Methods and Quality Assurance for Chesapeake Bay Water Quality Monitoring Programs

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Mary Ellen Ley, USGS/CBP QA coordinator, and workgroup members wrote the initial drafts, integrated the reviews, and finalized the document to ensure they represent the consensus of the Partner agencies. Durga Ghosh assisted in the final review process. The assembly and organization of the document was a result of the desktop publishing efforts of Melissa Merritt, Staffer, Chesapeake Resource Consortium.

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Section A. Overview

1. Purpose of this Document

The purpose of this document is to provide field and laboratory methods and associated quality control (QC) requirements for use in Chesapeake Bay water quality monitoring programs, which will result in the generation of known and comparable data quality. State agency staff or subcontractors (Participants) will conduct field measurements and collect and analyze water and sediment samples for specific physical, chemical, and biological parameters according to the procedures in this document.

Conformance with these procedures provides a solid foundation for a wide variety of scientific and management investigations, e.g., attainment of water quality standards, characterization of the health of the Chesapeake Bay and its tributaries, evaluation of long-term trends, effectiveness of management actions, ecosystem processes and water quality model development and calibration.

2. Organization of this Document

2.1 This document includes the requirements and recommendations for field measurements, field sampling, and laboratory analysis in support of Chesapeake Bay water quality monitoring programs. The first chapters provide general information regarding technical and contractual requirements, and the remaining chapters specify field and laboratory requirements for Chesapeake Bay Program tidal and nontidal monitoring programs.

Specifically, this document is organized as follows:

2.1.1 **Chapter 1** is a summary of the technical and contractual requirements established to ensure attainment of Chesapeake Bay Program monitoring objectives.

2.1.2 **Chapter 2** defines the Quality Assurance (QA) protocols and procedures for water quality monitoring programs that have been developed and agreed upon by Chesapeake Bay Program Partners.

2.1.3 **Chapter 3** is reserved for reporting requirements, data management procedures and software recommendations. Data deliverables and turnaround times required by the EPA Chesapeake Bay Program Office (CBPO) will be included.

2.1.4 **Chapter 4** presents specific methods and procedures for monitoring water quality in Chesapeake Bay mainstem and tidal tributaries. The procedures cover sample collection, field measurements, laboratory analyses and the associated QC requirements for each.

2.1.5 **Chapter 5** describes the field protocols and procedures for the Chesapeake Bay Nontidal Water Quality Network. It covers sample collection, field measurements and QC requirements.

2.1.6 **Chapter 6** contains the laboratory methods used by Chesapeake Bay Program laboratories.

3. Quality Assurance Policies

3.1 State agencies and academic institutions performing monitoring activities under EPA grants and cooperative agreements (i.e. Participants), must ensure the integrity of the data generated so that they are legally defensible.

3.1.1 Data integrity is defined as data that are complete, consistent, and without errors, and maintained with a data management system capable of preventing information corruption and unauthorized data modification.

3.1.2 Field and laboratory actions must be sufficiently documented to permit historical reconstruction of who, what, where, when and how the data was generated.

3.2 Each participant shall develop and operate a comprehensive quality management system that incorporates the applicable methods and quality control requirements contained in this document.

3.2.1 State agencies receiving monitoring funds will establish and maintain Quality Assurance Project Plans (QAPPs) that conform to Chesapeake Bay Program monitoring objectives and protocols.

3.2.2 Participant laboratories will establish a laboratory quality management system and document the policies and standard operational procedures in a Laboratory Quality Manual.

3.3 All procedures shall be carried out by qualified field and laboratory personnel who are trained in the specific analytical and reporting procedures outlined in this document. Additional requirements for maintaining generally acceptable practices and good laboratory practices are provided throughout this document.

3.4 Standard laboratory practices and procedures described herein are derived from authoritative methods recognized by the Environmental Protection Agency. Where Chesapeake Bay Program (CBP) analytical methods differ from EPA-approved methods, Participant laboratories have demonstrated and documented that the modifications either improve method performance or have a negligible effect. Equivalency studies are fully documented and available on the CBP Data Integrity Workgroup webpage at: http://www.chesapeakebay.net/groups/group/analytical_methods_and_quality_assurance_workgroup.

3.5 Laboratory and field SOPs must be consistent with Chesapeake Bay Program methods. If CBP methods fail to provide sufficient detail, Participants are encouraged to check the references listed below and cited throughout this document. Nontidal sampling procedures should be consistent with the USGS National Field Manual for water quality data.

3.5.1 *Standard Methods for the Examination of Water and Wastes*, (APHA) 20th, 21st or 22nd edition.

3.5.2 Methods *for the Determination of Inorganic Substances in Environmental Samples (*Aug. 1993). EPA/600/R-93/100.

3.5.3 *Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices- 2nd edition* (Sept. 1997). EPA/600/R-97/072.

3.5.4 U.S. Geological Survey, variously dated, *National field manual for the collection of water-quality data*: U.S. Geological Survey Techniques of Water-Resources

Investigations, book 9, chaps. A1-A9. Available online at http://pubs.water.usgs.gov/twri9A.

4. Participation in CBP Workgroup Meetings

4.1 Attendance of state representatives at Bay Program workgroup meetings, e.g., CBP Data Integrity Workgroup (DI Workgroup) and Integrated Monitoring Networks Workgroup, is recommended by the EPA Chesapeake Bay Program Office (CBPO).

4.2 The DI Workgroup is concerned with field and analytical methodology, quality assurance and data management issues. Membership includes laboratory directors, field managers, and State program managers conducting work for the Chesapeake Bay Program. The workgroup's goal is to ensure that analytical and field protocols provide consistent water quality data from tidal and nontidal waters of the Chesapeake Bay watershed.

4.3 Data quality and methods issues may originate from DI Workgroup members or be identified by other workgroups who observe anomalies during data analysis. The Workgroup seeks to identify discrepancies among Participants and take measures to improve the situation, frequently by refining and/or aligning procedures among Participants. The forum allows the different organizations to share the way they do things and individuals to present new and innovative ideas, potentially for their use.

4.4 Data comparability issues may also arise from regular DI Workgroup reviews of inter-laboratory performance samples. Participants shall conduct follow-up investigations to determine possible causes of inter-organization differences in split sample, blind audit or USGS reference sample results.

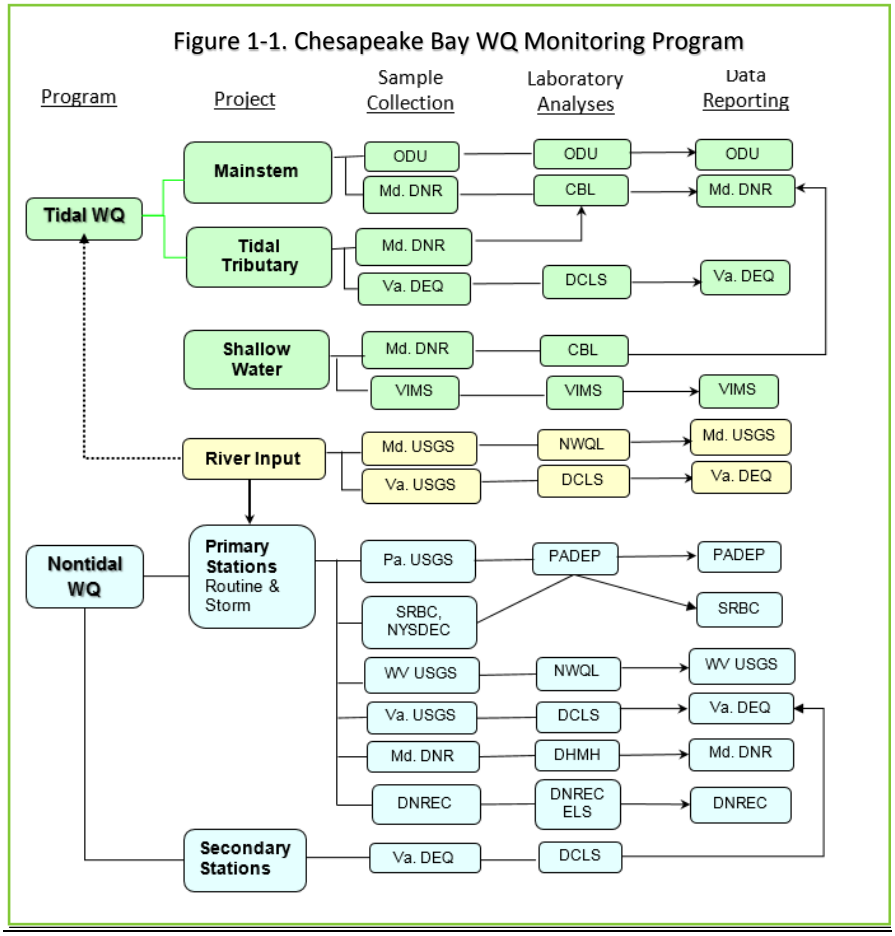
4.5 The CBP Integrated Trends Analysis Team conducts Bay-wide water quality data analyses and stresses the collection and management of comparable environmental data of known quality through continued communication and transfer of technologies among the agencies and institutions involved in the Chesapeake Bay water quality monitoring programs. Another group, the Integrated Monitoring Networks Workgroup, evaluates existing monitoring programs to ensure that sampling designs, parameters and methods generate the appropriate data to meet management and monitoring objectives.

5. State, Federal and Academic Participants

Chesapeake Bay Water Quality Monitoring Program Participants

CBL	Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science
CBPO	U.S. EPA Chesapeake Bay Program Office
DCLS	Virginia Division of Consolidated Laboratory Services
DNREC (ELS)	Delaware Dept. of Natural Resources and Environmental Control, Environmental Lab Services
MDNR	Maryland Department of Natural Resources
ODU	Old Dominion University Water Quality Laboratory
PADEP	Pennsylvania Department of Environmental Protection
NYSDEC	New York State Department of Environmental Conservation
USGS	United States Geological Survey (Md., Pa., Va. & WV Water Science Centers)
SRBC	Susquehanna River Basin Commission
VDEQ	Virginia Department of Environmental Quality
VIMS	Virginia Institute of Marine Science
WVDEP	West Virginia Department of Environmental Protection

Figure 1-1, Chesapeake Bay WQ Monitoring Program Participants, represent State and Federal Agencies participating in various Chesapeake Bay Program water quality monitoring activities.



Section B. Summary of Requirements

1. Task Areas

1.1. For each monitoring program component, Participants perform certain tasks. These tasks include: (1) development of a sampling schedule; (2) performance of field measurements; (3) collection, preparation and transport of field samples; (4) laboratory receipt and preparation of field samples; (5) chemical analyses of field samples; (6) adherence to QA/QC procedures outlined in this document; and (7) maintaining records, managing data and reporting results.

All efforts must be made to produce data that are comparable to data collected previously and currently by other Chesapeake Bay Program grant recipients and participants.

These tasks are specifically outlined as follows.

1.1.1. **Task 1. Development of sampling schedules**

1.1.1.1. Participants will develop sampling schedules that comply with the frequency and location requirements established in this document. The mainstem and tidal tributary sampling schedules shall be developed in close coordination among Virginia DEQ, CBPO, and Maryland DNR.

1.1.1.2. Participants will ensure compliance with the sampling schedule through the development and use of contingency plans.

1.1.1.3. Participants will visit each sampling station as specified in Chapters 4 and/or 5; verify and record site location according to procedures specified in each Chapter.

1.1.1.4. Participants will transport the sampling crew and all necessary sampling and field measurement equipment and ensure that they are capable of meeting the specified data quality objectives.

1.1.2. **Task 2. Performance of field measurements**

1.1.2.1. Participants will measure physical and chemical parameters as described in Chapters 4 and/or 5.

1.1.3. **Task 3. Collection, preparation, and transport of field samples**

1.1.3.1. Participants will develop and implement sample handling and preservation procedures as prescribed in Chapter 2.

1.1.3.2. Participants will collect samples as described in Chapter 4 and 5.

1.1.3.3. Participants will prepare and preserve samples as described in Chapter 4 and 5.

1.1.3.4. Participants will transport samples as described in Chapter 4 and 5.

1.1.4. **Task 4. Laboratory receipt and preparation of field samples**

1.1.4.1. Participants will develop and implement laboratory handling and custody procedures as described in Chapter 2.

1.1.4.2. All CBP monitoring samples shall be received and logged by the sample custodian or designee.

1.1.4.3. Participants will provide adequate storage for CBP samples awaiting analysis.

1.1.4.4. Participants will follow the applicable sample preparation procedures outlined in this document.

1.1.5. **Task 5. Chemical analysis of field samples**

1.1.5.1. Samples shall be analyzed by the techniques described in the methodologies given in Chapters 6.

1.1.5.2. Samples must be analyzed within the maximum specified holding times.

1.1.6. **Task 6. Adherence to the QA/QC procedures outlined in this document**

1.1.6.1. Participants will adhere to all QA procedures described in Chapter 2 and all applicable QC procedures described in Chapters 4, 5 and 6. Records documenting the use of the specified QC protocols shall be maintained in accordance with the document control procedures described in Chapter 2.

1.1.6.2. Participants will establish a Quality Assurance Project Plan (QAPP) with the objectives consistent with Chesapeake Bay Program monitoring objectives. The QAPP shall incorporate the QC procedures, any necessary corrective action, and all documentation required during data collection, as well as the quality assessment measures performed by management to ensure acceptable data production.

1.1.6.3. Participant laboratories will establish a comprehensive quality management system and document the policies and operational procedures in a Laboratory Quality Manual. The Quality Manual will address the components contained in Chapter 2.

1.1.6.4. Participant laboratories will analyze Chesapeake Bay Coordinated Split Samples, CBP Blind Audit and USGS Standard Reference Samples for the parameters and sample types analyzed for the CBP. The results from these laboratory inter-comparison samples may be used for evaluating laboratory performance.

1.1.6.5. "Compliant performance" is defined as that which yields correct compound identification and concentration values as determined by the CBPO, as well as meeting the method requirements for analysis, quality assurance/quality control, data reporting and other deliverables; including sample custody, sample documentation, and SOP documentation.

1.1.7. Task 7. Records, Data Management and Reporting

1.1.7.1. The laboratory shall establish a record keeping system that allows the history of the sample and associated data to be readily understood through the documentation. This system shall produce unequivocal, accurate records that document all laboratory activities associated with the testing such as equipment, personnel, methods, analytical standards, calibration, sample receipt, data verification, etc.

1.1.7.2. Samples will be analyzed and the data reported to EPA or the State Agency within the time specified in the cooperative agreement. The Participant will prepare electronic and hardcopy data according to the procedures specified by the CBPO and will report electronic and hardcopy data according to the formats, order, and turnaround times required.

2. Personnel Requirements

2.1 Participants will provide technical expertise for the EPA grant or State/EPA cooperative agreement.

2.2 Participants will designate and use key personnel to perform the functions described below. The CBPO reserves the right to review personnel qualifications and experience, and take action as appropriate.

2.3 Participants should have an organization with well-defined responsibilities for each individual in the management system to ensure sufficient resources are available to meet the conditions of the grant or cooperative agreement, and to maintain a successful operation. To establish this capability, the Participant will designate personnel to carry out the following responsibilities. Functions include, but are not limited to, the following:

2.3.1 Project Manager

The project manager is employed by the State or Federal agency. S/he is responsible for all aspects of the grant or assistance agreement, from sample collection through data delivery, and shall be the primary contact for the Participants and CBPO. The project manager shall be responsible for the technical and management aspects of the agreement and shall ensure that all requirements are met.

Recommended qualifications for Project Manager are: a) Bachelor's degree in chemistry, biology, or other scientific/engineering discipline and b) Two years of field experience, two years of laboratory experience, and one year of project management experience.

2.3.2 Field Supervisor/Chief Scientist

The field supervisor is responsible for all field activities performed on behalf of the State agency, including the training and supervision of new staff. Recommended qualifications are: a) Bachelor's degree in chemistry, biology or other scientific/engineering discipline and b) Two years of field experience, or a combination of supervisory and field experience.

2.3.3 Water Quality Laboratory Supervisor

The Water Quality Laboratory Supervisor is responsible for all technical efforts of the laboratory performed on behalf of the State agency. Recommended

qualifications are: a) Bachelor's degree in chemistry or other scientific/engineering discipline and b) Four years of water quality laboratory experience, or a combination of supervisory and laboratory experience.

2.3.4 Quality Assurance Officer

The State Agency Quality Assurance Officer is responsible for overseeing the quality assurance aspects of contract data and reporting directly to upper management to meet all terms and conditions of the State/EPA cooperative agreement.

Recommended qualifications are: a) Bachelor's degree in chemistry or any scientific/engineering discipline and b) Two years of field and laboratory experience, including at least one year of applied experience with QA principles and practices in sampling and analytical procedures.

2.3.4 Sample Custodian

The Laboratory Sample Custodian is responsible for receiving the water quality samples (logging, handling, and storage). The Field Office Sample Custodian is responsible for handling and temporary storage of samples in the field office.

Recommended qualifications are: a) High School Diploma with four or more science courses and b) One year of experience receiving and logging scientific samples. Additional education and/or experience may be substituted for these requirements.

2.3.5 Data System Manager

The Data system Manager is responsible for the management and quality control of all computing systems (hardware, software, documentation, and procedures), generating, updating, and quality controlling automated deliverables to meet all terms and conditions of the State/EPA cooperative agreement.

Recommended qualifications are: a) Bachelor's degree with four or more intermediate courses in programming, information management, database management systems, or systems requirements analysis and b) Two years of experience in data or systems management or programming including one year of experience with software utilized for data management and generation of data deliverables.

2.3.6 Field personnel

Field personnel are responsible for field measurements and collection of monitoring samples in accordance with this document. Recommended qualifications for field personnel: a) Bachelor's degree in chemistry or any scientific/engineering discipline, and/or b) Coursework or experience sampling surface waters and collecting in-situ measurements using the field instrumentation and sampling devices cited in this document. Experience may be waived if a person will work under the close supervision of experienced staff.

