Overview of the ISSC Laboratory Method Review Process

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ISSC Laboratory Committee Chair

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Introduction to the ISSC Laboratory Committee

- Current Composition
  - 28 Committee Members
    - 17 Voting Members
    - 11 Advisors
  - 10 State Representatives (2 SE, 2 Mid-Atlantic, 3 NE, 2 Gulf, 1 Pacific)
  - 2 International Representatives (Canada, New Zealand)
  - 2 “Industry” Representatives
  - 14 Federal Representatives (1 EPA, 4 NOAA, 9 FDA)

Introduction to the ISSC Laboratory Committee

- Subcommittees
  - Biotoxins
  - Microbiology
  - Checklists
  - Method Validation
  - Method Portal
  - Engagement

Introduction to the ISSC Laboratory Committee

- Meeting Schedule
  - Subcommittees
    - Monthly Standing Meetings
    - Typically 1 to 1.5 Hours
  - Full Committee
    - Monthly Standing Meetings (3rd Tuesday of the Month)
    - 2 Hours

Laboratory Committee Method Review Process

Preproposals

- ISSC Constitution, Bylaws, and Procedures (link)
  - Procedure XV – Procedure for the Approval of Analytical Methods for the NSSP

- "Prior to NSSP adoption, all laboratory methods shall be evaluated by the ISSC. Persons interested in submitting a method for inclusion in the NSSP must submit a pre-proposal outlining the following:
  - Description of Method
  - Proposed Use of Method
  - Time Table for SLV

- **Timeline = 1 to 3 months after receipt by LC**

Proposal Liaison

- “Submitters of pre-proposals receiving approval will be requested to submit a full proposal to the ISSC and a liaison from the Laboratory Committee will be assigned”

Full Proposal / Single Lab Validation

- Proposal submission should include:
  - Summary of the Need for the Method
  - Purpose and Intended Use of the Method
  - Method Documentation (i.e. equipment, sample collection requirements, test procedures, QA/QC)
  - Study Data – Tests To Evaluate Performance Characteristics of the Method
  - Data Evaluation and Discussion
  - Laboratory Evaluation Checklist

- A submission protocol and a summary of required elements are available on the ISSC website (link)

Single Laboratory Validation (SLV) Protocol

For Submission to the Interstate Shellfish Sanitation Conference (ISSC)

For Method Approval

Critical Information: Applicants shall attach all procedures, with materials, methods, calibrations and interpretations of data with the request for review and potential approval by the ISSC. The ISSC also recommends that submitters include peer-reviewed articles of the procedure (or similar procedures from which the submitting procedure has been derived) published in technical journals with their submittals. Methods submitted to the ISSC Laboratory Committee for acceptance will require, at a minimum, 6 months for review from the date of submission.

Note: The applicant should provide all information and data identified above as well as the following material, if applicable:

Justification for New Method

- Name of the New Method.
- Specify the Type of Method (e.g., Chemical, Molecular, or Culture).
- Name of Method Developer / Submitter.
- Developer / Submitter Contact Information [e.g., Address and Phone Number(s)].
- Date of Submission.
- Purpose and Intended Use of the Method.
- Need for the New Method in the NSSP, Noting Any Relationships to Existing Methods.
- Method Limitations and Potential Indications of Cases Where the Method May Not Be Applicable to Specific Matrix Types.
- Other Comments.

Method Documentation

- Method Title.
- Method Scope.
- References.
- Principle.
- Analytes/Measurands.
- Proprietary Aspects.
- Equipment.
- Reagents.
- Media.
- Matrix or Matrices of Interest.
- Sample Collection, Preservation, Preparation, Storage, Cleanup, etc.
- Safety Requirements.
- Other Information (Cost of the Method, Special Technical Skills Required to Perform the Method, Special Equipment Required and Associated Cost, Abbreviations and Acronyms Defined and Details of Turn Around Times [Time Involved to Complete the Method]).
- Test Procedures, (Be Specific and Provide Easy-to-Follow Step-by-Step Procedures and indicate critical steps.).
- Quality Control (Provide Specific Steps.).
- Validation Criteria (Include Accuracy / Trueness, Measurement Uncertainty, Precision [Repeatability and Reproducibility], Recovery, Specificity, Working and Linear Ranges, Limit of Detection, Limit of Quantitation / Sensitivity, Ruggedness, Matrix Effects and Comparability (if intended as a substitute for an established method accepted by the NSSP).
- Data and Statistical Analyses Performed for Each Validation Criterion Tested (Be Specific and Provide Clear Easy-to-Follow Step-by-Step Procedures.).
- Calculations and Formulas Used for Each Validation Criterion Tested.
- Results for Each Validation Criterion Tested.
- Discussion of Each Validation Criterion Tested.
- Summary of Results.
- Laboratory Evaluation Checklist for Use During Evaluations of Proper Method Implementation.

