Findings/Conclusions: The Laboratory Committee reviewed the guidance on State Shellfish LEOs and made amendments for clarity and feasibility of implementation.

Recommendations: The Laboratory Committee recommends that Task Force I adopt Proposal 17-112 as amended by Committee.

Charge 21: Proposal 17-114 Quality Systems Checklist

Findings/Conclusions: The Laboratory Committee reviewed the new quality checklist, discussed the intent which is already in practice, and made amendments for clarity.

Recommendations: The Laboratory Committee recommends that Task Force I adopt Proposal 17-114 as amended by Committee.

(101) Replicate is defined as two (2), or more, laboratory analyses conducted from the same sample ~~filters for thermostable direct hemolysin (tdh) analysis from the same~~ homogenate at the same dilution using the same method.

Section IV Guidance Documents – Chapter II. Growing Areas .20 Quantitative Analytical Method Verification

This guidance is provided to aid laboratories verifingy the performance of an NSSP Approved Method or Approved Limited Use Method quantitative single laboratory validated (SLV) method of analysis being transferred from the originating laboratory/submitter to the implementing laboratory before being placed in service by the implementing laboratory. When a laboratory implements an NSSP method for the first time, the methodThe following performance must be verified in that laboratory. The following performance criteria are to be verified: recovery, measurement uncertainty, precision (repeatability orand intermediate precision), linear range, limit of detection (LOD), limit of quantitation (LOQ), measurement uncertainty and comparability when applicable to a new or modified method used as a substitute/alternative to an established (NSSP) method.

Recovery and Measurement Uncertainty. Recovery is the fraction or percentage of an analyte(s)/measurand(s)/organism(s) of interest recovered after sample analysis. Measurement uncertainty expresses the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability.

Precision is the closeness of agreement between independent test results obtained under the stipulated conditions of repeatability (same laboratory, same analyst) or intermediate precision (same laboratory, different/multiple analysts). There are multiple components of precision: repeatability and intermediate precision. Repeatability is the measure of agreement of replicate tests carried out on the same sample in the same laboratory by the same analyst within short intervals of time. Intermediate precision reflects within-laboratory precision obtained under variable conditions, such as different days, different analysts, and/or different instrumentation.

Linear Range, Limit of Detection, and Limit of Quantitation. Linear range is the range within the working range where the results are proportional to the concentration of the analyte(s)/measurand(s)/organism(s) of interest present in the sample. The Limit of Detection (LOD) is the minimum concentration at which the analyte(s)/ organism(s) can be identified. LOD is matrix and analyte dependent. The Limit of Quantitation (LOQ)

Limit of Detection (LOD) is the minimum concentration at which the analyte(s)/measurand(s)/organism(s) of interest can be identified under the conditions of the test.

Limit of Quantitation (LOQ) is the minimum concentration of analyte(s)/measurand(s)/ organism(s) of interest that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.

Measurement Uncertainty is a single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. It takes into account all recognized effects operating on the result including overall precision of the complete method, the method and laboratory bias and matrix effects.

Comparability is the acceptability of a new or modified method as a substitute/alternative for an established (NSSP) method.

Suggested Test Procedure: Shellfish
Use samples free of the target analyte(s)/ measurand(s)/organism(s) of interest. For each shellfish type of interest use a minimum of 10-12 animalsshellfish per sample and prepare as a homogenate. For each sample take a minimum of six aliquots of the homogenate appropriately sized for the work and spike five

of the six aliquots with five different concentrations of the target analyte(s)~~/measurand(s)~~/organism(s)~~ of interest~~ spanning ~~50-150%~~beyond the desired~~ of the~~ working range~~/range of interest for the method under study~~ and including levels half, at, and twice the action level (or analytical level of interest). Do not spike the sixth aliquot of each sample~~; as~~ this is the sample blank. Process each aliquot including the sample blank to determine the concentration of the target analyte(s)~~/measurand(s)~~/organism(s) of interest. ~~Do three replicates f~~For each aliquot, excluding the sample blank, sub-aliquot for three replicate analysis.~~. Do only one blank per sample.~~ Repeat this process for each shellfish type of interest with a minimum of three samples~~ for each shellfish type of interest~~ collected from different growing areas, the same growing area harvested on different days or from different process lots. Use the same spike levels for each sample analyzed.

Comparability is the acceptability of a new or modified method as a substitute/alternative for an established (NSSP) method. (Should be included if intended as an alternative or a substitute for an established method accepted by the NSSP.)

Suggested Test Procedure: ~~**Comparability Testing of Shellfish for Methods Used as a Substitute/Alternative for an Established (NSSP) Method**~~

For each shellfish type of interest use a minimum of 10-12 shellfish per sample and prepare as a homogenate. For each sample take two aliquots and analyze one by the established (NSSP) method and the other by the ~~substitute/~~alternative method. Naturally ~~contaminated (~~incurred~~)~~ samples having a variety of concentrations spanning the range of the intended application of the method should be used in the comparison. Analyze a minimum of eight paired samples from different growing areas, the same growing area harvested on different days, from different process lots and covering different seasons as necessary. In cases where the occurance of the target analyte(s)~~/measurand(s)~~/organism(s) ~~of interest are~~ is intermittent~~ly present~~, spiked samples may be used as described above.

~~**Suggested Test Procedure: Water (growing water, wastewater, etc.)**~~

~~Use samples free of the target analyte(s)/measurand(s)/organism(s) of interest. For each sample take a minimum of six aliquots of the sample appropriately sized for the work and spike five of the six aliquots with five different concentrations of the target analyte(s)/measurand(s)/organism(s) of interest spanning 50-150% of the working range/range of interest for the method under study. Do not spike the sixth aliquot of each sample as this is the sample blank. Process each aliquot including the sample blank to determine the concentration of the target analyte(s)/measurand(s)/organism(s) of interest. Do three replicates for each aliquot excluding the sample blank. Do only one blank per sample. Repeat this process with a minimum of three samples choosing samples from different growing areas/wastewater plants, etc. Use the same spike level for each sample analyzed.~~

~~**Suggested Test Procedure: Comparability Testing of Water for Methods Used as a Substitute/Alternative for an Established (NSSP) Method**~~

~~For each sample take two aliquots and analyze for the target analyte(s)/measurand(s)/ organism(s) of interest by both the established (NSSP) method and the substitute/alternative method. Naturally contaminated (incurred) samples having a variety of concentrations spanning the range of the intended application of the method should be used in the comparison. Analyze a minimum of eight paired samples from different growing areas/wastewater plants, etc. covering different seasons as necessary. In case the target analyte(s)/measurand(s)/organism(s) of interest are intermittently present, spiked samples may be used as described above.~~

~~**Suggested Data Handling;** For microbiological methods use log transformed data.~~

~~Calculate the percent recovery by comparing the average recovery of the method to the average spike concentration.~~

~~Calculate the precision (repeatability, same laboratory, same analyst or intermediate precision, same laboratory, multiple/different analysts) by determining the coefficient of variation of the test data.~~

~~Calculate the linear range by plotting the test data versus the spike concentration and determining the correlation coefficient.~~

~~Calculate the limit of quantitation (LOQ) by plotting the coefficient of variation for the triplicates of each of five concentrations used per sample versus the spike concentration. There will be fifteen data points to be plotted. Using the equation of the line (y = mx + b) where m is the slope and b is the y-intercept, calculate the LOQ by setting y = 10% (0.1) and solving the equation for x (the LOQ).~~

~~Calculate the limit of detection (LOD) by dividing the limit of quantitation (LOQ) by 3.3 or by using the equation of the line and setting y = 33% (0.33) and solving the equation for x (the LOD).~~

~~Calculate the measurement uncertainty by subtracting the test results from the spike concentration that produced the result and determining the two-sided 95% confidence interval of these differences. This range represents the measurement uncertainty of the test data.~~

~~Calculate the two-sided 95% confidence interval estimate for the regression line (as a whole) relating the established (NSSP) method and the substitute/alternative method.~~

Suggested Method Acceptance: Compare the performance criteria calculated in the method verification study with the values obtained in the original single laboratory validation (SLV) submission by calculating the two-sided 95% confidence interval for the laboratory's mean recovery, estimated LOD and LOQ. If the ranges calculated for the recovery, LOD, LOQ and measurement uncertainty encompass (intersect) the values for the mean recovery, LOD, LOQ and measurement uncertainty obtained from the original SLV and the data is linear over the working range/range of interest with a precision/coefficient of variation which does not exceed that obtained in the original SLV, then it can be concluded that the method (which does not also require comparability testing) has been successfully transferred. For methods that also require comparability testing, the two-sided 95% confidence interval of the regression line relating the established (NSSP) method and the substitute/alternative method should encompass the slope of the regression line relating the two methods in the original SLV. This requirement in addition to the substitute/alternative method meeting the requirements for recovery, LOD, LOQ, measurement uncertainty, precision and linearity are necessary in order to conclude that the method has been successfully transferred.

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SHELLFISH LABORATORY EVALUATION CHECKLIST
Domoic Acid (Amnesic Shellfish Poisoning; ASP) HPLC-UV

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 "1"

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 '1 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, performance and rejection criteria established. e. Laboratory safety. f. Internal 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 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 trained in the operation and maintenance of a basic liquid chromatography system.**
			1.3 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 Laboratory Equipment
K	6		1.4.1 The pH meter has a standard accuracy of 0.1 unit. [Only applicable if using the sample cleanup procedure]
K	5		1.4.2 The pH meter is calibrated daily when in use. Results are recorded and records are maintained. [Only applicable if using the sample cleanup procedure]
K	8		1.4.3 Effect of temperature has been compensated for by an ATC probe, use of a triode or by manual adjustment. [Only applicable if using the sample cleanup procedure]
K	8		1.4.4 The pH meter manufacturer instructions are followed for calibration or a minimum of two standard buffer solutions is used to calibrate the pH meter.