Additional suggestions are given in the document *Knowledge and Skill Guidelines for Marine Technicians Who Work Aboard Research Vessels (2001)*, available on the world-wide web at:
http://www.marinetech.org/files/marine/files/Workforce/Marine%20Technicians.pdf

2.3.7 Water Quality Laboratory personnel

Water quality laboratory personnel are responsible for the analysis of samples in accordance with this document. Recommended qualifications include: a) Bachelor's degree in chemistry or any scientific/engineering discipline (or equivalent experience) and b) One year of experience analyzing nutrients and water quality parameters, specifically, with the instrumentation and methods cited in this document. Experience may be waived if a person will work under the close supervision of experienced staff.

2.3.8 **Database Manager**

The database manager is responsible for the operation and maintenance of software and programs generating, updating and quality controlling analytical databases and automated deliverables to meet all terms and conditions of the EPA grant or State/EPA cooperative agreement. Recommended qualifications are: a) Bachelor's degree with four or more intermediate courses in information management, information systems, database management systems, or systems requirements analysis and b) Two years of experience in data systems or including one year of experience with the software being utilized for data management and generation of data deliverables.

3. Facilities

3.1 The adequacy of the facilities is of equal importance as the technical staff to accomplish the required work as specified by this contract. Therefore, the Participant will provide the field and laboratory facilities described in this Section.

3.2 Field Facilities

3.2.1 **An adequate cruise vessel** to safely traverse the waterways and carry the field crew, field instrumentation, sampling equipment, sample preparation equipment and materials, freezers, refrigerators, or coolers with ice for cold-storage.

3.2.2 **Source of de-ionized (DI) organic-free water** to prepare reagent-grade, de-ionized water for calibration standards, field blanks and equipment rinsing.

3.3 Laboratory facilities

3.3.1 **Sample Receipt Area**

Adequate, contamination-free, well ventilated work space provided with chemical resistant bench top for receipt and safe handling of CBP samples.

3.3.2 **Storage Area**

Sufficient refrigerator space is necessary to maintain unused CBP samples for at least 60 days after data submission. NOTE: Samples or extracts and standards shall each be stored separately. Samples shall be stored in an atmosphere demonstrated to be free from all potential contaminants.

3.3.3 **Sample and Reagent Preparation Areas**

Adequate, contamination-free, well-ventilated work space provided with:

3.3.3.0 Benches with chemical resistant tops, exhaust hoods.

3.3.3.1 Source of de-ionized (DI) organic-free water.

3.3.3.2 Analytical balance(s) located away from drafts and rapid changes in temperature.

3.3.4 Standards and reagents

Participants will have in-house the appropriate standards and reagents to perform all procedures.

4. Instrumentation and Equipment

4.1 Participants will have the field and laboratory instrumentation and equipment described in this Section.

4.2 All equipment and instrumentation specified in this document should be in the possession of the Participant and maintained in good condition at all times. Participants will ensure that, in the event of instrument or equipment failure, backup instrumentation or equipment in good condition are available to perform field measurements, and sample collection, preparation, and analysis. It is recommended that surge protectors and a temporary backup power supply source are installed in order to protect analytical instruments, PCs, and other equipment in the event of a temporary electrical disruption or power surge.

4.3 In addition, Participants should have an in-house stock of instrument parts to ensure continuous operation to meet contract-specified holding and turnaround times.

4.4 At a minimum, Participants should have the following instruments and equipment to complete the work.

4.4.1 Field Instrumentation and equipment

4.4.1.1 Secchi disk: 20 cm. diameter

4.4.1.2 Multi-parameter sonde, equipped with calibrated probes to measure *in-situ* specific conductance, temperature, pH and DO. Depth sensor or calibrated line to measure sampling depth.

4.4.1.3 LI-COR® light attenuation sensors: Flat Cosine Underwater Quantum Sensor LI-192SA, air (deck) reference sensor LI-190SA, and Data Logger (e.g., LI-1000, LI-1400 or LI-1500)

4.4.1.4 Sample collection bottles.

4.4.1.5 System to collect below-surface samples (e.g., submersible pump or rosette).

4.4.2 Analytical instrumentation

4.4.2.1 Automated nutrient analysis system equipped with an auto-sampler, manifold, proportional pump, heating bath, colorimeter, photomultiplier and computer-based data system.

4.4.2.2 Particulate Carbon and Nitrogen Analyzer (CNH) equipped with a combustion tube, reduction tube, water trap, and nitrogen and carbon dioxide detectors.

4.4.2.3 TOC Analyzer which employs high temperature combustion, platinum catalyst, and a non-dispersive infrared detector.

4.4.2.4 Analytical balance located away from drafts and rapid changes in temperature.

4.4.2.5 Centrifuge.

4.4.2.6 Dual beam spectrophotometer with matched cuvettes.

4.4.2.7 Drying oven capable of maintaining 103–105°C.

4.4.2.8 Muffle furnace capable of maintaining 550°C.

4.4.2.9 pH meter and probe.

5. Health and Safety Considerations

5.1 Participants should be aware of the potential hazards associated with the handling and analyses of surface water samples as they may contain hazardous materials which could present a risk to human health and a hazard to field and laboratory instrumentation/equipment. It is the Participants' responsibility to take all necessary measures to ensure the health and safety of its employees, and to maintain its analytical instruments in good working condition.

5.2 Participants will comply with all applicable Occupational Safety and Health Administration (OSHA) requirements.

5.3 Research vessel personnel must comply with nationally-recognized safety protocols for the operation of research vessels. One set of protocols, Research Vessel Safety Standards of the University-National Oceanographic Laboratory System (UNOLS), may be found on the world-wide web at: UNOLS Research Vessel Safety Standards - Revised March 2009

5.4 All vessels used to collect samples from the Chesapeake Bay mainstem monitoring stations will be in full compliance with state and federal regulations regarding the use of tributyltin (TBT) anti-fouling paints.

5.5 All vessels used to collect samples will be in compliance with Section 312 of the Water Quality Act of 1987 which requires the installation of marine sanitation device (MSD) on all vessels with installed toilets operating in the navigable waters of the United States. Type III MSDs, which are designed to prevent the discharge of human waste from boats in any form, will be used.

6. Other Federal and State Requirements

6.1 The Participant will comply with all relevant State and Federal laws and regulations, including the Resource Conservation Recovery Act (RCRA) and the Clean Water Act (CWA).

6.2 For hazardous substances used in the sample preparation and analysis procedures described in this document (e.g., acetone and inorganic acids), appropriate state and federal regulations must be followed for their handling and disposal both in the laboratory and in the field.

CHAPTER 2. QUALITY ASSURANCE PROGRAM

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Section A. Introduction

Title I, §117 of the Clean Water Act requires that the Chesapeake Bay Program Office (CBPO) support the Chesapeake Executive Council by implementing and coordinating science, research, modeling, support services, monitoring and data collection. The CBPO has maintained and supported a research-quality monitoring program for Chesapeake Bay tidal waters since the late 1980s when standardized sampling, analytical and data management procedures were developed and coordinated with the then Maryland Office of Environmental Programs and the Virginia State Water Control Board. In the 1990s, standardized River Input Monitoring was initiated to measure the nutrient and sediment loadings from each of the watershed's nine largest rivers. Chesapeake Bay Nontidal water-quality monitoring was later expanded upstream into rivers and streams across the Bay watershed, with all six participating jurisdictions using comparable protocols.

The tidal and nontidal monitoring programs provide an uninterrupted record of high-quality data that is used to calculate status and trends of water quality constituents over time. Trend analyses in particular require very reproducible data that are collected at the lowest possible limits of detection. Changes in methods, laboratories, instruments, sampling sites, etc., may affect trends analyses so changes are carefully evaluated and approved to preserve the comparability of the data records over time.

Each participant in the Chesapeake Bay Tidal and Nontidal monitoring shall develop and implement a quality assurance (QA) program that is in accordance with the procedures and recommendations of this document. The purpose of this chapter is to establish data quality objectives and quality assurance protocols for incorporation into each organization's QA System, Quality Assurance Project Plans (QAPP) and Standard Operating Procedures (SOP).

Section B of this chapter describes the requirements and recommendations for a participant's quality management system and QA documentation. *Section C* covers the data quality objectives for field and laboratory operations. Section D is a summary of field quality control practices, with more detailed requirements for Tidal and Nontidal field procedures provided in Chapters 4 and 5 respectively.

Section E describes routine inter-laboratory comparison studies, performance testing and external audits. Finally, *Section F* provides guidance for laboratories to conduct side-by-side comparisons prior to making procedural modifications.

Section B. Quality Management

1. Quality Management Systems

1.1. Organizations receiving EPA funds for monitoring are required to establish and document a formal quality management system (QMS) to ensure the generation of reliable and defensible data. A QMS is comprised of the organizational structure, objectives, policies, principles, responsibilities, and steps for ensuring quality and accountability in its work processes, products and services. A QMS includes:

1.1.1. Field operations and support functions used to assure consistency and data integrity: training, procurement, information management, records, management reviews of operations and data quality, evaluation criteria and follow up response.

1.1.2. Protocols for identifying out-of-control sampling, field measurements, and analytical conditions; processes for implementing and documenting the necessary corrective actions; decision rules and mechanisms for communicating the outcome.

1.2. The Chesapeake Bay Program recommends that participating laboratories develop and maintain a quality system that is equivalent to the National Environmental Laboratory Accreditation Institute (TNI) standards; however, laboratory accreditation is not required.

1.3. Laboratory quality management systems should be fully documented in a Laboratory Quality Manual.

1.4. Additional information on the entire Chesapeake Bay Program Quality Assurance Program is on the web at: http://www.chesapeakebay.net/about/programs/qa.

2. Quality Management Plans

2.1. State agencies receiving EPA funds to conduct monitoring activities are required to document their quality management system in a Quality Management Plan (QMP).

2.2. EPA must review and approve the QMP prior to the initiation of environmental data collection and/or compilation activities. The document must be prepared in accordance with EPA QA/R-2: EPA Requirements for Quality Management Plans, which is available on the EPA Quality Program webpage (URL: http://www2.epa.gov/quality).

2.3. The QMP must be approved internally by the state QA Manager and the organization's senior management, and then be submitted to the EPA Project Officer at least 45 days prior to the initiation of data collection or data compilation. The U.S. EPA Region 3 Quality Assurance Manager approves the QMP.

2.4. An approved QMP is valid for up to five years unless there is a major program reorganization that affects quality assurance functions and structures in the organization.

3. Quality Assurance Project Plans

3.1. The Chesapeake Bay Quality Assurance Program requires the development and implementation of a Quality Assurance Project Plan (QAPP) for each of its monitoring activities. The QAPP must cover specific activities to be performed and procedures to be used by the Participant.

3.2. The purpose of the QAPP is to: 1) ensure that the level of needed data quality will be determined and stated before the data collection efforts begin and 2) ensure that all monitoring data generated and processed will reflect the quality and integrity established by the QAPP.

3.3. The QAPP is composed of standard elements that cover all aspects and activities of the monitoring, from planning, through implementation, to assessment. The document EPA Requirements for QA Project Plans (QA/R-5) fully describes the necessary elements which are outlined in Appendix 2-A.

3.4. Review and Approval of QAPPs

3.4.1. The EPA CBPO Project Officer and QA Coordinator will review and approve the QAPP at least to the "Conditionally Approved" level (meaning all technical issues having been resolved to the satisfaction of the CBPO) prior to data collection. The QAPP shall be reviewed and approved in the context of the Program's Data Quality Objectives (DQOs).

3.4.2. The CBP QA Coordinator shall review and evaluate the implementation of the plans during the operational phases of sampling and analyses. The CBP QA Coordinator shall also assess the actual performance of the planned activity and subsequent results according to the criteria described in the QAPPs.

3.5. EPA-approved QAPPs are posted on the CBP web pages for Tidal and Nontidal Quality Assurance. See http://www.chesapeakebay.net/about/programs/qa/tidal

4. Laboratory Quality System and Quality Manual

4.1. The purpose of the laboratory quality management system is to:

4.1.1. Establish and maintain data integrity, validity, and usability standards.

4.1.2. Ensure that sampling and analytical systems are maintained in an acceptable state of stability and reproducibility.

4.1.3. Detect problems through data assessment and establish corrective action procedures to ensure that the sampling, analytical, and measurement processes are reliable.

4.1.4. Document all aspects of the sampling, analytical, and measurement processes in order to provide data that are technically sound and legally defensible.

4.2. The laboratory quality management system should be documented in a Laboratory Quality Manual (QM). All policies and procedures governing the laboratory's quality system shall be documented in the QM, and all laboratory personnel shall follow the policies and procedures established by the quality manual.

4.3. The QM should present in specific terms, the policies, organization, objectives, and specific QA and QC activities designed to achieve the data quality requirements recommended in this document. Where applicable, Standard Operating Procedures (SOPs) pertaining to each element should be included or incorporated by reference as part of the QM. The QM should be available during on-site laboratory evaluations.

4.4. See Appendix 2-A of this chapter for an outline of key elements in the laboratory quality manual.

5. Standard Operating Procedures

5.1. A SOP is a written document which provides directions for the step-by-step execution of an operation, test, or action which is commonly accepted as the method for performing certain routine or repetitive tasks. These tasks include operations such as sampling, sample tracking, analysis, glassware preparation, instrument calibrations, preventive and corrective maintenance, and data reduction and analysis. SOPs should be expressed in terms of fixed protocols which must be followed. Where options exist, these should be clearly described, and criteria for the selection of alternatives must be included. SOPs should be written such that the actual practices are recorded.

5.2. SOPs should be clear, comprehensive, up-to-date, and sufficiently detailed to permit duplication of results by qualified analysts. All SOPs should reflect activities as they are currently performed in the field and laboratory. In addition, all SOPs should be:

5.2.1. Consistent with the field and laboratory methods contained in this document and/or established by Chesapeake Bay Program Workgroups.

5.2.2. Consistent with applicable federal and state regulations and guidelines.

5.2.3. Adequate to establish traceability of standards, instrumentation, samples, and monitoring data.

5.2.4. Simple, so that any user with appropriate general education, experience, and training can duplicate the task as historically performed.

5.2.5. Consistent with a) sound scientific and engineering principles, b) instrument manufacturers' instruction manuals and c) good laboratory practices.

5.2.6. Complete enough so the user or auditor follows the directions in a logical step-wise manner through the sampling, analysis, and data handling processes.

5.3. Benefits of SOPs

5.3.1. Adherence to SOPs minimizes measurement bias and increases reliability.

5.3.2. SOPs provide a record of the performance of all tasks at any fixed point in time.

5.3.3. SOPs increase the opportunity for thorough review of procedures with appropriate sign-off by management.

5.3.4. SOPs serve as training documents for new employees, resulting in consistent performance of tasks.

5.3.5. SOPs provide a historical record of changes made to the method over time.

5.4. The degree of adherence to the approved SOPs should be determined during systems audits. It is recommended that all SOPs be reviewed periodically and revised to reflect changes in procedures. The Laboratory Manager should provide the most current SOPs to the Agency program manager for inclusion in the Agency's QAPP.

5.5. Laboratory method SOPs should follow a standard format such as the example below.

5.5.1. Title Page (method name, number, version, effective date, document control number)

5.5.2. Scope and Application (matrices, analytical range, etc.)

5.5.3. Summary of Test Method

5.5.4. Definitions

5.5.5. Interferences

5.5.6. Safety

5.5.7. Equipment and Supplies

5.5.8. Reagents and Standards

5.5.9. Sample Preservation and Storage

5.5.10. Quality Control (from EPA 2012, 40 CFR Part 136, § 136.7)

5.5.10.1. Demonstration of Capability

5.5.10.2. Method Detection Limit

5.5.10.3. Laboratory Reagent Blank

5.5.10.4. Laboratory Control Sample

5.5.10.5. Matrix Spike and Matrix Duplicate

5.5.10.6. Control Charts (or other trend analysis of QC results)

5.5.10.7. Corrective Action (root cause analysis)

5.5.10.8. QC Acceptance Criteria

5.5.11. Calibration and Standardization

5.5.12. Sample Preparation and Analysis

5.5.13. Analytical Procedure

5.5.14. Calculations (automated and manual) and Reporting

5.5.15. Method Performance Summary

5.5.16. References

5.5.17. Tables, Diagrams, etc.

6. Procedural Change Authorization

6.1. The CBP Quality Assurance Coordinator must be notified of the intent to make any substantial or long-term change to a procedure or method, either in the field or laboratory. These changes include items such as instrument type and sampling stations.

6.2. The effects of any change in analytical instruments, reagents, calibration, digestion procedure, etc., should be quantified, documented and submitted to the CBP QA Coordinator prior to implementing. Section 2.F of this chapter provides detailed instructions for performing method validation and comparability studies.

6.3. All modifications should be documented using the Chesapeake Bay Monitoring Program Procedure Modification Tracking Form (PMTF) (Figure 2.1). The completed PMTF should be submitted to the State agency Monitoring Coordinator, CBP Quality Assurance Coordinator and CBP Water Quality Database Manager.

6.4. Minor changes in field or laboratory procedures, including detection limit changes, should be documented in the CIMS metadata and data submission tables.

6.5. Minor events and problems encountered during Chesapeake Bay mainstem cruises may be reported in the CBP Monitoring Cruise Report and submitted to the State agency, who will then forward the information to the CBPO.

6.6. Modifications due to emergencies during a sampling cruise are authorized by the Chief Scientist with priorities for safety and completion of the cruise. The change should be documented within 30 days after the cruise, in either the PMTF or the Monitoring Cruise Report, depending on size or potential impact of the deviation on the data.

