VALIDATION CRITERIA

Comparability is the acceptability of a new or modified analytical method as a substitute for an established method in the NSSP. To be acceptable the new or modified method must be sufficiently rugged to withstand the relatively minor day to day changes likely to occur in routine use. Comparability must be demonstrated for each substrate or tissue type of interest by season and geographic area if applicable.

Ruggedness of the new or modified method is the ability of the new or modified method to withstand relatively minor changes in analytical technique, reagents or environmental factors likely to arise in different test environments.

Procedure for testing the ruggedness of new or modified methods: 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. For each sample take two aliquots of either the growing water sample or shellfish homogenate appropriately sized for your work. Spike both aliquots with a suitable concentration of the target analyte/measurand/organism of interest. Process both aliquots of the sample as usual to determine new method/modified method concentration for the target analyte/measurand/ organism of interest. For the second aliquot of each sample, however, use a different batch or lot of culture media and/or test reagents as appropriate to process this aliquot. For growing waters, do ten (10) samples collected from a variety of growing waters. 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 two batches or lots of culture media and/or test reagents to process each sample such that "batch or lot 1" is used to process the first aliquot of each sample and "batch or lot 2" is used to process the second aliquot of each sample. Use a range of concentrations which spans the range of the new method or modified method's intended application to spike sample aliquots. Both aliquots of the same sample must be spiked with the same concentration of the target analyte/measurand/organism of interest. Process samples over a period of several days if possible.

Data for demonstrating the ruggedness of the new or modified method:

Sample type _____

Sample	Conc. "Batch or lot 1" Media and/or Reagents	Conc. "Batch or lot 2" Media and/or Reagents
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

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

Data handling to demonstrate the ruggedness of the new or modified method

In the day to day operations of the laboratory there will be changes in the batches/lots of culture media and/or test reagents used to process samples. Environmental factors are also likely to change over time. None of these factors, however, should adversely impact test results if the new or modified method as implemented is sufficiently rugged to be used routinely for regulatory monitoring.

To determine whether the new or modified method as implemented is sufficiently rugged to withstand the types of changes anticipated to occur in routine use, a two-sided t-test at a significance level (α) of .05 will be used on the

data to ascertain if results obtained using different culture media and/or test reagent batches/lots under slightly varying environmental conditions are significantly affected by such minor changes. Either a paired t-test or Welch's t-test will be used depending upon the shape of the distributions produced by the data for each batch/lot and their respective variances. Use log transformed data for the results obtained from microbiological methods. The appropriate t-test to be used for the analysis is determined in the following manner.

1. Test the symmetry of the distribution of results from both batch/lot 1 and batch/lot 2.
2. Calculate the variance of both batch/lot 1 and batch/lot 2 data.
3. Values for the test of symmetry for either batch/lot 1 or batch/lot 2 outside the range of -2 to +2 indicate a significant degree of skewness in the distribution.
4. A ratio of the larger of the variances of either batch/lot 1 or batch/lot 2 to the smaller of the variances of either batch/lot 1 or batch/lot 2 greater than 2 indicates a lack of homogeneity of variance.

5. Use either the paired t-test or Welch's t-test for the statistical analysis based on the following considerations.
    - If the distributions of the data from batch/lot 1 and batch/lot 2 are symmetric (within the range of -2 to +2) and there is homogeneity of variance, use a paired t-test for the statistical analysis.
    - If the distributions of the data from batch/lot1 and batch/lot 2 are symmetric (within the range of -2 to +2) but there is a lack of homogeneity of variance in the data, use Welch's t-test for the statistical analysis.
    - If the distribution of the data from batch/lot 1 and batch/lot 2 are skewed (outside the range -2 to +2) and the skewness for both groups is either positive for both or negative for both and there is homogeneity of variance in the data, use the paired t-test for the statistical analysis.
    - If the distribution of the data from batch/lot 1 and batch/lot 2 are skewed and the skewness for both groups is either positive for both or negative for both; but, the data lacks homogeneity of variance, use Welch's t-test to statistically analyze the data.

Data summary for demonstrating the ruggedness of the new or modified method:

Value for the test of symmetry of the distribution of batch/lot 1 _____

Value for the test of symmetry of the distribution of batch/lot 2 _____

Variance of batch/lot 1 data _____

Variance of batch/lot 2 data _____

Ratio of the larger to the smaller of the variances of batch/lot 1 and batch/lot 2 _____

Is there a significant difference between batch/lot 1 samples and batch/lot 2 samples
_____Y/N____