ISSC Single Laboratory Method Validation Guidelines

Suggested.Procedures.for.Validating.Quantitative.Methods.for.Biotoxins

Introduction: The purpose of laboratory method 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 within the Program. This document provides a summary of the types of information and data that the Interstate Shellfish Sanitation Conference (ISSC) Laboratory Committee (LC) requests from submitters for the validation of quantitative biotoxin methods. Included are the method performance criteria that should be examined as part of a validation package, suggested testing procedures, suggested data handling procedures, and an outline of how it is recommended that the data be presented in a final submission. In some circumstances, data being generated as part of this study can be used to evaluate multiple performance parameters. These areas of overlap will be highlighted throughout these guidelines.

Method validations are matrix specific, so each set of experiments described below should be repeated for each shellfish matrix category of interest. Alternatively, once a method has been validated and adopted into the program for a single matrix, method developers can then utilize matrix extensions to validate the method on additional shellfish matrices. Shellfish matrix categories and information on matrix extensions can be found in the NSSP Guide for the Control of Molluscan Shellfish, Section IV – Guidance Documents, Chapter III: Laboratory, .03 – Laboratory Method Matrix Extensions. Validation experiments can be completed using a single species from each category of interest, or can make use of a mix of species from within each category, as long as the total number of samples meets the criteria described in the guidelines below.

It should be noted that these guidelines represent one way to design and implement a validation study. If a planned validation study deviates from these guidelines, the method submitter is encouraged to consult with the LC through their assigned method liaison to determine if the alternative procedures are acceptable prior to initiation of the study. Any deviations from these suggested procedures should be highlighted in the final submission, along with a rationale for why an alternative procedure was used.

Validation Criteria

Matrix.Effects – An alteration of the analyte response due to interfering and often unidentified components in the sample matrix.

Accuracy—Trueness - The closeness of agreement between a test result and an accepted reference value. Trueness is the closeness of the mean of the measured values to the accepted reference value (i.e., bias). Good accuracy/trueness suggests the appropriateness of the method and the laboratory's performance of it for the intended work.

Recovery – The fraction or percentage of an analyte recovered following sample analysis. The recovery of the target analyte must be consistently fit for purpose over the range of concentrations of importance to the application to be of benefit in the intended work.

Linear.Range – The range where the results are proportional to the concentration of the analyte present in the sample.

Limit.of.Detection – The minimum concentration at which the analyte can be identified.

Limit.of.Quantitation – The minimum concentration of the analyte that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.

Measurement.Uncertainty – The possible range of values around the measured result within which the true value is expected to be with a stated degree of probability. A narrow range of values indicates that the method as implemented by the laboratory produces reliable results.

Repeatability – 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.

Precision – The closeness of agreement between independent test results obtained under stipulated conditions.

Intermediate.Precision – Within laboratory precision obtained under variable conditions such as different days, different analysts, or different instrumentation.

Specificity~~–S~~electivity – The ability of a method to measure what it is intended to measure.

Ruggedness~~–R~~obustness – The ability of a particular method to withstand relatively minor changes in analytical technique, reagents, or environmental factors likely to arise in different test environments.

Cross_Reactivity – In immunoassays, the degree to which an antibody binds to compounds that are structurally similar to the target analyte, essentially measuring how well the antibody can detect related compounds. This criterion may also apply to activity-based assays (e.g. receptor-binding assays) in which the response of related toxin congeners may vary.

Comparability – The acceptability of a new or modified analytical method as an alternative or substitute for an established method in the NSSP.

Validation Criteria – Experiment #1

Matrix.Effects.Evaluation

Suggested Procedure:

If you have determined that matrix effects are not a concern for your method, please include data or text explanation supporting that assertion within the final submission package.

For methods that need to be evaluated for matrix effects, matrix-matched standards (MMS) should be prepared by taking five sub-aliquots from a sample blank extract, and spiking each extract sub-aliquot with a different concentration of the target analyte(s) spanning the desired working range and including levels at half, at, and above the action level if these three levels are within the method linear working range. Generate a calibration curve using these five MMS from a sample and compare with neat standards (in solvent) to assess matrix effects. A minimum of ten samples collected from a variety of growing areas, the same growing area harvested on different days, or from different process lots, should be analyzed following these same procedures, using the same spike levels to generate MMS for each sample.

