

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 (i.e. interfering organisms) are analyzed in the presence of the analyte/measurand (target 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 target organism. 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 determinable level (by the MPN based method under study) of the target 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. Do not spike the third aliquot. This is the sample blank. Process each aliquot, the blank, the aliquot spiked with the target organism and the aliquot spiked with the target organism plus the suspected interfering organism as usual to determine the method MPN. 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.

Data:

Name of suspected interfering organism #1 _____

Sample type _____

Sample blank MPN _____

MPN of aliquot spiked
with target organism

MPN of aliquot spiked with target
organism and suspected
interfering organism

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. The **Specificity index (SI)** is calculated as indicated below:

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

All MPN count data is converted to logs. Samples spiked with the target organism and the target organism in the presence of a suspected interfering organism 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 for matrix effects are done using the 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, a two-sided t-test is used. For each suspected interfering organism calculate the average **Specificity Index (SI)** for each of the 5 replicates analyzed for each sample by obtaining the average log MPN for both target organisms only and target organisms in the presence of suspected interfering organisms and using the formula below.

$SI_{avg} = \frac{\text{Avg log MPN of sample spiked with target organism only}}{\text{Avg log MPN of sample spiked with both target and suspected interfering organism}}$

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 tested.

Data Summary:

Interfering organism #1 _____	SI _{avg} _____	Significant difference from 1 _____
Interfering organism #2 _____	SI _{avg} _____	Significant difference from 1 _____
Interfering organism #3 _____	SI _{avg} _____	Significant difference from 1 _____
Interfering organism #n _____	SI _{avg} _____	Significant difference from 1 _____