CHAPTER VI

ANALYTICAL METHODS & QUALITY CONTROL

Section A. Introduction

Section B. Definitions and Terms

Section C. Laboratory QA/QC

1. Sample Preservation and Holding Times
2. Sample Receiving
3. Sample Storage and Disposal
4. Support Equipment and Supplies
   - 4.1 Analytical Balances
   - 4.2 Reagent Water
   - 4.3 Artificial Seawater
   - 4.4 Glassware
   - 4.5 Drying Ovens and Desiccators
   - 4.6 Reagents and Standards
5. Instrument Calibration
6. Method Performance Checks
7. Control Charts
8. Method Detection Limits
9. Practical Quantitation Limits
10. References

Section D. Analytical Methods

1. Alkaline Persulfate Digestion for Nitrogen and Phosphorus, Total and Dissolved
2. Ammonia
3. Chlorophyll-*a* and Pheophytin
4. Dissolved Organic Matter Absorption Coefficient (CDOM)
5. Nitrate + Nitrite
6. Nitrite
7. Organic Carbon, Total and Dissolved
8. Orthophosphate, Total and Dissolved
9. Particulate Nitrogen and Particulate Carbon
10. Particulate Phosphorus Digestion

11. Total Suspended Solids
12. Fixed Suspended Solids
13. Silicates

Section E. Method Tables (Reserved)

SECTION A
INTRODUCTION

1. Scope

   1.1 Chapter VI contains analytical methods and QC protocols for laboratories that provide data for the CBP Tidal and/or Nontidal Water Quality Monitoring Programs.

   1.2 The procedures herein are intended to give the basic, minimum requirements and recommendations. Laboratory SOPs should be consistent with the procedures of this Chapter, but provide more detail.

   1.3 As mentioned in Chapter II.B.5, the comparability of CBP data is improved through the use of standard analytical methods and quality controls. Deviations from these methods may be permitted only if the laboratory demonstrates that the changes will not negatively affect the quality of future data. (Chapter II.F)

2. Clean Water Act Methods and Laboratory Accreditation Requirements

   2.1 The CPB methods in this Chapter are considered the authoritative source for Chesapeake Bay Program laboratory methods; they should be used for reviewing SOPs, conducting on-site audits and NELAC assessments.

   2.2 Several methods in this chapter are not approved methods under 40 CFR Part 136, Analytical Methods for CWA, but have been carefully developed, evaluated and determined to be the most appropriate, particularly for estuarine samples.

   2.3 Roughly half of the laboratories providing data to the CBP are certified under the National Environmental Laboratory Accreditation Conference (NELAC) Institute. Many requirements and recommendations in this document are consistent with the related NELAC Institute standards. However, there are many NELAC requirements not included in this document.