For LC-MS methods, analyte sensitivity can drift over time, and this drift must be assessed during the validation. One potential method of assessment is to alternate injections between neat standards and the five MMS from a sample (yielding a neat standard calibration curve and an MMS calibration curve for each sample) in one LC-MS sequence to determine matrix effects for all ten samples. Implementation of matrix effects evaluation can also be done on different days along with routine samples as long as solvent standard and MMS calibration curves are run side by side. For non-LC-MS methods, only one calibration curve from solvent standards per day is necessary to complete the table. For the purposes of completing Table 1, Matrix Effects (%) = (Slope of MMS/Slope of Solvent Standards – 1) × 100. Adjust method parameters to ensure the ten tested samples exhibit the same range of Matrix Effects if necessary. Determine whether the MMS or solvent standard calibration curve should be used for determination of toxins in samples based on the results of matrix effects.

Table 1:

Sample #	1	2	3	4	5	6	7	8	9	10
Matrix effects										

Validation Criteria – Experiment #2

Criteria.To.Be.Assessed¿Accuracy–Trueness·.Recovery·.Linear.Range·.Limit.of.Detection·.Limit.of.Quantitation.

(Full-method repeatability, intermediate precision, recovery precision, and measurement uncertainty are assessed in Experiment #3 using independent spiking and extraction replicates.)

Suggested Procedure:

Use shellfish free of the target analyte(s). For each shellfish matrix of interest, use a minimum of 12 animals per sample and prepare as a homogenate. Select a minimum of 10 samples collected from a variety of growing areas, the same growing area harvested on different days, or from different process lots.

For each of the 10 samples, following the appropriate process for the method being validated, prepare one spiked aliquot at each of five target analyte concentrations spanning the intended working range, including levels at half, at, and above the action level (at least 1.5x, as appropriate for the method and toxin being assessed) (Table 2). In cases where a toxin class has multiple congeners with varying toxicity, a cocktail of congeners with total toxin equivalents at the appropriate concentrations can also be used. Additional spikes in the lower part of the range may be required to obtain linear range, limit of detection, and limit of quantitation. Spike the analytes(s) into shellfish homogenate and vortex well before toxin extraction. Additionally, create a single unspiked replicate of each sample to serve as the sample blank.

*For three selected representative samples prepare three independent spiking and extraction replicates at three decision-relevant levels. The first result from each of these replicate subsets will be used in experiment #2, and the additional replicates will be used in later experiments.

Analyze each spiked aliquot once to determine the concentration of the target analyte(s). Additional spikes in the lower part of the range may be required to obtain linear range, limit of detection, and limit of quantitation.

Table 2:

Sample	Spike 1	Spike 2	Spike 3	Spike 4	Spike 5	Blank
Sample 1	1 extract; 1 analysis	1 extract; 1 analysis	3 independent extracts	3 independent extracts	3 independent extracts	Blank
Sample 2	1 extract; 1 analysis	1 extract; 1 analysis	3 independent extracts	3 independent extracts	3 independent extracts	Blank
Sample 3	1 extract; 1 analysis	1 extract; 1 analysis	3 independent extracts	3 independent extracts	3 independent extracts	Blank
Sample 4	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	Blank
Sample 5	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	Blank
Sample 6	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	Blank
Sample 7	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	Blank

Sample	Spike 1	Spike 2	Spike 3	Spike 4	Spike 5	Blank
Sample 8	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	Blank
Sample 9	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	1 extract; 1 analysis	Blank
Sample 10	1 extract; 1 analyses	1 extract; 1 analyses	1 extract; 1 analyses	1 extract; 1 analyses	1 extract; 1 analyses	Blank

Data Handling and Analysis:

a. Accuracy/Trueness

i. Use Experiment #2 data from all 10 samples and five spike levels. For each sample-level combination, use the measured result from the independently spiked and extracted aliquot.
   a. For the replicate subset, utilize the first result from each spike concentration and sample for the Experiment #2 matrix summary. (Retain the three independent extraction results for Experiment #3 precision analyses.)

ii. For each spike level, calculate Relative Error using the sample-level measured values from all 10 samples. As appropriate for the method being assessed, calculate relative bias as well. Summarize results by spike level across the 10-sample matrix set, and provide an overall summary across all spike levels to characterize performance throughout the intended working range.

