Proposal No. **17-104**

Proposal for Task Force Consideration at the ISSC 2017 Biennial Meeting	a. ☒ Growing Area b. ☐ Harvesting/Handling/Distribution c. ☐ Administrative
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Proposal Subject	Guidance for verifying the performance of a 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.
Specific NSSP Guide Reference	Section IV Guidance Documents – Chapter II. Growing Areas
Text of Proposal/ Requested Action	Section IV Guidance Documents – Chapter II. Growing Areas .20 Quantitative Analytical Method Verification This guidance is provided to verify the performance of a 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. The following performance criteria are to be verified: recovery, precision (repeatability or 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** is the fraction or percentage of an analyte(s)/measurand(s)/organism(s) of interest recovered after sample analysis. **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). **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. **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 12 shellfish 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% 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 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 level for each sample analyzed.

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 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 case the target analyte(s)/measurand(s)/organism(s) of interest are intermittently 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.
Public Health Significance	With the number of new analytical methods being adopted for use in the NSSP, it is necessary to have a standardized approach to verify the successful transfer of the method from the originating laboratory/SLV submitter to the implementing laboratory before the method is placed in service.
Cost Information	Not Available