

VALIDATION CRITERIA

Specificity is the ability of the method to measure only what it is intended to measure. To determine method specificity samples containing suspected interferences (interfering organisms/compounds/toxins) are analyzed in the presence of the analyte/measurand/targeted organism of interest.

Procedure: This procedure is applicable for use with either growing waters or shellfish tissue. Make every effort to use samples free of the targeted 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 under study) of the targeted 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 targeted analyte/measurand/organism of interest and the aliquot spiked with the targeted analyte/measurand/organism of interest in the presence of the suspected interfering organism/compound/toxin as usual to determine the method concentration for the targeted analyte/measurand/organism of interest. Do five (5) replicates for each aliquot excluding the sample blank. Do one sample blank per analysis. Repeat this process for all suspected interfering organisms/compounds/toxins.

Data:

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

Sample type _____

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

Concentration of aliquot spiked
with targeted analyte/measurand/
organism of interest

Concentration of aliquot spiked
with targeted analyte/measurand/
organism of interest and
suspected interfering organism/
compound/toxin

Replicate 1

2

3

4

5

Repeat for each suspected interfering organism tested.

DATA HANDLING

The **Specificity index** will be used to test the specificity of the method in the presence of suspected interfering organisms/compounds/toxins. The **Specificity index (SI)** is calculated as indicated below:

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

All microbiological count data must be converted to logs before analysis. Samples spiked with both the targeted analyte/measurand/organism of interest and the targeted anaalyte/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 corrections that may be necessary to microbiological data for matrix effects are done using log transformed data.

The Specificity index should equal one (1) in the absence of interferences. To test the significance of a Specificity index other than one (1) for any suspected interfering organism/compound/toxin, a two-sided t-test is used. For each suspected interfering organism/compound/toxin calculate the average **Specificity Index (SI)** for the 5 replicates

analyzed for each sample by obtaining the average concentration for both the aliquot containing the targeted analyte/measurand/organism of interest only and the aliquot containing the targeted analyte/measurand/organism of interest in the presence of suspected interfering organisms/compounds/toxins and using the formula below.

$$SI_{avg} = \frac{\text{Avg concentration of sample spiked with target of interest only}}{\text{Avg concentration of sample spiked with both target and suspected interferences}}$$

Perform a two-sided t-test at the .05 significance level to determine if the average Specificity index (SI) obtained from the 5 replicates of each analysis differs from one (1).
Repeat for all interfering organisms/compounds/toxins tested.

Data Summary:

Interfering organism/compound/toxin #1 _____ SI_{avg} _____
Significant difference from 1 _____

Interfering organism/compound/toxin #2 _____ SI_{avg} _____ Significant difference from 1 _____

Interfering organism/compound/toxin #3 _____ SI_{avg} _____
Significant difference from 1 _____

Interfering organism/compound/toxin #n _____ SI_{avg} _____ Significant difference from 1 _____