			The first must be near the electrode isopotential point (pH 7). The second must be near the expected sample pH (i.e., pH 2, 4 or 11) as appropriate. Standard buffer solutions are used once and discarded. [Only applicable if using the sample cleanup procedure]
K	5, 11		1.4.5 Electrode acceptability is determined daily or with each use following either slope or millivolt procedure. [Only applicable if using the sample cleanup procedure]
K	6, 2		1.4.6 The balances being used provide an appropriate sensitivity at the weights of use, at least 0.1 g for laboratory precision balances and 0.1 mg for analytical balances.
K	8		1.4.7 The balance calibration is checked monthly using NIST class S, ASTM class 1 or 2 weights or equivalent. Results are recorded and records are maintained.
K	1		1.4.8 Refrigerator temperature is maintained between 0 and 4 ºC.
K	8		1.4.9 Refrigerator temperature is monitored at least once daily. Results are recorded and records maintained.
K	4, 15		1.4.10 Freezer temperature is maintained at -10 ºC or below.
K	8		1.4.11 Freezer temperature is monitored at least once daily. Results are recorded and records maintained.
C	13		**1.4.12 All in-service thermometers are properly calibrated and immersed.**
K	5		1.4.13 All glassware is clean.
K	4		1.4.14 A high performance liquid chromatography system (HPLC) equipped with the following is used: a. mobile phase system delivering a pulse-free flow of 1.0 mL/min, b. solvent degasser, c. autosampler (refrigerated preferred) with loop suitable for 20 µL injections, d. temperature controlled column compartment capable of controlling temperature at 40 ºC, e. ultraviolet detector/diode array detector able to achieve the required sensitivity at a wavelength (A) of 242 nm, and f. a data collection system (e.g., computer, integrator).
K	2		1.4.15 Autopipettors are calibrated for the appropriate volumes used and checked annually for accuracy. Results are recorded and records are maintained.
K	4		1.4.16 A solid phase extraction (SPE) vacuum manifold capable of holding 3 mL cartridges is used. [Only applicable if using the sample cleanup procedure]
O	4		1.4.17 A centrifuge capable of holding 50 mL polypropylene tubes is used.
			1.5 Reagents and Reference Solution Preparation and Storage
C	4, 15		**1.5.1 All solvents and reagents used are analytical or LC grade materials.**
O	8		1.5.2 Water contains < 100 CFU/ml as determined monthly using the heterotrophic plate count method. Results are recorded and records are maintained. (Not required for bottled reagent grade or HPLC grade water when used immediately upon opening. If the bottle of water is not used entirely immediately, the water must be tested as above prior to continued use.)
K	8		1.5.3 Reagents are properly stored and labeled with the date of receipt, date opened or date prepared and expiration date.
C	4, 15		**1.5.4 The mobile phase system used to analyze domoic acid consists of: 10%**

			aqueous acetonitrile (v/v) and 0.1% trifluoroacetic acid (TFA).
O	4		1.5.5 Mobile phase is filtered before use if the HPLC does not have a degasser.
C	7		**1.5.6 Only certified reference materials are used for standard solutions. Source of the reference standard: _____**
K	4, 15		1.5.7 A cartridge wash solution is made up of 1 volume acetonitrile to 9 volumes of water (i.e., 10% aqueous acetonitrile). [Only applicable if using the sample cleanup procedure]
K	4		1.5.8 Citrate buffer (0.5 M, pH 3.2) is made up by dissolving 40.4 g citric acid monohydrate and 14 g triammonium citrate in 400 mL water, then adding 50 mL acetonitrile and diluting the total to 500 mL with water [or equivalent buffer]. [Only applicable if using the sample cleanup procedure]
C	**7**		**1.5.9 NRC CRM Zero-Mus or a negative control is used as a blank to ensure that there is no carry over between samples/standards. Source of the negative control:_____**
C	**7**		**1.5.10 All primary standards are stored appropriately as per supplier recommendations.**
C	**7**		**1.5.11 All standards used are within expiration date.**
C	**2**		**1.5.12 All standards are prepared either gravimetrically or using positive displacement pipettes.**
C	**4, 15**		**1.5.13 Working standards are made up from primary standard by dilution with the toxin-free, extraction solvent (i.e., 50% aqueous methanol). Dilution with toxin-free, cartridge wash solution (aqueous acetonitrile) is allowed if using the diluted crude sample or the sample cleanup procedure.**
C	**7**		**1.5.14 Zero-Mus is stored according to manufacturer's instructions.**
C	**2**		**1.5.15 Quality Control shellfish tissues are stored frozen.**
			1.6 Collection and Transportation of Samples
O	6, 1		1.6.1 Shellstock are collected in clean, waterproof, puncture resistant containers.
K	6, 1		1.6.2 Samples are appropriately labeled with the collector's name, type of shellstock, the harvest area, and time and date of collection.
C	6, 1		**1.6.3 Immediately after collection, shellstock samples are placed in dry storage (ice chest or equivalent) which is maintained between 0 and 10 °C with ice or cold packs for transport to the laboratory.**
K	14, 2		1.6.4 Time from collection to initiation of the extraction should not exceed 24 hours. However, if significant delays are anticipated or if they occur, the laboratory has an appropriate contingency plan in place to handle the samples. For samples shipped live in accordance with 1.6.3, the contingency plan ensures samples remain within allowable temperature tolerances and animals are alive upon receipt. The contingency plan also addresses field and/or laboratory processing that ensures the integrity of the sample or extract until initiation of the assay. For example, samples are washed, shucked, drained and processed as follows: a. refrigerated or frozen until extracted; b. homogenized and frozen until extracted; or c. extracted, the supernatant decanted, and refrigerated or frozen until assayed.

C	2		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 ASP TOXINS			
			2.1 Preparation of Sample
C	6, 1		**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 (e.g., 3 geoduck gut balls).**
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.
C	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.
K	6		2.1.8 Pieces of shell and drainage are discarded.
C	2, 6		**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 Sample Extraction
K	4,6		2.2.1 Sample homogenates are extracted as soon as possible (preferably the same day) or stored in the freezer at -10 ºC or below.
C	4		**2.2.2 Four (4) grams of homogenized sample is weighed into a 50 mL polypropylene centrifuge tube and subsequently extracted.**
C	**4**		**2.2.3 The sample homogenate is extracted with 16 mL extraction solvent (1:1 methanol:water [also referred to as 50% aqueous methanol]).**
K	4, 15		2.2.4 Homogenate/extract mixture is centrifuged and filtered before analysis.
K	4, 15		2.2.5 The filtered extract is injected into the HPLC or loaded into the autosampler immediately.
K	4		2.2.6 When crude samples are ~~injected~~diluted, dilutions ~~of the crude extracts are used. Dilutions of the crude samples~~ are made by diluting 1 mL of filtered sample supernatant into a 5 mL volumetric flask and diluted with water to 5 mL.
K	4, 15		2.2.7 Crude extracts are sealed tightly and stored at -10 ºC or below.
			2.3 Sample Cleanup (Optional)
O	4, 15		2.3.1 Three (3) mL SAX cartridges (500 mg silica derivatized with quaternary ammonium silane) are used for cleanup.
K	4		2.3.2 The SAX cartridge is conditioned with 6 mL methanol, followed by 3 mL water, followed by 3 mL extraction solvent (1:1 methanol:water).
C	**4, 15**		**2.3.3 The cartridge is not allowed to run dry during conditioning through sample loading.**
K	4, 15		2.3.4 Five (5) mL of filtered extract is loaded onto the cartridge and flowed slowly (~1 drop/s) until sample meniscus reaches the top of cartridge packing, discarding effluent.
K	4, 15		2.3.5 Five (5) mL of wash solution (1:9 acetonitrile:water) is loaded to the

			cartridge and washed slowly (~1 drop/s) until meniscus reaches the top of cartridge packing, discarding effluent.
K	4		2.3.6 0.5 mL of citrate buffer (0.5 M, pH 3.2) is loaded to the cartridge and flowed slowly (~1 drop/s) until meniscus reaches the top of cartridge packing, discarding effluent.
K	4, 15		2.3.7 A 2 mL volumetric tube is placed under the cartridge and any domoic acid is eluted into the tube by loading and flowing as much citrate buffer as needed slowly (~ 1 drop/s) until the 2 mL mark is reached on the tube.
C	**4, 15**		**2.3.8 The solution is thoroughly mixed before withdrawing an aliquot for analysis.**
K	4, 15		2.3.9 The cleaned up extract is injected into the HPLC or loaded into the autosampler immediately.
			2.4 Analysis
C	2		2.4.1 **A standard calibration curve (of at least six concentrations) is performed daily. Results are recorded and records are maintained.**
K	4, 15		2.4.2 Twenty (20) µL of extract is injected for analysis.
K	2		2.4.3 Samples are stored in the sample compartment of the autosampler at 4 °C during analysis. Otherwise samples must be analyzed within 9 hours if the autosampler is held at room temperature.
K	4, 15		2.4.4 A column heater is used and the temperature is maintained at 40 °C during the analysis.
C	4		2.4.5 **The appropriate analytical column is used: 25 cm x 4.6 mm id packed with 5 µm Vydac 201TP octadecylsilica or equivalent.**
K	2		2.4.6 The column is stored following the manufacturer's instructions when not in use.
O	2		2.4.7 If a precolumn in-line filter and/or a compatible guard column (e.g., 201GCC54T) are/is used, rejection criteria are established to determine when to change the filter/guard column.
C	**2**		2.4.8 **Dead volume in the system is minimized by the use of short lengths of connecting tubing of small internal diameter ~~(<20 cm of 0.01 in id)~~ between the sample injector and the column and between the column and detector.**
			2.5 System Suitability
C	**2**		**2.5.1 The correlation coefficient for the linear regression of the calibration standards must be ≥~~:'.~~ 0.990 for domoic acid.**
C	3		**2.5.2 The resolution and retention time criteria must ensure complete baseline resolution of L-tryptophan and domoic acid.**
K	2		2.5.3 Peak asymmetry is routinely monitored to evaluate the performance of the column. Results are recorded and records maintained.
C	2		**2.5.4 The column is replaced when a measure of peak asymmetry becomes <0.9 or >1.3.**
C	2,4		**2.5.5 Daily injection schedules must include the adequate frequency of injection standards and extraction blanks based on an assessment of individual standard toxin variability and lack of carry over.**
C	2		**2.5.6 Repeated injections of calibrated standards/samples agree within ±5% (as determined through the use of the coefficient of variation).**
			2.6 Calculation of Sample Toxicity