Relative Error (%) = [(Measured value - Reference value) / Reference value] x 100

Relative Bias (%) = [(Mean measured value - Reference value) / Reference value] x 100

b. Recovery

Use Experiment #2 data from all 10 samples and five spike levels to calculate Recovery across representative matrices.

i. For each sample-level combination, calculate Recovery (%) using the sample-level measured value.
Recovery (%) = (Measured value / Reference value) x 100
   a. For the replicate subset, utilize the first result from each spike concentration and sample for the Experiment #2 matrix summary. (Retain the three independent extraction results for Experiment #3 precision analyses.)

ii. For each spike level, summarize Recovery across the 10-sample matrix set by reporting mean Recovery (%), standard deviation, relative standard deviation, and range.

Variance-based estimates of repeatability, intermediate precision, recovery precision, and measurement uncertainty are evaluated in Experiment #3 using independent replicate extractions.

c. Linear Range

i. Perform the linear regression analysis for the standard calibration curves. Perform the linear regression analysis for each spiked sample at all spike levels with Y-axis using the mean response and X-axis using the mean measured concentration at each spike level.
   a. For the three selected samples at the three decision-relevant levels, use the first result from each spike concentration and sample.

ii. Compare the linear regression analysis obtained from the standards and the spiked samples, determine the linear working range for the samples. If the linear working range covers a wide range of the target analyte concentration, evaluate whether a weighting factor is appropriate

d. Limit of Detection

There are several approaches for determining LOD, depending on the method being assessed (ICH harmonized Tripartite guideline, Validation of analytical procedures: text and methodology Q2(R1), 1994, 1996, 2005). As one option, LOD and LOQ may be estimated from Experiment #2 if the low-level spikes and blanks provide adequate data near the expected detection and quantitation limits. If not, perform an additional low-level study using concentrations near the expected LOD/LOQ.

i. Based on Signal-to-Noise Ratio. This approach can only be applied to analytical procedures that exhibit baseline noise. A signal-to-noise ratio of 3 is generally considered acceptable for estimating the LOD.

ii. Based on the Standard Deviation of the Response and the Slope. LOD = 3.3σ/S. S is the slope of the calibration curve. σ is the standard deviation of the response, which can be estimated based on the standard deviation of the blank or the calibration curve. When calibration curve is used for LOD estimate, a special calibration curve should be performed using samples containing the target analyte in the range of LOD. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

e. Limit of Quantitation

There are several approaches for determining LOQ, similar to LOD.

i. Based on Signal-to-Noise Ratio. This approach can only be applied to analytical procedures which exhibit baseline noise. A signal-to-noise ratio of 10 is typically considered acceptable for estimating the LOQ.

ii. Based on the Standard Deviation of the Response and the Slope. LOD = 10σ/S. S is the slope of the calibration curve. σ is the standard deviation of the response, which can be estimated based on the standard deviation of the blank or the calibration curve. When calibration curve is used for LOQ estimate, a special calibration curve should be performed using samples containing the target analyte in the range of LOQ. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

Validation Criteria – Experiment #3

Criteria.To.Be.Assessed¿Measurement.Uncertainty·.Repeatability.(Within_Run.Precision)?and. Intermediate.Precision

Note: Experiment #2 and Experiment #3 are intentionally linked to avoid duplicate analyses. The three selected samples and three decision-relevant spike levels prepared as independent replicate extractions in Experiment #2 serve as Run 1 / Day 1 / Analyst 1 of Experiment #3.

Suggested Procedure:

Select three representative samples from Experiment #2. At each of three decision-relevant concentrations, approximately 0.5x the action level / limit, 1x the action level / limit, and above the action level / limit (at least 1.5x, as appropriate for the method and toxin being assessed), prepare three independently spiked and extracted replicates. This is Run 1 and is generated during Experiment #2. Repeat this complete procedure (sample preparation, spiking, extraction, and analysis) a second time, processing by different analysts or by the same analysts on a different day, for a total of three times, producing three independent validation runs.

Table 3:

Sample	Level	Run 1 from Exp. 2	Run 2	Run 3
Sample 1	0.5x limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 1	1x limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 1	Above limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 2	0.5x limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 2	1x limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 2	Above limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 3	0.5x limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 3	1x limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3
Sample 3	Above limit	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3	Rep 1, Rep 2, Rep 3

Data Handling and Analysis

A. Repeatability (Within-Run Precision)

Repeatability is estimated from the three independently prepared replicates within each analytical run.
For each sample and spike level:

Calculate the within-run standard deviation (Sr).

Calculate the Relative Standard Deviation (RSDr).

RSDr (%) = (Sr / Mean Measured Value) × 100

Report RSDr for each sample and spike level.