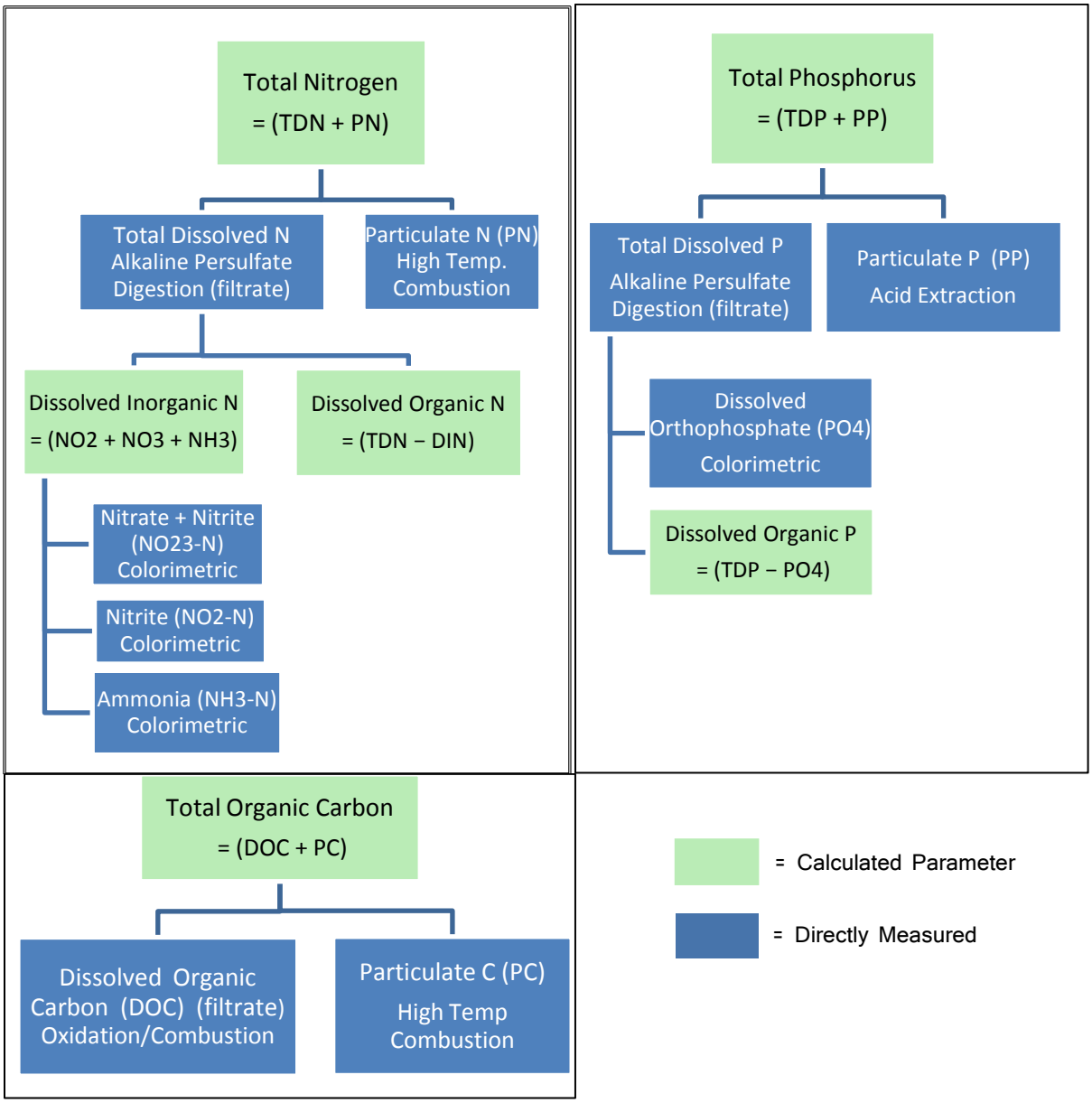
3. Parameter and Method Schemes

   3.1 Tidal Analytical Methods – The methods focus on the directly-measured parameters in Figure 1.

   3.2 Nontidal Analytical Methods – In most cases, the same method may be used for Tidal and Nontidal samples. However, there are several nontidal agencies whose whole water samples are analyzed directly for TN, TP and/or TOC. In most of these cases, the method for the total dissolved parameter is used on a whole water sample.

4. Section D contains the analytical methods for the parameters listed in Table VI.1

Figure 1 Nitrogen, Phosphorus & Carbon Parameters from Tidal Monitoring Stations



SECTION B
DEFINITIONS and TERMS

1. Batch[1] – Environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents.

   1.1 A **preparation batch** is composed of 1-20 samples, all analyzed on the same day.

   1.2 An **analytical batch** is composed of prepared environmental samples (extracts, digestates or concentrates), which are analyzed together as a group. An analytical batch may be greater than 20 samples.

2. Calibration Standard – A solution prepared from a primary dilution standard solution or stock standard solution containing the analyte. Calibration standards are used to calibrate the instrument response with respect to analyte concentration. Most methods require that calibration standards be carried through the entire analytical procedure, including digestion, combustion, etc.

3. Calibration Check Standard - a quality control sample used to verify initial instrument calibrations. Traceability shall be to a national standard when commercially available.

4. Certified Reference Material (CRM) - A reference material for which one or more property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. CRMs produced by the U.S. National Institute of Science and Technology are called *Standard Reference Materials* (SRMs).

5. Continuing Calibration Verification (CCV) Sample – A calibration standard, check standard or LCS that is analyzed periodically, no less than one per preparation batch and at the end of the analyses. The purpose of the CCV is to ensure that the calibration of the instrument is still valid.

6. Control Limit - The variation in a process data set expressed as ± X standard deviations from the mean and placed on a control chart to indicate the upper and lower acceptable limits of process data and to judge whether the process is in or out of statistical control.

7. Demonstration of Capability[1] – A procedure to establish the ability of an analyst to generate analytical results of acceptable accuracy and precision.

8. Duplicate Analysis -The analysis or measurement of the analyte of interest, performed as identically as possible on two subsamples of a sample. The subsamples are prepared in a manner such that they are thought to be essentially identical in composition.

9. Laboratory Control Sample[1] (LCS) – sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst specific precision and bias or to assess the performance of all or a portion of the measurement system.

