VALIDATION CRITERIA

1. **Accuracy/Trueness** is the closeness of agreement between test results and the accepted reference value. To determine method accuracy, the concentration of *Vibrio vulnificus* or *Vibrio parahaemolyticus* as measured by QPCR MPN is compared to a reference concentration.

2. **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.

Procedure: For each shellfish type of interest use a minimum of 10-12 animals per sample. For each sample take two (2) aliquots of homogenate appropriately sized for your work and spike one(1) of the two (2) aliquots with a suitable concentration of either *Vibrio vulnificus* or *Vibrio parahaemolyticus* as appropriate. Do not spike the second aliquot. This is the sample blank. Determine the concentration of *Vibrio vulnificus* or *Vibrio parahaemolyticus* used to spike each sample by plating on appropriate agar. Process both aliquots of sample homogenate as usual to determine the QPCR MPN. Do twenty (20) samples for each shellfish tissue type of interest. **Use a range of concentrations (from low but detectable by QPCR MPN through 10^7) to spike sample homogenates**. Use samples from a variety of growing areas, the same growing area harvested on different days or from different process lots.

Data:

Sample	Plate count (CFU)	Sample blank, QPCR MPN	Spiked sample, QPCR MPN
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

DATA HANDLING

1. **Accuracy/Trueness** – Data handling

The accuracy/trueness of a method consists of two distinct components, the portion due to the method itself regardless of the laboratory performing it and the portion contributed by the laboratory's performance. In a single laboratory method validation, it is impossible to distinguish the contribution of each to the overall accuracy/trueness of the method. Consequently, what is being estimated is the accuracy/trueness of the method as implemented by the laboratory performing the analysis. Good accuracy/trueness suggests the appropriateness of the method and the laboratory's performance of it for the intended work. Poor accuracy/trueness on the other hand indicates the unsuitability of the method and/or the laboratory's performance of it for the intended work. Accuracy/trueness will be determined by calculating the closeness of agreement between the test results and an accepted reference value obtained by plate count.

Procedure: To determine the accuracy/trueness of the method as implemented by the laboratory over the range in concentrations important in post harvest processing operations or for implementation of the Interim Control Plan, the data is worked-up in the following manner.

1. If necessary use the sample blank to correct the QPCR MPN of the spiked sample for matrix effects.
2. Convert plate counts and QPCR MPNs to logs.
3. Calculate the average plate count of the data in logs.
4. Calculate the average QPCR MPN of the data in logs.
5. Divide the average QPCR MPN in logs by the average plate count in logs.
6. Multiply the quotient by 100. This provides an estimate in percent of the accuracy/trueness of the method as implemented by the laboratory over the range in concentrations of *Vibrio vulnificus* or *Vibrio parahaemolyticus* of importance in post harvest processing operations or for implementation of the Interim Control Plan.

2. **Measurement uncertainty** – Data handling

Measurement uncertainty can be determined by subtracting the QPCR MPN results for each sample from the accepted reference values for the samples as determined by the accompanying plate count and calculating the 95% confidence interval of these differences. The confidence interval of these differences represents the range in values within which the true measurement uncertainty lies. A narrow range in values indicates that the method as implemented by the laboratory produces reliable results. A wide range in values suggests that the method and/or the laboratory's implementation of it may not be suitable for the intended work.

Procedure: Use the log transformed data for both the plate count and the QPCR MPN results. Calculate the two-sided 95% confidence interval for the difference in log counts between the reference (plate count) and the QPCR MPN method.

1. Let $\alpha = .05$ and $1-\alpha = .95$ %, the confidence interval for the test.
2. Subtract the (corrected if necessary) log QPCR MPN for each sample tested from its accompanying log plate count value.

3. Calculate the average (X_{avg}) of these values. This is the average difference between the reference and test values.
4. Calculate the standard deviation (s) of these values. This is the standard deviation of the difference in counts between the reference and test method.
5. Look up $t = t_{1-\alpha/2}$ for n-1 degrees of freedom (n =19) in the Table of the distribution of *t*.
6. Calculate x_u and $x_{L:}$

$$x_u = X_{(avg)} + t(s/n^{0.5})$$
$$x_L = X_{(avg)} - t(s/n^{0.5})$$

7. Take the antilog of x_u and x_L.
8. The interval from x_L to x_u is the 95% confidence interval of the average difference between the reference and test method. This is the measurement uncertainty of the method as implemented.