B. Intermediate Precision

Intermediate Precision evaluates the variability introduced by different analytical runs performed on different days, by different analysts, or both. For each sample and spike level, perform a one-way ANOVA using analytical run as the grouping factor.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	p-value	Fcrit
Between Runs	Sb	2	$M_b=S_b/2$	Mb/Mw		
Within Runs	Sw	6	$M_w=S_0/6$			
Total	Sb + Sw	8				

Calculate:

Repeatability for Sample 1

Standard deviation: Sr = SQRT(M_w)

Relative standard deviation RSD_r = (S_r/average of all 9 replicates with sample 1)×100

Intermediate Precision standard deviation for Sample 1:

Standard deviation: S_{IP} = SQRT((M_b-M_w)/n+M_W)

Relative standard deviation RSD_{IP} = (S_{IP}/average of all 9 replicates with sample 1)×100

Since variability including analysts or days cannot be less than within analysts or days, if S_R is less than S_r, S_{IP} is set equal to S_r.

Repeat calculation of S_r and S_{IP} for sample 2 & 3.

C. Measurement Uncertainty

Calculate the standard deviation for each sample and spike level. Estimate measurement uncertainty as approximately ±2 times the standard deviation (S_{IP}). Summarize the resulting uncertainty estimates across samples at each spike level by reporting the range of at each spike level.

D. Summary of Method Precision

Summarize the following for each of the three samples and spike levels:

Mean Recovery (%)
RSDr (%)
RSD_{IP} (%)
Measurement Uncertainty (± 2SD)

These values represent the performance of the complete analytical method over the concentration range of greatest regulatory importance.

Validation Criteria – Experiment #4

Criteria To Be Assessed¿Specificity–Selectivity (not required for LC MS_MS methods? as they combine LC and MS–MS selective techniques for toxin identification and quantification)

Suggested Procedure:

Use shellfish free of the target analyte(s). For each shellfish matrix of interest, use a minimum of 10-12 animals per sample and prepare as a homogenate. For each sample, take a minimum of three aliquots of the homogenate appropriately sized for the work. For one aliquot, create five replicates spiked at a low but determinate level of the target analyte. For the second aliquot, create five replicates at the same low level of the target analyte and also spike with a moderate to high level of a suspected interfering toxin or compound. Use the third aliquot to create a single unspiked replicate of the sample to serve as the sample blank. Spike the analyte(s) into shellfish homogenate and vortex well before extraction. Process replicates of each sub-aliquot and the sample blank to determine the concentration of the target analyte(s). Repeat this process for all suspected interfering toxins or compounds identified.

Table 4. For Each Potential Interfering Toxin or Compound:

	Sample Spiked with Low but Determinate Level of Target Analyte	**Sample Spiked with Target Analyte and Suspected Interfering Compound**	**Sample Blank**
Replicate 1			
Replicate 2			
Replicate 3			
Replicate 4			
Replicate 5			

Data Handling and Analysis:

a. Calculate the Specificity Index (SI) as follows:

$$SI = \frac{Sample\ Spiked\ With\ Target\ of\ Interest\ Only}{Sample\ Spiked\ With\ Both\ Target\ and\ Suspected\ Interference}$$

b. SI should equal one in the absence of interferences. To test the significance of an SI other than one, a two-sided t-test at the 0.05 significance level is performed to determine if the arithmetic mean of the SI differs from one. Repeat for all interfering compounds/toxins tested.

Validation Criteria – Experiment #5

Criteria.To.Be.Assessed¿Ruggedness–Robustness

Note: Data from Experiment #2 at the action level can be used to fulfill the "Following Method" data requirement described below in the section about assessing the effects of modifications to analysis parameters. Additional replicates of those samples will be needed in order to address the "Method with Modification" component of this test.

Suggested Procedure:

This experiment is to determine whether the method, as implemented, is sufficiently rugged to withstand the types of changes anticipated to occur in routine use. Identify critical performance parameters (i.e., lot of reagents, temperature, incubation time, pH, chemical stability of target analyte during storage and analysis, etc.) and compare results generated with and without modification to these parameters. Only one modification at a time should be examined.

