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.

4. Measurement Uncertainty: 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.

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 two aliquots of the sample homogenate appropriately sized for the work and spike one with the target analyte(s) at the action level. Do not spike the second aliquot as this is the sample blank. Process each aliquot to determine the concentration for the target analyte(s). Repeat this process with a minimum of 15 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.

5. Comparability: Comparability is the acceptability of a new or modified analytical method as an alternative or a substitute for an established method in the NSSP. To be acceptable the new or modified method must not produce a significant difference in results when compared to the officially recognized NSSP method. Comparability must be demonstrated for each substrate or type of interest by season and geographic area, if applicable.

Suggested procedure: For each shellfish type of interest use a minimum of 10-12 animals per sample and prepare as a homogenate. For each sample take two aliquots and analyze one by the officially recognized NSSP method and the other by the alternative test method. Naturally incurred samples having a variety of concentrations which span the range of the intended application of the method should be used in the comparison. Analyze a minimum of 20 paired samples, covering each season and a variety of growing areas. In cases where the occurrence of the target analyte(s) is intermittent, spiked samples can be used as described above for, but each spiked aliquot should be sub-aliquoted for analysis by both the officially recognized NSSP method and the alternative/test method.

Additional Information

References (Provide references that are pertinent and supplemental to the validation data submitted; these may include peer-reviewed publications in which the method was validated and/or applied, validation packages submitted to other entities, etc. Do not provide references in lieu of data in the "Validation Data" section.)

Standard Operating Procedure (SOP) (Provide a detailed procedure adequate for replication in additional laboratories.)

Laboratory Evaluation Checklist (Provide any additions and/or modifications to the current method checklist for laboratory evaluation based on inclusion of the new matrix/ces.)

Overview of Quality Systems (Provide an overview of the quality assurance/quality control systems utilized in the developer(s)/submitter(s) laboratory.)