   Note: For the LCS to also be used as the initial calibration verification check standard, it must be obtained from a second source and be traceable to a national standard.

10. Laboratory Reagent Blank (or Method Blank) – An aliquot of reagent water or reagent-grade artificial sea water that is carried through the entire analytical procedure, including exposure to all glassware, equipment, reagents, digestion, combustion, etc. The purpose of the laboratory reagent blank is to determine the level of contamination associated with the analysis of samples.

11. Limit of Detection (LOD)[1] – A laboratory's estimate of the minimum amount of an analyte in a given matrix that an analytical process can reliable detect in their facility. Also called the Method Detection Limit (MDL).

12. Limit of Quantitation (LOQ)[1] – The minimum concentration of an analyte that can be reported with a specified degree of confidence. Also called the Method Quantitation Limit (MQL) or Practical Quantitation Limit (PQL).

13. Matrix[1] – The substrate of a test sample. There are two Chesapeake Bay Program water quality matrices: a) Saline/Estuarine and b) Fresh surface water (fresh).

14. Matrix Spike - An aliquot of sample to which a known quantity of analyte is added in such a manner as to minimize the change in the matrix of the original sample. The matrix spike is analyzed exactly like a sample to determine whether the sample matrix contributes bias to the analytical results. The concentration of analyte in the sample must be measured in a separate aliquot in order to calculate the percent recovery.

15. Method Blank – See Laboratory Reagent Blank.

16. Proficiency Test (PT) Sample[1] – A sample or solution of method analyte(s) whose concentration is unknown to the laboratory. The purpose of PT samples is to test whether the laboratory can produce analytical results within a specified acceptance level. Chesapeake Bay Program laboratories analyze USGS Reference Samples (Nutrients) and Blind Audit Samples from the University of Maryland Chesapeake Biological Laboratory.

17. Quality Control Sample[1] (QCS) – A sample used to assess the performance of all or a portion of the measurement system. The QCS may be one of any number of samples, such as a Certified Reference Material, a laboratory control sample (LCS) or a matrix spike.

18. Reagent Water – Deionized or distilled water (>10 mΩ) that is demonstrated to be free of analytes of interest. This water is used for the preparation of blanks, reagents and standards.

19. Stock Standard Solution – A known concentration solution containing one or more method analytes, prepared in the laboratory using ACS Reagent Grade materials (or equivalent), purchased from a reputable commercial source.

[1] Management and Technical Requirements for Laboratories Performing Environmental Analyses, Module 4: Quality Systems for Chemical Testing. The NELAC Institute Standard EL-V1M4-2011

SECTION C
LABORATORY QA/QC

1. Sample Preservation and Holding Times

Laboratories must ensure that samples are properly preserved, stored and analyzed within the required holding times. Preservation and holding time requirements for Chesapeake Bay Program water quality analyses appear in Table VI.1 below. Deviations from these requirements are allowed if comparability data on record show equivalent results.

Table VI.1: PRESERVATION & HOLDING TIMES for TIDAL & NONTIDAL PARAMETERS

Parameter	Tidal WQ[a]		Nontidal WQ[b]	
	Preservation	**Max. Holding Time** (days)	**Preservation**	**Max. Holding Time**
Total dissolved phosphorus	Freeze ≤ –20°C	28	Cool, ≤ 6°C	28 days
Dissolved orthophosphate	Freeze ≤ –20°C	28	Cool, ≤ 6°C	48 hrs.
Particulate phosphorus (filters)	Freeze ≤ –20°C	28	Freeze, ≤ –20°C	28 days
Nitrite	Freeze ≤ –20°C	28	Cool, ≤ 6°C	48 hrs.
Nitrate + nitrite	Freeze ≤ –20°C	28	Cool, ≤ 6°C, H_2SO_4 to pH<2	28 days
Ammonia	Freeze ≤ –20°C	28	Cool, ≤ 6°C, and H_2SO_4 to pH<2	28 days
Total dissolved nitrogen	Freeze ≤ –20°C	28	Cool, ≤ 6°C	28 days
Particulate nitrogen (filters)	Freeze ≤ –20°C	28	Freeze, ≤ –20°C	28 days
Particulate carbon (filters)	Freeze ≤ –20°C	28	Freeze, ≤ –20°C	28 days
Dissolved and total organic carbon	Freeze ≤ –20°C	28	Cool, ≤ 6°C, and H_2SO_4 to pH<2	28 days
Chlorophyll *a* / Pheophytin (filters)	Freeze ≤ –20°C	28	Freeze, ≤ –20°C	28 days
Suspended solids, Total and Fixed – Field filtered samples	Freeze ≤ –20°C	28	Not Applicable	–
Suspended solids, Total and Fixed – Whole water samples	Cool, ≤ 6°C	7	Cool, ≤ 6°C	7 days
Suspended Sediment Concentration	Not Applicable	–	Cool & Dark	120 days
Silicates	Cool, ≤ °C	28	Cool, ≤ 6°C	28 days