To assess the effect of modifications to extraction parameters, the following procedure should be followed: Use shellfish free of the target analyte(s). For each shellfish matrix of interest, use a minimum of 10-12 animals per sample and prepare as a homogenate. Take two aliquots of the homogenate appropriately sized for the work. Following the appropriate process for the method being validated, spike one aliquot with the target analyte(s) at the action level. Aliquot 1 should be processed following the method procedure. Aliquot 2 should be processed following the method procedure, with modification to a single critical performance parameter. Spike the analyte(s) into the shellfish homogenate and vortex well before extraction. Repeat this process for a minimum of 10 samples collected from a variety of growing areas, the same growing area harvested on different days, or from different process lots.

Table 5:

	Following Method	Method with Modification
Sample 1		
Sample 2		
Sample 3		
Sample 4		
Sample 5		
Sample 6		
Sample 7		
Sample 8		
Sample 9		
Sample 10		

Data Handling and Analysis:

For each sample, calculate the paired difference between the result obtained following the method as written and the result obtained following the modified method.

Paired Difference = (Modified Method Result – Method Result)

Examine the distribution of the paired differences. If the paired differences are approximately symmetric and free of substantial outliers, analyze the data using a paired t-test. If the paired differences exhibit marked skewness or contain substantial outliers, analyze the data using the Wilcoxon signed-rank test.

Data Summary:

a. mean result under method conditions
b. mean result under modified conditions
c. mean paired difference
d. p-value
e. Test used
f. Is there a significant difference between batch/lot 1 samples and batch/lot 2 samples (Y/N)

Validation Criteria – Experiment #6

Criteria.To.Be.Assessed¿Cross.Reactivity.and.Extraction.Efficiency.of.Multiple.Target.Analyte.Congeners.(If.Necessary)

For antibody- or activity-based assays that may detect congeners within a toxin class to varying degrees, cross-reactivity and extraction efficiency should be assessed for each congener, if possible.

Suggested Procedure:

a. Cross reactivity

Use shellfish free of the target analyte(s). For each shellfish matrix of interest, use a minimum of 10-12 animals per sample and prepare as a homogenate. Follow the appropriate process for the method being validated to create sufficient blank (unspiked) replicate extracts to complete the steps below.

Prepare matrix-matched standards (MMS) by taking five sub-aliquots from a sample blank extract and spiking each extract sub-aliquot with a different concentration of the target analyte spanning the desired working range as described in Experiment 1. The target analyte is the toxin congener specified in the method for calibration and sample quantitation.

Spike three replicate sub-aliquots from a sample blank extract with a (single) different congener (Congener 1) at the action level for the toxin class. Determine the concentration of Congener 1 in each replicate using the matrix-matched target analyte standards as the calibration solution. Repeat the above process for each available analyte congener.

Table 6a. Cross reactivity in sample matrix

	Replicate 1	Replicate 2	Replicate 3	Sample blank
Congener 1 MMS				
Congener 2 MMS				
Congener 3 MMS				
Congener 4 MMS				
Congener 5 MMS				
Congener 6 MMS				
Congener 7 MMS				

b. Extraction efficiency

Extraction efficiency should be evaluated for those congeners demonstrating cross-reactivity in the assay. Use shellfish free of the target analyte(s). For each shellfish matrix of interest, use a minimum of 10-12 animals per sample and prepare as a homogenate. Following the appropriate process for the method being validated, create three replicates of an aliquot spiked with Congener 1 at the action level for the toxin class. Spike the congener into the shellfish homogenate and vortex well before extraction. Additionally, create a single unspiked replicate of the sample to serve as the sample blank.

Prepare matrix-matched standards (MMS) of Congener 1 by taking five sub-aliquots from the sample blank extract and spiking each extract sub-aliquot with a different concentration of Congener 1, spanning the desired working range as described in Experiment 1.

Determine the concentration of Congener 1 using the corresponding Congener 1 MMS as the calibration solution to determine method extraction efficiency. Repeat the above process for each analyte congener available.

Table 6b. Extraction Efficiency and Matrix Effects

	Replicate 1	Replicate 2	Replicate 3	Sample blank
Congener 1				
Congener 2				
Congener 3				
Congener 4				
Congener 5				
Congener 6				
Congener 7				

Data Handling and Analysis:

Calculate the average and standard deviation of the three replicates of each congener examined. Calculate cross-reactivity (from 6a) and extraction efficiency (from 6b) as a percentage of the spike concentration added.

Table 6c:

	Congener cross reactivity (%) ± S.D. (6a)	Extraction Efficiency (%) ± S.D. (6b)
Congener 1		
Congener 2		
Congener 3		
Congener 4		
Congener 5		
Congener 6		
Congener 7		

Validation Criteria – Experiment #7

Criteria.To.Be.Assessed¿Comparability.(If.Necessary)

Note: Comparability only needs to be assessed if the proposed method is intended as an alternative or substitute for an established method that has already been adopted by the NSSP.

