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 or measurand 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 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 MPN based method under study) concentration of the target organism of interest. Spike the second aliquot of the growing water sample or shellfish homogenate with a medium concentration of the target organism of interest. Spike the third aliquot of the growing water sample or shellfish homogenate with a high (but determinable by the MPN based method under study) concentration of the target 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 counts important to the application of the method (working range). Determine the concentration of the target organism of interest used to spike each aliquot by plating on appropriate agar. Process each aliquot including the sample blank as usual to determine the method MPN. 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	Plate count (CFU)/MPN of Blank	Spiked Sample MPN
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 organism of interest. B refers to the sample blank.

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

DATA HANDLING

Precision

The MPN provides the means by which these methods become quantitative in application. As an MPN, they are limited in their maximum level of precision to that achievable through the combination of tubes and dilution ratios employed. In practice, MPN methods are limited to the maximum precision described by the equation $\mathbf{0.5487/n^{0.5}(D)}$ where n is the number of tubes in each dilution and D is the log of the dilution ratio used. In order for these MPN based methods to be effective, they should not be significantly more variable than the MPN test at their basis over the entire range of concentrations important for the intended application (working range).

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. Calculate the precision of the MPN based test under study from the equation $0.5487/n^{0.5}(D)$; where n = the number of tubes in each dilution and D is the log of the dilution ratio used.
2. Convert plate counts and MPNs for the spiked samples to logs.
3. If necessary, use the sample blank to correct the MPNs of the spiked samples for matrix effects.
4. 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		

5. 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, compare the standard deviation (Mean Square$^{0.5}$) of the ANOVA variance component, Total to the standard deviation for the MPN test being used [$0.5487/n^{0.5}(D)$] by performing a one-sided t-test at the .05 significance level to determine if the variability of the MPN method under study exceeds the variability permissible based on the combination of tubes and dilutions used.

Recovery

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

1. Convert plate count and MPN data for the spiked samples to logs.
2. If necessary, use the sample blank to correct the MPNs of the spiked samples for matrix effects.
3. For each sample determine the average in logs of the replicate MPN counts at each concentration such that there is only one log value, the average of the two replicate counts at each concentration.
4. For each sample subtract the average MPN count in logs from its associated log plate count value at each concentration.

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 MPN based method under study 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 MPN based method under study 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. Convert plate count and MPN data for the spiked samples into 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 in logs by summing over concentrations and dividing by 30.
4. Calculate the average MPN in logs by summing over concentrations and replicates and dividing by 60.
5. Divide the average MPN in logs by the average plate count in logs and 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**
- Does the variability of the MPN method under study exceed the variability permissible for the MPN method based on the combination of tubes and dilutions being used? **NA/Y/N**
- Is the one way analysis of variance to determine the consistency of recovery of the MPN based 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/_____%**