Total Phosphorus	Not Applicable	–	Cool, ≤ 6°C and H_2SO_4 to pH<2. Or freeze	28 days
Total Nitrogen	Not Applicable	–	Cool, ≤ 6°C [c]. Or freeze	28 days
Total Kjeldahl Nitrogen	Not Applicable	–	Cool, ≤ 6°C, H_2SO_4 to pH<2	28 days

[a] Virginia River Input Monitoring (RIM) samples are preserved according to Tidal WQ specifications.
[b] Maryland RIM samples are preserved according to Nontidal WQ specifications.
[c] Samples may be acidified but require pH adjustment prior to lab analysis by the alkaline persulfate method.

2. Sample Receiving

2.1 The laboratory must establish procedures for the receipt, identification and custody of samples.

2.1.1 The laboratory shall designate a sample custodian and staff responsible for receiving samples.

2.1.2 The condition of the shipping and sample containers must be inspected and documented upon receipt by the sample custodian or his/her representative.

2.1.3 The sample custodian and representatives will maintain records of sample receipt and condition.

2.1.4 Each sample container must be labeled with a unique identifier that is cross-referenced with the corresponding field documentation.

2.2 The laboratory must establish a sample acceptance policy and procedures that include:

2.2.1 Procedures to verify that the samples have been properly preserved by checking the temperature and pH, and ensuring that no leakage or cross-contamination has occurred, and,

2.2.2 A policy for analyzing incorrectly preserved samples and the associated qualifier codes that would accompany data from these samples.

3. Sample Storage and Disposal

3.1 Storage temperatures of refrigerators and freezers must be checked each day of normal laboratory operation and recorded in a temperature log.

3.2 Samples shall be stored according to the conditions specified in Table VI.1 unless the laboratory has demonstrated that an alternative preservation method yields equivalent results, Deviations from these conditions shall be approved by AMQAW and the CBP QA Officer or his/her designee prior to making the change.

3.3 Samples should be stored in an atmosphere free of all potential contaminants, away from analytical standards, reagents and food.

3.4 Samples may be disposed after the data have been validated by the laboratory and reported to the State agency.

3.5 Records documenting all phases of sample handling from collection to final analysis should be maintained for at least five years.

4. Support Equipment and Supplies

4.1 The analytical balance is the most important piece of equipment in an analytical laboratory. The sensitivity of the balance must be at least 0.1 milligram.

4.1.1 Mount the balance on a heavy table away from laboratory traffic, drafts and temperature changes. Routinely check the level of the balance; adjust level when necessary.

4.1.2 Check calibration of the balance each day of use with NIST-traceable weights that represent the working range, e.g., a high and low weight. Make sure the balance temperature is equilibrated with room temperature.

4.1.3 The balance should be serviced and fully calibrated annually by a certified vendor.

4.2 Reagent Water

4.2.1 Use only deionized or distilled water (>10 mΩ) that is demonstrated to be free of analytes of interest.

4.2.2 Check the resistivity of the reagent water each day of use and record in logbook.

4.3 Artificial Sea Water (ASW) – A prepared standard solution of low nutrient synthetic seawater. The following formulation yields a 36 psu salinity solution which may be diluted to match the salinity of the samples. This formulation is not recommended for nitrate, nitrite or ammonia analyses because magnesium may interfere.

Using analytical reagent grade reagents, dissolve 31g of sodium chloride NaCl (CAS No. 7647-14-5), 10g of magnesium sulfate, $MgSO_4 \cdot 7H_2O$ (CAS No. 10034-99-8) and 0.05g sodium bicarbonate ($NaHCO3 \cdot H_2O$) (CAS No. 144-55-8) in 1 liter of reagent water.

4.4 Glassware

4.4.1 Class A Volumetric lab ware such as pipettes, burettes, graduated cylinders and volumetric flasks shall be used unless otherwise specified in the procedure.

4.4.2 Auto-pipette volumes should be verified quarterly using gravimetric or spectrophotometric methods.

4.5 Drying Ovens, Muffle Furnace and Desiccators

4.5.1 Check the temperature of drying ovens each day of operation using a thermometer traceable to NIST. Adjust temperature if needed and record observations and actions in a logbook.

