Section IV: Guidance Documents, Chapter III. Laboratory, @.02 Methods, .04 Laboratory Method Platform Extensions

Laboratory methods approved for use within the National Shellfish Sanitation Program (NSSP) must be validated for their intended use before being adopted into the program. Each method approval is specific to the equipment, materials, and laboratory procedures used as part of the original validation study.

In some circumstances, modifications to validated methods are necessary to improve accessibility, adapt to new equipment or technology, or adapt to changes in availability or suitability of materials utilized in the original method. Additional studies must be done to demonstrate that these modified methods perform equivalently to the approved method before they can be used within the NSSP. In some situations, the modifications are significant enough that a full Single Lab Validation (SLV) is necessary. In other situations, a smaller extension study may be appropriate.

In the case of Real-Time PCR Methods, extension studies can be used to demonstrate equivalent method performance when the method modification involves any of the following:

1) New Platform/Instrument
    a. New company, same chemistry
    b. Same company, new model
2) Analysis changes (i.e. use of auto-thresholds instead of set thresholds)
3) Changes in detection hardware (i.e. optical system updated, closed vs. automated system)
4) Changes in cycle conditions (i.e. temperature or time)
5) Changes in extraction protocols
6) Changes in enzyme
7) Changes in reagents
8) Changes in primer/probe (i.e. new manufacturer, new structure, new type, new fluorophore)

If a method modification falls into any of these categories, then a platform extension study should be conducted and the data submitted to the ISSC for review following the process outlined in the ISSC Constitution, Bylaws, and Procedures, Procedure XV, starting with the required pre-proposal, and moving on to the validation study.

For guidance on performing a platform extension study for a Real-Time PCR Method, please refer to the platform extension guidelines available on the ISSC website.

Guidance has not yet been developed on what types of biotoxin method modifications can be validated through a platform extension. If you believe that your proposed biotoxin method modification can be validated through a platform extension, please contact the ISSC Laboratory Committee for consultation on how to proceed before undertaking the validation.

****Note: Pages 2-7 of this document are intended to be hosted on the Laboratory section of the ISSC website along with the other method validation guidelines and SOPs****

ISSC Platform Extension Guidelines

Suggested Procedures for Validating a Real-Time PCR Method Platform Extension

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 additional instrument platforms. These recommendations are intended for methods which have already undergone single laboratory validation (SLV) and are being considered for use with a different instrument or platform, which requires further validation.

Platform Extension Pre-Study Requirement

Prior to initiation of the platform extension study, an initial study should be conducted to determine the likelihood of successful completion of the platform extension. This initial study should be conducted in the absence of matrix (i.e. a pure culture isolate).

Pure culture of the target organism should be grown and diluted serially 10-fold to beyond the limit of detection. Dilutions should be spread plated on a non-selective media in triplicate, and the results should be averaged to determine the concentrations. Equivalent dilutions of three independently grown cultures of the same organism, each analyzed in triplicate, should be used to generate standard curves.

From these standard curves, the following performance criteria should be evaluated:

1) Method Sensitivity – Based on the lowest detectable copy number.
2) Reaction Efficiency – Based on the formula $E = 100 \times (-1+10^{(-1/slope)})$.
3) Repeatability – Based on the standard deviation of generated C_t values.

To be acceptable, method sensitivity should be <20 CFU/rxn, reaction efficiency should be 90-110%, and repeatability should average ≤ 0.5 C_t. If these criteria are met, then the full Platform Extension Study should be conducted in the appropriate matrix(ces) as described below. If these criteria are not met, the method should be further modified to produce acceptable results on the new platform, and a full SLV may be required.

Platform Extension Study

Note: These guidelines are designed for use by a laboratory that has access to the original method platform as well as the intended new platform. If the original platform is not available, it is recommended that the laboratory attempt to collaborate with a laboratory that has the original platform available, or that retains samples analyzed using the original platform, to generate the necessary data.