(For guidance: refer to the checklists in the National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish 2017, Guidance Documents, Chapter II - Growing Areas, .15 Evaluation of Laboratories by State Laboratory Evaluation Officers Including Laboratory Evaluation Checklists.)

ISSC Method Application and Single Lab Validation Summary of Required Elements for Acceptance of a Method for Use in the NSSP

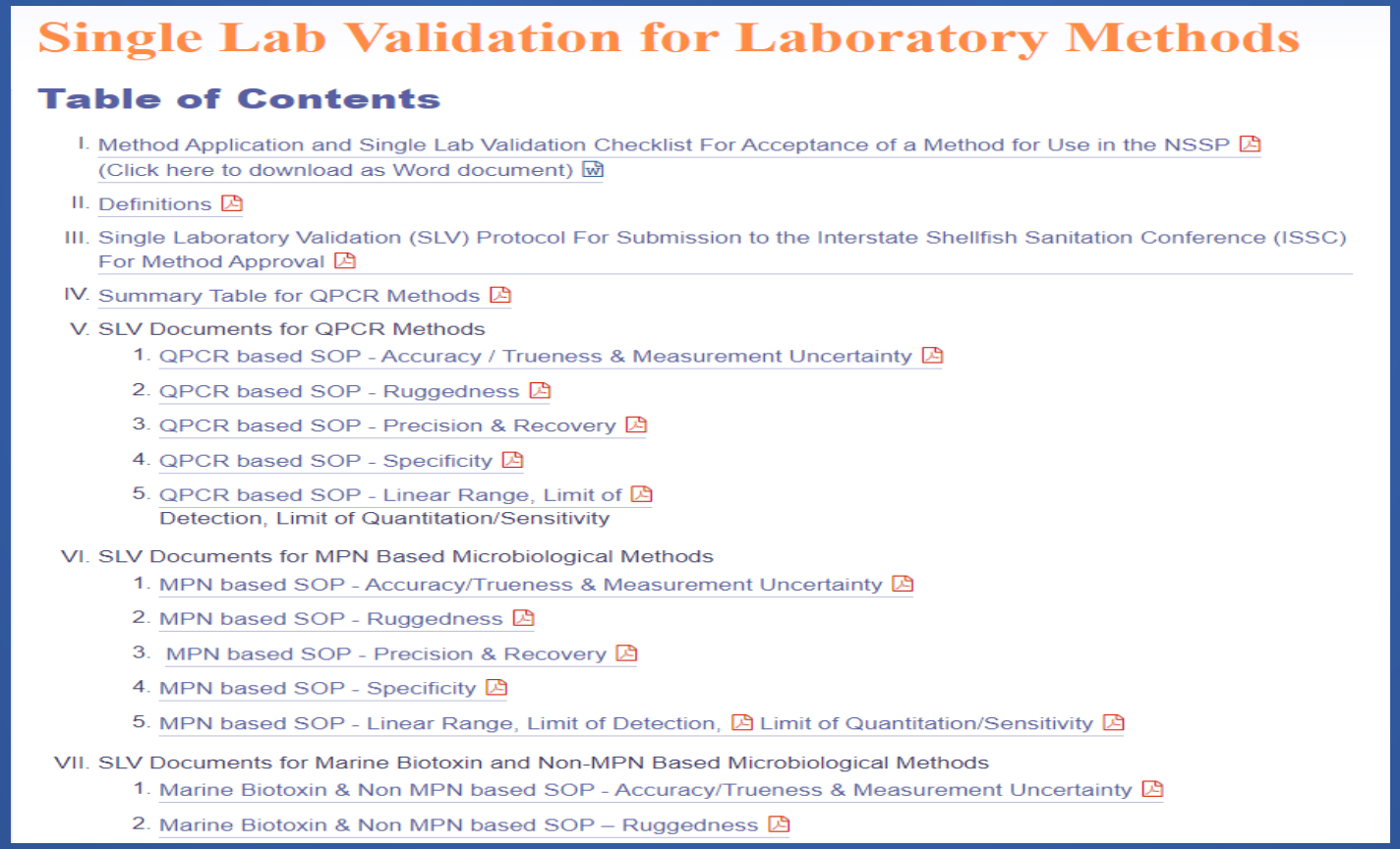
The purpose of single laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that the analytical method under consideration for adoption by the NSSP is fit for its intended use in the Program. A summary of required elements has been developed which explores and articulates the need for the method in the NSSP; provides an itemized list of method documentation requirements; and, sets forth the performance characteristics to be tested as part of the overall process of single laboratory validation. For ease in application, the performance characteristics listed under validation criteria in this document have been defined and accompany the summary of required elements as part of the process of single laboratory validation. Further a generic protocol has been developed that provides the basic framework for integrating the requirements for the single laboratory validation of all analytical methods intended for adoption by the NSSP. Methods submitted to the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Committee for acceptance will require, at a minimum, six (6) months for review from the date of submission.