4.5.2 Check the temperature of muffle furnace annually using a thermometer traceable to NIST. Adjust temperature if needed and record observations and actions in a logbook.

4.5.3 Check accuracy of digital temperature display readings at least once a year with a thermometer

traceable to NIST. If temperature of thermometer is different, develop a correction factor for displayed readings and record corrected values in the logbook.

4.5.4 Desiccators must contain a chemical drying agent and color indicator to show that the drying agent is active.

4.6 Reagents and Standards

4.6.1 Reagents and standards must meet ACS Reagent Grade specifications and requirements. If ACS-grade reagents are not commercially available then the lab must demonstrate that the reagents used are free from the contaminant of interest.

4.6.2 Standard solutions must be prepared and diluted with reagent water by using Class A volumetric pipettes and flasks. Weigh solid standard materials on a calibrated analytical balance to 0.0001 g.

4.6.3 All reagents must be stored in the appropriate bottles and labeled with the following information:

Identity (e.g., 15N NaOH)
Preparation Date
Expiration Date
Concentration (e.g., mg N/L)
Initials of Preparer

4.6.4 Do not use chemicals past the manufacturer's expiration date. If a purchased chemical has no expiration date on the original label, then there is no age limit. However, laboratories may set a standard expiration date for these instances (e.g., 5 years from date received).

4.6.5 The laboratory shall maintain records on the preparation of reagents and standards which to demonstrate traceability. Preparation records must include the date of preparation, expiration date and preparer's initials.

4.6.6 It is recommended that stock standard solutions be assigned a unique identifier to be associated with the calibration record. NELAC certified laboratories must assign a unique identifier to both stock and working standards.

4.6.7 When an instrument prepares working standards from a concentrated solution, check the concentrations with manual dilutions of a certified reference material.

4.7 Centrifuge – The removal of turbidity for Chlorophyll extracts requires a centrifuge capable of 675 g. The relationship between RPM and g is as follows:

$$g = (1.118 \times 10^{-5})\, R \cdot S^2,$$

where g is the relative centrifugal force, R is the radius of the rotor in centimeters, and S is the speed of the centrifuge in revolutions per minute. Values of RCF in units of times gravity (× g) for common micro-centrifuge rotor radii are found in standard conversion tables.

It is recommended that the centrifuge RPMs be verified every 1-3 years.

4.8 Thermometers

4.8.1 NIST-certified thermometers must be fully calibrated by NIST at least once every 5 years. Laboratories must verify the calibration annually by ice-point determination.

4.8.2 Working thermometers must be verified annually at the temperatures at which they are used, against a certified calibration standard thermometer, traceable to NIST.

4.8.3 Label or tag the working thermometer with an identification number, date of calibration, calibration temperature and correction factor.

5. Instrument Calibration

5.1 Details of the initial instrument calibration procedures including calculations, integrations, background corrections, acceptance criteria and associated statistics, must be included or referenced in the laboratory SOP.

5.2 Prepare a series of calibration standards by diluting suitable volumes of primary dilution standards with reagent water or artificial seawater according to the salinity of the samples.

5.2.1 The concentration range of the calibration standards should bracket the expected concentrations of samples, not to exceed two orders of magnitude.

5.2.2 The concentrations of the standards should be evenly spaced across the calibration range.

5.2.3 The number of calibration standards and frequency of preparation for each test are to be specified in the laboratory's SOP. Recommended methods for Chesapeake Bay Program laboratories appear in Section D of this chapter.

5.2.4 The lowest calibration standard must be at or below the lowest quantitation or reporting level (e.g. MQL or PQL).

5.2.5 Calibration standards must be subject to the same sample preparation and analysis steps as samples, e.g., digestion, combustion, etc.

5.3 Initial Calibration (Adapted from TNI Standard EL-V1M4-2011)

5.3.1 Each day, prior to the analysis of samples, establish the linear working range of the instrument with at least three calibration standards per decade, the lowest of which must be at or below the lowest quantitation level (e.g., PQL).

5.3.2 Include a standard with zero analyte concentration to estimate the y-intercept. (Do not force curve through zero because instruments auto-correct for the y-intercept.)

5.3.3 Verify the initial instrument calibration prior to analysis with a certified reference material (CRM) or LCS that is traceable to a national standard. If a CRM is commercially unavailable, the initial calibration verification (ICV) sample may be prepared from material obtained from a second

manufacturer or an independently prepared lot.

5.3.4 Criteria for the acceptance of calibration curves must be established, e.g., correlation coefficient or response factor, and be appropriate to the calibration technique employed. If the initial calibration or verification results are outside established acceptance criteria, corrective actions must be performed and all associated samples reanalyzed. If sample reanalysis is not feasible, do not report the data.