C	4, 15		2.6.1 **The toxicity of the individual toxins is calculated as follows:** μg/g domoic acid (DA) = DA injected $\times \frac{V}{W} \times (F)$ **where: DA injected = the concentration in µg/ml of the extract injected;** **V = total volume of homogenate and extraction solvent (mL);** **W = weight (g) of tissue homogenate extracted (e.g., 4 g); and** **F = dilution factor (e.g., if SAX cleanup or crude sample dilution are performed).** **The concentration of DA injected may be determined using the nearest standard or the equation of the day's standard curve.**
C	4, 15		2.6.2 **Calculated domoic acid concentrations include the sum of domoic acid and ~~isomer/~~epimer peaks, when the epimer represents 5% or more of the peak area.**
C	12		2.6.3 **Any value at or above 20 ppm (mg/kg or µg/g) domoic acid is actionable.**

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5. Association of Official Analytical Chemists (AOAC). 1991. *Quality Assurance Principles for Analytical Laboratories.* AOAC, Arlington, VA.
6. American Public Health Association. 1970. *Recommended Procedures for the Examination of Sea Water and Shellfish*, 4th Edition. APHA, Washington, D.C.
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12. U.S. Food and Drug Administration (FDA) and Interstate Shellfish Sanitation Conference (ISSC). 2015. *NSSP Guide for the Control of Molluscan Shellfish.* FDA/ISSC, Washington, D.C. and Columbia, S.C.
13. U.S. Department of Commerce. 1976. NBS Monograph 150. U.S. Department of Commerce, Washington, D.C.
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LABORATORY:			**DATE OF EVALUATION:**
SHELLFISH LABORATORY EVALUATION CHECKLIST **SUMMARY OF NONCONFORMITIES**			
Page	**Item**	**Observation**	**Documentation Required**

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	

AMNESIC SHELLFISH POISON (ASP or domoic acid) COMPONENT: PARTS I AND II

A. Results Total # of **Critical (C)** Nonconformities Total # of Key (K) Nonconformities Total # of Critical, Key, and Other (O) Nonconformities	_____ _____ _____

B. Criteria for Determining Laboratory Status of the ASP (domoic acid) Component

1. **Conforms Status: The ASP 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 ASP 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 ASP 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.**
   - 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/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:

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
5001 CAMPUS DRIVE
COLLEGE PARK, MD 20740-3835
TEL. 240- 402-2151/2055/4960 FAX 301-436-2601
CFSANDSSLEOS@FDA.HHS.GOV

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:**

OTHER OFFICIALS PRESENT:	**TITLE:**

Items which do not conform are noted by: Conformity ~~i~~ts noted by a "√"

C- Critical K - Key O - Other NA- Not Applicable

Check the applicable analytical methods:

	MPN Real-time PCR method for *Vibrio vulnificus* detection in Oysters [PART III] SmartCeycler II
	MPN Real-time PCR method for *Vibrio parahaemolyticus* detection in Oysters [PART III] SmartCeycler II and AB 7500 Fast

PART I – Quality Assurance			
		ITEM	
CODE	**REF**		
		1.1 Quality Assurance (QA) Plan	
K	4, 6	1.1.1 Written Plan (Check √ those items which apply).	
			a. Organization of the Laboratory.
			b. Staff training requirements.
			c. Standard operating procedures (SOPs).
			d. Internal quality control measures for equipment, their calibration maintenance, repair, performance and rejection criteria established.
			e. Laboratory safety.
			f. Internal performance assessment.
			g. External performance assessment.
C	**4**		**1.1.2 The QA plan is implemented.**
K	6		1.1.3 The Laboratory participates in a proficiency testing program annually. Specify the program(s): _____
		1.2 Educational/Experience Requirements	
C	**State's Human Resources Department**		**1.2.1 In state/county laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology or equivalent discipline with at least two years of laboratory experience.**
K	State's Human Resources Department		1.2.2 In state/county laboratories, the analysts meet the state/county educational and experience requirements for processing samples in a public health laboratory.
C	**USDA Microbiology & EELAP**		**1.2.3 In commercial laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology or equivalent discipline with at least two years of laboratory experience.**
K	USDA Microbiology & EELAP		1.2.4 In commercial laboratories, the analysts must have at least a high school diploma and at least three months of experience in laboratory sciences.
		1.3 Work Area	
O	4, 6		1.3.1 Adequate for workload and storage.
K	6		1.3.2 Clean, well lighted.
K	6		1.3.3 Adequate temperature control.
O	6		1.3.4 All work surfaces are nonporous, easily cleaned and disinfected.
K	6		1.3.5 Microbiological quality of the air contains fewer than 15 colonies/plate for a 15 minute exposure determined monthly. The results are recorded and records maintained.
		1.4 Laboratory Equipment	
K	5		1.4.1 To determine the pH of prepared media and reagents, the pH meter has a standard accuracy of 0.1 pH units
K	9		1.4.2 pH electrodes consisting of pH half-cell and reference half-cell or equivalent combination electrode free from (Ag/AgCl) or contains an ion exchange barrier preventing passage of Ag ions into the medium which may affect the accuracy of the pH reading. ~~The pH electrodes being used consist of a pH half cell and reference half cell or equivalent combination electrode/triode free from silver/silver~~
K	6		1.4.3 The effect of temperature on the pH is compensated for by an internal/external ATC probe or by manual adjustment (*Circle the appropriate type of adjustment*).
K	4		1.4.4 The pH meter is calibrated daily or with each use as per product literature. Results are recorded and records maintained.
K	6		1.4.5 A minimum of two standard buffer solutions are used to calibrate the pH meter. The first is near the electrode isopotential point (pH 7).

			The second is near the expected sample pH (i.e. pH 4 or pH 10). Standard buffer solutions are used once and discarded.
O	4		1.4.6 Electrode acceptability is determined daily or with each use by the millivolt procedure or through determination of the slope (*Circle the method used*).
K	5		1.4.7 The balances used provide a sensitivity of at least 0.1g at the weights of use.
K	6		1.4.8 Balance calibrations are checked monthly according to manufacturer's specifications using NIST Class S or ASTM Class 1 or 2 weights or equivalent. The accuracy of the balance is verified at the weight range of use. Results are recorded and records maintained.
K	6		1.4.9 Refrigerator temperatures are monitored at least once daily on workdays. Results are recorded and records maintained.
K	1		1.4.10 Refrigerator temperatures are maintained between 0 and 4°C, except for reagent refrigerators which are maintained between 2 and 8°C.
C	**7**		**1.4.11 Freezer temperature is maintained at -15~~20~~°C or below.**
O	7		1.4.12 Freezer temperature is monitored at least once daily on workdays. Results are recorded and records maintained.
C	**5**		**1.4.13 The temperature of the incubator is maintained at 35+/-2.0°C.**
K	6		1.4.14 Thermometers used in the air incubators are graduated at no greater than 0.5°C increments.
K	5		1.4.15 Working thermometers are located on top and bottom shelves of use in the air incubator or appropriately placed based on the results of spatial temperature checks.
K	4, 6		1.4.16 Air incubator temperatures are taken twice daily on workdays. Results are recorded and records maintained.
C	**3**		**1.4.17 All working thermometers are appropriately immersed.**
C	**2, 20**		**1.4.18 Working thermometers are either: calibrated mercury-in-glass thermometers, calibrated non-mercury-in-glass thermometers, or appropriately calibrated electronic devices, including Resistance Temperature Devises (RTDs) and Platinum Resistance Devices (PTDs).**
C	**6, 20**		**1.4.19 A standards thermometer has been calibrated by NIST or a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at the points 0 ~~,~~and 35~~,~~ ~~54 and 55°C (54C for Vp and 55C for Vv).~~ These calibration records are maintained.**
K	3, 5		1.4.20 Standard thermometers are checked annually for accuracy by ice point determination. Results are recorded and maintained. Date of most recent determination:_____
C	**2, 20**		**1.4.21 Either mercury-in-glass thermometers, non-mercury-in-glass thermometers having the accuracy (uncertainty), tolerance and response time of mercury or low drift electronic resistance thermometers with an accuracy of ≤0.05°C are used as the laboratory standards thermometer (*Circle the thermometer type used*).**
K	3, 8		1.4.22 All working thermometers are checked annually against the standards thermometer at temperature(s) of use. Results are recorded and records maintained.
O	6		1.4.23 Appropriate pipet aids are available and used to inoculate samples.
K	2		1.4.24 Micropipettors are calibrated annually at appropriate volumes used ~~annually~~ and checked for accuracy quarterly. Results are recorded and records maintained.
~~K~~	~~5~~		~~1.4.25 Pipets used to inoculate samples and prepare reagents deliver~~

