VALIDATION CRITERIA

Accuracy/Trueness is the closeness of agreement between test results and the accepted reference value. To determine method accuracy/trueness, the concentration of the target organism of interest as measured by the MPN based method under study is compared to a reference concentration.

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: This procedure is applicable for use with either growing waters or shellfish tissues. Make every effort to use samples free of the targeted organism of interest. For each shellfish type of interest use a minimum of 10-12 animals per sample. For each sample take two (2) aliquots of either the homogenate or growing water sample appropriately sized for your work and spike one(1) of the two (2) aliquots with a suitable concentration of the target organism of interest. Do not spike the second aliquot. This is the sample blank. Determine the concentration of the target organism of interest used to spike each sample by plating on appropriate agar. Process both aliquots of sample as usual to determine the method MPN. For growing waters do twenty (20) samples collected from a variety of growing areas. For shellfish do twenty (20) samples for each shellfish tissue type of interest collected from a variety of growing areas, the same growing area harvested on different days or from different process lots. **Use a variety of concentrations spanning the range of counts (the working range) of importance in the application of the method to spike sample homogenates or growing water samples**. Both the low and high level spike concentrations must be determinable by the MPN based method under study.

Data:
Working Range _____
Sample Type _____
Agar used to determine spike concentration _____
Organism used for spiking _____

Sample	Plate count (CFU)	Sample blank, MPN	Spiked sample, MPN
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			

Sample	Plate count (CFU)	Sample blank, MPN	Spiked sample MPN
14			
15			
16			
17			
18			
19			
20			

For shellfish samples, repeat for each tissue type of interest.

DATA HANDLING

Accuracy/Trueness

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 potential 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 a reference value obtained by plate count.

To determine the accuracy/trueness of the method as implemented by the laboratory over the range in concentrations important to the intended application of the method, the data is worked-up in the following manner.

1. Convert plate counts and MPNs to logs.
2. If necessary use the sample blank to correct the MPNs of the spiked samples for matrix effects.
3. Calculate the average plate count of the data in logs.
4. Calculate the average MPN of the data in logs.
5. Divide the average 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 importance to the intended application of the method.

Measurement uncertainty

Measurement uncertainty can be determined by subtracting the MPN results for each sample from the reference values for the samples as determined from the accompanying plate counts in logs 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.

Use the log transformed data for both the plate count and the MPN results. If necessary use the sample blank to correct the MPNs of the spiked sample for matrix effects and calculate the two-sided, 95% confidence interval for the difference in log counts between the reference (plate count) and the MPN method under study. This range in counts represents the measurement uncertainty of the method as implemented by the laboratory.

Data Summary:

Calculated % accuracy/trueness _____
Calculated measurement uncertainty _____