5.3.5 Other Calibration Requirements

5.3.5.1 Sample results must be quantitated from the initial instrument calibration curve and may not be quantitated from any continuing instrument calibration verification sample.

5.3.5.2 Standards may not be "dropped" from the calibration curve to meet the acceptance criteria. However, it is permissible to omit high and/or low end calibration points from the curve if all sample results reported fall within the "new" working range.

5.3.5.3 If replicate standards are prepared, use their mean concentration to establish the curve.

5.3.5.4 Only values below the highest calibration standard are considered valid. Samples may be diluted quantitatively to bring the concentration within the calibrated range.

5.3.5.5 Measured concentrations below the LOQ or reporting limit shall be reported as having less certainty and shall be reported using the appropriate problem code.

5.4 Continuing Calibration Verification

5.4.1 A continuing calibration verification standard is to be analyzed *with each preparation batch*, i.e. no less than one per 20 CBP samples, and at the end of the sample run.

5.4.2 The CCV standard must be within 90-110% of the known analyte concentration.

5.4.3 Low-level accuracy may be demonstrated by analyzing a standard corresponding to the PQL.

5.4.4 For calibration curves spanning two orders of magnitude, a high and low CCV is recommended.

5.5 Calibration Records

5.5.1 Sufficient raw data records must be retained for at least 5 years to permit reconstruction of the initial instrument calibration, e.g., calibration date, test method, instrument, analysis date, each analyte name, analyst's initials or signature; preparation of standards, concentration and response, calibration curve or response factor; or unique equation or coefficient used to reduce instrument responses to concentration.

5.5.2 It is recommended that each calibration record contain or display the unique ID of the calibration standard(s) used. This is a requirement for NELAC certified laboratories.

6. Method Performance Checks

6.1 Method Blank (i.e., Laboratory Reagent Blank)

6.1.1 A method blank shall be analyzed at the beginning and end of each preparation batch, i.e., no less than one per 20 CBP samples. The method blank is carried through all steps of sample preparation and analysis, along with samples in the preparation batch.

6.1.2 Results of the method blank are used to check for possible contamination of samples in the preparation and analysis of samples.

6.1.3 If the method blank concentration ≥ PQL, or more stringent criteria, laboratory or reagent contamination should be suspected. Re-analyze another aliquot of blank solution. If the problem remains, investigate and take appropriate corrective action before continuing analyses.

6.1.4 Reanalyze all samples related to the high blank and report the new values. If unable to reanalyze the samples, the analyst will discuss the situation with the program manager and decide to either: 1) reject the results and report only the problem code, or 2) report the original sample results with the appropriate a problem code,

6.2 Laboratory Control Sample

6.2.1 At least one laboratory control sample (LCS) shall be analyzed for every preparation batch (i.e., no less than one LCS per 20 CBP samples). The LCS concentration must be within the calibration range of the method and be carried through all steps of sample preparation and analysis, along with samples in the preparation batch.

6.2.2 Results of the LCS are used to evaluate the performance of the total analytical system, including all preparation and analytical steps. The acceptance criterion for the LCS is ± 10% of the known or certified value.

6.2.3 If the LCS is > 10% of the known concentration, reject all results back to the last acceptable LCS. Investigate the cause of the problem and take corrective actions as necessary.

6.2.4 Reanalyze all samples in the preparation batch and report the new values. If unable to reanalyze the samples, the analyst will discuss the situation with the program manager and decide to either: 1) reject the results and report only the problem code, or 2) report the original sample results with the appropriate a problem code

6.3 Laboratory Replicates

6.3.1 Laboratory replicate analyses provide a measure of laboratory precision. At least one duplicate should be analyzed for every 20 CBP samples, including field duplicates.

6.3.2 Prepare duplicates by taking two aliquots from a well homogenized sample. The duplicate sample must be subjected to all steps in the analytical process, including digestion, dilution, etc. More replicates may be analyzed and reported if desired.

6.3.3 Precision may be estimated by calculating the relative percent difference (RPD) or the coefficient of variation (CV), however, the RPD is preferred metric.