			~~accurate aliquots and are tested for accuracy with each new lot received.~~
		1.5 Labware and Glassware Washing	
K	5		1.5.1 Utensils, containers, glassware and plasticware are clean borosilicate glass, stainless steel or other noncorroding material.
K	5		1.5.2 Culture tubes are new and of a suitable size to accommodate the volume for - nutritive ingredients and sample.
K	5		1.5.3 Dilution bottles and tubes are made of borosilicate glass or plastic and closed with secure caps or screw caps with nontoxic liners.
K	5		1.5.4 Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
K	5		1.5.5 In washing reusable pipets, glassware and labware, a succession of at least three fresh water rinses plus a final rinse of deionized water is used to thoroughly rinse off all detergent.
C	**2**		**1.5.6 An alkaline or acidic detergent is used for washing glassware/labware.**
C	**6**		**1.5.7 With each load of labware/glassware washed, the contact surface of several dry pieces from each load are tested for residual detergent (acid or alkali as appropriate) with aqueous 0.04% bromothymol blue (BTB) solution. Results are recorded and records maintained.**
		1.6 Sterilization and Decontamination	
K	5		1.6.1 The autoclave is of sufficient size to accommodate the workload.
K	4		1.6.2 Routine autoclave maintenance is performed and the records maintained.
C	**6, 20**		**1.6.3 The autoclave provides a sterilizing temperature of 121± 2°C as determined for each load using a calibrated maximum registering thermometer. As an alternative, an appropriate temperature monitoring device is used in place of the maximum registering thermometer when these are unavailable due to the ban on mercury.**
K	6		1.6.4 An autoclave standards thermometer has been calibrated by a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at 121°C. Calibration at 100°C, the steam point is also recommended but not required.
K	10		1.6.5 The autoclave standards thermometer is checked every five years for accuracy at either 121°C or at 100°C, the steam point if the thermometer has been previously calibrated at this temperature. Date of most recent determination: _____
K	1		1.6.6 Working autoclave thermometers are checked against the autoclave standards thermometer at 121°C yearly. Date of last check:_____
K	6		1.6.7 Spore strips/suspensions appropriate for use in an autoclave media cycle are used monthly according to manufacturer's instructions to evaluate the effectiveness of the sterilization process. Results are recorded and the records maintained.
O	6		1.6.8 Heat sensitive tape is used with each autoclave batch.
K	6		1.6.9 Autoclave sterilization records including length of sterilization, total heat exposure time and chamber temperature are maintained. Type of record: Autoclave log, computer printout or chart recorder tracings (*Circle the appropriate type or types*).

K	6		1.6.10 For dry heat sterilized material, the hot-air sterilizing oven provides heating and sterilizing temperatures in the range of 160 to 180°C.
K	5		1.6.11 A thermometer capable of determining temperatures accurately in the range of 160 to 180°C is used to monitor the operation of the hot air sterilizing oven.
K	8		1.6.12 Records of temperature and exposure times are maintained for the operation of the hot-air sterilizing oven.
K	6		1.6.13 Spore strips/suspensions appropriate for use in dry heat are used quarterly to evaluate the effectiveness of the sterilization process in the hot-air oven. Results are recorded and records maintained.
K	5		1.6.14 Reusable pipets are stored and sterilized in aluminum or stainless steel containers.
K	5		1.6.15 Reusable pipets (in canisters) are sterilized in a hot-air oven at 170°C for 2 hours.
C	**2**		**1.6.16 The sterility of reusable pipets is determined with each load sterilized. Results are recorded and records maintained.**
C	**2**		**1.6.17 The sterility of autoclave sterilized disposable pipet tips and microcentrifuge tubes is determined with each load sterilized. Results are recorded and records maintained.** **If presterilized pipet tips and microcentrifuge tubes are purchased certificate should be maintained and sterility confirmed as in 1.6.18.**
C	**2**		**1.6.18 The sterility of presterilized disposable pipets, pipet tips and microcentrifuge tubes is determined with each lot received. Results are recorded and records maintained.**
K	8		1.6.19 Spent broth cultures and agar plates are properly decontaminated before disposal.
		1.7 Media Preparation	
K	13, 14		1.7.1 Alkaline peptone water (APW) is prepared from the individual components and pH adjusted appropriately.
K	6		1.7.2 Media components are properly stored in a cool dry place.
O	6		1.7.3 Media components are labeled with the analyst's initials, date of receipt and date opened.
O	6		1.7.4 Dehydrated media are labeled with date of receipt and date opened.
C	**6**		**1.7.5 Caked or expired media or media components are discarded.**
C	**6**		**1.7.6 Reagent water for media and diluent preparation is analyzed for residual chlorine monthly and is at a non-detectable level (≤0.1 ppm). Results are recorded and records maintained**
K	6		1.7.7 Reagent water for media and diluent preparation contains <100 CFU/mL as determined monthly using the heterotropic plate count method. Results are recorded and records maintained.
K	5		1.7.8 The volume and concentration of media in the tube is suitable for the amount of sample inoculated.
C	**6**		**1.7.9 ~~The total time of exposure of media~~Media broths ~~to~~ are not in the autoclave ~~temperatures does not exceed~~for more than 60 minutes.**
C	**1**		**1.7.10 Media and diluent sterility is determined for each load sterilized. Results are recorded and records maintained.**
C	**1**		**1.7.11 Media productivity is determined using media-appropriate positive and negative control cultures for each lot of dehydrated media received or with each batch of media prepared when the medium is made from its individual components.**
C	**6**		**1.7.12 The pH of the prepared media is determined after sterilization to ensure that it is consistent with manufacturer requirements and/or method tolerance. Results are recorded and records are maintained.**
		1.8 Storage of Prepared Culture Media	

K	5		1.8.1 Prepared culture media are stored in a cool, clean, dry place where excessive evaporation and the danger of contamination is minimized.
K	8		1.8.2 Stored media are labeled with the storage expiration date or sterilization date.
K	5		1.8.3 Storage of prepared culture media at room temperature does not exceed 7 days.
K	2		1.8.4 Storage under refrigeration of prepared broth media with loose fitting closures does not exceed 1 month.
K	6		1.8.5 Storage under refrigeration of prepared culture media with screw- cap closures does not exceed 3 months.
K	11		1.8.6 All prepared broth media stored under refrigeration is warmed to room temperature prior to use, without exceeding incubation temperature.
PART II –Samples			
		2.1 ~~Collection and Transportation of Samples~~Sample Collection, Transportation and Receipt	
C	**2, 6**		**2.1.1 A representative sample is collected and a chain of custody documenting the history of the sample(s) from collection to final disposal has been established.**
K	5		2.1.2 ~~Oyster~~ Shellfish samples as received are collected in clean, waterproof, puncture resistant containers loosely sealed or are rejected for regulatory analysis.
K	5		2.1.3 ~~Oyster~~ Shellfish samples as received are labeled with the collector's (or if PHP, company/processor and collector's) name, the source, the time and date of collection or are rejected for regulatory analysis.
C	**5**		**2.1.4 Immediately after collection, shellfish samples are placed in dry storage (ice chest or equivalent) which is maintained between ~~0~~ 2 and 10°C with ice or cold packs for transport to the laboratory. Once received, the samples are placed under refrigeration unless processed immediately. ~~Add 2.1.5~~**
C	**1**		**2.1.5 Analysis of the samples is initiated as soon as possible after collection, but not to exceed 36h. If processing IQF samples, samples are defrosted under refrigeration for no longer than 36h once removed from the freezer.**
		2.2 Preparation of Samples for Analysis	
K	2, 6		2.2.1 Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes~~, prior to use~~.
O	2		2.2.2 Blades of shucking knives are not corroded.
K	5		2.2.3 The hands of the analyst are thoroughly washed with soap and water or ~~donned in~~ new gloves are donned, immediately prior to cleaning the shells of debris.
O	2		2.2.4 The faucet used for rinsing the shellfish does not contain an aerator.
K	5		2.2.5 Shellfish are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
K	5		2.2.6 Samples are allowed to drain in a clean container or on clean towels prior to opening
K	5, 15		2.2.7 Immediately prior to shucking, the hands or gloved hands of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol. The gloves if worn are latex, nitrile and/or stainless steel mesh to protect analyst's hands from injury.
C	**5**		**2.2.8 Shellfish are not shucked through the hinge.**
C	**5**		**2.2.9 The contents of the sample (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
C	**5**		**2.2.10 A representative sample of at least 12 shellfish is used for analysis**
C	**2, 5**		**2.2.11 A quantity of meat and liquor is sufficient to cover the blender blades or additional oysters are used in order to ensure sample homogeneity.**