Name of the New Method	
Name of the Method Developer/Submitter	
Developer Contact Information	

Required Elements	Brief Description
A. Need for the New Method	
1. Clearly define the need for which the method has been developed.	
2. What is the intended purpose of the method?	
3. Is there an acknowledged need for this method in the NSSP?	
4. What type of method? i.e. chemical, molecular, culture, etc.	
B. Method Documentation	
1. Method documentation includes the following information:	
Method Title	
Method Scope	
References	
Principle	
Any Proprietary Aspects	
Equipment Required	
Reagents Required	
Sample Collection, Preservation and Storage Requirements	
Safety Requirements	
Clear and Easy to Follow Step-by-Step Procedure	
Quality Control Steps Specific for this Method	
Laboratory Evaluation Checklist for Use During Evaluations of Proper Method Implementation	
C. Validation Criteria	
1. Accuracy / Trueness	
2. Measurement Uncertainty	
3. Precision Characteristics (repeatability and reproducibility)	
4. Recovery	
5. Specificity	
6. Working and Linear Ranges	
7. Limit of Detection	
8. Limit of Quantitation / Sensitivity	
9. Ruggedness	
10. Matrix Effects	
11. Comparability (if intended as a substitute for an established method accepted by the NSSP)	
D. Other Information	
1. Cost of the Method	
2. Special Technical Skills Required to Perform the Method	
3. Special Equipment Required and Associated Cost	
4. Abbreviations and Acronyms Defined	
5. Details of Turn Around Times (time involved to complete the method)	
6. Provide Brief Overview of the Quality Systems Used in the Lab	
Submitters Signature	Date:
Submission of Validation Data and Draft Method to Committee	Date:
Reviewing Members	Date:
Accepted	Date:
Recommendations for Further Work	Date:
Comments:	

SOPs For Data Generation

- Also available on the ISSC Website are SOPs for generating the data necessary for the Lab Committee to evaluate method performance (link)



Single Lab Validation for Laboratory Methods

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VALIDATION CRITERIA

Accuracy/Trueness is the closeness of agreement between test results and the accepted reference value. To determine method accuracy/trueness, the concentration of the target organism of interest as measured by the MPN based method under study is compared to a reference concentration.

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.

Procedure: This procedure is applicable for use with either growing waters or shellfish tissues. Make every effort to use samples free of the targeted organism of interest. For each shellfish type of interest use a minimum of 10-12 animals per sample. For each sample take two (2) aliquots of either the homogenate or growing water sample appropriately sized for your work and spike one(1) of the two (2) aliquots with a suitable concentration of the target organism of interest. Do not spike the second aliquot. This is the sample blank. Determine the concentration of the target organism of interest used to spike each sample by plating on appropriate agar. Process both aliquots of sample as usual to determine the method MPN. For growing waters do twenty (20) samples collected from a variety of growing areas. For shellfish do twenty (20) samples for each shellfish tissue type of interest collected from a variety of growing areas, the same growing area harvested on different days or from different process lots. **Use a variety of concentrations spanning the range of counts (the working range) of importance in the application of the method to spike sample homogenates or growing water samples**. Both the low and high level spike concentrations must be determinable by the MPN based method under study.