The following equation is used to calculate RPD:

$$\text{RPD}(\%) = \left(\frac{|\text{A1} - \text{A2}|}{(\text{A1} + \text{A2})/2}\right) * 100$$

where: A1 = Sample result
A2 = Duplicate sample result (or matrix spike duplicate)

6.3.4 The following equations are used to calculate the coefficient of variation (also called the relative standard deviation).

$$\text{CV}(\%) = \frac{\text{SD}}{\text{MEAN}} * 100; \quad \text{And} \quad \text{SD} = \sqrt{\frac{(X - \overline{X})^2}{(N - 1)}}$$

Where: CV = Coefficient of variation
SD = Standard deviation
Mean = Mean of duplicate (or replicate) sample results
N = Number of samples
X = Sample result
$\overline{X}$ = Mean of duplicate (or replicate) samples

6.4 Matrix Spike (not required for chlorophyll, PN, PC or TSS)

6.4.1 A matrix spike is used primarily as a means of evaluating bias that may result from the analysis of a particular matrix when using a specific procedure. The saline, aqueous matrix has been demonstrated to impart bias in some analyses. Sample spike analysis involves the introduction of a known amount of the analyte of interest into one of two aliquots from a well homogenized sample and a calculation of spike recovery.

6.4.2 The spike concentration must be at least four times the calculated MDL.

6.4.3 The sample is spiked prior to all steps in the analytical process, particularly when a digestion is involved.

6.4.4 Proper assessment requires that the integrity of the sample matrix be maintained. The original sample must not be diluted more than 10% due to the spike process.

6.4.5 The analytical system response from the sample plus the spike should be in the same range as the sample set undergoing analysis, ideally approximating 50-75% of a full scale response.

6.4.6 A matrix spike should be analyzed once for every 20 CBP samples.

6.4.7 Matrix spikes cannot be performed on lab or field blanks.

6.4.8 The percent recovery of analyte from the matrix spike sample is calculated using the following equation:

$$Matrix\ Spike\ Recovery = \frac{SSR - SR}{SA} \times 100$$

where: SSR = Spike sample result
SR = Sample result
SA = Spike added

6.4.9 If the spike recovery is outside the range designated in Table VI.2, the spike analysis is repeated after checking for obvious sources of error. At a minimum, this involves an immediate repeat of the instrumental analysis. If the result is still beyond acceptance limits and the analytical process employed a digestion step, the matrix spike should be reanalyzed including redigestion. If the recovery of the repeated sample spike is outside the acceptance range, the recovery problem may be matrix related.

If possible, take steps to identify and remove the interference. If unable to correct the problem, report the concentration with the problem code "Possible Matrix Interference".

7. Control Charts

7.1 Real-time quality control charts for precision and accuracy should be developed and maintained for each parameter and appropriate concentration ranges, using the most recent 12 months of data or the last 30 data points. More points may be used if deemed necessary.

7.1.1 Control charts are centered at the arithmetic mean. Unless otherwise specified in the method, the upper and lower control limits are defined at $\pm$ 3 standard deviations from the mean and the upper and lower warning limits are defined at $\pm$ 2 standard deviations from the mean.

7.1.2 Typical precision and accuracy acceptance windows for are provided within each method and in Table VI.2 below.

7.1.3 Once control charts have been established, they should be used to determine if a given analytical or measurement process is out of control and corrective actions initiated.

7.1.3.1 A process is out of control if 3 or more data points are outside either control limit.

7.1.3.2 Immediate corrective action is necessary for any process identified as being out of normal control limits. Where possible, this should include reanalysis.

7.1.3.3 A warning of possible systematic error is indicated if 7 successive data points fall away from the mean on the same side of the center line, if 7 or more data points fall outside of either warning limit, or if a discernible trend develops.

7.2 Control charts for method blanks and laboratory control samples are recommended.

8. Method Detection Limits

8.1 Verify the method detection limit (MDL) for each analyte using low-level estuarine or fresh water containing the analyte at approximately the current detection limit. The water sample may be spiked with reagent water if necessary to make the concentration approximately 1 – 5 times the current method detection limit.

8.2 Alternatively, determine the MDL in reagent (blank) water by preparing a laboratory standard (i.e., analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the current method detection limit.

8.3 Analyze at least seven replicate aliquots of water *which have been prepared individually* (e.g., digested) and subjected to the entire analytical method. It is recommended that some of the replicates be analyzed on a different day to include the day-to-day variability in the MDL determination.

8.4 Perform all calculations defined in the method and report concentrations in appropriate units.

8.5 If a blank measurement is required to calculate a particular analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

8.6 Calculate the MDL as follows: (Online calculator available at: http://www.chemiasoft.com/mdl_by_epa.html)

MDL = (t)(S), where:

S = the standard deviation of the replicate analyses,
t = Student's t value for n-1 degrees of freedom at the 99% confidence limit; where
t = 3.143 for six degrees of freedom

8.7 MDLs should be determined every 12 months or whenever a significant change in method, instrument, operator, or instrument response occurs, or a new matrix is encountered.