K	2, 13		2.2.12 ~~Either a 1:1 dilution can be made at this point, or proceed directly to~~ The sample can be processed directly or a 1:1 dilution of shellfish:diluent made. If a dilution is made, the sample is weighed to the nearest 0.1 gram and an equal amount, by weight, of diluent is added.
K	13		2.2.13 Sterile phosphate buffered saline (pH 7.4) is used as the sample diluent.
C	**5**		**2.2.14 Samples are blended for 60 to 120 seconds until homogenous.**
PART III- PCR method for Vibrio vulnificus and Vibrio parahaemolyticus detection in Oysters			
		3.1 APW Enrichment	
K	5		3.1.1 Sterile phosphate buffered saline (PBS) is used as the sample diluent.
C	**5, 15**		**3.1.2 The 1:10 dilution is prepared gravimetrically with PBS. All successive dilutions are prepared volumetrically.** **For example, if an initial 1:1 dilution of the sample was used for blending, the 1:10 dilution is prepared by adding 20 g of sample homogenate to 80 ml~~L~~ of PBS. If the homogenate was not diluted, the 1:10 dilution is prepared by adding 10g of sample homogenate to 90 ml of PBS.**
C	**17**		**3.1.3 Appropriate sample dilutions are inoculated into APW.** **Specify dilution(s) used _____** **Specify number of tubes per dilution _____**
C	**2, 15**		**3.1.4 For *V. parahaemolyticus* analysis, a tdh+, trh+ *V. parahaemolyticus* culture diluted to <10^3 per ml is used as a positive process control. A non ~~*Vibrio.*~~ *parahaemolyticus* ~~V. Vulnificus~~ culture is used as a negative process control.** **For *V. vulnificus* analysis, a *V. vulnificus* culture diluted to <10^3 per ml is used as a positive process control. A non ~~*Vibrio.*~~ *vulnifius* ~~V. parahaemolyticus~~ culture is used as a negative process control.** **The process control cultures accompany the samples throughout incubation, isolation, and confirmation. Records are maintained.**
C	**13**		**3.1.5 Inoculated APW enrichment tubes are incubated at 35+/-2°C.**
C	**13**		**3.1.6 Tubes are read after 18 – 24 hours of incubation. Clear tubes are negative. Turbid tubes are positive and shall be further processed.**
		3.2 PCR Reagents	
C	**14, 15**		**3.2.1 Lyophilized primers and probes are stored according to manufacturers instructions.**
K	14, 15		3.2.2 Fluorescent probes are stored in light occluding tubes or containers.
C	**14, 15, 18, 19**		**3.2.3 The PCR forward and reverse primers and probes are appropriate for the platform.~~used target.~~** **For Total and Pathogenic Vp Real-time PCR Method** tdh_269-20: 6FAM-5'-TGACATCCTACATGACTGTG-3'-MGBNFQ trh_133-23: NED/—TET-5'-AGAAATACAACAATCAAAACTGA-3'- tlh_1043: JOE/TEXAS RED-5'- CGCTCGCGTTCACGAAACCGT -3'- IAC_109: CY5-5'- TCTCATGCGTCTCCCTGGTGAATGTG -3'- BHQ2 trh_20F: 5'-TTGCTTTCAGTTTGCTATTGGCT-3' trh_292R: 5'-TGTTTACCGTCATATAGGCGCTT-3' tdh_89F:5'-TCCCTTTTCCTGCCCCC-3' tdh_321R: 5'-CGCTGCCATTGTATAGTCTTTATC-3' tlh_884F: 5'-ACTCAACACAAGAAGAGATCGACAA-3' tlh_1091R: 5'-GATGAGCGGTTGATGTCCAAA-3' IAC_46F: 5'-GACATCGATATGGGTGCCG-3' IAC_186R: 5'-CGAGACGATGCAGCCATTC-3'

			For Vv Real-time PCR Method vvhF 5'-TGTTTATGGTGAGAACGGTGACA-3' vvhR 5'-TTCTTTATCTAGGCCCCAAACTTG-3'~~'~~ ~~VvP 5' CCGTTAACCGAACCACCCGCAA 3' add dye, q and iac~~
C	**14, 18**		**3.2.4 Lyophilized forward and reverse primers, and probes, are hydrated with TE buffer to produce a 0.1 mM stock solution.**
C	**14, 18**		**3.2.5 Using molecular grade, nuclease free water, primer and probe stock solutions are diluted to produce a 0.01 mM working solution.**
C	**14, 18**		**3.2.6 ~~Storage of r~~Reconstituted primers and probes are stored in a -20°C manual defrost freezer for up to~~does not exceed~~for the expiration date or 1 year~~5 freeze**
C	**~~16~~2 1,**		**3.2.7 Platinum *Taq* DNA is stored in -20°C manual defrost freezer until first use. After first use, ~~it is~~can be stored between 2-8°C.**
C	**~~16~~2 1, 22**		**3.2.8 PCR reagents (dNTPs, buffer, MgCl2, fluorescent dyes) are stored in -20°C manual defrost freezer until first use. After first use, they ~~are~~ can be stored between 2-8°C.**
		3.3 DNA Extraction	
C	**14, 18**		**3.3.1 All microcentrifuge tubes and pipet tips are sterile.**
C	**14, 18**		**3.3.2 Pipet tips have aerosol barriers.**
K	14, 18		3.3.3 Latex or nitrile gloves are worn throughout the extraction and PCR preparation process.
K	14, 18		3.3.4 All work surfaces, centrifuge racks and equipment used in PCR analysis are disinfected immediately prior to DNA extraction, Master Mix preparation and PCR analysis.
C	**14, 18**		**3.3.5 Aseptic technique is observed throughout the extraction and PCR analysis.**
C	**14, 18**		**3.3.6 One thousand (1000) µL aliquots from each positive APW enrichment tube, including the process controls, are extracted.**
C	**14, 18**		**3.3.7 Positive APW aliquots are placed in sterile microcentrifuge tubes and heated at 95-100~~+/- 5~~°C for 10 minutes.~~-~~**
K	14, 18		3.3.8 A set of positive and negative process controls are included with each batch of samples in a heating block/boiling bath.
C	**14, 18**		**3.3.9 After boiling, tubes are chilled in ice or immediately frozen in a manual defrost freezer for future analysis. Boil preps may be refrigerated not to exceed 72 hours.**
K	14, 18		3.3.10 Frozen extracts are analyzed within 6 months of frozen storage.
		3.4 Preparation of the Master Mix for PCR	
C	**14, 16, 18**		**3.4.1 Nuclease-free microcentrifuge tubes and pipette tips, with filters, are used in Master Mix preparation.**
C	**14, 16, 18**		**3.4.2 For each reaction, add the specified amount of water, buffer, MgCl2, dNTPs, specific primers, nuclease probes, *Taq*, and internal control DNA is added.**
K	14, ~~16~~21, 18		3.4.3 The Master Mix is gently vortexed to mix constituents and then briefly spun, immediately prior to dispensing aliquots to reaction tubes or plates.
C	**14, 16, 18**		**3.4.4 Twenty-three (23) µL of Master Mix is used for each PCR reaction.**
C	**14, 16, 18**		**3.4.5 Master Mix must be used on the day of preparation or stored at –20°C until time of use.**
		3.5 PCR	
C	**14, 19**		**3.5.1 If previously frozen, the DNA extracts are completely thawed at temperatures no warmer than room temperature.~~ at room temperature.~~ Immediately prior to use, DNA extracts are centrifuged at >5,000xg for 2 minutes to remove particulate matter and cell debris.**
C	**14, 19**		**3.5.2 Two (2) µL of DNA template is added to each reaction tube or plate well containing 23µL of Master Mix for a total PCR reaction volume of 25µL.**

K	14, 19		3.5.3 Two (2) µL of molecular grade, nuclease free water is added to a reaction tube or plate well containing 23µL of Master Mix for each batch of Master Mix prepared as a no template control.
C	**14, 19**		**3.5.4 Two (2) µL of DNA template extracted from the negative process control culture is added to a reaction tube or plate well containing 23µL of Master Mix.**
C	**14, 19**		**3.5.5 Two (2) µL of DNA template extracted from the positive process control culture is added to a reaction tube or plate well containing 23µL of Master Mix**.
O	14, 19		3.5.6 Two (2) µL of DNA template extracted from the positive control culture (prepared separately from the positive process control) is added to a reaction tube or plate well containing 23µL of Master Mix as the positive PCR control.
K	14, 19		3.5.7 Immediately prior to loading the reaction tubes or plates into the instrument they are centrifuged for 3-5 seconds to ensure that all reagents and the DNA template are in the bottom of the tube to optimize the PCR amplification process.
C	16		**3.5.8 After centrifugation, tubes or plates are inserted into the instrument.**
		3.6 PCR Amplification	
C	**14, 19**		**3.6.1 The appropriate instrument platform is used for the protocol.**
K	16		3.6.2 Manufacturer's instructions are followed in operating the instrument.
C	**14, 19**		**3.6.3 The PCR cycle parameters used are appropriate for the protocol.**
K	14, 19		3.6.4 Optical calibrations for the dyes being used are current, per the instrument manufacturer's recommendations.
C	**14, 19**		**3.6.5 The analysis settings are adjusted as specified in the protocol.**
		3.7 Computation of Results	
K	14, 19		3.7.1 All runs in which the positive control generates a Ct value for the target(s) of interest and the negative control reaction generates no Ct value for the target(s), but a Ct value for the internal control are considered valid.
C	**2**		**3.7.2 Data is quality checked by the analyst.**
C	**14, 19**		**3.7.32 All reactions in a valid run which generate a Ct value for the target(s) of interest with a sigmoidal amplification curve are considered to be positive.**
C	**16**		**3.7.34 Any sample which does not demonstrate a sigmoidal amplification curve may have a reported positive/negative determination that is discrepant from the instrument if appropriately justified using the raw fluorescent data.**
K	16		3.7.54 All reactions in a valid run which do not generate a Ct value for the target(s) of interest, but do generate a Ct value for the internal control are considered negative.
C	**16**		**3.7.65 Any reaction in which no Ct value is generated for the target(s) of interest or the internal control is considered invalid and should be re-tested.**
C	**13**		**3.7.76 Upon determination of positive reactions, refer to the original positive dilutions of APW and record MPN values as derived from the calculator in Appendix 2 of the FDA Bacteriological Analytical Manual (BAM).**
K	13		3.7.87 For APW enrichment, results are reported as MPN/g of sample.