Suggested Procedure:

Naturally incurred samples are preferred for this analysis, although equivalent spiked samples can be used if naturally incurred samples are unavailable. For each shellfish matrix of interest, use a minimum of 10-12 animals per sample and prepare as a homogenate. For each sample, take two aliquots, analyze one using the officially recognized NSSP method, and analyze the second using the proposed method. A minimum of thirty paired samples should be analyzed, including a variety of toxin concentrations that span the range of the intended application of the method, including primarily determinate results. Samples should come from a variety of different geographic areas or different seasons.

Table 7:

	NSSP Method	Proposed Method
Sample 1		
Sample 2		
Sample 3		
Sample 4		
Sample 5		
Sample 6		
Sample 7		
Sample 8		
Sample 9		
Sample 10		
Sample 11		
Sample 12		
Sample 13		
Sample 14		
Sample 15		
Sample 16		
Sample 17		
Sample 18		
Sample 19		
Sample 20		

	NSSP Method	Proposed Method
Sample 21		
Sample 22		
Sample 23		
Sample 24		
Sample 25		
Sample 26		
Sample 27		
Sample 28		
Sample 29		
Sample 30		

Data Handling and Analysis:

Two methods of analysis are considered to be comparable when no significant difference can be demonstrated in their results. Since samples may cover a wide concentration range of the target analyte, regression line analysis is used for comparing the new method with the established method. Perform regression analysis with y-axis for the new method and x-axis for the established method, obtain the results of product-moment correlation coefficient (r), the slope (b) and the intercept (a) along with their lower and upper 95% confidence limit of the linear regression line from Regression analysis table (e.g., Excel-data analysis-regression). The ideal values for b = 1 and a = 0 should be included in the 95% confidence limits.

Data Reporting

Upon completion of the necessary experiments and data analysis, a written report summarizing the findings should be completed for submission with the SLV. The report should include the following:

1) Method title
2) Method submitter(s) and contact information
3) Intended use
4) Rationale for this method in the NSSP – Does this method meet an immediate or continued need, improve analytical capability, reduce cost, ease implementation, etc.?
5) Method principle/basis – Receptor binding assay, immunoassay, LC-MS, etc.
6) Target matrix/matrices – Describe the shellfish matrix or matrices that will be validated using the matrix category table available at NSSP Guide for the Control of Molluscan Shellfish, Section IV – Guidance Documents, Chapter III: Laboratory, .03 – Laboratory Method Matrix Extensions. Include details on the specific species to be used to represent each matrix category.
7) Target toxin(s)
8) Existing certifications of the method
9) Equipment required – Provide a list of specialized equipment needed to perform the method.
10) Reagents required – Provide a list of specialized chemicals, reagents, etc. needed to perform the method.
11) Proprietary aspects
12) Laboratory safety requirements – Describe the safety measures, beyond those of routine laboratory practices, required to perform the method.
13) Method cost – Provide an estimate of start-up costs, cost per analysis, etc.
14) Sample throughput – Provide a description of how many samples can be analyzed by this method in a given time frame.
15) References – Provide references that are pertinent and supplemental to the validation data submitted. This may include peer-reviewed publications in which the method was validated and/or applied, validation packages submitted to other entities, etc.
16) Standard Operating Procedure – Provide a detailed method procedure that is adequate to allow for replication of the method in additional laboratories.
17) Draft Method Evaluation Checklist – Following the format of other laboratory evaluation checklists (available at https://www.issc.org/nssp/2023-nssp-guide-control-molluscan-shellfish), and using the Standard Operating Procedure as a starting point, develop a draft checklist that laboratory evaluation officers could utilize to evaluate laboratory implementation of the method.
18) Overview of Quality Systems – Provide an overview of the quality assurance/quality control systems utilized in the developer(s)/submitter(s) laboratory. This should include any laboratory accreditation or conformance status, if available, or the laboratory Quality Assurance Manual.
19) Analysis of each parameter described in the "Validation Criteria" section below, including data tables/figures, summary of calculations, and final results highlighted, and an explanation of why each result demonstrates that the method fit for purpose.

20) All raw data used in the analyses should be presented in table form as appendices to the written report using the spreadsheets included in Appendix 1 of this document.