VALIDATION CRITERIA

Precision is the closeness of agreement between independent test results obtained under stipulated conditions.

Recovery is the fraction or percentage of an analyte/measurand/organism of interest recovered following sample analysis.

Procedure: This procedure is applicable for use with either growing waters or shellfish tissue. 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 four (4) aliquots of either the shellfish homogenate or growing water sample appropriately sized for the work. Spike one of the four aliquots with a low (but determinable by the method under study) concentration of the target analyte/measurand/organism of interest. Spike the second aliquot of the growing water sample or shellfish homogenate with a medium concentration of the target analyte/measurand/organism of interest. Spike the third aliquot of the growing water sample or shellfish homogenate with a high (but determinable by the method under study) concentration of the target analyte/measurand/organism of interest. Do not spike the fourth aliquot of the growing water sample or shellfish homogenate. This is the sample blank. Spiking levels must cover the range in concentrations important to the application of the method (working range). For microbiological methods determine the concentration of the target organism of interest used to spike each aliquot by plating in/on appropriate agar. Process each aliquot including the sample blank as usual to determine the method concentration for the target analyte/measurand/organism of interest. Do two (2) replicates for each of the three (3) spiked aliquots. Replicate analysis is unnecessary for the sample blank. Do only one sample blank per sample. For growing waters, do ten (10) samples collected from a variety of growing areas. For shellfish, do ten (10) 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 the same spiking levels for each of the ten (10) samples analyzed in this exercise (i.e. 10^1, 10^3 and 10^5).

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

Sample	Spike conc/Plate count/Conc of blank	Conc in spiked sample from analysis
1L		$1L_a$
		$1L_b$
1M		$1M_a$
		$1M_b$
1H		$1H_a$
		$1H_b$
1B		
2L		$2L_a$
		$2\ L_b$
2M		$2M_a$
		$2M_b$
2H		$2H_a$
		$2H_b$
2B		
"		"
"		"
"		"
"		"
10L		$10L_a$
		$10L_b$
10M		$10M_a$
		$10M_b$
10H		$10H_a$
		$10H_b$
10B		

L, M and H refer to low, medium and high concentrations respectively. L_a, L_b, M_a, M_b, H_a and H_b refer to the replicate determinations of the sample aliquots spiked with low (L), medium (M) and high (H) concentrations of the target analyte/measurand/organism of interest. B refers to the sample blank.

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

DATA HANDLING

Precision

To determine the precision of the method as implemented by the laboratory over the range in concentrations important to the intended application of the method, the data is manipulated in the following manner:

1. Convert the plate counts and spiked sample results for the microbiological methods to logs.
2. If necessary, use the sample blank (converted to logs for the microbiological methods) to correct the results from the spiked samples for matrix effects.
3. Perform a nested or hierarchical analysis of variance (ANOVA) on the corrected spiked sample data using the following variance components.

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Samples	9		
Concentrations in samples	20		
Determinations within concentrations	30		
Total	59		

4. Calculate the variance ratio (F) at the 95% confidence interval for the variance components, concentrations in samples/determinations within concentrations. If the variance ratio is significant this indicates that the precision of the method as implemented by the laboratory is not consistent over the range in concentrations important to the intended application.

If the variance ratio is not significant, calculate the coefficient of variation of the spiked sample data by:

1. Calculating 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.
2. Calculate the standard deviation of the spiked sample data by taking the square root of the nested ANOVA variance component, **Total**.
3. Divide the standard deviation of the spiked sample data by the average concentration of the analyte/measurand/organism of interest calculated for the spiked samples. For microbiological methods log transformed data is used for this calculation; and,
4. Multiply the quotient above by 100. This is the coefficient of variation of the method over the range of concentrations of importance in the application of the method as implemented by the laboratory.

Recovery

The recovery of the target analyte/measurand/organisms of interest must be consistently good over the range of concentrations of importance to the application of the method under study to be of benefit in the intended work. To determine whether recovery by the method as implemented by the laboratory is consistent over the range in concentrations important to the application of the method, the data is manipulated in the following manner:

1. Convert plate count and spiked sample results for the microbiological methods 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. For each sample determine the average of the replicates at each concentration such that there is only one value, the average of the two replicates at each concentration tested.
4. For each sample subtract the average for the replicates from its associated spike concentration/plate count value.
5. Perform a one way analysis of variance (ANOVA) on the data formatted by sample concentration with the following variance components:

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Concentration	2		
Error	27		
Total	29		

6. Calculate the variance ratio (F) at the 95% confidence interval for the mean square for concentration divided by the mean square for error. If the variance ratio or F test is significant at the 95% confidence interval, perform Tukey's Honestly Significant Difference (HSD) to compare recovery by concentration. A significant F test suggests that recovery of the method as implemented by the laboratory is not consistent over the range in concentrations important to the application of the method and may not be suitable for the work intended.

   If the variance ratio or F test is not significant at the 95% confidence interval, conclude that the recovery is consistent over the range in concentrations important to the application of the method and calculate the overall percent recovery of the method as implemented by the laboratory.

To determine the percent recovery of the method as implemented by the laboratory, the data is manipulated in the following manner:

1. Use log transformed data for microbiological methods.
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 spike concentration/plate count by summing over concentrations and dividing by 30.
4. Calculate the average concentration of analyte/measurand/organism of interest in the spiked samples from the analysis by summing over concentrations and replicates and dividing by 60.
5. Divide the average concentration of analyte/measurand/organism of interest from the analysis of the spiked samples by the average concentration from the spike/plate counts then multiply by 100. This is the percent recovery of the method as implemented by the laboratory.

Data Summary:

- Is the variance ratio at the 95% confidence interval for the variance components, concentrations in samples/determinations within concentrations significant? **Y/N**
- If the variability of the method as implemented by the laboratory is consistent over the range in concentrations important to its intended applications, what is the coefficient of variation? **NA/_____%**
- Is the one way analysis of variance to determine the consistency of recovery of the method under study significant? **Y/N**
- At what concentrations is the one way analysis of variance significant? **NA/_____**
- What is the overall percent recovery of the MPN based method under study? **NA/_____%**