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LABORATORY:	**DATE of EVALUATION:**

SHELLFISH LABORATORY EVALUATION CHECKLIST

SUMMARY of NONCONFORMITIES

Page	Item	Observation	Documentation Required

Page____of _____

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	
MICROBIOLOGICAL COMPONENT: (Part I-III)	
A. Results Total # of Critical (C) Nonconformities in Parts I-III Total # of Key (K) Nonconformities in Parts I-III Total # of Critical, Key and Other (O) Nonconformities in Parts I-III	

B. Criteria for Determining Laboratory Status of the Microbiological Component:

1. **Does Not Conform Status**: The Microbiological component of this laboratory is not in conformity with NSSP requirements if:

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

   b. The total # of Key nonconformities is **≥ 13 or**_____

   c. The total # of Critical, Key and Other is **≥ 18** _____

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

C. Laboratory Status (*circle appropriate*)

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

Acknowledgment by Laboratory Director/Supervisor:

All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before_____.

Laboratory Signature: _____ Date:_____

LABORATORY:		
Page	Item	Observation

Amended Language Proposal No. **17-112**

Section IV Guidance Documents – Chapter II Growing Areas .15 Evaluation of Laboratories by State Shellfish Laboratory Evaluation Officers Including Laboratory Evaluation Checklists amend language.

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 the Authority ~~he/she should~~ must 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 the Authority ~~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~~ must ~~be~~:
    a. ~~A~~ Be a state employee;
    b. Have a minimum of two years of shellfish laboratory experience or a laboratory background~~;~~ with a minimum of three years bench level experience with the method~~s~~ types that will be evaluated ~~e.g. mouse bio-assays, fermentation tube MPNs, HPLC, ELISAs, Functional Assays;~~.
    c. ~~Preferably h~~ Have ~~laboratory evaluation~~ experience performing laboratory evaluations or supervising a laboratory; 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 FDA National Laboratory Standard

1. The FDA National Laboratory Standard/s will be responsible for standardizing all LEOs.
2. The FDA National Laboratory Standard will conduct certifications/recertifications. The Standardization evaluation process will consist of a minimum of ~~two (2)~~ one (1) practice evaluation~~s~~ in areas under consideration for certification and one (1) formal standardization evaluation. The evaluation will be checklist specific and the State Shellfish LEO will be standardized to evaluate the methods only for which they have been certified.
3. FDA Standard Operating Procedure for Laboratory Evaluations will be provided to every LEO candidate for the purpose of evaluation standardization.

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.
3. The Authority will provide, or ensure adequate opportunity for, State Shellfish LEOs to maintain communication with FDA LEOs, as needed, to provide guidance and updates relevant to the NSSP laboratory evaluation program and any changes to their State programs.

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 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.

State Shellfish Laboratory Evaluation Officer's Responsibilities

1. Conduct on-site laboratory evaluations at least every three (3) years. However, more frequent evaluations are strongly encouraged and may be 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.
2. Provide appropriate post-evaluation follow-up for each laboratory evaluated, (i.e., monitoring corrective actions and resolutions of all nonconformities).
3. Prepare ~~timely~~ narrative evaluation reports within 30 days 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 to ~~on~~ the analytical results. Completed corrective actions should be included in the narrative report only if they were corrected~~completed~~ during the evaluation~~on-site~~. Recommendations for corrective action or, if applicable, suggestions to enhance laboratory operations should also be included in the narrative report.
4. Distribute completed evaluation reports with checklists to FDA LEOs and to the appropriate FDA ~~Regional~~ Shellfish Specialist.
5. Inform FDA Shellfish ~~Laboratory Evaluation Officers~~LEOs when a laboratory has been found to be in nonconforming status the same day ~~immediately upon~~ as the evaluation is completed~~closeout~~. A letter informing FDA National Laboratory Standard of upgraded status by way of a separate Completed Corrective Action Memo will be sent, should one be necessary.

6. Coordinate proficiency testing at least yearly for all laboratories in the State supporting the microbiology component of the NSSP.
7. Prepare annually (in December) a summary list of all laboratories, ~~and~~ qualified analysts, and methods performed in each NSSP laboratory and transmit it to the FDA Shellfish LEOs.

8.

Certification Process

Certification of qualified individuals 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)~~ vibrio detection and/or marine ~~B~~biotoxin components of the NSSP depending on their qualifications and the needs of the state shellfish sanitation program. ~~and at the discretion of FDA.~~ Certification is dependent upon the ~~per~~prospective 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 course and field standardization.

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~~ a minimum of ~~two~~ one practice and one final onsite evaluations ~~with an~~ the FDA National Laboratory Standard. ~~Shellfish Laboratory Evaluation Officer and preparation of the corresponding narrative evaluation reports.~~ For the final standardization assessment, the onsite evaluation, all "Critical" nonconformities cited, or lack thereof, must be in agreement between the FDA National Laboratory Standard and the State LEO candidate. Additionally, for "Key" and "Other" nonconformities, the evaluation checklists completed by the prospective State Shellfish LEO candidate and the FDA National Laboratory Standard should be in 90% agreement.

~~2.~~3. During all joint field evaluations the State Shellfish LEO Candidate will be the lead evaluator. He or she will be responsible for requesting documents, assessing records, and conducting the evaluation. FDA Standard Operating Procedure for inspection will be followed regarding assessment requests. The Candidate shall also conduct the "exit" interview and discuss all significant findings with management.

~~3.~~4. The narrative evaluation report must be prepared by the State Shellfish LEO candidate for each joint but independent evaluation conducted. 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~~ to the analytical results. Recommendations for corrective action, or if applicable, suggestions to enhance laboratory operations should be included in this narrative report(s).

~~4.~~5. Final ~~F~~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 30 ~~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~~ National Laboratory Standard.

~~5.~~6. Field standardization is ~~based on a~~ pass/fail ~~system~~.

~~6.~~7. After successfully completing the Field Standardization Exercise, the State Shellfish LEO Candidate will be granted the title of Laboratory Evaluation Officer. A certificate recognizing that accomplishment will be

forwarded to the State Shellfish LEO Candidate, along with formal notification to the State Shellfish LEO Candidate's supervisor, within thirty (30) days.

~~Certification~~

~~1. 1. Certification is dependent upon the perspective State Shellfish LEO satisfying~~
~~2. 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.~~

~~3. 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.~~

~~4.~~1. ~~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; including attending the Shellfish Program Laboratory Methods and Evaluation Procedures Course. If the LEO candidate is unsuccessful in his/ her final standardization attempt he/ she must repeat ~~the~~ two (2) practice evaluations before attempting the ~~and one (1)~~ final standardization evaluation again. If failure continues after the second attempt, the candidate will not be eligible for a third attempt at standardization without the expressed permission of the National Laboratory Standard.
3. The requesting Authority may withdraw the prospective State Shellfish LEO from consideration.

Recertification

1. Recertification normally occurs every ~~five (5)~~ six (6) 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 National Laboratory Standard. ~~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.

Standardization Maintenance

1. Maintenance will be provided in the form of updated Laboratory Evaluation Officer courses, updated field standardization guides, and other guidance/technical assistance activities on an as needed basis.
2. State Shellfish LEOs will be required to attend the Shellfish Program Laboratory Methods and Evaluation Procedures Course every three years ~~or~~if ~~when~~ it is offered by FDA

Revocation of Certification

1. State Shellfish LEOs 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.
5. The National Laboratory Standard will document the reason(s) for revocation of the LEO certification. This information shall be forwarded to the Candidate's supervisor and a copy shall be placed in the FDA file. All evidence and conclusions reached by the FDA shall be documented in writing by the Standard and shall be retained for three (3) years in accordance with the Freedom of Information Act.

PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
5001 CAMPUS DRIVE
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TEL. 240- 402-2151/2055/4960 FAX 301-436-2601
CFSANDSSLEOS@FDA.HHS.GOV

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:**

OTHER OFFICIALS PRESENT:	**TITLE:**

Conformity is noted by a (Y), no (N), or not applicable (N/A) for each checklist item. Please note that for all N/A indications, you must document the reason why this requirement is N/A on a separate record. Record comments related to any requirement on the space provided in the summary of nonconformities. All nonconformities must be identified and explained. Quality System must be in place for onsite laboratory evaluation to be scheduled.

