VALIDATION CRITERIA

5. **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 of interest (*Vibrio vulnificus*, *Vibrio parahaemolyticus*, etc).

Procedure: For each shellfish tissue type of interest use a minimum of 10-12 animals per sample. For each sample take three (3) aliquots of homogenate appropriately sized for your work and spike two (2) of the three (3) with a low but determinable level (by QPCR MPN) of *Vibrio vulnicus* or *Vibrio parahaemolyticus* as appropriate. Take one of these two (2) aliquots and 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 either *Vibrio vulnificus* or *Vibrio parahaemolyticus* and the aliquot spiked with *Vibrio vulnicus* or *Vibrio parahaemolyticus* plus the suspected interfering organism as usual to determine the QPCR 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 blank, QPCR MPN _____

	Aliquot spiked with Vv or Vp, QPCR MPN	Aliquot spiked with Vv or Vp and interfering organism, QPCR MPN
Replicate 1		
2		
3		
4		
5		

Repeat for each suspected interfering organism tested.

DATA HANDLING

5. **Specificity** – 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.

$$\textbf{Specificity index (SI)} = \frac{\textbf{Sample spiked with analyte}}{\textbf{Sample spiked with analyte and suspected interference}}$$

Samples spiked with analyte and analyte in the presence of suspected interferences may have to be corrected for matrix effects before determining the Specificity index (SI). A sample blank must accompany the analysis and is used for this purpose.

Rationale: 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.

Procedure: For each suspected interfering organism calculate the Specificity index (SI) for each of the 5 replicates analyzed for each sample using the formula below.

$$SI = \frac{\text{Log QPCR MPN of sample spiked with analyte}}{\text{Log QPCR MPN of sample spiked with analyte and suspected interference}}$$

Perform the two-sided t-test to determine if the average specificity index (SI) obtained from the 5 replicates of each analysis differs from one (1).

1. Let the significance level, $\alpha = .05$
2. Look up $t_{1-\alpha/2}$ for n-1 degrees of freedom (n = 4) in the Table of the distribution of *t*.
3. Calculate the average Specificity index (SI_{avg}) from the data for each analysis.
4. Calculate the standard deviation (s).
5. Calculate u, $u = t_{1-\alpha/2}\, s/n^{0.5}$, n = # of replicates per analysis.
6. If $[SI_{avg} - m_o] > u$, where $m_o = 1$ decide that the average Specificity index (SI_{avg}) differs from one (1) and that the method may not be specific for the analyte (i.e. *Vibrio vulnificus* or *Vibrio parahaemolyticus*)

Repeat this analysis 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 _____