The purpose of the platform extension study is to generate data that can be analyzed to determine if a modified platform performs in an equivalent manner to the original method platform. Unlike with other ISSC validations, however, this suggested procedure does not directly assess performance characteristics for the modified method. Instead, characteristics like Accuracy, Sensitivity, Specificity, Precision, and overall Comparability are indirectly assessed by comparing the results generated by the original platform with results generated by the modified platform to ensure equivalent performance. This assessment evaluates the following:

- The modified method should correctly detect the presence or absence of a target pathogen or DNA sequence at an equivalent level to the original method.
- The modified method should have false negative and false positive rates equivalent to or better than the original method.
- The modified method should reliably detect the presence of the analyte at the Limit of Detection (LOD) that was determined through the original SLV.
- The modified method should demonstrate a comparable cycle threshold (Ct) to the original method.

To generate this data, the following experiments should be conducted, with all samples analyzed on both the validated platform and the proposed platform. The data from both experiments will be compiled into one complete dataset for analysis.

Experiment 1: Use shellfish free of the target organism(s). For each shellfish matrix of interest, use a minimum of 12 animals and prepare as a homogenate. Take four aliquots of the sample homogenate appropriately sized for the work and spike the first aliquot at or near the LOD for the validated method. Spike the second aliquot at a concentration 0.5 to 1 log higher and spike the third aliquot at the highest level of interest. Do not spike the fourth aliquot, as this will be used as the sample blank. To ensure the accuracy of the spikes, determine the concentrations of the organisms in each spike by plating (in triplicate and calculating the average) on a non-selective media.

For each aliquot, excluding the sample blank sub-aliquot for three replicate analyses. Process each replicate, as well as the blank (a total of 10 MPNs) according to the NSSP validated protocol. Run each extract on both the validated platform and the proposed platform. Repeat this process for a minimum of three shellfish samples collected from different growing areas, the same growing area harvested on different days, or from different process lots. Use the same target spike levels on each of the three shellfish samples. (Figure 1)

Experiment 2: Use naturally incurred samples. For each shellfish matrix of interest, use a minimum of 12 animals per sample and prepare as a homogenate. Process each sample using the established NSSP method and run each extract on both the validated platform and the proposed platform. Repeat this process for a minimum of five shellfish samples collected from different growing areas, the same growing area harvested on different days, or from different process lots. (Figure 2)

Data Analysis

Note: Because this analysis is assessing the relative performance of the two PCR platforms, each MPN tube subsequently tested via PCR counts as an individual "sample" for all the assessments below.

Data analysis for a platform extension should answer the following questions:

1) Does the modified method correctly detect the presence or absence of a target pathogen or DNA sequence at an equivalent level to the original method?
2) Does the modified method have false positive and false negative rates equivalent to or better than the original method?
3) Does the modified method reliably detect the presence of the analyte at the Limit of Detection that was determined through the original SLV?
4) Does the modified method demonstrate comparable cycle thresholds (Cts) to the original method?
5) Is this modified method comparable to the original method?

Several of the analyses below make use of this contingency table to determine the values to input into the equations. Complete this table utilizing the compiled results from experiments 1 and 2.

Contingency Table

	Validated PCR (+)	Validated PCR (-)	Total
Modified Assay (+)	A (True Positive)	B (False Positive)	A+B
Modified Assay (-)	C (False Negative)	D (True Negative)	C+D
Total	A+C	B+D	N (Total Samples)

Cohen's Kappa Coefficient (K) quantifies the level of agreement between the methods above what is expected by chance alone. Calculate K using this online calculator: https://www.graphpad.com/quickcalcs/kappa1/

Percent Positive Agreement (Estimated Sensitivity) = 100 x (A/(A+C))
Percent Negative Agreement (Estimated Specificity) = 100 x (D/(B+D))
Overall Percent Agreement (Total Accuracy) = 100 x ((A+D)/N)

McNemar's Test determines if the differences between discordant pairs (i.e. original method vs. modified method) are significant. Run McNemar's Test using the contingency table to complete this online calculator: https://www.graphpad.com/quickcalcs/mcnemar1/