Figure 2.1

CHESAPEAKE BAY MONITORING PROGRAM PROCEDURE MODIFICATION TRACKING FORM

PMTF # _____ **□ APPROVED □ DENIED**

This form is used to request approval for modifications and to document approved modifications made to Chesapeake Bay Program Office procedures or methods. It is not a substitute for timely contact with the CBPO Quality Assurance Officer or his/her designee, who may be reached at 1-800-968-7229. A detailed method description including the proposed modification must be attached to this form prior to submittal to CBPO.

DATE SUBMITTED		DATE APPROVED	
REQUESTOR NAME		ORGANIZATION	
NEWLY PROPOSED MODIFICATION [ ]	FIELD-APPROVED MODIFICATION [ ]	APPROVED BY: DATE:	
TYPE OF PROCEDURE / METHOD	SAMPLING [ ] FIELD MEASUREMENT [ ]	ANALYTICAL [ ] OTHER [ ] SPECIFY:	REPORTING [ ]
DURATION	PERMANENT [ ] TEMPORARY [ ]	EFFECTIVE DATE: START DATE: END DATE:	
PROCEDURE/METHOD DESCRIPTION			
MODIFICATION DESCRIPTION			
JUSTIFICATION FOR MODIFICATION			
ANALYTICAL PARAMETERS THAT MAY BE AFFECTED BY THIS CHANGE			
AFFECTED QA PLAN(S) (TITLE, REVISION, & DATE)			
AFFECTED CRUISE(S)			
PMTF COMPLETED BY	NAME:		DATE:

STATE APPROVAL: NAME _____ TITLE _____

SIGNATURE _____ DATE _____

CBPO APPROVAL: NAME _____ TITLE _____

SIGNATURE _____ DATE _____

7. Sampling Handling and Custody

7.1. Procedures should be established that ensure that samples are properly collected, preserved, transported, stored and analyzed within the required holding times.

7.2. The laboratory must establish and operate a system for assuring positive identification of samples and documentation of all samples received. To ensure sample integrity, procedures for sample identification, sample receiving, and custody should be developed, instituted and documented.

7.3. Sample Identification

7.3.1. To assure traceability of samples while in possession, there should be a specified method for maintaining identification of samples in the field and throughout the laboratory.

7.3.2. Each sample and sample preparation container should be labeled with a unique identifier that is cross-referenced with the corresponding documentation.

7.3.3. Sample receiving, storage and disposal requirements are described in Chapter 6, Sections C.2 & C.3.

8. Document Control

8.1. The goal of the document control program is to assure that all documents and electronically stored information from a specified cruise are accountable, secure, and completely retrievable. Document control is recommended for each activity to include electronic as well as hardcopy documentation. Accountable documents should include but not be limited to: field and laboratory logbooks, sample work sheets, bench sheets, and other documents relating to the sample or sample analyses. The following document control procedures have been established to assure that all field and laboratory records are assembled and stored for delivery to the CBPO or are available upon request from the CBPO.

8.2. Preprinted Forms and Logbooks

8.2.1. All documents produced which are directly related to the sampling, preparation, and analysis of CBPO samples should be maintained for inspection by the CBPO. All observations and results not recorded on preprinted forms should be entered into permanent logbooks. When data from a cruise are compiled, all original field and laboratory forms and copies of all cruise-related logbook entries should be included in the documentation package.

8.2.2. Pre-printed field and laboratory forms should contain the name of the field crew/laboratory and be dated (month/day/year) and signed by the person responsible for performing the activity at the time an activity is performed.

8.2.3. Logbook entries should be dated (month/day/year) and signed by the person responsible for performing the activity at the time an activity is performed.

8.2.4. Logbook entries should be in chronological order.

8.2.5. Pages in unbound logbooks should be sequentially numbered.

8.2.6. Data sheets or logs should be maintained to enable a reconstruction of the sample collection or analysis in question.

8.2.7. Corrections to supporting documents and raw data should be made by drawing a single line through the error and entering the correct information. Corrections and additions to supporting documents and raw data should be dated and initialed. No information should be obliterated or rendered unreadable. All notations should be recorded in ink. Unused portions of documents should be crossed out.

8.3. Storage of Files

Field and laboratory documents will be maintained in a secure location for a period of five years from the date of sample delivery.

9. Consistency of Documentation

9.1. A document control officer responsible for the organization and assembly of the data package should be assigned.

9.2. All copies of field and laboratory documents should be complete and legible.

9.3. Before releasing test results, the document control officer should assemble and cross-check the information on sample tags, custody records, laboratory bench sheets, personnel and instrument logs, and other relevant data to ensure that data pertaining to each particular sample or sample delivery group is consistent throughout the data submittal package.

9.4. All documents relevant to each cruise, including logbook pages, bench sheets, screening records, re-preparation records, records of failed or attempted tests, and custody records should be inventoried.

10. Contingency Plans and Health and Safety Plans

Participants should develop and implement the following additional plans:

10.1. A contingency plan covering the availability and/or plan for a backup vessel.

10.2. A contingency plan for key field instrumentation failure.

10.3. A Health and Safety Plan in accordance with all applicable State and Federal regulations.

Section C. Data Quality Indicators and Objectives

1. Introduction

Data Quality Objectives (DQOs) are qualitative and quantitative statements that specify the quality of data required supporting specific CBPO decisions. DQOs specify the level of uncertainty that a decision maker is willing to accept in results derived from monitoring data. The level of uncertainty is largely a function of sampling frequency and spatial density.

Sampling frequency and fixed station locations for the Chesapeake Bay Tidal Monitoring Program were established by comparing the power and robustness of various fixed station sampling designs (Alden, 1994). It was determined that 14 monitoring events, or "cruises" were sufficient for calculating long-term annual trends with acceptable confidence. As funding permits, additional cruises are added to capture major climatic and biological events. Approximately 100 mid-channel sampling locations or "stations" represent the different regions of the estuary and were selected to represent the Chesapeake Bay segmentation/ characterization scheme, which is based on circulation patterns, salinity and geomorphology.

Sampling frequency and fixed station locations for the Chesapeake Bay Nontidal Monitoring Program are described in Chapter 5, Nontidal Water Quality Monitoring.

2. Data Quality Indicators

2.1 Data Quality Indicators (DQIs) are quantifiable attributes applicable to an entire data set. The principal indicators of data quality are precision, bias, accuracy, representativeness, comparability, completeness, and sensitivity.

2.2 For both field and lab measurements, performance criteria (i.e., acceptance limits) are established for each Data Quality Indicator to ensure that reported data are sufficient for their intended use. These criteria are called Measurement Quality Objectives (MQOs).

2.3 The performance criteria given in this section are associated with the methods contained in this document. Laboratories may develop their own internal criteria based on recent internal performance data, which often results in different acceptance thresholds than those given here.

2.4 Quality Objectives for activities are established for comparability, representativeness, precision, accuracy, and completeness, using the following protocols.

2.4.1 The sampling procedures and sample locations recommended in this document are used to ensure sampling comparability and representativeness of data generated to meet the CBP needs.

2.4.2 Actual precision of the overall sampling and analytical procedures is assessed through replicate field samples and may be expressed as relative percent difference (RPD). *In-situ* measurements are not replicated. Sampling precision can be estimated by comparing overall precision to the analytical precision.

2.4.3 Positive bias from contamination is checked through the analysis of field blanks. Field-spiked samples are not required for CBP nutrient monitoring.

2.4.4 Completeness of sampling is calculated based on the ratio of actual samples collected vs. samples that were planned, and is expressed as percent completeness.

2.5 Quality Objectives for ***in-situ*** field measurements are expressed in terms of comparability, representativeness, completeness, and minimum reporting limits using the criteria listed in Table 2.2.

2.5.1 Comparability and representativeness of field measurement data are ensured through adherence to the CBP methodologies and quality assurance protocols.

2.5.2 Completeness of field measurement data is calculated based on the ratio of measurements made to measurements planned, and is expressed as percent completeness.

2.6 Reporting limits for field instruments may be based on the manufacturer's stated sensitivity or precision.

2.7 Quality objectives for analytical **laboratory data** are expressed in terms of comparability, representativeness, precision, accuracy, bias, completeness, and detection limits, using the following protocols.

2.7.1 Accuracy, expressed as percent recovery, is based on the analysis of spiked samples and certified reference materials.

2.7.2 Completeness of analytical data is calculated based on the ratio of samples that are analyzed to the number of samples received, and is expressed as percent completeness.

2.7.3 Method detection limits (MDLs) and practical quantitation limits (PQLs) should be determined for all parameters using the procedures in *Chapter 6*, Sections C.8 & C.9, which are based on the 40 CFR Part 136 procedure.

2.7.4 Real-time quality control charts for precision and accuracy should be developed to monitor changes in random and systematic errors. Charts may be constructed for each parameter and applicable concentration ranges using the most recent 12 months of data, or at least the last 30 data points.

3. Performance Criteria (MQOs) for Field Methods

3.1. The combined precision of sampling and analysis is estimated for each parameter from field split samples (sample types FS1 and FS2) that are processed exactly the same as regular samples. Alternatively, concurrent samples (sample types S1 and S2) may be used.

3.1.1. Field precision may be estimated by comparing the overall precision to the laboratory precision.

3.1.2. Dissolved parameters are expected to have higher precision due to the homogeneous nature of the sample.

3.2. Positive bias in sampling and analytical processes is estimated from field blanks (FB). Field blanks are processed in the field exactly the same as regular samples.

3.2.1. Field bias may be estimated by comparing the overall bias to the laboratory bias.

3.2.2. The analysis of a sample of unprocessed field blank water is called, source water blank (SWB) may provide useful information for bias estimates.

3.2.3. Performance criteria for overall precision, bias and completeness are provided in Table 2.1 below.

Table 2.1 Quality Objectives for Overall Precision, Bias and Completeness – Tidal and Nontidal Parameters

PARAMETERS	Procedure (Chapters 4 & 5)	PRECISION (FS1 & FS2)	BIAS (Field Blanks)	COMPLETE-NESS
Tidal Water Quality	4.A.4	Particulate[1]: ≤ 30% RPD	≤ PQL or Reporting limit	95%
		Dissolved: ≤ 20% RPD		
Nontidal Water Quality	5.C.4	Particulate[1]: ≤ 30% RPD	≤ PQL or Reporting limit	90%
		Dissolved: ≤ 20% RPD		
		Whole Water[2]: ≤ 20% RPD		

[1] Particulate parameters include TSS, Particulate Nitrogen, Particulate Carbon and Particulate Phosphorus.
[2] Whole water parameters include Total Nitrogen, Total Phosphorus and Total Organic Carbon.

4. Performance Criteria (MQOs) for Field Measurements

4.1. Performance criteria guidelines for field instrument post-calibration tolerance, completeness, precision and minimum reporting limits are provided in Table 2.2 below.=

Table 2.2 Quality Objectives for In-situ Field Measurements

PARAMETER	Procedure (Chapter 4)	POST-CALIBRATION TOLOERANCE	COMPLETE-NESS	PRECISION/ REPORTING LIMIT
pH	4.B.3	± 0.2 units	95%	0.1 pH unit
Dissolved Oxygen	4.B.3	± 0.3 mg DO/L	95%	0.1– 0.2 mg DO/L
Secchi Depth	4.B.5	NA	95%	0.05 - 0.1 meter
Specific Conductance	4.B.3	± 5% of calibration standards	95%	1 umho/cm
Salinity	4.B.3	NA	95%	0.1 psu
Light Attenuation	4.B.6	NA	95%	0.05% @ 100% light
Water Temperature	4.B.3	NA	95%	0.1°C
Depth	4.B.3	NA	95%	meter

5. Performance Criteria (MQOs) for Laboratory Analyses

5.1 Precision, bias, accuracy and detection limit objectives for laboratory water quality analyses are provided below in Table 2.3.

5.2 Laboratory precision objectives apply only to laboratory replicates whose concentrations are all above the practical quantitation limit (PQL). Precision objectives for concentrations near the PQL are to be developed and specified by the laboratory.

Table 2.3. Quality Objectives for Laboratory Analyses (Tidal and Nontidal WQ Parameters)

PARAMETER	Reference Procedure (Chapter 6)	LAB PRECISION (above PQL)	PERCENT RECOVERY	TIDAL MDL (mg/L)	NONTIDAL	
					MDL (mg/L)	RL (mg/L)
Total Dissolved Phosphorus	6.D.1	≤ 20% RPD	80 - 120%	0.003	0.006	0.01
Total & Dissolved Ortho-Phosphate	6.D.8	≤ 20% RPD	80 - 120%	0.002	0.004	0.005
Particulate Phosphorus	6.D.10	≤ 30% RPD	80 - 120%	0.003[a]	0.003[a]	0.01
Nitrite	6.D.6	≤ 20% RPD	80 - 120%	0.002	0.002	0.01
Nitrite + Nitrate	6.D.5	≤ 20% RPD	80 - 120%	0.002	0.005	0.01
Ammonia	6.D.2	≤ 20% RPD	80 - 120%	0.004	0.005	0.01
Total and Total Dissolved Nitrogen	6.D.1	≤ 20% RPD	80 - 120%	0.05	0.05	0.10
Particulate Nitrogen	6.D.9	≤ 30% RPD	90 - 110% (CRM)	0.020[a]	0.020[a]	0.030
Particulate Carbon	6.D.9	≤ 30% RPD	90 - 110% (CRM)	0.09[a]	0.30[a]	0.50
Total & Dissolved Organic Carbon	6.D.7	≤ 20% RPD	80 - 120%	0.30	0.30	0.50
Chlorophyll-*a*	6.D.3	≤ 20% RPD	N/A	1.0 µg/L	1.0 µg/L	2.0 µg/L
Pheophytin	6.D.3	≤ 20% RPD	N/A	1.0 µg/L	1.0 µg/L	2.0 µg/L
Total Suspended Solids	6.D.11	≤ 30% RPD	N/A	3.0	3.0	5.0
Fixed Suspended Solids	6.D.12	≤ 30% RPD	N/A	3.0	3.0	5.0
Silicate	6.D.13	≤ 20% RPD	80 - 120%	0.02	0.03	0.3

[a] MDL is based upon a 250 mL sample volume. Other volumes yield different MDLs.

Section D. Field Quality Control

1. Annual Calibration

1.1. An annual calibration is an extensive and thorough calibration using standards or instruments traceable to certified (e.g. National Institute of Standards and Technologies) instruments or standards. Annual calibrations of *in-situ* instruments may be performed by a manufacturer or a specialized service contractor.

1.2. Annual calibrations will be performed on each field instrument, with the exception of LiCor® meters, where calibration is recommended annually, and required every two years.

2. Routine Calibration

2.1. Field staff shall calibrate *in-situ* instruments before and after each sampling event, deployment, or multiple-day cruise to ensure that the field instrument response is comparable to the response that existed at the annual calibration.

2.2. Instrument calibrations are performed according to manufacturers' specifications.

2.2.1. Routine calibration is required for *in-situ* dissolved oxygen (DO), pH, and conductivity measurements (but not for depth, PAR and temperature).

2.2.2. For dissolved oxygen, a calibration check is recommended at the beginning of each sampling day. If daily DO checks deviate by ≥ 0.30 mg DO/L from the expected value, the sensor must be recalibrated. If a calibration check (daily or post-calibration) is ≥ 0.50 mg DO/L, censor all data corresponding back to the last calibration check using the CIMS WQ Problem Code "V" (Sample results rejected due to QC criteria).

2.2.3. Calibration of Dataflow and extended *in-situ* deployments are performed for DO, pH, conductivity, chlorophyll and turbidity measurements. (See Participant's QAPP for the most recently approved procedures.)

2.3. SOPs for calibration should describe the preparation and use of the standard reference solution(s). If commercially prepared standards are used, cite the commercial source(s) in the field SOP and mark the date received on the bottle or calibration log.

2.4. When the calibration check indicates that a significant change occurred during a cruise, the instrument should be serviced and re-calibrated as described in the annual calibration.

2.5. If a daily or post-calibration check does not meet tolerances, qualify all data corresponding to the last calibration performed.

3. Calibration Samples

Calibration samples are water samples or independent *in-situ* measurements used to develop a statistical relationship between an *in-situ* measurement and the parameter of concern. One

example is the collection of grab chlorophyll *a* samples for converting *in-situ* fluorescence measurements into chlorophyll *a* estimates. A second example is the collection of PAR measurements for converting *in-situ* turbidity measurements into corresponding light attenuation coefficients (Kd).

4. Quality Indicators and Objectives for Field Measurements

Measurement quality objectives for field measurement post-calibration tolerance, completeness, precision and minimum reporting limit are provided in Table 2.2, which is copied below.

Table 2.2 Quality Objectives for In-situ Field Measurements

PARAMETER	CHAPTER 4 REFERENCE	POST-CALIBRATION TOLERANCE	COMPLETE-NESS	PRECISION / REPORTING LIMIT
pH	4.B.3	± 0.2 units	95%	0.1 pH unit
Dissolved Oxygen	4.B.3	0.3 mg DO/L	95%	0.1– 0.2 mg DO/L
Secchi Depth	4.B.5	NA	95%	0.1 meter
Specific Conductance	4.B.3	± 5% of std.	95%	1 umho/cm
Salinity	4.B.3	NA	95%	0.1 psu
Light Attenuation	4.B.6	NA	95%	0.05% @ 100% light
Water Temperature	4.B.3	NA	95%	0.1°C
Depth	4.B.3	NA	95%	0.5 meter

5. Field Replicate Samples

5.1. **Field Split (FS1 & FS2):** Two representative portions are taken from one homogeneous sample and processed identically. The data from the field split samples are an indicator of reproducibility (precision) in the sample preparation and analysis steps.

5.2. **Field Duplicate, Co-located (S1 & S2):** A field duplicate is a sample taken at the same sample location and depth as a CBP sample. The sample and its duplicate shall be taken in quick succession of each other. The data from field duplicates may be used to estimate overall precision or to deduce sampling precision.