Parts of the Quality Checklist	
Part I	Quality Management: Laboratory Operations and Responsibilities for Quality Systems
Part II	Quality Assurance: The Process of Documenting and Maintaining a Quality System
Part III	Quality Control: Documentation for Quality System Defensibility

PART I – Quality Management: Laboratory Operations and Responsibilities for National Shellfish Sanitation Program Laboratory Quality Systems		
		ITEM
Conformance Comments	**Ref**	
		1.1 Components of the Laboratory Quality System
	1,3,6,9	1.1.1 The laboratory has an overall Quality System supported by quality management structure, quality assurance processes and quality control functions.
	1,3,6,9	1.1.2 Management and technical structure exist to support the Quality System.
	1,3,6,9	1.1.3 Quality documentation is required by the laboratory. These include a Quality Assurance (QA) Manual (or otherwise named) and Standard Operating Procedures (SOPs) to support the quality assurance process of the laboratory.
	1, 9	1.1.4 The documents used to implement the quality assurance process and records used to verify quality control (QC) function of the laboratory are reviewed and controlled.
	9	1.1.5 An established process of Quality System assessment and technical proficiency are documented with results retained until the next review.
	9	1.1.6 Resolution, management review and prevention of nonconformities are a documented component of the Quality System.
		1.2 Laboratory Management Structure and Quality Systems
	1,3,6,9	1.2.1 The laboratory's structure is clearly organized with supervisory chain delineated.
	9	1.2.2 The laboratory has ensured that its management and personnel are free from any undue internal and external commercial, financial and other pressures and influences that may adversely affect the quality of their work.
	9	1.2.3 The laboratory has documentation of dedicated personnel with the authority and resources required to carry out their duties, including implementing and maintaining the Quality System of the laboratory.
	1, 9	1.2.4 The laboratory's designated quality personnel ensure adherence to the quality system, including SOPs and QC. These staff have clear documented authority to initiate actions to prevent or minimize departures from quality system and monitor the corrective action process.
	9	1.2.5 The laboratory has documentation of a designated quality system manager, responsible for monitoring all aspects of the quality system to assure defensibility. This person shall have unrestricted access to FDA Shellfish Laboratory Evaluation Officers (LEOs) and the highest levels of the laboratories management. In the case of a single person laboratory, FDA LEOs will assist with developing a monitoring plan.
	1, 9	1.2.6 A documented system is in place to ensure that appropriate review of and communication regarding the elements of the quality system are established among the laboratory staff and laboratory management.
		1.3 Laboratory Personnel and Roles in a Quality System
	1,3, 9	1.3.1 The roles and responsibilities of all personnel are defined in the QA

			manual, read by all staff and the acknowledgments of these responsibilities are retained.
	9	1.3.2	The laboratory policy and the training procedures for personnel are documented and relevant to the scope of the current activities in the laboratory. If the laboratory intends to add methods to their scope, training SOPs must also be added with successful completion by the analyst(s) that will perform the method(s). In the case of a single person laboratory, method proficiency verification must be retained during the life of the methods use in the laboratory.
	9	1.3.3	The laboratory shall maintain a personnel file/ record of any relevant authorization(s), qualifications, trainings, and/or proficiencies for each analyst. This information shall be available upon request as verification of staff training and shall be retained for all staff until two years after they are no longer employed by the laboratory.
	1, 3, 9	1.3.4	The laboratory has documented that all personnel involved in testing have read and understand the applicable SOPs and associated quality documentation and implement the policies and procedures required for the performance of their technical function.
PART II – Quality Assurance: The Process of Documenting and Maintaining a Quality System			
		2.1 Quality Assurance Process: QA Manual, SOPs and Document Control	
	1, 9	2.1.1	The QA manual shall include or make reference to all laboratory SOPs and any supporting procedures, including technical procedures.
	1, 9	2.1.2	SOPs are controlled documents and include detailed, written instructions to achieve uniformity of test methods and quality control procedures, such that items that might affect the quality or defensibility of the outcome are mitigated.
	1, 9	2.1.3	SOPs and the QA Manual are controlled documents, such that specific individuals are designated within the laboratory with editorial control. These individuals are identified in the QA Manual.
	1, 9	2.1.4	Each time an SOP or the QA manual has changed, the new version will be marked as such and will be distributed to the laboratory with older versions removed from circulation.
	1, 9	2.1.5	Staff training requirements are documented in the QA manual and the training procedure is included.
		2.2 Quality Manual Items	
	1, 9	2.2.1	Quality Assurance Manual contains: □ Table of Contents; □ Organizational chart; □ A description of the Quality System and procedure for implementation and maintenance; □ Policy and procedure for resource management (human resources, competence and training, work environment and safety), description of responsibilities; □ Policy and procedures for rejection criteria; □ Policy and procedures for calibration of equipment and Equipment file items such as maintenance; □ Policy and procedure for traceability and required documentation, □ Policy and procedure for internal audits; □ Policy and Procedure for data analysis and control of nonconforming work; and □ Policy for corrective actions (CAs) and preventative actions (PAs).
	1,3,6,9	2.2.2	The organizational chart clearly depicts laboratory structure with quality and technical personnel listed.

	1, 9	2.2.3	The policy for human resources provisions includes hiring and assignment of staff, competence and responsibilities for positions, and a procedure of training for each technical competence, including proficiencies required.
	1, 3, 4, 6, 9	2.2.4	Policies for work environment and safety protocols, analytical methods, and quality control performed for the National Shellfish Sanitation Program (NSSP) are included or referenced in the QA Manual and shall be provided upon request.
	1, 9	2.2.5	A policy regarding appropriate equipment file maintenance and retention (e.g., calibration records, maintenance documentation, manuals of operation) is included in the QA Manual.
	1, 9	2.2.6	The SOP for calibration and maintenance of equipment is kept or referenced in the QA Manual and shall be provided upon request.
	1, 9	2.2.7	The SOP for traceability of analytical results is included or referenced in the QA Manual and shall be provided upon request. This traceability procedure includes a documented procedure for the unique identification of samples and the process for chain of custody verification.
	1, 9	2.2.8	The QA Manual has a policy and a procedure for internal quality audits. These audits are planned and scheduled annually or as needed. The policy states auditors do not audit their own work. In the case of a single person laboratory, FDA LEOs will assist with an audit plan.
	1, 9	2.2.9	The QA Manual contains a policy for data analysis to require that all analyses performed have been carried out correctly, documented, controls were used accurately and the results meet specified requirements.
	1, 9	2.2.10	The QA Manual contains a procedure for the control of nonconforming work in the case of : □ identification, documentation, evaluation, segregation (where practical), disposition of nonconforming sample/analyte/result and customer notification; □ assigning responsibility for the review and the authority for disposition of nonconforming sample/analyte/result; □ a nonconforming result correction and the re-verification/calibration of the affected equipment after the correction to demonstrate conformity (if necessary); and □ handling a nonconforming result when it is detected, after delivery to the customer.
	1, 9	2.2.11	The QA manual contains a procedure for preventative actions in which laboratory staff identify potential nonconformities in audit results, quality records, or customer complaints through a review process. Steps are then determined to identify preventive actions to implement. The necessary changes are made to SOPs and this exercise is recorded, and records maintained.
	1, 3, 6, 9	2.2.12	The QA manual has a policy and a procedure for developing corrective action(s) to eliminate the cause of identified nonconformities in order to prevent recurrence. Corrective actions describe the nonconformities, define the process for evaluating the need for actions to ensure that nonconformities do not recur (root cause analysis), explain the process to implement the corrective action(s) needed, and the resultant outcome. There is also a procedure to monitor progress of any ongoing corrective actions and the resolution.
	1, 3, 4,	2.2.13	The QA Manual contains a policy stating laboratory management

	6, 9	shall ensure and document the competence of staff independently operating equipment resulting in a documented measurement, analysis result, quality control value/result, determination of data value for sample result, and review/closure of corrective action for efficacy.
	1, 9	~~2.2.5~~2.2.14 The policy for sample rejection criteria includes what the laboratory will accept and reject based on NSSP requirements and chain of custody.
	1, 3, 4, 6, 9	~~2.2.6~~2.2.15 The laboratory shall have sample acceptance procedures that include safe handling, transport, and storage to prevent contamination or deterioration and to protect the sample integrity. These procedures are provided to customers.
	1, 3, 4, 6, 9	~~2.2.7~~2.2.16 The laboratory has procedures for handling nonconforming samples and who will be contacted in the case of sample rejection.
	~~1, 9~~	~~2.2.8 A policy regarding appropriate equipment file maintenance and retention (e.g., calibration records, maintenance documentation, manuals of operation) is included in the QA Manual.~~
	~~1, 9~~	~~2.2.9 The SOP for calibration and maintenance of equipment is kept or referenced in the QA Manual and shall be provided upon request.~~
	~~1, 9~~	~~2.2.10 The SOP for traceability of analytical results is included or referenced in the QA Manual and shall be provided upon request. This traceability procedure includes a documented procedure for the unique identification of samples and the process for chain of custody verification.~~
	~~1, 9~~	~~2.2.11 The QA Manual has a policy and a procedure for internal quality audits. These audits are planned and scheduled annually or as needed. The policy states auditors do not audit their own work. In the case of a single person laboratory, FDA LEOs will assist with an audit plan.~~
	~~1, 9~~	~~2.2.12 The QA Manual contains a policy for data analysis to require that all analyses performed have been carried out correctly, documented, controls were used accurately and the results meet specified requirements.~~
	~~1, 9~~	~~2.2.13 The QA Manual contains a procedure for the control of nonconforming work in the case of :~~ ~~identification, documentation, evaluation, segregation (where practical), disposition of nonconforming sample/analyte/result and customer notification;~~ ~~assigning responsibility for the review and the authority for disposition of nonconforming sample/analyte/result;~~ ~~a nonconforming result correction and the re-verification/calibration of the affected equipment after the correction to demonstrate conformity (if necessary); and~~ ~~handling a nonconforming result when it is detected, after delivery to the customer.~~
	~~1, 9~~	~~2.2.14 The QA manual contains a procedure for preventative actions in which laboratory staff identify potential nonconformities in audit results, quality records, or customer complaints through a review process. Steps are then determined to identify preventive actions to implement. The necessary changes are made to SOPs and this exercise is recorded, and records maintained.~~
	~~1, 3, 6, 9~~	~~2.2.15 The QA manual has a policy and a procedure for developing corrective action(s) to eliminate the cause of identified nonconformities in order to prevent recurrence. Corrective actions describe the nonconformities, define the process for evaluating the~~

