VALIDATION CRITERIA

Linear Range is the range within the working range where the results are proportional to the concentration of the analyte or measurand present in the sample.

Limit of Detection is the minimum concentration at which the analyte or measurand can be identified.

Limit of Quantitation/Sensitivity is the minimum concentration of the analyte or measurand that can be quantified with an acceptable level of precision and accuracy under the conditions of the test.

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 at least six (6) aliquots of either the growing water sample or shellfish homogenate appropriately sized for your work and spike five (5) of the six (6) aliquots with five (5) different concentrations (i.e. 10^a, 10^b…10^n) of the target organism of interest spanning the working range/range of interest of the method under study. Do not spike the sixth or last aliquot of each sample. This is the sample blank. Determine the concentration of the target organism of interest used to spike each aliquot of each sample by plating on appropriate agar. Process each aliquot including the sample blank by the method MPN. Do two (2) replicates for each aliquot excluding the sample blank. Do only one 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 (10^a, 10^b…10^n).

Data:
Sample type _____
Working range/Range of interest _____
Spiking levels _____
Agar used to determine spike concentration _____
Organism used for spiking _____

Spike level	**0****	**10^a**	**10^b**	**10^c**	**10^d ….. 10^n**
Sample 1					
Plate count (cfu)*					
Log MPN, replicate 1					
Log MPN, replicate 2					
Sample 2					
Plate count (cfu)*					
Log MPN, replicate 1					
Log MPN, replicate 2					

Spike level	**0****	**10^a**	**10^b**	**10^c**	**10^d ….. 10^n**
Sample 3					
Plate count (cfu)*					
Log MPN, replicate 1					
Log MPN, replicate 2					
Sample 4					
Plate count (cfu)*					
Log MPN, replicate 1					
Log MPN, replicate 2					
Sample 5					
Plate count (cfu)*					
Log MPN, replicate 1					
Log MPN, replicate 2					

Sample 6
Plate count (cfu)*
Log MPN, replicate 1
Log MPN, replicate 2

Sample 7
Plate count (cfu)*
Log MPN, replicate 1
Log MPN, replicate 2

Sample 8
Plate count (cfu)*
Log MPN, replicate 1
Log MPN, replicate 2

Sample 9
Plate count (cfu)*
Log MPN, replicate 1
Log MPN, replicate 2

Sample 10
Plate count (cfu)*
Log MPN, replicate 1
Log MPN, replicate 2

***Plate count converted to logs**
****** Unspiked sample blank**
10^a, 10^b, 10^c, 10^d, and 10^n represent the spiking levels

For shellfish samples repeat for each tissue type of interest.

DATA HANDLING

Linear Range

In an MPN the more target organisms present the greater the number of tubes and dilutions expected to be positive. The more positive tubes in each dilution, the higher the MPN count will be. Thus a linear relationship must exist between the number of target organisms and the method of detecting positives. In this case a linear relationship must exist between the number of target organisms present introduced through spiking and the MPN based method used to detect and quantify them.

Procedure:
If necessary use the sample blank to correct the replicate MPNs of the spiked samples for matrix effects. To determine if a linear relationship exists between the number of target organisms present in the samples and the MPN generated by the method as implemented by the laboratory, calculate the correlation coefficient (Pearson's r) and test for significance by performing a two-sided t-test at the .05 significance level. If Pearson's r is significant a linear relationship is supported between the number of target organisms present and the MPN based method used to detect and quantify them.

If Pearson's r is significant plot the data for the log MPN of the samples on the y-axis versus the plate count in logs on the x-axis and calculate the equation of the line which results.

Limit of Detection

In an MPN based test, one organism should be capable of producing a positive test. Consequently one target organism cell should be the limit of detection of any MPN based method.

Procedure:
Assuming that the relationship between the number of target organisms present and the MPN based method used to detect and quantify them is linear, the equation of the line describing this relationship can be used to determine the limit of detection of the method as implemented.

The equation of the line is given by the formula, **y = mx +b** where: **y** is the log MPN of the samples; **m** is the slope of the line describing the relationship between the number of target organisms present and the MPN of the method as implemented; **x** is the number of target organisms spiked into the sample and **b** is the y-intercept of the line. In order to determine the limit of detection of the method as implemented, set **x** in the above equation equal to one (1), the theoretical limit of detection of the MPN based method and solve the equation for **y**. Convert **x** = 1 to its log value which is zero (0). The terms **mx** in the equation then cancel out and **y** is equal to **b**, the y-intercept of the line. Take the antilog of **b**, the y-intercept. This value is the limit of detection of the method as implemented. If the limit of detection as implemented is a value other than one (1), it must be determined whether this value is significantly different from one. To do this, the 95% confidence interval estimate for the y-intercept is determined. If this confidence interval estimate encompasses the y-intercept derived from the data of the line, then it can be concluded that the limit of detection of the method as implemented is one cell consistent with the MPN requirement that a single cell should be able to produce a positive test.

Limit of Quantitation/Sensitivity
If the method as implemented by the laboratory is capable of detecting one (1) cell then the limit of quantitation/sensitivity is easily calculated. Because the method under study is MPN based, the bacterial concentration that can be quantified with an acceptable level of precision and accuracy depends on the number of tubes used for each dilution and the dilution ratio employed. As an example, use of a 3-tube MPN and a dilution ratio of 0.01, 0.001 and 0.0001 will result in a limit of quantitation/sensitivity of 3.6 MPN/gram or 36 MPN per ml.

Data Summary:
Correlation coefficient (Pearson's r) calculated is _____
The equation for the line obtained is _____.
Is Pearson's r significant Y/N
Antilog of the y-intercept (limit of detection) _____
95% confidence interval estimate for the y-intercept (limit of detection) if applicable_____
Limit of quantitation/ sensitivity of the method as implemented _____