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 targeted analyte/measurand/organism of interest as measured by the analytical 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 target analyte/measurand/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 known concentration of the target analyte/measurand/organism of interest. Do not spike the second aliquot. This is the sample blank. For microbiological methods determine the concentration of the target organism of interest used to spike each sample by plating on/in appropriate agar. Process both aliquots of sample as usual to determine the method concentration for the target analyte/measurand/organism of interest. 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 concentrations 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 yield determinate values when analyzed by the method under study.

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

Sample	Spike conc/plate count	Sample blank conc	Spiked sample conc from analysis
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Sample	Spike conc/plate count	Sample blank conc	Spiked sample conc from analysis
11			
12			
13			
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 either a known reference value or a reference value obtained by plate count for microbiological methods.

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 to logs.
2. If necessary use the sample blank (converted to logs for microbiological methods) to correct the results from the spiked samples for matrix effects.
3. Calculate the average reference concentration of the analyte/measurand used to spike the samples; or, for microbiological methods calculate the average plate count of the data in logs. The average plate count represents the average reference concentration for the microbiological method.
4. Calculate the average concentration of the analyte/measurand/organism of interest in the spiked samples. For microbiological methods log transformed data is used for this calculation.
5. Divide the average concentration calculated from the spiked samples by the average reference concentration.
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 results for each spiked sample from the reference value for the sample 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 microbial results obtained from the spiked samples. If necessary use the sample blank (converted to logs for microbiological methods) to correct the spiked sample for matrix effects and calculate the two-sided, 95% confidence interval for the difference in concentrations between the reference and the spiked samples. This range in counts represents the measurement uncertainty of the method as implemented by the laboratory.

Data Summary:

Calculated % accuracy/trueness _____
Calculated measurement uncertainty _____