			~~need for actions to ensure that nonconformities do not recur (root cause analysis), explain the process to implement the corrective action(s) needed, and the resultant outcome. There is also a procedure to monitor progress of any ongoing corrective actions and the resolution.~~
	~~1, 3, 4, 6, 9~~	~~2.2.16~~	~~The QA Manual contains a policy stating laboratory management shall ensure and document the competence of staff independently operating equipment resulting in a documented measurement, analysis result, quality control value/result, determination of data value for sample result, and review/closure of corrective action for efficacy.~~
PART III- Quality Control: Documentation for Quality System Defensibility			
		3.1 Documentation	
	1, 9	3.1.1	The laboratory investigates proficiency testing (PT) programs for areas of continual improvement and actively addresses problematic results through the prescribed corrective action process.
	1, 9, 10	3.1.2	The laboratory personnel performing sampling and testing participate in PT programs and exercises when available. If no PT exists, participation in interlaboratory comparisons is considered.
	1, 3, 6, 9, 10	3.1.3	Corrections to quality control records, bench sheets and reports follow the requirements below: □ A single line is drawn through the incorrect information; □ The correct information is written next to the incorrect information; □ The person responsible for the correction initialed the information; □ If not obvious, the reason for correction has been included; and □ If corrections are necessary in an electronic document, old information must be retained in some form, the person making the change must be identified, the date of the change noted, and the reason for the change noted.
	1, 3, 6, 9, 10	3.1.4	All records, required to be retained for two years (or length of time as dictated by State law), shall be legible and shall be stored in such a way that they are readily retrievable to prevent damage or loss.
	1	3.1.5	All records and documents must be written in indelible ink.
		3.2 Method Performance Validation	
	1, 3, 6, 9	3.2.1	The laboratory will internally validate new methods to confirm with objective evidence that the intended protocols are demonstrated and outcomes are fulfilled.
	1, 9	3.2.2	Methodologies do not deviate from the validated method and the laboratory's internal validation shall remain on file in the laboratory.
	1, 3, 6, 9, 10	3.2.3	The laboratory shall report the method chosen in writing to the customer.
	1, 4, 9	3.2.4	Methodologies and protocols are selected based on NSSP requirements and samples are processed as per the citation in the current Model Ordinance.
	~~1, 4, 9~~	~~3.2.5~~	~~Methodologies and protocols are selected based on NSSP requirements, and samples are processed as per the citation in the current Guide for the Control of Molluscan Shellfish.~~
		3.3 Environmental Conditions	
	1, 3, 4, 5, 6, 9, 10	3.3.1	Laboratory facilities for analysis, including lighting and environmental conditions such as temperature and humidity, shall support accurate performance of the tests.
	1, 3, 4, 5, 6, 9, 10	3.3.2	The laboratory shall monitor, control, and record environmental conditions as required by the relevant specifications, methods and procedures, or where they influence the outcome of results (e.g., biological sterility, dust, humidity, electrical supply, temperature,

			vibration).
	1, 3, 4, 6, 9, 10	3.3.3	Laboratory personnel shall stop testing when the environmental conditions jeopardize the results of analyses.
	1, 3, 4, 6, 9, 10	3.3.4	Personnel shall ensure good housekeeping in the laboratory.
		3.4 Equipment	
	1, 3, 4, 6, 9, 10	3.4.1	The laboratory shall have instructions and/ or SOPs on the use and operation of all relevant equipment, and on the handling and preparation of items for testing, where the absence of such could jeopardize the outcome of analysis or influence results.
	1, 9, 10	3.4.2	All equipment in the laboratory is labelled with the manufacturer's name, identification number, and serial number or other unique identification that is traceable.
	1, 9, 10	3.4.3	Equipment files contain reports and certificates of all calibrations, the due date of next calibration, dates and results of any maintenance, adjustments, damage, malfunction, and modification or repair to the equipment.
	1, 9, 10	3.4.4	If equipment (e.g., thermometer, balance) was sent out of the laboratory for service, performance has been verified prior to use again in the laboratory.
		3.5 Temperature Measuring Devices	
	1, 8, 9, 10	3.5.1	~~Serial number~~Unique identifier, ice point date (if applicable) and any correction factor is recorded on in use temperature measuring device (TMD).
	1, 8, 9, 10	3.5.2	TMDs are calibrated as per the NSSP requirements and ice points/steam points are performed annually on Standards thermometers.
	1, 8,	3.5.3	TMDs calibration certificates are retained for three consecutive calibration cycles.
	1, 8, 9, 10	3.5.4	Where calibrations give rise to a set of correction factors, the laboratory shall have procedures to ensure these records are retained until the next check is performed.
	1, 8, 9, 10	3.5.5	Range and graduations of all TMDs are appropriate for the designated use. Dial thermometers are not used in the laboratory.
	8, 9, 10	3.5.6	For electronic TMDs, probe/sensor is uniquely labeled and placement within unit being monitored follows manufacturer's instructions to ensure accurate readings, as devices vary.
	1, 8, 9, 10	~~3.5.6~~3.5.7	Temperature Monitoring Systems (wired/wireless) must record temperature reading from each sensor/probe in the piece of equipment being monitored at the same or greater frequency and accuracy as stipulated for mercury in glass thermometers, as per manufacturer specifications.
		3.6 Disposables and Pipettors	
	1, 3, 4, 6, 9, 10	3.6.1	Pipettors, accuracy checked, fixed volume or electronic are calibrated according to NSSP requirements.
	1, 3, 10	3.6.2	Pipettors are etched with identification (imprinted serial numbers acceptable) and tagged with last date of accuracy check.
	1, 2, 3, 4, 6, 9, 10	3.6.3	Appropriate pipettor tips are used and sterility checks are performed on an appropriate quantity.
	1, 2, 3, 4, 6, 9, 10	3.6.4	Sterility checks on disposables are performed according to a cited QC practice, within a designated SOP. (e.g., laboratory may cite and implement a recognized standard of sterility testing, they may test 10% of a "lot" or any 3 in a box.)
		3.7 Test Record/Bench Sheet Requirements	

	1, 3, 4, 6, 9, 10	3.7.1	Test records/bench sheets shall contain information to facilitate repeatability under conditions as close as possible to the original including QC information (or reference) for media and supplies used.
	1, 9, 10	3.7.2	Test records/bench sheets must show date, time and temperature of samples at the start of analysis and contain the name or initials of the analyst performing the test for each group of samples.
	1, 4, 9, 10	3.7.3	Test records/bench sheets must include sterility controls or a reference to the document containing sterility controls for disposables and dilution buffer.
	1, 4, 9, 10	3.7.4	Test records/bench sheets must include media productivity (positive and negative) controls or a reference to the document containing media productivity controls.

REFERENCES

1. ~~Good Laboratory Practice.~~Title 21, Code of Federal Regulations, Part 58, *Good Laboratory Practice for Nonclinical Laboratory Study*. U.S. Government Printing, Washington, D.C. *Technical Programs – Criteria for Laboratories Performing Food Testing*. AOAC, Arlington, Va.
2. U.S. Department of Commerce. 1976. *NBS Monograph 150*. U.S. Department of Commerce, Washington, D.C.
3. Association of Official Analytical Chemists (AOAC). 1991. Quality Assurance Principles for Analytical Laboratories. AOAC, Arlington, VA.
4. Interstate Shellfish Sanitation Conference (ISSC). 2017. ISSC, Columbia, SC.
5. The NELAC Institute (TNI). 2003 National Environmental Laboratory Accreditation Conference (NELAC) STANDARD QUALITY SYSTEMS. July 2005. Weatherford, TX.

~~5. Title 21, Code of Federal Regulations, Part 58, *Good Laboratory Practice for Nonclinical Laboratory Study*. U.S. Government Printing, Washington, D.C. *Technical Programs – Criteria for Laboratories Performing Food Testing*. AOAC, Arlington, Va.~~

6. U.S. Environmental Protection Agency (EPA). 1975. *Handbook for Evaluating Water Bacteriological Laboratories*. EPA – 670/9-75-006. U.S. EPA, Cincinnati, Ohio.
7. U S. Food and Drug Administration (FDA). 1995. *Bacteriological Analytical Manual*. U.S. FDA, 8th Edition, AOAC, Arlington, VA.
8. National Institute of Standards and Technology Special Publication 250-23, 128 pages (Sept. 1988)U.S. Government Printing office, Washington, D.C. Library of Congress Catalog Number: 88-6000580.
9. The International Organization for Standardization and the International Electrotechnical Commission. Online: https://www.iso.org/obp/ui/#iso:std:iso-iec:17025:ed-2:v1:en accessed June 6, 2017.
10. National Conference on Interstate Milk Shipments. Cultural Procedures, 2400 Form. Online: http://ncims.org/programs/ accessed June 6, 2017.

LABORATORY:	**DATE of EVALUATION:**

SHELLFISH LABORATORY EVALUATION CHECKLIST

SUMMARY of NONCONFORMITIES

Page	Item	Observation	Documentation Required

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE/POINT OF CONTACT:	

NSSP Quality System Evaluation: (Part I-III)

A. Criteria for Determining Laboratory Status of the Quality System Component:

1. **Laboratory must satisfy all sections of the Quality System prior to onsite evaluation**:

a. The total # of nonconformities in Part I _____

b. The total # of nonconformities in Part II _____

c. The total # of nonconformities in Part III _____

B. Laboratory Status (*circle appropriate*)

Does Not Conform **Conforms**

Acknowledgment by Laboratory Director/Supervisor:

All Corrective Actions will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____ so onsite evaluation can be scheduled.

Laboratory Signature: _____ Date:_____

LEO Signature: _____ Date:_____

LABORATORY:

Page	Item	Observation