Data:
Working Range _____
Sample Type _____
Agar used to determine spike concentration _____
Organism used for spiking _____

Sample	Plate count (CFU)	Sample blank, MPN	Spiked sample, MPN
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			

Sample	Plate count (CFU)	Sample blank, MPN	Spiked sample MPN
14			
15			
16			
17			
18			
19			
20			

For shellfish samples, repeat for each tissue type of interest.

DATA HANDLING

Accuracy/Trueness

The accuracy/trueness of a method consists of two distinct components, the portion due to the method itself regardless of the laboratory performing it and the portion contributed by the laboratory's performance. In a single laboratory method validation, it is impossible to distinguish the contribution of each to the overall accuracy/trueness of the method. Consequently, what is being estimated is the accuracy/trueness of the method as implemented by the laboratory performing the analysis. Good accuracy/trueness suggests the appropriateness of the method and the laboratory's performance of it for the intended work. Poor accuracy/trueness on the other hand indicates the potential unsuitability of the method and/or the laboratory's performance of it for the intended work.
Accuracy /trueness will be determined by calculating the closeness of agreement between the test results and a reference value obtained by plate count.

To determine the accuracy/trueness of the method as implemented by the laboratory over the range in concentrations important to the intended application of the method, the data is worked-up in the following manner.

1. Convert plate counts and MPNs to logs.
2. If necessary use the sample blank to correct the MPNs of the spiked samples for matrix effects.
3. Calculate the average plate count of the data in logs.
4. Calculate the average MPN of the data in logs.
5. Divide the average MPN in logs by the average plate count in logs.
6. Multiply the quotient by 100. This provides an estimate in percent of the accuracy/trueness of the method as implemented by the laboratory over the range in concentrations of importance to the intended application of the method.

Measurement uncertainty

Measurement uncertainty can be determined by subtracting the MPN results for each sample from the reference values for the samples as determined from the accompanying plate counts in logs and calculating the 95% confidence interval of these differences. The confidence interval of these differences represents the range in values within which the true measurement uncertainty lies. A narrow range in values indicates that the method as implemented by the laboratory produces reliable results.

Use the log transformed data for both the plate count and the MPN results. If necessary use the sample blank to correct the MPNs of the spiked sample for matrix effects and calculate the two-sided, 95% confidence interval for the difference in log counts between the reference (plate count) and the MPN method under study. This range in counts represents the measurement uncertainty of the method as implemented by the laboratory.

Data Summary:
Calculated % accuracy/trueness _____
Calculated measurement uncertainty _____

Following Proposal Submission…

- ISSC Executive Office will assign the proposal to the Laboratory Committee
- The Laboratory Committee Chair will provide the proposal materials to the full Laboratory Committee, and will assign the initial review of the proposal to the appropriate subcommittee

Subcommittee Review

- Small group of specific subject matter experts, along with the liaison and other interested parties.

- Will review the full submission, including method documents, data generated, data analysis, lab evaluation checklist, etc., and will make a recommendation to the full Laboratory Committee.

- **Timeline = Typically 1 to 6 months following receipt by LC**

Laboratory Committee Review

- Subcommittee recommendation and summary of deliberations presented at next full Laboratory Committee Meeting.
- Laboratory Committee will vote on the Subcommittee Recommendation:
  - Recommend Acceptance
  - Recommend “No Action”
  - Provide questions, comments, summary of additional data or analyses needed, etc.