5.3. The recommended frequency for collecting Field Split or Field Duplicate samples is according to the CBP monitoring program:

5.3.1. **Mainstem Replicates:** Collect a field split or a field duplicate once for every 20 samples.

5.3.2. **Tidal Tributary Replicates:** Each sampling group should collect field splits once per month, from both surface and bottom depths.

5.3.3. **Non-tidal Replicates:** The minimum number of field replicates is 2 duplicate pairs per station per year, about 10% of the samples. Sample collection groups (Addendum, Table 4) with more than 12 stations may limit the number of

duplicate pairs to 24 per year (i.e., 2 per month), with at least 1 duplicate pair collected at each station.

5.3.4. See Section B.6 and B.7in *Chapter 5, Nontidal Water Quality Monitoring*, for further requirements.

6. Field Blanks

6.1. A field blank is an aliquot of deionized water, free of the analytes of interest, which is poured into a sample container in the field and treated as a sample in all respects, including exposure to sampling site conditions, processing, filtration, preservation, storage and all analytical procedures.

6.2. Because a field blank is treated exactly like an environmental sample at the laboratory it will include any contamination introduced during transport, laboratory handling and analysis.

6.3. The purpose of the field blank is to demonstrate that[1]:

6.3.1. The sampling equipment has been adequately cleaned to remove contamination introduced by samples obtained at previous sites;

6.3.2. Sample collection and processing have not resulted in contamination; and that

6.3.3. Sample handling and transport have not introduced contamination.
[1]Quality-Control Design for Surface Water Sampling in the National Water-Quality Assessment Program (USGS OFR 97-223).

6.4. The frequency for preparing field-filtered blanks is according to the CBP monitoring program:

6.4.1. **Mainstem Monitoring:** Prepare one field-filtered blank each day.

6.4.2. **Tidal Tributary Monitoring:** Prepare at least one field-filtered blank per month.

6.4.3. **Non-tidal Monitoring:** Prepare at least one field blank per station each year for primary stations. The blanks should be taken from a variety of locations, flow conditions and months to obtain a representative sampling of blanks across time, space and flow conditions.

6.4.4. See *Chapter 5, Nontidal Water Quality Monitoring*, Section B.6, for further requirements.

6.5. If the concentration of a field blank exceeds the PQL, reporting limit or the lowest analytical standard in the calibration curve, field and/or laboratory contamination should be suspected and corrective action initiated.

6.6. Corrective action includes an investigation of possible contamination sources (e.g., instrument calibration check, field blank water, sample containers, etc.) and procedural modifications if necessary.

6.7. Blank values are NOT to be subtracted from sample results unless specified by the laboratory standard operating procedure.

7. Sampling Equipment Blanks

7.1. Equipment blanks indicate the effectiveness of the sampling equipment cleaning procedure. The equipment blank may be processed in the field office after the equipment has been cleaned.

7.2. An equipment blank is required once per year or whenever new equipment is used for the first time.

7.3. The equipment blanks consist of reagent-grade, deionized water that has been passed sequentially through each component of the sample processing and collection equipment, e.g., submersible pump and hose, Rosette bottles, sampling containers, churn splitter, filtration unit, etc.

7.4. An analysis of the unfiltered reagent grade water used to prepare the blanks maybe helpful in interpreting the results if contamination is found.

7.5. If the concentration of the equipment blank exceeds the lowest analytical standard in the calibration curve, prepare blanks of just the sampling equipment to isolate the cause of the contamination.

Section E. External Performance Assessments

1. Split Sample Program – Interlaboratory Comparisons

1.1. Background and Objectives

All laboratories participating in Chesapeake Bay Water Quality monitoring programs are required to participate in the Chesapeake Bay Coordinated Split Sample Program (CSSP). The CSSP was established in June 1989 by a recommendation of the Analytical Methods & QA Workgroup to the CBP Monitoring Subcommittee. The major objective of this program is to establish a measure of data comparability among Participants in the monitoring programs.

There are two CSSP sample types; the first is a saline water matrix, collected from the mainstem of the Bay. The second is a fresh water tributary sample, collected from the Potomac River. CSSP samples are collected four times a year for each type, and transported to the laboratories for processing the following morning.

1.2. Summary of Criteria

1.2.1. The Participant will participate in the applicable component(s) of the CSSP.

1.2.2. SOPs that are developed and used should be in accordance with the document titled *Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines, Revision 4* (December 2010), plus any revisions specified by the CBP QAO.

1.2.3. For each of the CSSP stations and on a quarterly basis, Participants receive and analyze three (fresh water) or four (saline) sub-samples. Laboratories analyze each sub-sample as a discrete sample for the analyses which they routinely perform in support of basin-wide data collection programs. One of the three sub-samples should be used to generate laboratory duplicates and a laboratory spike. These QC samples should be analyzed concurrently with the associated CSSP sub-samples.

1.2.4. The routine submission of split sample data is the responsibility of each laboratory and its in-house data management organization.

1.2.5. To supplement the analyses of the CSSP sub-samples and the respective QC sample, a certified standard reference material (CRM) for each parameter should be analyzed where available. The analysis of standard reference materials provides a strong measure of comparability among all laboratories and within one laboratory's analytical system over time, and is a critical element of any diagnostic efforts associated with the CSSP.

2. Performance Testing

2.1. The University of Maryland Chesapeake Biological Laboratory prepares blind audit samples for most water quality parameters. Blind audit samples are distributed semi-annually to participating laboratories.

Annual reports are available online at http://nasl.cbl.umces.edu/qaqc/blindaudit.html

2.2. Laboratories also participate in the USGS Standard Reference Sample study for nutrients. Lab managers are advised to analyze both high and low concentrations unless one concentration far exceeds their normal ranges of operation.

3. Audits of Data Quality

3.1. State agency staff or contractors shall review field blank and field duplicate data to assess the quality of sampling activities. Associated data that exceeds acceptance limits must be qualified via problem codes and corrective actions initiated to identify and correct the problem.

3.2. Analytical and measurement data should be reviewed at each level of collection, compilation and reporting to check the quality of measurement and analytical activities, respectively.

3.3. Participants prepare and submit a summary with each data submittal. The summary must include an explanation for each data point that did not meet the QC criteria established for each method, and deviations that occurred during the generation of the data.

3.4. The CBP Data Upload and Evaluation Tool (DUET) electronically performs over 160 checks on the data. Participants will be informed if any of the submitted data do not fall within the prescribed QC limits. Any errors found will be corrected by the Participant at no additional cost to the CBPO.

3.5. The CBP Grant Project Officer has the ultimate responsibility to accept or reject each data submittal.

4. On-Site Audits

4.1. The CBP QAO or representative will conduct periodic on-site evaluations of field and laboratory activities. The frequency of these on-site audits may be increased depending on the Participant's performance. On-site evaluations are carried out to monitor their ability to collect and analyze samples according to the DQOs established by the CBP Monitoring Program.

4.2. The CBP QA Coordinator and a State representative will inspect the Participant's field and laboratory facilities to verify the adequacy and maintenance of instrumentation, the continuity of personnel meeting experience and/or education requirements, and the acceptable performance of analytical and QC procedures. The Participant should expect that items to be monitored will include but not be limited to the following:

- Size and appearance of the facility.
- Quantity, condition, availability, and scheduled maintenance and performance of instrumentation.
- Availability, appropriateness, and use of field and laboratory SOPs.
- Field and laboratory staff qualifications, experience, and personnel training programs.
- Reagents and sample storage facilities.
- Reagent and test solutions preparation logbooks and raw data.

- Field and laboratory bench sheet and logbook maintenance and review.
- Review of the sample analysis/data package inspection procedures.

4.3. Prior to an on-site evaluation, various documents pertaining to performance of the Participant is integrated in a profile package for discussion during the evaluation. Items that may be included are previous on-site reports, laboratory evaluation sample scores, review of data, QA materials, and data trend reports.

4.4. The CBP QAO or representative will discuss his/her findings with the Participant in the presence of a representative from the State agency. During the debriefing, the auditor will present his/her findings and recommendations for corrective actions to field and laboratory personnel.

4.5. Following an on-site evaluation, audit reports which discuss deficiencies found during the on-site evaluation will be forwarded to the Participant. The Participant must respond to the audit report within 30 days of the report and, concurrently, the report must be sent to the CBP QAO and the State representative.

4.5.1. If the Participant fails to take appropriate corrective action to resolve the deficiencies discussed in the on-site reports, any further sampling or analytical activities will not be conducted.

Section F. Comparability Studies

1. Background

Chesapeake Bay Program (CBP) data are used to calculate long-term trends in contaminants, which require precise data that are comparable over long periods of time. Seemingly insignificant changes in procedures may cause step-trends over time. To prevent this the CBP requires that the effects of any change in instruments, reagents, calibration, digestion procedures, etc., be quantified, documented and submitted to the CBP QA Coordinator prior to implementing the change.

Although EPA allows certain changes in methods without official approval under 40 CFR Part 136.6, the Chesapeake Bay Program requires that some of the modifications allowed under §136.6 be submitted to and approved by the CBP QA Officer prior to implementation (see Section F.2.7 below).

2. Demonstrating Equivalency of Method Modifications

2.1. Method modifications requirements of EPA 40 CFR §136.6:

2.1.1. The underlying chemistry and determinative technique are essentially the same;

2.1.2. The modified method produces equivalent performance for the analyte(s) of interest and

2.1.3. The equivalent performance is documented.

2.2. Side-by-side comparability studies must be performed using identical subsamples that are analyzed by each method or technique.

2.2.1. The different methods should be performed on the same day to minimize changes between the sample pairs.

2.2.2. Side-by-side analyses should be conducted on different days, weeks or even months to incorporate the day-to-day variability of each method.

2.3. When validating new procedures, laboratories must adhere to the standardized QC procedures detailed in the CBP method and incorporate these criteria into the method.

2.4. Laboratories must use a reference matrix (usually, reagent water) and field samples for the validation study. If a laboratory intends to apply the method to more than one matrix type, the laboratory must validate the method on field samples of each matrix type. Fresh water and saline waters are considered different matrices.

2.5. The new method must meet or exceed the performance measures of the original method. These measures include MDL, spike, duplicate and blank results; calibration checks, standard reference material, calibration correlation coefficients, etc.

2.6. Modifications to procedures for method-defined analytes such as TSS and chlorophyll are not allowed, nor are changes that would result in measurement of a different form or species of an analyte.

2.7. Modifications that require validation and CBP approval include:

2.7.1. Changes in sample preparations such as digestions, distillations, and extractions.

2.7.2. New instrumentation, type of detector, or a change from manual discrete instrumentation to an automated system.

2.7.3. Changes in reagents, reaction times and temperatures.

2.8. Minor modifications that do not require a validation study or CBP approval include:

2.8.1. Changes in the calibration range.

2.8.2. Adjusting sample sizes or diluting samples to optimize method performance.

2.8.3. Changes in pH adjustment reagents or buffer reagents, provided that the changes do not produce interferences.

2.8.4. Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter or modifying the temperature program for a specific GC column.

2.8.5. Replacement of instrument components purchased from, or recommended by the original manufacturer.

3. Validation of Method Modifications

3.1. Method Compilation

3.1.1. Prior to conducting a validation study, the laboratory should document (or reference) the exact procedures that will be used for the new method. The new method should be followed as written. If changes are necessary during the course of validation, then the date and rationale for the changes should be noted. All measures of performance must be repeated following a change in procedure.

3.2. Method Detection Limit Study

3.2.1. The lab must use the procedures specified in the modified method to perform a method detection limit (MDL) study in accordance with the procedure given at 40 CFR Part 136. Each laboratory must perform its MDL study as described in Chapter 6, Section C.8, on an instrument that is calibrated to encompass the projected reporting limit.

3.3. Calibration

3.3.1. Following completion of the MDL study, re-calibrate the instrument to include a standard less than or equal to the reporting limit. The laboratory must demonstrate that the linearity criterion and the MDL of the modified method are as good as, or better than those of the original method.

3.4. Initial Precision and Recovery

3.4.1. After successfully calibrating the instrument, perform an initial precision and recovery (IPR) analyses using the procedures specified in the EPA reference method. The IPR consists of analyses of four replicates of reagent water spiked with the analytes of interest.

3.4.2. For each analyte, the precision of analysis of the replicates, as determined by the standard deviation or relative standard deviation (RSD) of the measurements, should be less than the standard deviation or RSD specified in quality control (QC) acceptance criteria in the method. Similarly, for each analyte, the average percent recovery of the measurements should fall within the range of percent recovery specified in the method. If either the precision or recovery test is failed, the test is repeated until the laboratory is able to meet precision and recovery requirements.

3.4.3. Include a minimum of one blank in the initial demonstration, and the concentration of the analyte(s) in the blank should be less than the level(s) specified in the method. Repeat the initial demonstration with the modified method as an integral part of the method, until the QC acceptance criteria in the method for precision and recovery and for the blank are met. Otherwise, the modification will not be permitted. Maintain records that document that the initial demonstration was performed on the modified method and those requirements for precision and recovery and the blank were met.

3.5. Field Sample Validation

3.5.1. After successful completion of IPR analyses, the method modification is to be validated on the matrix type(s) chosen for the validation study.

3.5.2. Validation must include the analysis of paired subsamples using the current method and the modified method. This will permit an estimation of bias between the methods.

3.5.3. The numbers of analyses should be 100 samples per matrix, and cover the typical ranges of seasonal concentration and spatial differences.

3.5.4. Ideally, the two methods will be conducted on the same day to minimize differences between the sample pairs.

3.5.5. Analytical batches should be run on different days, weeks or even months to incorporate the day-to-day variability of each method.

3.6. Ongoing Precision and Recovery

3.6.1. The laboratory must demonstrate that it can meet the precision and recovery QC acceptance criteria of the original method. Each batch of samples which includes field samples, but not the IPR samples, must include ongoing precision and recovery QC samples.

3.7. Calibration Verification

3.7.1. The laboratory must verify calibration as described in the method. The field samples discussed in Section 3.5 above must be analyzed in a separate batch of determinations from the initial calibration sequence, so that calibration verification is performed. Calibration verification sample results of the modified method must

meet the acceptance criteria of the original method. Recommend at least 5 CCV samples of several (3 to 5) different concentrations.

3.8. **Contamination Level in Blanks**

3.8.1. The laboratory must prepare and analyze at least one method blank with each sample batch during which the matrix samples are prepared and analyzed. The actual number of blank samples analyzed by each laboratory must meet or exceed the frequency specified in the method. The laboratory modified method must demonstrate that it can meet the QC acceptance criterion for blanks that is specified in the method.

4. Statistical Analyses

4.1. A *paired t-test* is best for the comparison of two different methods on samples of different concentrations, especially if the differences between pairs are normally distributed. A two-sided test with a p-value of 0.01 is recommended.

4.2. A *Wilcoxon Signed-Rank test* is recommended when the differences between the sample pairs are not normally distributed. Alternatively, the *Kruskal-Wallis* may be used.

4.3. An *Analysis of Variance* (SAS® PROC GLM, a General Linear Model) is recommended if one wants to compare the effects of interferences on the different methods. Samples with the potential to interfere must be identified to conduct this analysis.

Note: A regression analysis that compares one method directly against another is NOT recommended since neither method can be assumed to have no error. Such an analysis would result in a "regression dilution effect". However, some labs have used linear regression when the paired t-test showed a significant difference and a correction factor was needed for an unavoidable method change.

5. Documentation

5.1. See Table 2.4, *Documentation of Method Equivalency*

Table 2.4. Documentation of Method Equivalency

	New Method	Current Method	Reference Method
1. Title and Description *List & attach SOPs for new and current methods.*			
2. Procedural differences			
3. Concentrations of calibration standards			
4. Initial Precision & Recovery			
5. Calibration Verification *- Initial Cal Verification Result - Ongoing Cal Verification Res*			
6. Method Detection Limit			
7. Correlation coefficient of calibration curve			
8. Sample matrix and concentration range for each *(fresh and saline waters are separate matrices)*			
9. Paired t-test results *(per each matrix) A two-sided t-test with p-value of 0.01*			
10. Wilcoxin Signed-Rank test *(if paired differences are not normally distributed)*			
11. Other Statistics			
12. Certified reference material results *with certified values*			
13. PT sample and results *(USGS, ERA, CBP blind audit, etc.)*			
14. Method blank results			
15. Spiked sample results *(Sample conc. and % recovery of each spike)*			
16. Duplicate sample results *(Rep 1, Rep 2 values and RPDs)*			
17. Raw Data sample pairs *(Submit Excel file or equivalent)*			
18. Analyte carry-over			

Section G. References

Alden et al. 1994. An Assessment of the Power and Robustness of the Chesapeake Bay Program Water Quality Monitoring Program: Phase II - Refinement Evaluation. Chesapeake Bay Program Office, Annapolis, MD. http://archive.chesapeakebay.net/pubs/calendar/MONSC_06-05-09_Handout_4_10309.pdf

Chesapeake Bay Program. 2010. Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines, Revision 4, December 2010. Chesapeake Bay Program Office, Annapolis, MD. http://archive.chesapeakebay.net/pubs/quality_assurance/CSSP_Guidelines_12-17-10.pdf

U.S. Environmental Protection Agency. 2012. 40 CFR Part 136 – Guidelines Establishing Test Procedures for the Analysis of Pollutants under the Clean Water Act; Analysis and Sampling Procedures. Federal Register/Volume 77 / No. 97/ May 18, 2012/ Rules and Regulations http://www2.epa.gov/cwa-methods/cwa-methods-regulatory-history.