8.8 MDLs should always be calculated using the same calibration curve that would be used for typical sample analysis. If more than one instrument or calibration range is used in the lab, determine the MDL on the instrument that will be used to report low-level results, under realistic conditions.

8.9 A table of MDL and PQL values shall be submitted annually. When values change, a revised table of MDL and PQL values and their effective dates should be included with the next data submittal.

9. Practical Quantitation Limits

9.1 The method quantitation limit (MQL or PQL) is approximately 3.18 (MDL), or:

MQL = 10 (S), where S = the standard deviation of the replicate analyses as described in subsection C.8.6 above.

9.2 The lowest calibration standard concentration must be at or below the PQL (see subsection C.5.2.4).

9.3 Analytical results between the MDL and PQL shall be quantified. Report the numerical value and qualify with the appropriate qualifier code "G".

Students' *t* Values at the 99 Percent Confidence Level

Number of replicates	Degrees of freedom (n-1)	$t_{(n-1,.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602

Table VI.2. FREQUENCY OF ROUTINE CALIBRATION, BLANK AND QC SAMPLES

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Action
Instrument Calibration	Each analysis day unless otherwise specified in method	Using all standards in curve, r ≥ 0.995. Linear at PQL	Repeat full calibration
Initial Calibration Verification (ICV)	After calibration standards, prior to sample analysis	90-110% recovery of known concentration	Recalibrate and verify prior to analysis.
Lab Control Sample (LCS) – 2nd source CRM	After calibration, prior to sample analysis	90-110% recovery of known concentration	Recalibrate and verify prior to analysis.
Method Blank	Beginning and end of preparation batch (20 samples)	Reject sample results if blank ≥ PQL	Reanalyze another aliquot of blank solution. Investigate sources of contamination.
Method Quantitation Limit (MQL/ PQL) Standard	After calibration, prior to analysis, unless otherwise specified in method	Method-specific	Reanalyze standard. If unacceptable, increase the concentration and analyze. If higher std. meets criterion, raise reporting limit of batch.
Continuing Calibration Verification (CCV)	After every 10 samples and end of run	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV.
Spike Sample	One per 20 samples	Analyte-specific See Table II.3	Spike another sample aliquot and analyze. Suspect matrix interference; remove interference if possible.
Duplicate Sample	One per 20 samples	RPD (or CV) ≥ 3 std. deviations of annual average difference.	Reanalyze sample; reject result (or batch) if ≥ 3std. deviations of annual average difference.

10. References

10.1 The NELAC Institute (2011). Volume 1, Management and Technical Requirements for Laboratories Performing Environmental Analyses, Module 2: Quality Systems General Requirements. The NELAC Institute Standard EL-V1M4-2011

10.2 The NELAC Institute (2011). Management and Technical Requirements for Laboratories Performing Environmental Analyses, Module 4: Quality Systems for Chemical Testing. The NELAC Institute Standard EL-V1M4-2011

10.3 40 CFR, [part] 136 Appendix B. Definition and Procedure for the Determination of the Method Detection Limit. Revision 1.11.

10.4 U.S. EPA (1997). Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices – 2nd Edition. EPA/600/R-97/072, September, 1997

10.5 Salley, B.A., J.G. Bradshaw and B.J. Neilson. 1986. Results of comparative studies of preservation techniques for nutrient analysis on water samples. VIMS, Gloucester Point, VA., 23062. 32pp

10.6 Macdonald, R.W. and F.A. McLaughlin. 1982. The effect of storage by freezing on dissolved inorganic phosphate, nitrate and reactive silicate for samples from coastal and estuarine waters. Water Research. 16: 95-104.

10.7 Thayer, G.W. 1970. Comparison of two storage methods for the analysis of Nitrogen and Phosphorus fractions in estuarine water. Ches. Sci. 11:3, 155-158.

SECTION D
ANALYTICAL METHODS

1. Alkaline Persulfate Digestion for Nitrogen and Phosphorus, Total and Dissolved
2. Ammonia
3. Chlorophyll-*a* and Pheophytin
4. Dissolved Organic Matter Absorption Coefficient (CDOM)
5. Nitrate + Nitrite
6. Nitrite
7. Organic Carbon, Total and Dissolved
8. Orthophosphate, Total and Dissolved
9. Particulate Nitrogen and Particulate Carbon
10. Particulate Phosphorus Digestion
11. Total Suspended Solids
12. Fixed Suspended Solids
13. Silicates