- **Timeline = 1 to 2 months after subcommittee recommendation**

Overall Timeline

- Typically 1 to 2 Years From Receipt of Preproposal to Final Approval, Although That Timeline Can Vary Significantly.
- Example:

Preproposal Received By Lab Committee - February 2016

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Approved - April 2016

↓

Full SLV Received By Lab Committee - April 2017

↓

Approved at Biennial Meeting - October 2017

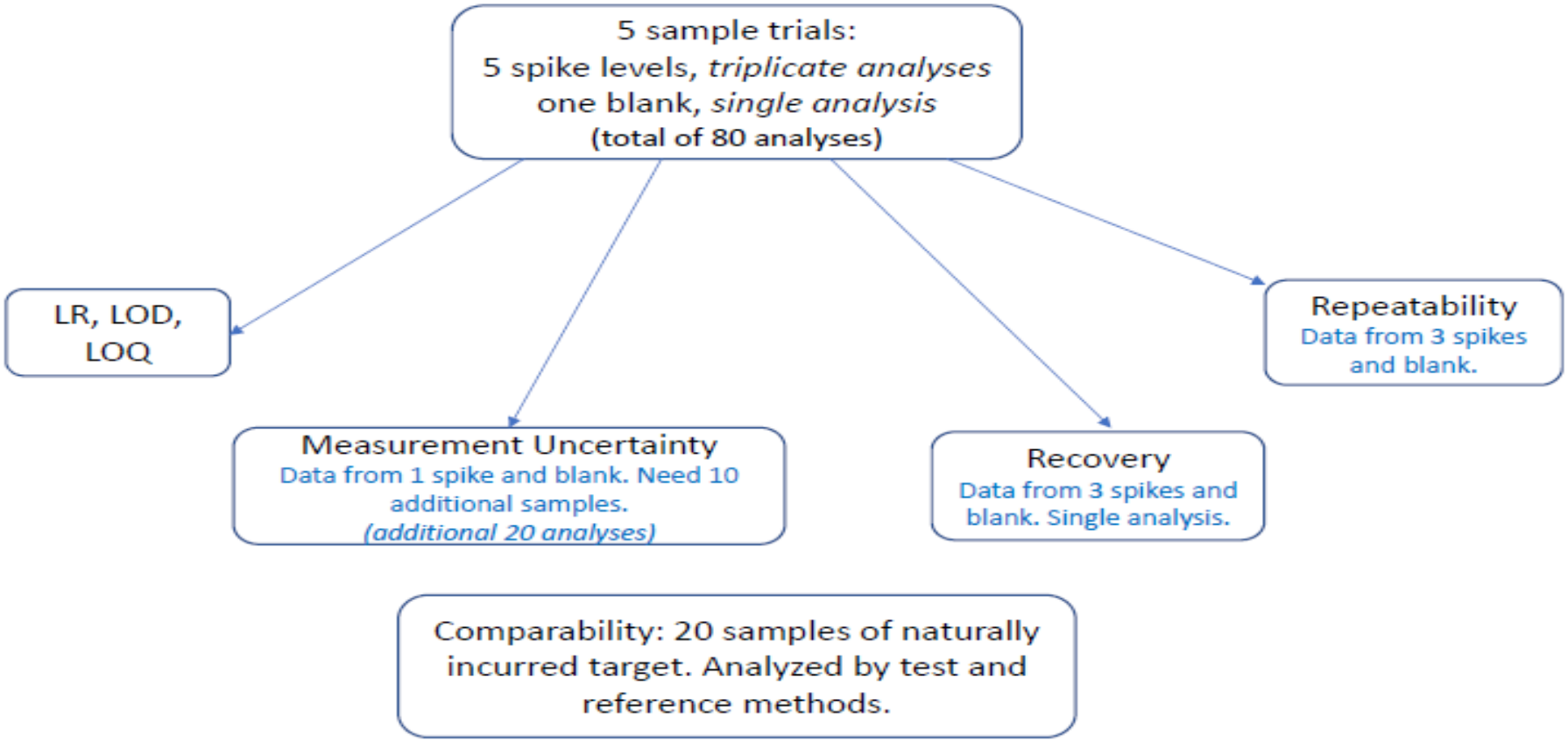
- Others much longer
  - Multiple reviews
  - Difficulty generating follow up data
  - Staff changes/retirements
  - Funding limitations

Matrix Extension Guidelines

- ISSC Constitution, Bylaws, and Procedures – Procedure XV (10.)

