VALIDATION CRITERIA

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

Limit of Detection is the minimum concentration at which the analyte/measurand/organism of interest can be identified.

Limit of Quantitation/Sensitivity is the minimum concentration of the analyte/measurand/organism of interest 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 analyte/measurand/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 analyte/measurand/organism of interest spanning 50 – 150% of the working range/range of interest for the method under study. Do not spike the sixth or last aliquot of each sample. This is the sample blank. For microbiological methods determine the concentration of the target analyte/measurand/organism of interest used to spike each aliquot of each sample by plating in/on appropriate agar. Do not use aliquots of the same master solution/culture to spike all the samples in this exercise. A separate master solution /culture should be used for each sample. Process each aliquot including the sample blank as usual to determine method concentration for the target analyte/measurand/organism of interest. Do three (3) 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 _____
Range in spiking levels used _____
Agar used to determine spike concentration _____
Organism used for spiking _____

Aliquot	**0***	**1**	**2**	**3**	**4**	**5**
Sample 1						
Spike conc./plate count						
Response, replicate 1						
Response, replicate 2						
Response, replicate 3						

Aliquot	**0***	**1**	**2**	**3**	**4**	**5**
Sample 2						
Spike conc./plate count						
Response, replicate 1						
Response, replicate 2						
Response, replicate 3						

Sample 3
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

Sample 4
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

Sample 5
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

Sample 6
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

Sample 7
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

Sample 8
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

Aliquot	**0***	**1**	**2**	**3**	**4**	**5**

Sample 9
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

Sample 10
Spike conc./plate count
Response, replicate 1
Response, replicate 2
Response, replicate 3

*** Unspiked sample blank**

Response is the signal data (absorbance, florescence, Ct value), colonies, plaques, etc resulting from the analysis.

For shellfish samples repeat for each tissue type of interest.

DATA HANDLING

Linear Range

To determine the range within the working range where the results are proportional to the concentration of the target analyte/measurand/organism of interest present, 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 microbiological methods) to correct the results from the spiked samples for matrix effects.
3. Divide the response obtained for each replicate tested by the concentration of the spiked analyte/measurand/organism of interest which gave rise to it. Use log values for the microbiological data.
4. Plot the data obtained above on the y-axis against the log of the concentration of the spiked analyte/measurand/organism of interest which gave rise to the respective data point on the x-axis. Connect the points. This is the relative response line.
5. Calculate the mean of the values obtained (in step 3) when the response for each replicate tested is divided by the concentration of the spiked analyte/measurand/organism of interest which gave rise to it.
6. Plot this value on the y-axis of the graph obtained in step 4 at each log concentrations of the analyte/measurand/organism of interest spiked into the samples. Connect the points to form a horizontal line. This constitutes the line of constant response
7. Multiply the value obtained in step 5 by 0.95 and 1.05.
8. Plot these values on the y-axis of the graph obtained in steps 4 and 6 at each log concentration of the analyte/measurand /organism of interest spiked into the samples. Connect the points to form two horizontal lines which bracket the line of constant response.
9. The method is linear up to the point where the relative response line (obtained in step 4) intersects either of the lines obtained above.
10. The linear range of the method as implemented by the laboratory is comprised of the range in concentrations obtained by taking the antilogs of the concentrations of the spiked analyte/measurand/organism of interest bracketed within the horizontal lines of the plot obtained in step 8 above.

Limit of Detection and Limit of Quantitation/Sensitivity

To determine the minimum concentration at which the analyte/measurand/organism of interest can be identified and subsequently quantified with an acceptable level of precision and accuracy under the conditions of the test, the data is manipulated in the following manner.

1. Calculate the coefficient of variation or relative standard deviation for each concentration of analyte/measurand/organisn of interest spiked into the samples. Use the log transformed data for manipulating microbiological results.
2. Plot the coefficient of variation/relative standard deviation on the y-axis for each concentration of analyte/measurand/organism of interest spiked into the samples and plotted on the x-axis. Use log transformed concentration values for the microbiological data.
3. Fit the curve and determine from the graph the concentration of analyte/measurand/organism of interest which gave rise to a coefficient of variation/relative standard deviation of 10%. This is the limit of quantitation/sensitivity of the method as implemented by the laboratory.
4. Divide the value for the limit of quantitation/sensitivity obtained from step 3 above by 3.3 or determine the concentration of analyte/measurand/organism of interest that gave rise to a coefficient of variation/relative standard deviation of 33%. This value is the limit of detection of the method as implemented by the laboratory.

For single laboratory validation, the concepts of "blank + 3σ" and "blank + 10σ" generally suffice for determining the limit of detection and the limit of quantitation/sensitivity. Since the blank is in theory zero (0), then the limit of detection and the limit of quantotation /sensitivity become 3σ and 10σ respectively. An absolute standard deviation of 3 and 10 equates to a coefficient of variation/relative standard deviation of 33% and 10% respectively. Accordingly the limit of detection and the limit of quantitation/sensitivity become the concentration of analyte/measurand/organism of interest which give rise to these values.

Data Summary:

Linear range of the method as implemented _____
The limit of detection of the method as implemented _____
The limit of quantitation/sensitivity of the method as implemented _____