U.S. Environmental Protection Agency. 1983. Chesapeake Bay: A Framework for Action, Appendix F – A Monitoring and Research Strategy to Meet Management Objectives. Chesapeake Bay Liaison Office, Annapolis, MD. http://www.chesapeakebay.net/content/publications/cbp_12405.pdf

U.S. Environmental Protection Agency. 2001a. EPA Requirements for Quality Management Plans (QA/R-2), EPA240/B-01/002, March 2001. http://www2.epa.gov/quality/epa-qar-2-epa-requirements-quality-management-plans

U.S. Environmental Protection Agency. 2001b. EPA Requirement for Quality Assurance Project Plans (QA/R-5),

EPA240/B-01/003, March 2001. http://www2.epa.gov/quality/epa-qar-5-epa-requirements-quality-assurance-project-plans

U.S. Environmental Protection Agency. 2007. Solutions to Analytical Chemistry Problems with Clean Water Act Methods, March 2007 http://www.epa.gov/waterscience/methods/files/pumpkin.pdf

U.S. Environmental Protection Agency. 2010. Quality Management Plan for the Chesapeake Bay Program. Chesapeake Bay Program Office, Annapolis, MD. http://archive.chesapeakebay.net/pubs/quality_assurance/CBPO_QMP_2010_final.pdf

U.S. Environmental Protection Agency. 2016. Definition and Procedure for the Determination of the Method Detection Limit. Revision 2. EPA 821-R-16-006, December 2016. (From 40 CFR, Part 136.)

APPENDIX 2-A.
Quality Assurance Project Plans and Laboratory Quality Manuals

1. Quality Assurance Project Plan

The QA Project Plan shall be composed of standard elements that cover the entire project from planning, through implementation, to assessment. The document EPA Requirements for QA Project Plans (QA/R-5) fully describes the necessary elements which are summarized below.

A. PROJECT MANAGEMENT

The elements in this group address the basic area of project management, including the project history and objectives, roles and responsibilities of the participants. These elements ensure that the project has a defined goal, that the participants understand the goal and the approach to be used and that the planning outputs have been documented.

B. DATA GENERATION AND ACQUISITION

The elements in this group address all aspects of project design and implementation. Implementation of these elements ensure that appropriate methods for sampling, measurement and analysis, data collection or generation, data handling, and QC activities are employed and are properly documented.

C. ASSESSMENT AND OVERSIGHT

The elements in this group address the activities for assessing the effectiveness of the implementation of the project and associated QA and QC activities. The purpose of assessment is to ensure that the QA Project Plan is implemented as prescribed.

D. DATA VALIDATION AND USABILITY

The elements in this group address the QA activities which occur after the data collection phase of the project is completed. Implementation of these elements ensures that the data conform to the specified criteria, thus achieving the project objectives.

A1 Title and Approval Sheet
A2 Table of Contents
A3 Distribution List
A4 Project/Task Organization
A5 Problem Definition/Background
A6 Project/Task Description
A7 Quality Objectives and Criteria
A8 Special Training/Certification
A9 Documents and Records

B1 Sampling Design (Experimental Design)
B2 Sampling Methods
B3 Sample Handling and Custody
B4 Analytical Methods
B5 Quality Control
B6 Equipment Testing & Maintenance
B7 Instrument & Equipment Calibration
B8 Inspection & Acceptance of Supplies
B9 Non-direct Measurements
B10 Data Management

C1 Assessments & Response Actions, i.e., corrective action
C2 Reports to Management

D1 Data Review, Verification & Validation
D2 Verification & Validation Methods
D3 Reconciliation with User Requirements

2. Laboratory Quality Manual (Example Format)

1.0 Quality Assurance Policies
1.1 Quality Assurance Policy Statement
1.2 Proficiency Test Program
1.3 Review of Requests for the Acceptance of New Work

1.4 Document Control System (for bench sheets, log books, SOPs, etc.)

2.0 Organization and Responsibilities

2.1 Organizational Chart
2.2 Management
2.3 Laboratory Director, Associate Laboratory Director
2.4 Technical Staff (Include IT, Analysts, etc.)
2.5 Information Management System
2.6 Training
2.7 Laboratory Capabilities

3.0 Quality Assurance Indicators

3.1 Determining Control Limits for:
- Precision & Accuracy
- Representativeness
- Completeness
- Comparability

3.2 Procedure for Method Detection Limit Studies

4.0 Sample Handling

4.1 Sample Tracking
4.2 Sample Acceptance Policy
4.3 Sample Receipt Protocols
4.4 Sample Storage Conditions
4.5 Chain of Custody
4.6 Sample Disposal

5.0 Calibration Procedures and Frequency

5.1 Traceability of Calibration
5.2 Instrument Calibration (initial and continuing)

6.0 Test Methods and Standard Operating Procedures

6.1 Reference Method (authoritative source)
6.2 Demonstration of Method Capability
6.3 Method Detection Limit
6.4 Changes and Modifications

7.0 Quality Control Checks

7.1 Internal Quality Control Samples
7.2 Instrument-Specific Quality Control Checks
7.3 Standard Reference Materials

8.0 Data Reduction, Review, Reporting and Records

8.1 Data Reduction and Review
8.2 Secondary Data Review
8.3 Report Format and Contents
8.4 Records Management and Control

9.0 Performance and System Audits and Frequency

9.1 Internal Laboratory Audits
9.2 Managerial Review
9.3 Third Party Audits

Chapter 3. DATA MANAGEMENT AND REPORTING

Section A. Introduction

Section B. General Requirements

1. Reporting Water Quality Data
2. Significant Figures, Qualifiers
3. Metadata / documentation

Section C. Database Requirements

1. Software
2. DUET Tables and Formats
3. Reporting Field Duplicate Results
4. Reporting Field Blank Results
5. Lookup Tables

Section D. DUET Submittals

1. Schedule for Data Deliverables
2. Data Submission Process

Section E.DUET Data Reviews & Checks

1. Range Checks
2. Completeness Checks
3. Consistency Checks
4. Problem Codes
5. Bias Problem Codes
6. Precision Problem Code

Section F.References

Appendix 3-A

Tables

TABLE 3.1. DUET DATA SUBMITTAL TABLES

Section A. Introduction

The Chesapeake Environmental Data Repository (CEDR) was introduced in 2014 and involves the merging of the Water Quality and the Living Resources (tidal benthic, nontidal benthic, and tidal plankton) data holdings into a single schema database. All data in CEDR is available to the public. The DataHub is the Chesapeake Bay Program's primary tool for searching and downloading environmental data for the Chesapeake Bay watershed. The 2015 re-design of the DataHub user interface provides a single portal that permits CEDR data downloads (water quality and living resources) as well as the in-vivo fluorescence, point source, and toxics CBP data holdings. The latter three data holdings are not in the CEDR schema at this point, but there have been discussions on bringing them into CEDR in the future. The CBP DataHub UI can be found at http://data.chesapeakebay.net/Home.

This chapter describes the process that all water quality data submittals must go through before inclusion in the CEDR database. It is intended to assist data providers with the tools needed to process, report and submit data.

Water quality data submittals, comprised of all field measurement, analytical, and appropriate QC data from all stations sampled, are submitted through the CBP Data Upload and Evaluation Tool (DUET). All data submittals to DUET must be in electronic format and use Microsoft Access 2000 or higher. DUET performs a series of 170 QAQC checks pertaining to the timeliness, completeness, and quality of the submitted data. After the submittal passes all of the data checks and no issues of concern are found, the data provider and CBP data manager must agree to accept the submittal and the dataset is imported into CEDR.

Section B. General Requirements

1. Reporting Water Quality Data

1.1. Participants shall submit a complete data package for each cruise. The data package must adhere to the policies for Chesapeake Bay Program Data Submission. http://archive.chesapeakebay.net/cims/Guidance%20for%20Data%20Management%20Nov%202006.pdf

1.1.1. The data package or served data must comprise all field measurement, analytical, and appropriate QC data from all stations sampled during the cruise.

1.1.2. Submitted data must be formatted in ACCESS database files as specified in the CBP Water Quality Database Design and Data Dictionary. http://www.chesapeakebay.net/documents/3676/cbwqdb2004_rb.pdf

1.1.3. The data shall utilize the CBP common data dictionary for defining all data elements and units of measure.

1.2. Field data and laboratory analysis information shall be submitted through the CBP Data Upload and Evaluation Tool (DUET). http://duet.chesapeakebay.net/

1.2.1. The CBP IT contractor developed the DUET Users Guide to assist data providers in file management using this tool. A series of video tutorials are available for the various functions (upload file, reload file, replace file, file status) of DUET.

http://www.chesapeakebay.net/documents/3676/duet_user_guide_v2_1_03dec2013.pdf

1.3. Numeric Data

1.3.1. Numeric data are to be reported with the same number of significant figures as the original measurement.

1.3.2. If possible, all validated data should be reported, including instrument readings below method detection limits and negative readings. A value of zero may be reported only if the measured or recorded value is zero.

1.3.3. Values below the method detection limit (MDL) must have the less than detection flag "<" added to the qualifier field in the WQ_DATA table and be censored to the MDL. Qualify values between the MDL and the Practical Quantitation Level (PQL) (or reporting limit) by adding a "G" code to the qualifier field.

1.3.4. Below-detection values are reported without censoring in the Value field of the WQ_DATA_BMDL Table, and are available only to approved CBP data analysts.

1.4. Data should utilize the CBP Method Codes which are listed in the Data Dictionaries. If a new method code is needed, the Participant will submit a request to the appropriate CBP Data Manager.

1.5. All submitted or served data shall be accompanied by metadata. Metadata is descriptive information about the data, often referred to as documentation, which fully conforms to the Federal Geographic Data Committee's requirements for metadata. (www.fgdc.gov/metadata). Metadata submitted to the CBP shall follow the CIMS Metadata Reporting Guidelines. http://archive.chesapeakebay.net/cims/metasep.pdf

1.6. All data must conform to the consistent set of common station names for reporting monitoring station locations. The Station Names table, maintained on the Chesapeake Bay Program web site, serves as the master list. If a new station name is needed, the Participant will submit a request to the CBP Data Manager to coordinate the development of the new station name.

1.7. Hardcopy records and source documents for both the cruise event data and the laboratory analysis data should be kept for a period of 5 years after sample collection.

1.8. Participants shall be responsible for correcting any errors found by the CBP Data Manager and resubmitting the data in the required format to the CBPO. If a Participant is the source of the error, they shall correct the errors found for no additional cost for a period of 5 years after sample collection.

Section C. Database Requirements

1. Software

1.1. All data submittals to DUET must be in electronic format and use Microsoft Access 2000 or higher.

2. DUET Tables and Formats

2.1. All DUET data submittals must contain the seven tables shown in Table 3.1 in their Microsoft Access database. It is not a requirement that each of these tables be populated, but they must be present in the submittal even if empty. Many submittals consist of only a populated WQ_DATA and WQ_EVENT table. The table structure for these two tables are shown in Table 3.2 and 3.3.

Table 3.1: Tables will be checked during the DUET quality assurance checks.

Table Name	Description
WQ_CHLOROPHYLL	Contains information about the optical densities that are used to calculate chlorophyll and related concentrations.
WQ_CRUISES	Contains information about the cruises that were conducted in order to collect the data contained in the data submission.
WQ_DATA	Contains the depth dependent data and secchi depth collected during each monitoring event that occurred during each cruise.
WQ_DATA_BMDL	Contains the actual measurement that was below the method detection limits for a parameter.
WQ_EVENT	Contains information about each monitoring event that was conducted during the cruises.
WQ_KD	Contains light attenuation records
WQ_QAQC	Contains quality assurance / quality control records.

3. Reporting Field Duplicate Results

3.1. Field duplicate record values are reported in the WQ_DATA table. Each sample record is differentiated by a unique sample replicate type (i.e. S1, S2 or FS1, FS2) in the SAMPLE_ID field.

3.2. It is necessary for laboratories to report analytical results for field duplicates to at least 3 significant figures to obtain the most accurate estimates of precision. Low values near the MDL are typically reported to one or two significant figures but in the case of field duplicate samples, request that the lab submit unrounded, low-concentration data to at least 3 figures. For example, the value 0.005 has only one significant figure and the raw, unrounded version of this value is needed.

3.3. Another exception for field duplicate data involves those rare cases where one or both field duplicates fails a consistency check (e.g., TDP > TP). Data collection groups should evaluate both field duplicates for consistency and assign an appropriate problem code for failures, however, unlike normal data, failed duplicate results must be reported along with the problem code "NQ". This practice is necessary to obtain representative QC data, even if the sample data would normally be censored.

3.4. After the precision calculations are completed, DUET will censor the "NQ" coded data prior to uploading to CIMS. Similarly, the unrounded low concentration data will be rounded to the appropriate decimal place prior to the upload. All original duplicate sample data, including the unrounded and uncensored results, will be archived and made available through a request to the CBP NTN Project Data Manager.

4. Reporting Field Blank Results

4.1. Field blank (FB) results are reported in the SAMPLE_ID field in the WQ_DATA table. Equipment blanks (EB) and Source Water blanks (SWB) are reported in the SAMPLE_REPLICATE_TYPE field in the WQ_QAQC table. Other frequently used QC sample types are listed in Table 3.4.

4.2. Laboratories are to quantify and report all field blank results above the method detection limit (MDL) to ensure that low-level contamination is not a significant contributor to low-level WQ concentrations just above the reporting limit.

4.3. For FBs > MDL, data collectors and/or agency staff will investigate potential sources of contamination and assess the significance of the contamination. Corrective action is required for significant problems, especially for chronic detections above a reporting limit. Refer to Section E.5 for the CBP BIAS problem codes and descriptions for actions.

5. Lookup Tables

5.1. A DUET Submittal Lookup Guide has been developed to provide acceptable entries for the foreign key fields in the WQ_DATA, WQ_DATA_BMDL and WQ_EVENT tables. This guide provides codes for the tidal, nontidal, and non-traditional partner CBP programs. http://www.chesapeakebay.net/documents/3676/duet_lookup_tables_version3.1_28mar16.pdf

Table 3.2: WQ_DATA
The WQ_DATA table contains all of the processed depth-dependent data (water column profile data) collected during a monitoring event and submitted to the Chesapeake Bay Program Office. Secchi Depth is also included in this table at the request of the Data Management and Quality Assurance Workgroup.

FIELD	DESCRIPTION	TYPE	LENGTH
PROJECT	Code identifying the project under which the monitoring was conducted.	text	4
SOURCE	Code identifying the source that collected the water sample and/or the field measurement.	text	10
STATION	Code identifying the monitoring station where the data was obtained.	text	15
SAMPLE_DATE	The date that the sample or measurement was obtained.	Date/Time	
SAMPLE_TIME	The time that the sample or measurement was obtained.	Date/Time	
DEPTH	The depth (in meters) where the water sample or measurement was obtained.	single	
LAYER	Code identifying the water column layer where the sample or measurement was obtained.	text	5
SAMPLE_TYPE	Code identifying the how the sample or measurement was obtained.	text	5
SAMPLE_ID	Code identifying each sample when multiple samples/measurements are collected or a water sample is split into multiple samples.	text	7
PARAMETER	Code identifying the parameter name.	text	15
QUALIFIER	Code that identifies if the reported value was outside of the method detection limit.	text	5
VALUE	The reported value of the parameter.	single	
UNITS	Code identifying the units of measurement in which a parameter is reported.	text	10
METHOD	Code identifying the field/laboratory test procedure used to measure the parameter value.	text	5
LAB	Code identifying the lab where the sample was analyzed.	text	10
COMMENTS	Additional information about the data record.		
PROBLEM	Code identifying any sample analysis problems.	text	2
PRECISION_PC	Code identifying a precision issue with duplicate samples.	text	4
BIAS_PC	Code identifying a biased data record that can be attributed to either contamination of a filed blank or associated water quality sample.	text	4

Table 3.3: WQ_EVENT
The WQ_EVENT table contains all of the processed depth-independent data (e.g. weather codes), with the exception of SECCHI DEPTH, collected during a monitoring event.

FIELD	DESCRIPTION	TYPE	LENGTH
CRUISE	The CBP cruise on which the monitoring event occurred.	text	6
SOURCE	Code identifying the organization that collected the water sample and/or field measurement.	text	10
AGENCY	Code identifying the organization responsible for submitting the data record to the CBP.	text	10
PROJECT	Code identifying the project under which the monitoring was conducted.	text	4
PROGRAM	Code identifying the program under which the monitoring was conducted.	text	4
STATION	Code identifying the monitoring station where the data was obtained.	text	15
SAMPLE_DATE	The starting date of the monitoring event.	Date/Time	
SAMPLE_TIME	The starting time of the monitoring event	Date/Time	
TOTAL_DEPTH	The station's total depth (in meters) at the start of the sampling event.	single	
LOWER_PYCNOCLINE	The depth (in meters) of the bottom of the pycnocline.	single	
UPPER_PYCNOCLINE	The depth (in meters) of the top of the pycnocline.	single	
TIDE_STAGE	Code identifying the tide stage observed at the start of the monitoring event.	text	2
WAVE_HEIGHT	Code identifying the estimated wave height at the start of the monitoring event.	text	2
AIR_TEMP	The air temperature (in Celsius) measured at the beginning of the monitoring event.	single	
WIND_DIRECTION	Code identifying the prevailing wind direction at the start of the monitoring event.	text	3
WIND_SPEED	Code identifying the estimated wind speed at the start of the monitoring event.	text	2
PRECIP_TYPE	Code identifying the precipitation type observed at the start of the monitoring event.	text	2
CLOUD_COVER	Code identifying the estimated cloud cover at the start of the monitoring event.	text	2
GAGE_HEIGHT	The gage height (in feet) measured at the start of the monitoring event.	single	
PRESSURE	The barometric pressure (in millimeters mercury) recorded at the start of the monitoring event.	single	
EVENT_TYPE	Code identifying a NonTidal Network event type; three primary event types are routine, routine storm impacted, and storm.	text	5
EVENT_REMARK	Code identifying a NonTidal Network event sampling location based on flow conditions.	text	5
COMMENTS	Additional information about the sampling event (e.g. parameters not measured).		

Table 3.4: Frequently used sample replicate types for identifying data records. This field is referred to as SAMPLE_ID in the WQ_DATA and WQ_DATA_BMDL tables, and as SAMPLE_REPLICATE_TYPE in the WQ_QAQC table.

