## VALIDATION CRITERIA

**Comparability** is the acceptability of a new or modified analytical method as a substitute for an established method in the NSSP. To be acceptable, the new or modified method must be specific for the analyte/measurand/organism of interest. Comparability must be demonstrated for each substrate or tissue type of interest by season and geographic area if applicable.

**Specificity of the new or modified method** is the ability of this new or modified method to measure only what it is intended to measure. To determine the specificity of new or modified methods, samples containing suspected interferences (interfering organisms/compounds/toxins) are analyzed in the presence of the analyte/measurand/target organism of interest.

**Procedure for demonstrating the specificity of the new or modified method:** This procedure is applicable for use with either growing waters or shellfish tissue. Make every effort to use samples free of the target analyte/measurand/organism of interest. For each shellfish tissue type of interest use a minimum of 10-12 animals per sample. For each sample take three (3) aliquots of either the shellfish homogenate or growing water sample appropriately sized for the work and spike two (2) of the three (3) with a low but determinate level (by the method/modified method under study) of the target analyte/measurand/organism of interest. Take one of these two (2) aliquots and also spike it with a moderate to high level of a suspected interfering organism/compound/toxin if not naturally incurred. Do not spike the third aliquot. This is the sample blank. Process each aliquot, the sample blank , the aliquot spiked with the target analyte/measurand/organism of interest and the aliquot spiked with the target analyte/measurand/organism of interest in the presence of the suspected interfering organism/compound/toxin as usual to determine the method/modified method concentration for the target analyte/measurand/organism of interest. Do five (5) replicates for each aliquot excluding the sample blank. Do one (1) sample blank per analysis. Repeat this process for all suspected interfering organisms/compounds/toxins.

**Data for demonstrating the specificity of the new or modified method:**

Name of suspected interfering organism/compound/toxin #1 _____

Sample type _____

Sample blank concentration for the target analyte/measurand/organism of interest _____

| | Conc. of aliquot spiked with target analyte/measurand/organism of interest | Conc. of aliquot spiked with target analyte/measurand/organism of interest of interest in the presence of suspected interfering organism/compound/toxin |
|---|---|---|
| Replicate 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |

**Repeat for each suspected interfering organism/compound/toxin tested.**

**Data handling for demonstrating specificity of the new or modified method**
The specificity index will be used to test the specificity of the new or modified method in the presence of suspected interfering organisms/compounds/toxins. The specificity index (SI) is calculated as indicated below:

$$\text{Specificity index (SI)} = \frac{\text{Sample spiked with only target of interest}}{\text{Sample spiked with target in presence if suspected interferences}}$$

All microbiological count data must be converted to logs before statistical analysis. Samples spiked with both the target analyte/measurand/organism of interest and the target analyte/measurand/organism of interest in the presence of a suspected interfering organism/compound/toxin may have to be corrected for matrix effects before determining the Specificity index (SI). The sample blank accompanying the analysis is used for this purpose. Any correction that may be necessary to microbiological data for matrix effects are done using log transformed data.

The Specificity index (SI) should equal one (1) in the absence of interferences. To test the significance of a Specificity index (SI) other than one (1) for any suspected interfering organism/compound/toxin, a two-sided t-test at the .05 significance level is used. For each suspected interfering organism/compound/toxin calculate the average Specificity index ($SI_{avg}$) for the five (5) replicates analyzed for each sample by obtaining the average concentration for both the aliquot containing the target analyte/measurand/organism of interest only and the aliquot containing the target analyte/measurand/organism of interest in the presence of suspected interfering organisms/compounds/toxins and using the formula below.

$$SI_{avg} = \frac{\text{Avg. conc. of sample spiked only with target of interest}}{\text{Avg. conc. of sample spiked with target in the presence of suspected interferences}}$$

Perform the t-test to determine if the average Specificity index (SI) obtained from the five (5) replicates from each analysis differs from one (1). Repeat for all the suspected interfering organisms/compounds/toxins tested.

**Data summary for testing the specificity of the new or modified method:**

Interfering organism/compound/toxin #1 _____ $SI_{avg}$ _____
SI different from 1 _____

Interfering organism/compound/toxin #2 _____ $SI_{avg}$ _____
SI different from 1 _____

Interfering organism/compound/toxin #3 _____ $SI_{avg}$ _____
SI different from 1 _____

Interfering organism/compound/toxin #n _____ $SI_{avg}$_____
SI different from 1 _____