The **Bland-Altman Plot** is a visual method used to analyze the agreement between two different measurement methods/instruments. Utilizing the C_t values from Experiments 1 and 2, create a data table as follows:

a. X = mean of each pair of measurements (validated platform and proposed platform)
b. Y = difference between each pair of measurements

Example:

Sample	X	Y
1	19.9	1.2
2	19.8	1.1
3	23.4	-1.2
4	36.6	-0.3
5	21.1	0.8

Create a scatter plot using these X,Y coordinate pairs. Calculate the overall average of all the differences and draw a horizontal line on the plot at this value (Bias Line). Calculate the standard deviation (SD) of all the differences and draw horizontal lines at the Bias Line ± 1.96(SD) (Limits of Agreement).

Use the following information to determine the success of the platform extension study:

Cohen's Kappa (K)	**Strength of Agreement**
0.81 to 1.00	Almost Perfect
0.61 – 0.80	Substantial
0.41 – 0.60	Moderate
<0.40	Fair to Poor (Method Re-optimization Required)

The Cohen's Kappa (K) must be between 0.81 to 1.00, indicating a near perfect-to-perfect agreement between the platforms.

Percent positive agreement, percent negative agreement, and overall percent agreement should all exceed 95%.

The results of the McNemar's Test should have a p-value >0.05, indicating no significant difference in results between the two platforms.

The data points on the Bland-Altman Plot should be tightly clustered around the zero line, the bias line should be close to the zero line, and the separation between the limits of agreement should be small.

Reporting

The report included in the final proposal's submission should include all the following:

1) A completed "ISSC Method Application and Single Lab Validation Summary of Required Elements for Acceptance of a Method for Use in the NSSP" form, available through the ISSC website. Section C of that form does not need to be completed for a platform extension. Information to complete Sections A, B, and D should focus only on the method modifications being assessed, not on the original method.
2) Raw data and summary results from the pre-study.
3) All the raw data associated with the platform extension study
4) A summary of the data analysis, highlighting specific results.
5) A revised laboratory evaluation checklist accounting for the changes made.

Figure 1: Flow diagram of Experiment 1. (Repeat two (2) additional times for a total of three (3) independent experiments.