Sample Replicate Type	Sample Replicate Description
EB	Equipment And Filtration Blank
FB	Field Blank
FS1	Field Split Subsample 1
FS2	Field Split Subsample 2
LS1	Lab Split Subsample 1
LS2	Lab Split Subsample 2
M1	Field Measurement 1
M2	Field Measurement 2
S1	Sample 1
S2	Sample 2
SWB	Source Water (DI) Blank

Section D. Duet Submittals

1. Schedule for Data Deliverables

1.1. Mainstem and Tidal Tributary Data

1.1.1. Mainstem participants shall submit field data in electronic format via DUET to the CBPO within two months after the end of the cruise month. Virginia submits two months of mainstem data two months from the end of the second month of collection. Maryland tidal tributary submittals are due within three months after the end of the cruise month, while Virginia grant guidance allows for two submittals per year. This allows the data collector/data provider sufficient time to have all laboratory analyses completed and reported before submission to DUET.

1.2. Nontidal WQ Data

1.2.1. Nontidal Network (NTN) datasets are submitted for a water year cycle (October 1 – September 30) by 15 March of the following year. Maryland core-trend (nontidal out of network) project data are submitted with the tidal tributary data for a time period.

2. Data Submission Process

2.1. All water quality data submittals to CBP must be uploaded through DUET (Data Upload and Evaluation Tool). DUET is the revised version of the Data Upload and Quality Assurance Tool (DUQAT), replacing it in 2013. DUET allows a data submitter to upload data, view file status, and check the quality assurance report generated in real time.

2.2. DUET performs a suite of 170 QAQC fatal and nonfatal error checks on the data submittal. Fatal error checks pertain to data and database table integrity and will prevent a submittal from passing checks and being imported into the CBP database, while nonfatal error checks, including completeness and range checks, indicate possible issues of concern with the submittal. The nonfatal error checks will not prevent a submittal from being imported into the CBP database. Please refer to Appendix 3-A for a full list of the DUET fatal and nonfatal error checks. The submitted water quality data is processed by DUET, and generates routine reports with selected metadata on the following:

2.2.1. Timeliness of the Source Submissions and the Agency Uploads

2.2.2. Completeness of the submitted data, in relation to the data expected.

2.2.3. Quality of the submitted data, in relation to possible clerical errors, extreme values, logical relational expressions, and data accuracy (bias and precision).

2.3. After the data submittal has passed checks, the data submitter and CBP Data Manager review the quality assurance report for possible issues. The data submittal is accepted into the CBP database only after both parties agree that there are no issues of concern with the dataset.

Section E. Duet Data Reviews & Checks

1. Range Checks

DUET performs range checks to determine possible outlier records in a data submittal. P1 and P99 range checks are performed on individual parameters based on the historical data for each station. Once a week, the P1 and P99 stored procedures are run to adjust the historical values for any new records that have been added to the database. There are additional range checks performed to identify outliers/ decimal point errors in the WQ_DATA table for the five parameters (chlorophyll a, DO, salinity, secchi depth, and water temperature) used in the water quality attainment indicator:

WQ_DATA record(s) contain a SALINITY Reported Value < 0 or > 32.

WQ_DATA record(s) contain a DO Reported Value < 0 or > 20.

WQ_DATA record(s) contain a SECCHI Reported Value < 0 or > 3.

WQ_DATA record(s) contain a CHLA Reported Value < 0 or > 200.

WQ_DATA record(s) contain a WTEMP Reported Value < -1 or > 30.

2. Completeness Checks

A series of completeness checks are performed on all Nontidal Network (NTN) submittals. The NTN Data Providers for each state in the watershed do not necessarily submit the identical parameters, so each one has a unique set of completeness checks. These checks are run to determine if there are missing parameter records based on the event type of an event. It is typical of a storm event to contain additional parameters (primarily SSC related) than a routine event. This check is a nonfatal error as missing parameter records for an event are reported in the DUET QA report, but this will not prevent the dataset from being imported into CEDR. A tidal completeness check may be developed in the future.

3. Consistency Checks

DUET performs three consistency checks to determine if a parameter or combination of two parameters has a greater value than another parameter that should have a larger value. Data Providers are asked to perform any other consistency check that could affect their dataset. The three automated DUET consistency checks are:

[NO2F] > [NO23F]

[NO23F] + [NH4F] > [TDN]

[PO4F] > [TDP]

Depending on the level of analytical precision, the data provider should apply one of the four consistency check problem codes below to a record when applicable.

QQ Part Exceeds Whole Value Yet Difference is within Analytical Precision (e.g., PQL or Reporting Limit)

NQ Part Exceeds Whole Value and Difference is not within Analytical Precision

IQ Cannot Determine if Part Exceeds Whole Value and Whether or not Difference is within Analytical Precision

NV Negative Calculated Value is Valid Given Precision of Measured Water Quality Parameters; Actual Calculated Concentration Likely is Low; possibly less than PQLS of Measured Water Quality Parameters

4. CBP Problem Codes and Descriptions

Errors that occur in sampling and analysis are flagged in the database using problem codes. The data provider adds the problem code to the data record. Only one problem code can be applied to a record, so in the case that more than one may apply, the highest order code should be used. If a data provider decides not to report erroneous data, the data record must still be submitted without the value. An individual data record may be null only if the problem field is populated with a defined problem code. An undefined or null problem code for a null data record will produce a fatal error. The list below is the current problem codes and their descriptions.

A Laboratory Accident

AA Field Accident

B Chemical Matrix Interference

C Instrument Failure

CC Cannot Calculate Given Available Data

D Insufficient Sample

DD Sample Size Not Reported (Assumed)

E Sample Received After Holding Time

F Post-Calibration Failure Likely Due to Equipment Damage After Sampling; Data Appear Normal

FF Mean Reported due to Poor Replication between Pads

GG Sample Analyzed After Holding Time

I Suspect Value has been Verified Correct

IQ Cannot Determine if Part Exceeds Whole Value and Whether or Not Difference is Within Analytical Precision

J Incorrect Sample Fraction for Analysis

JJ Volume Filtered not Recorded (Assumed)

L LICOR® Calibration off by ≥ 10% per year. Use with CALC KD where PROB of LU, LS, LB exist in raw data

LB LICOR® Calibration off by ≥ 10% per year for both Air and Upward Facing Sensors

LS LICOR® CALIBRATION off by ≥ 10% per year for Air Sensor

LU LICOR® CALIBRATION off by ≥ 10% per year for Upward Facing Sensor

MM Over 20% of Sample Adhered to Pouch and Outside of Pad

N None

NN Particulates Found in Filtered Sample

NQ Part Exceeds Whole Value and Difference is Within Analytical Precision

NV Negative Calculated Value is Valid Given Precision of Measured Water Quality Parameters; Actual Calculated Concentration Likely is Low

P Provisional Data

Q Analyte Present; Reported value is estimated; CONC is below the range for quantitation

QQ Part Exceeds Whole Value yet Difference is Within Analytical Precision

R Sample Contaminated

RR No Sample Received by Lab from Field Office

SS Sample Rejected due to High Suspended Sediment Concentration

TP Torn Filter Pad

U Matrix Problem Resulting from the Interrelationship between Variables such as pH and Ammonia

V Sample Results Rejected due to QC Criteria

WW High Optical Density (750 nm); Actual Value Recorded

X Sample Not Preserved Properly

5. CBP BIAS Problem Codes and Descriptions

In 2013, a BIAS_PC field was added to the WQ_DATA table in an effort to identify a biased data record that can be attributed to either contamination of a field blank or associated water quality sample.

BB Spurious or persistent contamination that appears to affect blanks only. Contamination is related to the manner or equipment or supplies used to obtain the blank; such as contaminated source water.

BM Biased measurement; concentration could reflect contamination bias; associated field blank concentrations were within same magnitude and could contribute at least 10% to the measured water quality parameter concentration.

CB Spurious or persistent contamination; which appears to reflect the manner or equipment or supplies used to obtain blanks and associated water quality samples.

UB Concentration of field blank reflects initial or isolated occurrence of contamination; source of contamination under investigation.

6. CBP PRECISION Problem Code and Description

In 2013, a PRECISION_PC field was added to the WQ_DATA field to identify a precision issue with duplicate samples. The code will be applied by DUET based on the relative percent difference of a given parameter. DUET will calculate the Relative Percent Difference (RPD) using field duplicates to assess a combined field and laboratory precision. If RPD values exceed 30% for particulate parameters (i.e., PN, PP, PC, TSS, SSC and Chlorophyll), or 20% for dissolved and total N, P, or C parameters, and both reported values are above the reporting limit, the Precision Problem Code "HI" will be added to the PRECISION_PC field of the CEDR data record(s). These control limits are subject to change once sufficient duplicate data are generated from which to establish different precision objectives.

HI High relative percent difference in duplicate sample measurements.

Section F. References

Chesapeake Bay Program (1998). Chesapeake Information Management System Metadata Reporting Guidelines. Annapolis: U.S. Environmental Protection Agency.

Chesapeake Bay Program (2002). CIMS Data Upload & Quality Assurance Tool: Data User's Guide. Annapolis: U.S. Environmental Protection Agency.

Chesapeake Bay Program (2004). Water Quality Database – Database Design and Data Dictionary. Annapolis: U.S. Environmental Protection Agency.Chesapeake Bay Program (2006). Chesapeake Bay Program Guidance for Data Management. Annapolis: U.S. Environmental Protection Agency.

Chesapeake Bay Program (2012). Guide to Using Chesapeake Bay Program Water Quality Monitoring Data. Annapolis: U.S. Environmental Protection Agency (EPA 903-R-12-001).

Chesapeake Bay Program (2013). Data Upload and Evaluation Tool (DUET) User Guide. Annapolis: Vistronix, Inc.

Chesapeake Bay Institute (2016). DUET Tidal, Nontidal and Volunteer Monitoring Data Submittal Lookup Tables. Annapolis: U.S. Environmental Protection Agency.

APPENDIX 3-A

DUET FATAL Error Checks

Fatal Errors will stop the data submission from being loaded into the CIMS WQ Database.

DUET Fatal Error Checks		
0	WQ_DATA	record(s) contain undefined or null station codes.
0	WQ_DATA_BMDL	record(s) contain undefined or null station codes.
0	WQ_DATA	record(s) contain undefined or null sample type codes.
0	WQ_DATA_BMDL	record(s) contain undefined or null sample type codes.
0	WQ_DATA	record(s) contain undefined or null sample id codes.
0	WQ_DATA_BMDL	record(s) contain undefined or null sample id codes.
0	WQ_DATA	record(s) contain undefined or null layer codes.
0	WQ_DATA_BMDL	record(s) contain undefined or null layer codes.
0	WQ_DATA	record(s) contain undefined or null parameter codes.
0	WQ_DATA_BMDL	record(s) contain undefined or null parameter codes.
0	WQ_DATA	record(s) contain undefined or null method codes for the records parameter.
0	WQ_DATA_BMDL	record(s) contain undefined or null method codes for the parameter.
0	WQ_DATA	record(s) contain undefined problem codes.
0	WQ_DATA_BMDL	record(s) contain undefined problem codes.
0	WQ_DATA	record(s) contain undefined or null source codes.
0	WQ_DATA_BMDL	record(s) contain undefined or null source codes.
0	WQ_DATA	record(s) contain undefined or null unit codes for the record's parameter.
0	WQ_DATA_BMDL	record(s) contain undefined or null unit codes for the parameter.
0	WQ_DATA	record(s) contain a null value and a null problem code.
0	WQ_DATA	record(s) contain undefined qualifier codes.
0	WQ_EVENT	record(s) contain undefined or null agency codes.
0	WQ_QAQC	record(s) contain undefined or null agency codes.
0	WQ_DATA	record(s) are not associated with event records in the data submission or the CIMS Water Quality Database.
0	WQ_CHLOROPHYLL	record(s) contain undefined or null lab codes.
0	WQ_QAQC	record(s) contain undefined lab codes.
0	WQ_CHLOROPHYLL	record(s) contain undefined or null layer codes.
0	WQ_QAQC	record(s) contain undefined or null layer codes.

DUET Fatal Error Checks		
0	WQ_DATA	record(s) contain null sample dates.
0	WQ_DATA_BMDL	record(s) contain null sample dates.
0	WQ_DATA	record(s) contain null sample times.
0	WQ_DATA_BMDL	record(s) contain null sample times.
0	WQ_DATA	record(s) contain undefined or null project codes.
0	WQ_DATA_BMDL	record(s) contain undefined or null project codes.
0	WQ_CHLOROPHYLL	record(s) contain undefined or null SampleId codes.
0	WQ_EVENT	record(s) contain undefined or null station codes.
0	WQ_CHLOROPHYLL	record(s) contain undefined or null station codes.
0	WQ_QAQC	record(s) contain undefined or null station codes.
0	WQ_EVENT	record(s) contain undefined tide stage codes.
0	WQ_QAQC	record(s) contain undefined or null unit codes.
0	WQ_EVENT	record(s) contain undefined wave height codes.
0	WQ_EVENT	record(s) contain undefined wind direction codes.
0	WQ_EVENT	record(s) contain undefined wind speed codes.
0	WQ_EVENT	record(s) contain undefined event type.
0	WQ_QAQC	record(s) are not associated with WQ_EVENT records.
0	WQ_EVENT	record(s) contain undefined cloud cover codes.
0	WQ_DATA	record(s) contain null or negative depths.
0	WQ_DATA_BMDL	record(s) contain null or negative depths.
0	WQ_CHLOROPHYLL	record(s) contain null or negative depths.
0	WQ_QAQC	record(s) contain negative or null depths.
0	WQ_DATA_BMDL	record(s) contain duplicate key fields.
0	WQ_EVENT	record(s) contain duplicate key fields.
0	WQ_CHLOROPHYLL	record(s) contain duplicate key fields.
0	WQ_QAQC	record(s) contain duplicate key fields.
0	WQ_DATA_BMDL	record(s) contain key fields already in the water quality database.
0	WQ_EVENT	record(s) contain key fields already in the water quality database.
0	WQ_CHLOROPHYLL	record(s) contain key fields already in the water quality database.
0	WQ_QAQC	record(s) contain key fields already in the water quality database.
0	WQ_CHLOROPHYLL	record(s) contain null extract volumes.

DUET Fatal Error Checks		
0	WQ_DATA	record(s) contain undefined or null lab codes and a sample type of D.
0	WQ_DATA_BMDL	record(s) contain undefined or null lab codes and a sample type of D.
0	WQ_DATA	record(s) contain lab codes and a sample type of 'ISM'
0	WQ_CHLOROPHYLL	record(s) contain null light paths.
0	WQ_QAQC	record(s) contain undefined or null method codes.
0	WQ_DATA	records(s) contain incompatible sample type and sample id (replicate type) codes.
0	WQ_EVENT	record(s) contain undefined precipitation type codes.
0	WQ_CHLOROPHYLL	record(s) contain undefined problem codes.
0	WQ_QAQC	record(s) contain undefined problem codes.
0	WQ_EVENT	record(s) contain undefined or null program codes.
0	WQ_QAQC	record(s) contain undefined or null program codes.
0	WQ_EVENT	record(s) contain undefined or null project codes.
0	WQ_CHLOROPHYLL	record(s) contain undefined or null project codes.
0	WQ_QAQC	record(s) contain undefined or null project codes.
0	WQ_QAQC	record(s) contain undefined qualifier codes.
0	WQ_EVENT	record(s) contain null sample dates.
0	WQ_CHLOROPHYLL	record(s) contain null sample dates.
0	WQ_QAQC	record(s) contain null sample date times.
0	WQ_QAQC	record(s) contain undefined or null sample replicate type codes.
0	WQ_EVENT	record(s) contain null sample times.
0	WQ_CHLOROPHYLL	record(s) contain null sample times.
0	WQ_CHLOROPHYLL	record(s) contain undefined or null sample type codes.
0	WQ_CHLOROPHYLL	record(s) contain null sample volumes.
0	WQ_EVENT	record(s) contain undefined or null source codes.
0	WQ_CHLOROPHYLL	record(s) contain undefined or null source codes.
0	WQ_QAQC	record(s) contain undefined or null source codes.
0	WQ_DATA	record(s) contain duplicate key fields.
0	WQ_EVENT	record(s) contain a cruise that is not defined in the data submission or the CIMS Water Quality Database.
0	WQ_EVENT	record(s) contain a null cruise.
0	WQ_QAQC	record(s) contain a CRUISE that is not in the dataset or the database.

DUET Fatal Error Checks		
0	WQ_CRUISES	record(s) contain undefined or null agency codes.
0	WQ_KD	record(s) are not associated with event records in the data submission or the CIMS Water Quality Database.
0	WQ_CRUISES	record(s) contain null cruise numbers.
0	WQ_CHLOROPHYLL	record(s) contain depth for AP layer > than depth at B layer.
0	WQ_DATA	record(s) contain depth for AP layer > than depth at B layer.
0	WQ_DATA_BMDL	record(s) contain depth for AP layer > than depth at B layer.
0	WQ_CHLOROPHYLL	record(s) contain depth for AP layer > than depth at BP layer.
0	WQ_DATA	record(s) contain depth for AP layer > than depth at BP layer.
0	WQ_DATA_BMDL	record(s) contain depth for AP layer > than depth at BP layer.
0	WQ_CHLOROPHYLL	record(s) contain depth for BP layer > than depth at B layer.
0	WQ_DATA	record(s) contain depth for BP layer > than depth at B layer.
0	WQ_DATA_BMDL	record(s) contain depth for BP layer > than depth at B layer.
0	WQ_CHLOROPHYLL	record(s) contain depth for S layer > than depth at AP layer.
0	WQ_DATA	record(s) contain depth for S layer > than depth at AP layer.
0	WQ_DATA_BMDL	record(s) contain depth for S layer > than depth at AP layer.
0	WQ_CHLOROPHYLL	record(s) contain depth for S layer > than depth at B layer.
0	WQ_DATA	record(s) contain depth for S layer > than depth at B layer.
0	WQ_DATA_BMDL	record(s) contain depth for S layer > than depth at B layer.
0	WQ_CHLOROPHYLL	record(s) contain depth for S layer > than depth at BP layer.
0	WQ_DATA	record(s) contain depth for S layer > than depth at BP layer.
0	WQ_DATA_BMDL	record(s) contain depth for S layer > than depth at BP layer.
0	WQ_KD	record(s) contain null or negative depths.
0	WQ_CRUISES	record(s) contain duplicate key fields.
0	WQ_KD	record(s) contain duplicate key fields.
0	WQ_CRUISES	record(s) contain key fields already in the water quality database.
0	WQ_KD	record(s) contain key fields already in the water quality database.
0	WQ_CRUISES	record(s) contain null end date, or an end date > current date
0	WQ_EVENT	record(s) contain a lower pycnocline depth < upper pycnocline depth.
0	WQ_EVENT	record(s) contain a null lower pycnocline when upper pycnocline is not null
0	WQ_KD	record(s) contain undefined or null method codes.