- *"For methods already adopted into the NSSP, consideration of expanding a method to a new molluscan shellfish species is accomplished using the "ISSC Method Application Format for Biotoxin Methods Matrix Extension" and the "ISSC Method Application Format for Microbiology Methods Matrix Extension." The simplified, reduced approach to method validation for expanding an NSSP method to a new molluscan shellfish species is visually represented in the "Matrix Extension Guidelines" schematic."*

Matrix Extension Guidelines



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ISSC Method Application Format for Biotoxin Methods Matrix Extension

The purpose of laboratory validation in the National Shellfish Sanitation Program (NSSP) is to ensure that methods under consideration for adoption by the NSSP are fit for their intended use in the Program. This document provides a detailed outline of the types of information and data the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Committee (LC) requests from submitters for extension of current NSSP methods to cover additional matrices (i.e., molluscan shellfish species). These recommendations are intended for methods which have already undergone a single laboratory validation (SLV) and are being considered for use with a new matrix. Included are the method performance criteria that should be examined for inclusion in the validation package, along with LC recommendations for each criterion. Data generated for the more robust performance criteria may be used to satisfy multiple criteria, if applicable.

Method Overview
Method Title:
Method Submitter(s) and Contact Information:
Intended or Target Use: (approved, approved limited use, or emergency use)
Rationale for this Method in the NSSP: (Does the method meet an immediate or continued need or improve analytical capability?)
Method Principle/Basis: (receptor binding assay, immunoassay, LC-MS, etc.)
Target Matrix/Matrices: (list shellfish species by common and scientific names)
Target Toxin(s):
Existing Certification(s) of the Method: (AOAC, etc.)
Equipment Required: (Provide a list of specialized equipment needed to perform the method.)
Reagents Required: (Provide a list of specialized chemicals, reagents, etc. needed to perform the method.)
Proprietary Aspects: (Provide any aspects of the method that are proprietary or trade secret.)
Safety Requirements: (Describe the safety measures, beyond those of routine laboratory practices, required to perform the method, including personal protective equipment, fume hoods, etc.)
Method Cost: (Provide an estimate of cost per analysis, including start-up costs for specialized equipment, personnel, etc.)
Sample Throughput: (Provide a description of how many samples can be analyzed by this method in a given time frame; please specify under what conditions this throughput can be achieved.)

Validation Data
1. Recovery: Recovery is the fraction or percentage of an analyte recovered following sample analysis. To determine method accuracy/trueness/recovery, the concentration of the target analyte as measured by the analytical method under study is compared to a true value or accepted reference concentration. Consider using certified reference materials (if available). Suggested procedure: Use shellfish free of the target analyte(s); analyze intended blank matrix tissue for background interferents. For each shellfish type of interest use a minimum of 10-12 animals per sample and prepare as a homogenate. Take four aliquots of the sample homogenate appropriately sized for the work and spike one with the target analyte(s) at half the action level. Spike a second aliquot with the target analyte(s) at the action level. Spike the third aliquot with the target analyte(s) at twice the action level. Do not spike the fourth aliquot; this is the sample blank. Process each aliquot to determine the concentration for the target analyte(s). Repeat this process with a minimum of five samples for each shellfish type of interest collected from a variety of growing areas, the same growing area harvested on different days, or from different process lots. Additional samples may be required to examine the effects of seasonal and/or geographical differences in shellfish matrix components or analyte profiles on the method performance.
2. Repeatability: 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. Suggested procedure: Use shellfish free of the target analyte(s). For each shellfish type of interest use a minimum of 10-12 animals per sample and prepare as a homogenate. Take four aliquots of the sample homogenate appropriately sized for the work and spike one with the target analyte(s) at half the action level. Spike a second aliquot with the target analyte(s) at the action level. Spike the third aliquot with the target analyte(s) at twice the action level. Do not spike the fourth aliquot; this is the sample blank. For each aliquot, excluding the sample blank, prepare three sub-aliquots for analysis. Process each sub-aliquot, including the sample blank, to determine the method concentration of the target analyte(s). Repeat this process for each shellfish type of interest with a minimum of five samples collected from a variety of growing areas, the same growing area harvested on different days, or from different process lots. When available, shellfish with naturally incurred target analyte(s) should be included. Use a minimum of 10-12 animals per sample and prepare as a homogenate. For each shellfish type of interest, use three samples at a range of concentrations bracketing the action level (below, at or near, and above). For each sample homogenate prepare a minimum of three aliquots for analysis. Process each aliquot to determine the method concentration of the target analyte(s).
3. 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 present in the sample. The limit of detection is the minimum concentration at which the analyte can be identified. Limit of detection is matrix and analyte dependent. The limit of quantitation is the minimum concentration of the analyte that can be quantified with an acceptable level of precision and accuracy under the conditions of the test. Suggested procedure: Use samples free of the target analyte(s); analyze intended blank matrix tissue for background interferents. For each shellfish type of interest use a minimum of 10-12 animals 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), spanning beyond the desired working range and including levels half, at, and twice the action level. Do not spike the sixth aliquot of each sample; this is the sample blank. Process each aliquot, including the sample blank to determine concentration for the target analyte(s). For each aliquot, excluding the sample blank, sub-aliquot for three replicate analyses. Repeat this process for each shellfish type of interest with a minimum of five samples collected from a variety of growing areas, the same growing area harvested on different days or from different process lots. Use the same spike levels for each of the samples analyzed.