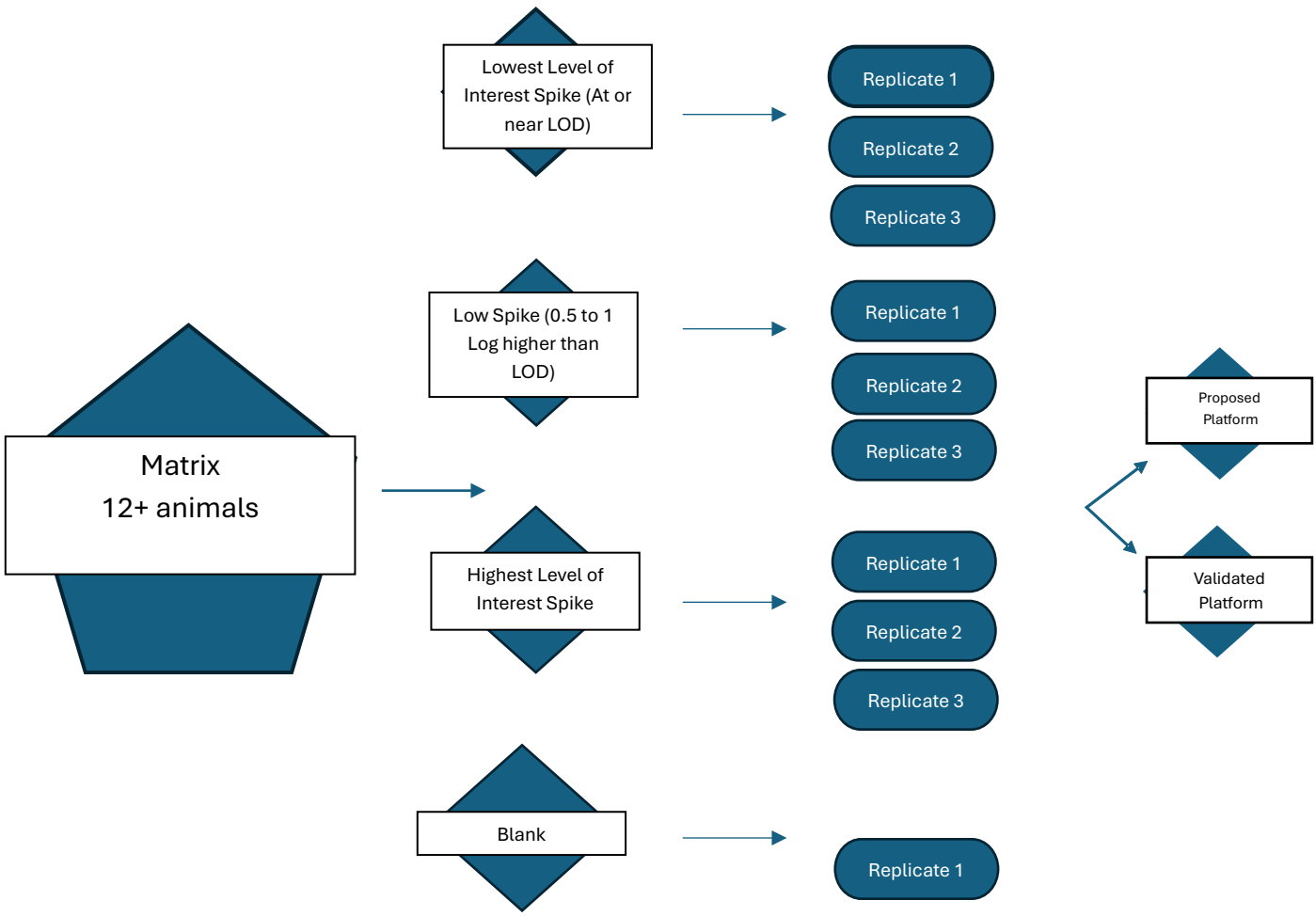
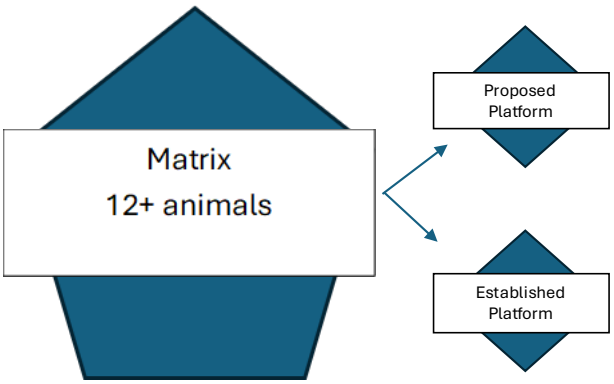


Figure 2: Flow diagram of Experiment 2. (Repeat four (4) additional times for a total of 5 independent experiments)



References:

1. Liu, K.C., Pires, G.S., Furseth, H.A., and Jinneman, K.C., **Report of a Single Laboratory Validation Study: Platform Extension of the Simplex Real-time PCR Method for Detection of Prohibited Materials in Animal Feed**., US Food and Drug Administration, LIB #4656
2. Saah, A.J., Hoover, D.R., **"Sensitivity" and "Specificity" Reconsidered: The Meaning of These Terms in Analytical and Diagnostic Settings**., *Annals of Internal Medicine*. 1997;126:91-94.
3. Association of Public Health Laboratories. **Laboratory test Verification and Validation Toolkit**. APHL website. Accessed August 14, 2026.
4. U.S. Food and Drug Administration Foods Program. (2023) **Guidelines for the validation of analytical methods for nucleic acid sequence-based analysis of food, feed, cosmetics, and veterinary products**. (Edition 1.2).
5. U.S. Food and Drug Administration Foods Program. (2019) **Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds**. (Edition 3.0).