DUET Fatal Error Checks		
0	WQ_CRUISES	record(s) contain undefined or null program codes.
0	WQ_CRUISES	record(s) contain undefined or null project codes.
0	WQ_KD	record(s) contain undefined or null project codes
0	WQ_KD	record(s) contain null sample dates.
0	WQ_KD	record(s) contain undefined or null sample id (sample replicate type) codes.
0	WQ_KD	record(s) contain null sample times.
0	WQ_CRUISES	record(s) contain undefined or null source codes.
0	WQ_KD	record(s) contain undefined or null source codes.
0	WQ_CRUISES	record(s) contain a start date > than the end date.
0	WQ_CRUISES	record(s) contain null start date, or a start date > current date.
0	WQ_EVENT	record(s) contain a lower pycnocline depth >= total depth.
0	WQ_EVENT	record(s) contain a upper pycnocline depth > = total depth.
0	WQ_KD	record(s) contain undefined or null unit codes.
0	WQ_EVENT	record(s) contain a null upper pycnocline when lower pycnocline is not null.
0	WQ_EVENT	record(s) contain undefined event remark.
0	WQ_KD	record(s) contain undefined or null station codes.
0	WQ_QAQC	record(s) contain undefined or null parameter codes.
0	WQ_DATA	record(s) contain undefined BIAS problem codes.
0	WQ_DATA	record(s) contain undefined PRECISION problem codes.

DUET NON FATAL ERROR CHECKS

The CIMS Water Quality Data Manager will review these results before approving a data submission.

DUET Non Fatal Error Checks		
0	WQ_CHLOROPHYLL	record(s) contain absorbances not between zero and one.
0	WQ_EVENT	record(s) contain an air temperature > 40 C or < -20 C.
0	WQ_EVENT	record(s) are not associated with WQ_DATA or WQ_LIGHT records. This might not be a problem.
0	WQ_DATA	record(s) with a "<" qualifer code are not associated with WQ_DATA_BMDL records.
0	WQ_DATA_BMDL	record(s) are not associated with WQ_DATA records with a "<" qualifier code.
0	WQ_DATA	record(s) are not associated with WQ_CHLOROPHYLL
0	WQ_DATA	record(s) contain depths > the historical maximum.
0	WQ_DATA_BMDL	record(s) contain depths > the historical maximum.
0	WQ_CHLOROPHYLL	record(s) contain depths > the historical maximum.
0	WQ_EVENT	record(s) occur at the same station within 30 minutes of one another.

DUET Non Fatal Error Checks		
0	WQ_CHLOROPHYLL	record(s) are not associated with WQ_DATA.
0	WQ_DATA	record(s) contain depths > associated event(s) total depth.
0	WQ_DATA_BMDL	record(s) contain depths > associated event(s) total depth.
0	WQ_CHLOROPHYLL	record(s) contain depths > associated event(s) total depth.
0	WQ_DATA	record(s) contain possible errors in coding of sample id.
0	WQ_CHLOROPHYLL	record(s) contain possible errors in coding of sample id.
0	WQ_EVENT	record(s) contain a null total depth.
0	WQ_EVENT	record(s) contain total depth > the historical maximum.
0	WQ_EVENT	records contain a total depth < maximum depth in the associated data records.
0	WQ_DATA	record(s) contain inconsistent relationships: [NO2F] > [NO23F] and PROBLEM not QQ.
0	WQ_DATA	record(s) contain inconsistent relationships: [NO23F] + [NH4F] > [TDN]
0	WQ_DATA	record(s) contain inconsistent relationships: [PO4F] > [TDP] and PROBLEM not QQ.
0	WQ_DATA	record(s) contain values below historical P1 values.
0	WQ_DATA	record(s) contain values above historical P99 values.
0	WQ_CRUISES	record(s) contain potential problems with CRUISE_ID.
0	WQ_CHLOROPHYLL	record(s) contain depth for M layer > than depth at B layer.
0	WQ_DATA	record(s) contain depth for M layer > than depth at B layer.
0	WQ_DATA_BMDL	record(s) contain depth for M layer > than depth at B layer.
0	WQ_CHLOROPHYLL	record(s) contain depth for S layer > than depth at M layer.
0	WQ_DATA	record(s) contain depth for S layer > than depth at M layer.
0	WQ_DATA_BMDL	record(s) contain depth for S layer > than depth at M layer.
0	WQ_KD	record(s) contain depths > the historical maximum.
0	WQ_DATA	record(s) contain a SALINITY Reported Value < 0 or > 32.
0	WQ_DATA	record(s) contain a DO Reported Value < 0 or > 20.
0	WQ_DATA	record(s) contain a SECCHI Reported Value < 0 or > 3.
0	WQ_DATA	record(s) contain a CHLA Reported Value < 0 or > 200.
0	WQ_DATA	record(s) contain a WTEMP Reported Value < -1 or > 30.

CHAPTER 4. MAINSTEM & TRIBUTARY FIELD PROCEDURES

Section A. Background and Description

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2. Monitoring Objectives
3. Sampling Design
4. Water Quality Parameters
5. Map of Mainstem and Tributary Station Locations

Section B. Field Measurement Procedures

1. Sampling Sites
2. Weather Conditions
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8. Decontamination

Section C. Sampling Procedures

1. Sampling Schedules
2. Sampling Equipment
3. Reagents
4. Sample Collection
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6. Quality Control Samples
7. Documentation and Records
8. Decontamination

Section D. References

Appendix 4-A: CBP Mainstem and Tributary Station Locations

FIGURES

TABLES

Section A. Background and Description

1. Management Objectives

The 1985 Chesapeake Bay Restoration and Protection Plan identified the need for restoration activities and a monitoring program to measure the success of these activities. In June 2000, the Chesapeake Bay Program adopted a new Bay agreement, Chesapeake 2000: A Watershed Partnership to guide the management and restoration of the Bay. In this "C2K"document, Bay Program Partners agreed upon multiple objectives, one of which was "to achieve and maintain the water quality necessary to support the aquatic living resources of the Bay and its tributaries". Water quality monitoring data are necessary to assess these objectives.

2. Monitoring Objectives:

2.1. The monitoring objectives for the Tidal Water Quality Monitoring Program are to:

2.2. Assess the habitat conditions for aquatic living resources and determine if these conditions meet tidal water quality criteria and standards designed to protect them from nutrient and sediment impacts;

2.3. Deduce the likely causes of nutrient and sediment impairments, and determine the best course of action necessary to meet the water quality criteria and standards;

2.4. Support continued refinement, calibration and validation of Chesapeake Bay Program models such as the Estuarine Water Quality and Sediment Transport Model; and

2.5. Provide a long-term consistent set of data that is available for public and private research.

3. Sampling Design

3.1. The Chesapeake Bay Mainstem and Tributary Monitoring Program focuses on inorganic and physical parameters in the open and deep tidal waters of the Chesapeake Bay estuary. Staffs from the Maryland Department of Natural Resources (MDNR), Virginia Department of Environment (VDEQ), and Old Dominion University (ODU) collect surface to bottom discrete measurements at over 100 mid-channel stations. The timing and methods of sample collection are closely coordinated between Maryland and Virginia to permit consistent data presentations across the region. Phytoplankton samples are collected concurrently with water quality samples.

3.2. Twenty-four field and laboratory parameters are monitored and include nutrients, suspended solids, dissolved oxygen, salinity, temperature and chlorophyll *a*. Field parameters are measured *in-vivo*, and discrete grab samples are processed (i.e., filtered) immediately onboard. A complete listing of mainstem and tributary water quality parameters is provided in Table 4.1.

3.3. The number of grab samples taken and the depth at which they are taken varies among stations. At many stations, four samples are collected: surface and bottom, and just above and below the pycnocline if one is present. In the absence of a pycnocline, samples are taken at the surface and bottom, and at 1/3 and 2/3 of the total depth. Shallower stations may have only two samples (surface and bottom) collected. Procedures for determining the

pycnocline and sampling depths are described in Chapter 4, Sections B.4, B.3.4 and C.4.

3.4. A map showing the locations of the Maryland and Virginia Water Quality Monitoring Stations is available on the Chesapeake Bay Program Website at: http://www.chesapeakebay.net/documents/3676/map_of_mainstem_and_tributary_monitoring_stations.pdf

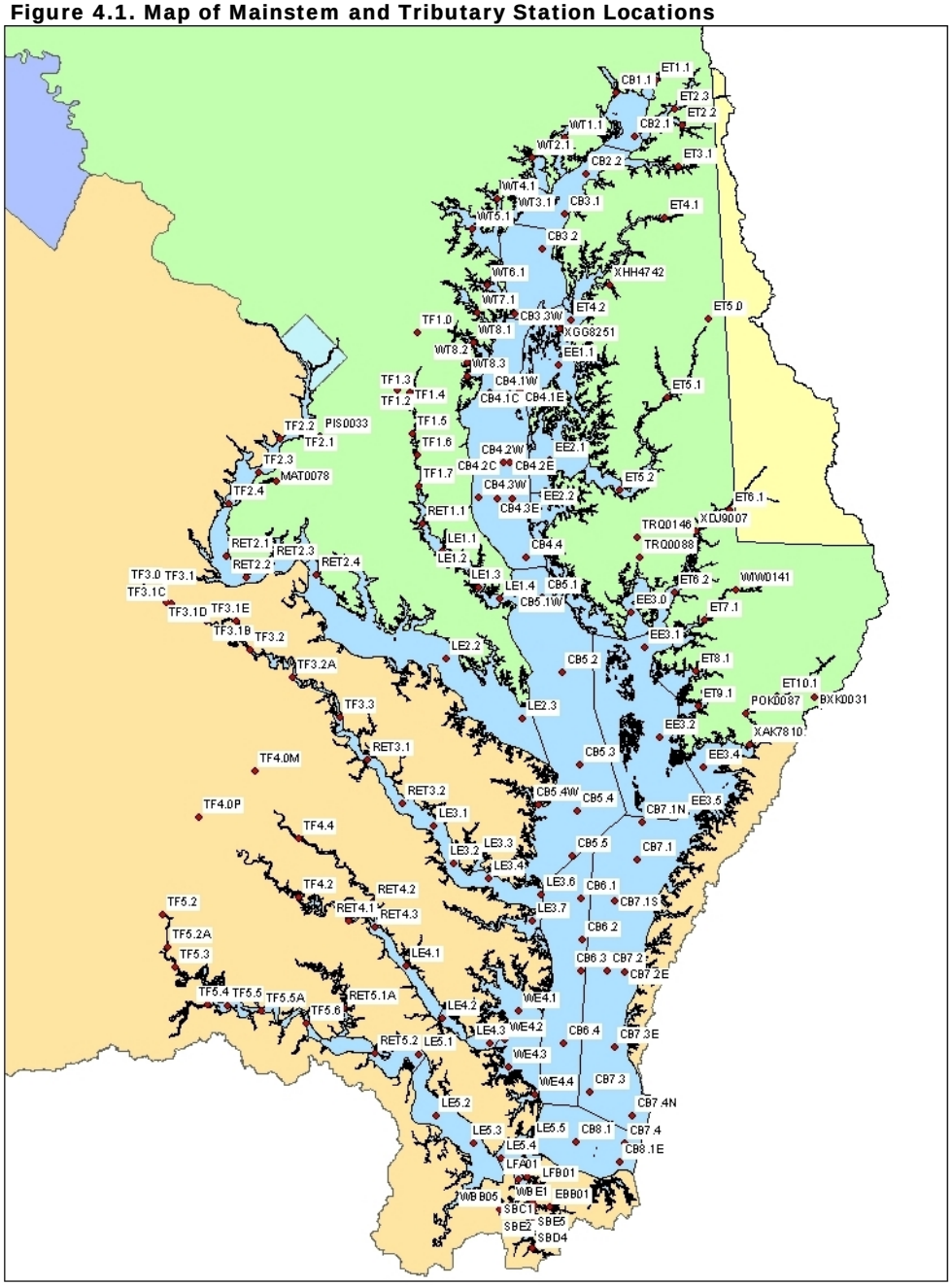
3.5. See Appendix A for the lat/long coordinates and a description of each station. This information is also available through the Chesapeake Bay Environmental Data Repository (CDEDR) under Water Quality Data at: http://data.chesapeakebay.net/WaterQuality.

6. Water Quality Parameters

TABLE 4.1 Mainstem and Tributary WQ Parameters

Laboratory Parameters	In-situ Measurements
Total Dissolved Phosphorus (TDP) Dissolved Orthophosphate (PO_4F) Particulate Phosphorus (PP) Nitrite (NO_2F) Nitrate + Nitrite ($NO_{23}F$) Ammonium (NH_4F) Total Dissolved Nitrogen (TDN) Particulate Nitrogen (PN) Particulate Carbon (PC) Total Suspended Solids (TSS) Volatile Suspended Solids* (VSS) Chlorophyll *a* (CHLA) Pheophytin (PHEO) Dissolved Organic Carbon* (DOC) Silica* (SiF) * Collected from a subset of stations	Dissolved Oxygen (mg/L) Temperature (°C) pH (su) Specific Conductance (µmhos/cm) Salinity (psu) Secchi Depth (m) Total Depth (m) Sample Depth (m) Photosynthetic Active Radiation (PAR) (µM = µmoles/second-meter^2)

Figure 4.1. Map of Mainstem and Tributary Station Locations



Section B. Field Measurement Procedures

1. Sampling Sites

1.1. Each fixed station has predetermined latitude and longitude coordinates. Locate the sampling location by GPS and position the vessel within 25 meters of that point. The engine may be turned off and the vessel either anchored or allowed to drift. Avoid drifting to shallower or deeper waters as this may result in real differences in water quality.

1.2. Record the actual initial GPS coordinates on the field sheet or in the GPS unit. If sampling conditions prevent collection of data at the predetermined coordinates, record the actual coordinates and depth in the "Details" field of the CIMS Event Table.

1.3. The total depth measurement from surface to bottom may be done in several ways: from the vessel depth finder, the pressure sensor of the sonde, or calibrated markings on lines attached to the sampling equipment.

2. Weather Conditions

Record weather and sea conditions at the time of sampling, i.e., cloud cover, air temperature, precipitation type, wind speed, wind direction, wave height and tidal current stage.

3. Physiochemical Profile

3.1. **Parameters**

An *in-situ* vertical profile is determined at every sampling station. The following parameters are measured from the bottom to the surface of the water column: water temperature, pH, dissolved oxygen, salinity, and secchi depth. The depth of each measurement and total depth are also measured. Light attenuation (PAR) is measured at mainstem stations and the Virginia tributary phytoplankton monitoring stations.

3.2. **Equipment**

3.2.1. A multi-parameter, water quality instrument, such as a YSI or Hydrolab multi-parameter sonde.

3.2.2. The instrument shall be equipped with the following sensors:

3.2.2.1. Dissolved oxygen sensor,

3.2.2.2. pH sensor,

3.2.2.3. Thermistor (temperature sensor), and,

3.2.2.4. Conductivity cell (salinity)

3.2.2.5. Note: Some sondes have pressure sensors which may be used to determine sampling depth.

3.2.3. The sonde must be outfitted with a data logger or computer to display the measurement values. If possible, record and store measurements electronically.

3.3. **Calibration:** (See also Chapter 2, Section D)

3.3.1. Pre-cruise calibrations must be performed for dissolved oxygen (DO), pH, and conductivity to standardize the response of each probe. Post-cruise calibration checks are performed to ensure that calibration was maintained over the course of sampling. For multiple day cruises, a daily, one-point DO calibration check is highly recommended.

3.3.2. Staff shall maintain logbooks for each instrument and/or sensor. The logbooks shall document all calibration, maintenance and servicing information.

3.3.3. Calibrations are performed indoors after the probes have stabilized to room temperature. Follow all manufacturer specifications for calibration and maintenance; fully document selected options in the calibration SOP.

3.3.4. If a post-calibration check does not meet acceptable tolerances, censor the corresponding data back to the last calibration check using the CIMS WQ problem code "V" (Sample results rejected due to QC criteria.)

If the post-calibration failure is due to an accident that occurred after sampling, qualify the data with the CIMS WQ problem code "F" (i.e., Post-calibration failure possible due to equipment damage after sampling, but data appear normal.)

3.3.5. **Conductivity:** The conductivity sensor must be calibrated against a reference solution, according to manufacturer's specifications. As a minimum, conductivity should be verified before and after each cruise.

3.3.6. **pH:** As a minimum, the pH sensor must be calibrated at the beginning and end of every cruise. Use two standard solutions of pH 4.0, pH 7.0 or pH 10.0 buffers, bracketing the expected values of pH. If the post-calibration drift is ± 0.2 pH units or more, censor the data with the CIMS WQ problem code "V" or report values with code "F".