Link: Pages 19-24 of this document

Method Approvals

Laboratory Method References

Publications & Reports

ISSC / FDA National Vibrio parahaemolyticus Workshop

Laboratory Method References

Education Materials

DOMESTIC NSSP LABORATORY LIST

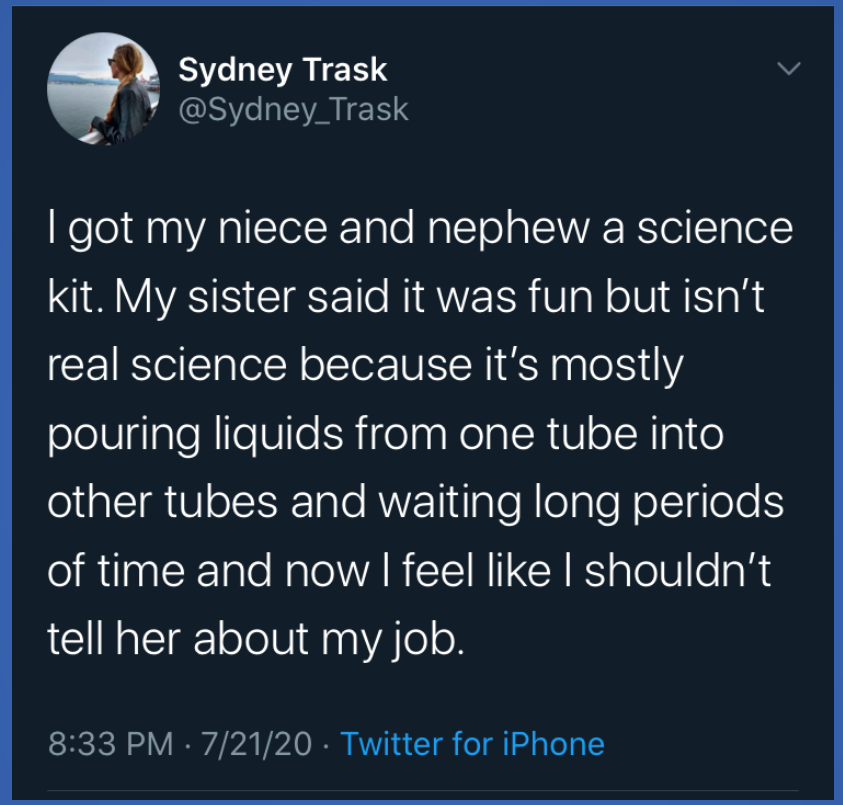
Method	NSSP Status	Target/Matrix/Limitations	Application (Purpose of Method)	Reference	Checklist Links
Approved Methods for Microbiological Testing					
APHA Decimal Dilution	Approved	Total Coliforms and Fecal Coliforms - G.A. waters and Shellfish	Growing Area Classification	Recommended Procedures, APHA 1970	Microbiology
12 tube Single Dilution	Approved	Total Coliforms and Fecal Coliforms - G.A. waters (cannot be used with Systematic Random Sampling) and Shellfish (end product depurated shellfish only)	Growing Area Classification and Depuration	Redman 1974, Proceedings of 8th National Shellfish Sanitation Workshop (J. Springer); NSSP Interpretation 17-IV-@.02-102	Microbiology
Other APHA (10 tube, 10 ml portions)	Approved	Total Coliforms - UV Treated Process water	Wet Storage and Depuration	Standard Methods, 18th edition, 1992 Section 9221	Microbiology
mEndo agar LES	Approved	Total Coliforms - UV Treated Process water	Wet Storage and Depuration	Standard Methods, 20th edition, 1998. Section 9222 B. ; Proposal 11-111	Microbiology
A-1M Decimal Dilution MPN	Approved	Fecal Coliforms - G.A. waters	Growing Area Classification	Method 978.23, AOAC 1990	Microbiology
A-1M 12 tube Single Dilution MPN	Approved	Fecal Coliforms - G.A. waters (cannot be used with Systematic Random Sampling)	Growing Area Classification	Method 978.23, AOAC 1990; NSSP Interpretation 17-III-@.02-100	Microbiology
A-1M without salicin	Approved	Fecal Coliforms - G.A. waters (requires comparability testing)	Growing Area Classification	Karolus et. al 2003	Microbiology
mTEC	Approved	Fecal Coliforms - G.A. waters	Growing Area Classification	Rippey et. al 1987 (grandfathered)	Microbiology
ETCP	Approved	Fecal Coliforms - Shellfish (Clams only)	Depuration	Cabelli and Heffernan 1970	Microbiology
Standard Plate Count	Approved	Total Bacteria - Shellfish	Shellfish transportation and handling	Recommended Procedures, APHA 1970	Microbiology
Approved Limited Use Methods for Microbiological Testing					
Modified Double Agar Overlay (MSC)	Approved Limited Use	MSC - Shellfish (Hard and Soft Clams and Oysters)	Tracking tool for point-sources; est. buffer zones	SLV (2009; Proposal 09-114 [soft shelled clams and oyster] ;13-120 [hard clams])	Microbiology

1	2	3	4	5	6	7	8
Oysters	***Hard Clams***	***Non-US Hard Clams***	***Geoducks****	***Soft Clams***	***Mussels***	***Estuarine Mussels (non-Mytilus)***	***Scallops*** **
Eastern Oyster (*Crassostrea virginica*)	Atlantic Surfclam (*Spisula solidissima*)	Wedge Shell Clam (*Donax cuneatus*)	Pacific Geoduck Clam (*Panopea generosa*; formerly *P. abrupta*)	Softshell Clam (*Mya arenaria*)	Blue Mussel (*Mytilus edulis*)	Asian Green Mussel (*Perna viridis*)	Sea Scallop (*Placopecten magellanicus*)
Edible Oyster (*Ostrea edulis*)	Ocean Quahog (*Arctica islandica*)	Asiatic Hard Clam (*Meretrix meretrix*)	Atlantic Geoduck Clam (*Panopea bitruncata*)		Mediterranean Mussel (*Mytilus galloprovincialis*)		Rock Scallop (*Crassodoma gigantea*)
Olympia Oyster (*Ostrea lurida*)	Northern Quahog (*Mercenaria mercenaria*)				California Mussel (*Mytilus californianus*)		Bay Scallop (*Argopecten irradians*)
Pacific Oyster (*Crassostrea gigas*)	Southern Quahog (*Mercenaria campechiensis*)				Chilean Mussel (*Mytilus chelensis*)		Peruvian Scallop (*Argopecten purpuratus*)
	Northern Razor Clam (*Siliqua patula*)				Korean Mussel (*Mytilus coruscus*)		
	Pacific Littleneck Clam (*Protothaca staminea*)						
	Butter Clam (*Saxidomus gigantea*)						
	Sunray Venus Clam (*Macrocallista nimbosa*)						
	Japanese Littleneck Clam (*Venerupis philippinarum*)						

*Geoducks are generally analyzed as whole animals for microbiological methods and gutballs only for biotoxin methods. If a different form of the animal is to be processed (i.e., gutball for micro method or whole animal for biotoxin method), it should be considered a separate matrix.

**Scallops can be analyzed as whole animal or muscle excluded. These different forms of the animal should be considered a separate matrix. Methods for muscle only will not be considered as the product is not within the NSSP.

Questions?



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