3.3.7. **Dissolved oxygen (DO):** The DO probe must be calibrated at the beginning and end of each multiple-day cruise according to manufacturer's specifications. Typically, Clark cell probes are calibrated against water-saturated air. Check the calibration at the beginning of each day.

If daily DO checks deviate by ≥ 0.30 mg DO/L from the expected value, the sensor must be recalibrated before using again. If a calibration check (daily or post-calibration) is ≥ 0.50 mg DO/L, censor all data corresponding back to the last calibration check using the CIMS WQ Problem Code "V".

3.3.8. **Thermistor:** Verify the accuracy of the thermistor reading at least once a year against a NIST certified thermometer over a range of temperatures. If the temperature is off by ≥ ± 1°C, have a service representative recalibrate the unit.

3.3.9. **Depth sensor:** Verify the accuracy of the sonde pressure transducer at the beginning of each sampling day at a known depth below the surface. The depth should be

accurate to 0.2 meters, or the tolerance given by the manufacturer.

3.3.10. **LiCor Meter:** An annual calibration is recommended; every 2 years is required.

3.4. **In-situ Data Collection (Multi-parameter sonde)**

3.4.1. Record the total depth from surface to bottom from the vessel depth finder, using a calibrated line or the sonde.

3.4.2. Take the surface reading at 0.5m (Md.) or 1.0m (Va.) below the surface. Take subsequent readings at 1m, 2m, and 3m depths below the surface, then at least every 2m intervals until reaching the nearest whole meter that is at least one meter off the bottom.

3.4.3. Take measurements every meter if:

3.4.3.1. The station total depth is ≤10m deep,
3.4.3.2. The change in DO is ≥1.0 mg/L every 2 m, or,
3.4.3.3. The change in specific conductance is ≥ 1,000 µmhos/cm every 2 m (1 µmhos/cm = 1 microsiemen/cm (µS/cm))

3.4.4. Lower the sonde to the desired depth, as displayed on the data logger or winch meter.

3.4.5. When all readings have stabilized, record the temperature, pH, DO, conductivity and salinity values on the field data sheet and in the data logger if available.

3.4.6. Repeat procedure for all required depths. Collect additional measurements at the calculated AP and BP depths if these depths were not included in the profile.

4. Pycnocline determination

The pycnocline is a region in which the water density changes appreciably with increasing depth and thus forms a layer of much greater stability than is provided by overlying surface waters. It is necessary to calculate the pycnocline in the field to determine the depths at which to collect grab samples.

The pycnocline calculated threshold value (CTV) is calculated using Equation 4.1. If the CTV is less than 500 micromhos/cm (or 500 µS/cm), then there is no discernible pycnocline. If the CTV is above 500 micromhos/cm, a pycnocline exists with boundaries at the first and last depths where the change in conductivity is greater than the CTV.

Eqn. 4.1

$$CTV = \frac{C_b - C_s}{D_b - D_s} \times 2$$

Where:

C_b = bottom conductivity (micromhos/cm),

C_s = surface conductivity (micromhos/cm),

D_b = depth of bottom conductivity measurement (m),

D_s = depth of surface conductivity measurement (m),

CTV = calculated threshold value (micromhos/cm)

5. Secchi depth

5.1. Secchi depth is a measure of the transparency of a water column.

5.2. **Equipment**: A 20 cm Secchi disk, held by a non-stretchable line that is marked in tenths of meters.

5.3. **Frequency:** The Secchi depth is determined at each station.

5.4. **Procedure:**

5.4.1. The Secchi depth must be determined from the *shady* side of the boat during daylight hours.

5.4.2. Slowly lower the Secchi disk until it is no longer visible and note the depth using the markings on the line.

5.4.3. Slowly raise the Secchi disk until it just becomes visible and note the depth using the markings on the line.

5.4.4. Record the average of the two Secchi readings to the nearest 0.1 meter.

5.4.5. Do not wear sunglasses or any devices that shades the eyes while performing this procedure.

5.5. **Quality Control** - Secchi

5.5.1. If the range of measurements for the two readings is greater than 0.5 m, repeat the entire measurement process, raising and lowering the disk several times around the "disappearing" point.

5.5.2. If the disk hits the bottom and remains visible, record the depth reading and note this in the comment section.

5.5.3. Calibrate the markings on the Secchi line periodically against a metal meter stick.

6. Light Attenuation (PAR)

6.1. **Equipment:** Manufactured by LI-COR, Inc.

6.1.1. LI-192SA, flat cosine Underwater Quantum Sensor

6.1.2. LI-190SA, air (deck) reference sensor.

6.1.3. Data Logger (e.g., LI-1000, LI-1400 or LI-1500).

6.2. **Parameters**:

6.2.1. **EPARU_Z:** Downwelling PAR, measured underwater (µM, micromoles/second-meter^2 = µE, microEinsteins).

6.2.2. **EPAR_S:** PAR measured in air, on deck or pier, recorded concurrently with each underwater depth reading (µM).

6.2.3. **SDEPTH:** Sample depth at which PAR reading is recorded (meters).

6.3. **Depths for Mid-Channel PAR**

6.3.1. Record the initial PAR just below the surface. If wave action is too great, take the initial PAR at 0.5 m.

6.3.2. As a general rule, record subsequent measurements at 1 meter increments for mainstem stations and at 0.5 m intervals for tributary stations deeper than 2 m. These may be increased to 2 m if the water is clear and deep.

6.3.3. Increments of 0.25 m are recommended for stations ≤ 2 m, or if water is turbid or colored.

6.3.4. Measurements are recorded until the meter reads ≤10 % of the initial subsurface value or if the bottom is reached.

6.4. **PAR Procedure**

6.4.1. PAR must be measured from the *sunny* side of a vessel or pier during daylight hours. Both the deck sensor and the submersed sensor must be placed away from the shadow of the vessel.

6.4.2. Check the sensor operation by connecting the deck sensor and underwater sensor to corresponding ports of the Li-Cor data logger, and turn the power on. Check battery level. Cover the sensor and check to see if the output is near zero. Place the sensor under a light source to assure that it has a positive reading. A negative reading indicates that the polarity of the sensor is reversed (it is plugged into the cable backwards). A very positive or negative reading under darkness indicates a possible short in the cable or some other electrical short.

6.4.3. In the field, mount the deck sensor in a location which is level and unobstructed by shadows. Ensure that the sensors are positioned properly on the deck sensor mounting and the lowering frame. Connect deck sensor and underwater sensor to corresponding ports of the meter, and turn the power on.

6.4.4. Set the instrument averaging constant to display results from previous 10 or 15 seconds.

6.4.5. Lower the frame until the sensor is just below the surface of the water; simultaneously record the deck sensor and the underwater readings. If wave action prevents this, take a deeper reading ≤ 0.5 m. Determine the measurement depth using a calibrated pole, non-stretching rope or depth sensor.

6.4.6. Lower the frame and take subsequent measurements at depths appropriate to the monitoring location and purpose of the project. Allow the instrument output to stabilize, and then record both deck and underwater readings at each depth.

6.4.7. The simultaneous deck and submersed readings are stored by pressing "Enter". Each record will be stored with a time stamp which will flash on the data logger readout. Record this time so that all readings can be subsequently retrieved at the end of the cruise. Alternatively, enter the station ID and the sampling interval into the data logger remarks function before starting the PAR readings.

6.5. Quality Control

6.5.1. The deck cell should have the highest PAR value, and the underwater sensor output should decrease as the sensor is lowered to greater depths. The deck sensor reading should be consistent throughout the profile. If the sunlight fluctuates during the profile it may be necessary to repeat the measurement.

6.5.2. Periodically check that the two sensors are linear and not out of calibration with respect to each other.

6.5.3. Calibration of the sensors by LICOR, Inc. is recommended every year, and required every two years.

6.6. Reporting PAR

The CIMS database stores PAR values from the deck sensor and each underwater measurement. Report the values under the following method codes:

6.6.1. **EPARU_Z F01**: Flat cosine sensor LI-192SA

6.6.2. **EPAR_S F01**: Deck (reference) sensor LI-190SA

6.6.3. **SDEPTH:** Sample depth at which PAR reading is recorded, in meters

7. Documentation and Records

Field Data Sheets, Calibration and Maintenance Logs must permit a complete historical reconstruction of the data. See Chapter 2, Section B.8 for detailed document control protocols.

8. Decontamination

8.1. **Multi-Parameter Sonde:** Rinse the sonde, probes and cable with tap water or DI water at the end of each sampling day. Cover with the storage cup containing ~ 1 inch of water to provide an air-saturated environment. For routine maintenance, follow the manufacturer's procedures.

8.2. **LiCor Sensors:** Rinse the underwater sensor and cable with tap or DI water at the end of each sampling day.

8.3. **Secchi Disk:** Clean the Secchi disk with a mild detergent solution at least once per month.

Section C. Sampling Procedures

1. Sampling Schedules

1.1. The Maryland Department of Natural Resources (MDNR) and Virginia Department of Environmental Quality (VDEQ) collaborate to develop an annual sampling or "cruise" schedule to ensure that samples are collected by both groups within reasonably the same time. The cruise schedule is submitted to the EPA Project Officer and published on the Chesapeake Bay Program website at: http://www.chesapeakebay.net/documents/3676/water_quality_monitoring_cruise_schedules_8407.pdf .

1.2. Weather and sea conditions permitting, all field measurements and sample collections for each mainstem cruise shall be completed during three days; and all field monitoring and sample collections for each tributary cruise shall be completed as closely as possible to the mainstem cruise dates.

1.3. Due to the large distance that must be covered in a single day of sampling, it is not practical to sample stations at a selected tidal stage. Instead, cruises shall begin on the designated date in the early morning and shall be completed by evening (usually 6 or 7 pm). Sampling should occur during daylight hours. In addition, all light dependent measurements, i.e., Secchi depth, light attenuation and fluorescence, must be measured during daylight hours.

2. Sampling Equipment

2.1. Collection bottles: Two to four pre-cleaned 4-liter polyethylene bottles per sampling station.

2.2. Submersible pump or Hydrocast sampling system (General Oceanics Model 1016 Rosette, or equivalent).

2.3. Laboratory sample bottles - Pre-cleaned or new HDPE bottles, stored protected from contamination. Avoid using sample bottles previously used for high concentration samples.

2.4. Sample filter containers – Plastic bags, aluminum foil or plastic dishes, appropriate for the parameter.

2.5. Filtration apparatus with vacuum pump. Syringe filters may be permitted for certain projects, but never for PC/PN.

2.5.1. *Chlorophyll* – Vacuum pressure < 10 in. Hg (< 5 psi).

2.5.2. *PC/PN* – Vacuum pressure < 10in. Hg (< 5 psi).

2.5.3. *TSS & Nutrients* – Unspecified

2.6. Filter Preparation – Whatman® GF/F, 0.7 µm pore size. Diameters other than those listed below may be used. See specific comments throughout the analytical methodology for concerns related to alternate sizes.

2.6.1. TSS & VSS: *Whatman® GF/F, 47 mm.*

Rinse filters three times with DI and transfer them to weighing pans. For TSS only, place in a drying oven at 103-105°C for 1 hour. For combined TSS/VSS, combust at 550 °C for 15 minutes in a muffle furnace. Cool in a desiccator and weigh. For at least 10% of the samples[1], repeat the cycle of drying or combustion until a constant weight is reached as defined in the method cited (e.g., ± 5% is required by Standard Methods)

2.6.2. **TSS only:** Same as 2.6.1, without combustion at 550°C.

2.6.3. **PN/PC:** Whatman® GF/F, 13 or 25 mm diameter – Filters must be pre-combusted at 500°C.

2.6.4. **Chlorophyll:** Whatman® GF/F, 47mm diameter – Pretreatment is unnecessary.

2.6.5. **Dissolved parameters:** Filters must be pre-rinsed to remove trace elements. Alternatively, use TSS filtrate.

2.6.6. **Particulate P:** Use either TSS or other pre-rinsed filters.

2.7. Refrigerator or a cooler with ice (≤ 6°C), and/or a freezer (≤ -20°C).

3. Reagents

3.1. Magnesium carbonate suspension for fresh water chlorophyll – Add 1 g of finely powdered $MgCO_3$ to 100 mL water.

3.2. Reagent water - ASTM Type II water, or equivalent.

[1] The CBP procedure deviates from Standard Methods, 20th edition, which requires re-drying and re-weighing 100% of the filters.

3.3. Hydrochloric acid (HCl) solution for cleaning equipment – Prepare a 5% or 10% HCl (v/v) solution. Be sure to add concentrated HCl to the water.

3.4. Reagent-grade HCl for preserving liquid samples.

4. Sample Collection

4.1. Collect samples after completing field measurements.

4.2. At stations where pycnocline sampling is designated and a pycnocline is detected, samples are collected 0.5 m (Md.) or 1.0 m (Va.) below the surface; 1.0 m above the upper boundary of the pycnocline; 1.0 m below the lower boundary of the pycnocline; and 0.5 – 1 m above the bottom. The MDNR bottom sample is taken from the nearest whole meter that is at least one meter off the bottom. See Chapter 4, Section B.4 for pycnocline definitions and alculations.

4.3. If there is no discernable pycnocline at designated stations, collections will be made at 0.5 m (Md.) and 1.0 m (Va.) below the surface; at the closest profile depth above one third of the distance between the surface depth, 1.0 m and the bottom sample depth; at closest profile depth below two thirds the distance between 1.0 m and the bottom sample depth; and 1.0 m above the bottom to the nearest 0.5 m.

4.4. At all other stations, samples are collected at the surface (0.5 m (Md.) and 1.0 m (Va.)) and ≥ 1m above the bottom.

4.5. Lower the submersible pump or Rosette to each sampling depth and fill one bottle per depth. Process the sample according to Section 5 below.

5. Sample Processing and Preservation

5.1. Sample processing and division for all samples should follow Figure 4.1. Filtration should be done immediately after sample collection to prevent chemical degradation and biological growth (Zimmerman and Keefe, 1997). If weather conditions are poor, it may be necessary to process samples on shore as soon as possible, within 2 hours of collecting the sample.

5.2. Wear disposable, powderless gloves while processing the samples.

5.3. **Chlorophyll and pheophytin processing [2]:**

5.3.1. Filtration for chlorophyll should be done in subdued light as soon as possible after collecting the sample. Gently shake the sample and pour quickly into a graduated cylinder, measure and record the volume. Transfer the aliquot to the filter tower and

[2] Deviations from EPA Method 446.0 are: a) Filtration pressure of 10 in. Hg is used instead of 6 in. Hg, b) Chlorophyll filters from tributary stations may be stored on ice for up to 24 hours, and c) Holding time prior to analysis is 28 days instead of 24 days.

apply the vacuum to concentrate the algae on the filter.

5.3.2. Filter sufficient sample (100-1,500 mL) to produce a green color on the filter pad. To avoid cell damage and loss of contents during filtration, do not exceed a vacuum of 10 in. Hg (≤ 5 psi), or a filtration duration greater than 10 minutes. Do not suck the filter dry with the vacuum; instead slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.

5.3.3. If sampling non-saline water, i.e., < 0.5 psu salinity, add 1mL of saturated MgCO3 solution during the last few seconds of filtering. MgCO3 is optional for higher salinity samples.

5.3.4. Remove the filter from the fritted base with forceps, fold once with the particulate matter inside and place it in a foil pouch or Petri dish. Wrap Petri dish in aluminum foil to protect the phytoplankton from light. Store the filters at ≤ -20°C. Short term storage up to 4 hours on ice is acceptable2 for processed filters but they should be stored as soon as possible at ≤-20°C.

5.3.5. Chlorophyll samples that cannot be concentrated immediately after collection may be chilled at ≤ 6°C in the dark for up to 2 hours before the plankton are concentrated, however any delay is strongly discouraged due to the possible growth of phytoplankton cells or degradation of pigments.

5.3.6. The filter may be stored in the dark at ≤ -20°C for up to 28 days before extracting the pigments. Studies have shown that extracts are stable for up to 3 months if they are extracted soon after collection (Wasmund, et al. 2006), however, analysis within 28 days is required.

5.4. **Particulate Carbon and Particulate Nitrogen:** A 25-500 mL sample is filtered under a vacuum (≤10 in. Hg or ≤ 5 psi) through a 13 mm GF/F filter. Do not rinse the residue on the filter with DI. Place the filters in Petri dishes or in foil pouches and keep on ice or in a freezer (at ≤ -20°C) for short-term storage and transport.

5.5. **Ammonia, Nitrite, Nitrite+ nitrate, Orthophosphate, Total Dissolved Nitrogen, Total Dissolved Phosphorus, Dissolved Organic Carbon, and Silicates:** Pour the filtrate from the particulate phosphorus and/or total suspended solids filtration into sample containers, keep on ice or in freezer (≤ -20°C) for short-term storage and transport, except samples for silicate analyses must be cooled and stored at ≤ 6°C. Ammonium and DOC samples should be frozen immediately, or acidified to pH ≤2, cooled and stored at ≤ 6°C. (Note: Use only HCl to acidify DOC samples to protect laboratory instruments.)

5.6. **Total Suspended and Volatile Solids:**

5.6.1. A 500 mL sample is filtered under vacuum through prepared 47 mm GF/F filters. It may be necessary to reduce the volume to keep the filtration time under 10 minutes.

5.6.2. Shake the sample vigorously and quickly fill a graduated cylinder to the desired volume to prevent solids from settling. Quantitatively transfer the subsample to the filtration apparatus and remove all traces of water by continuing to apply vacuum after the sample has passed through. Record the volume of sample filtered.

5.6.3. With the suction on, wash the filter, residue and inside wall of filter tower with three 10 mL portions of deionized water, allowing complete drainage between washing. Remove all traces of water from the filter by continuing to apply vacuum pressure after water has passed through.

5.6.4. If the filtrate is used for dissolved parameters, dispense the filtrate into sample containers prior to rinsing the filters.

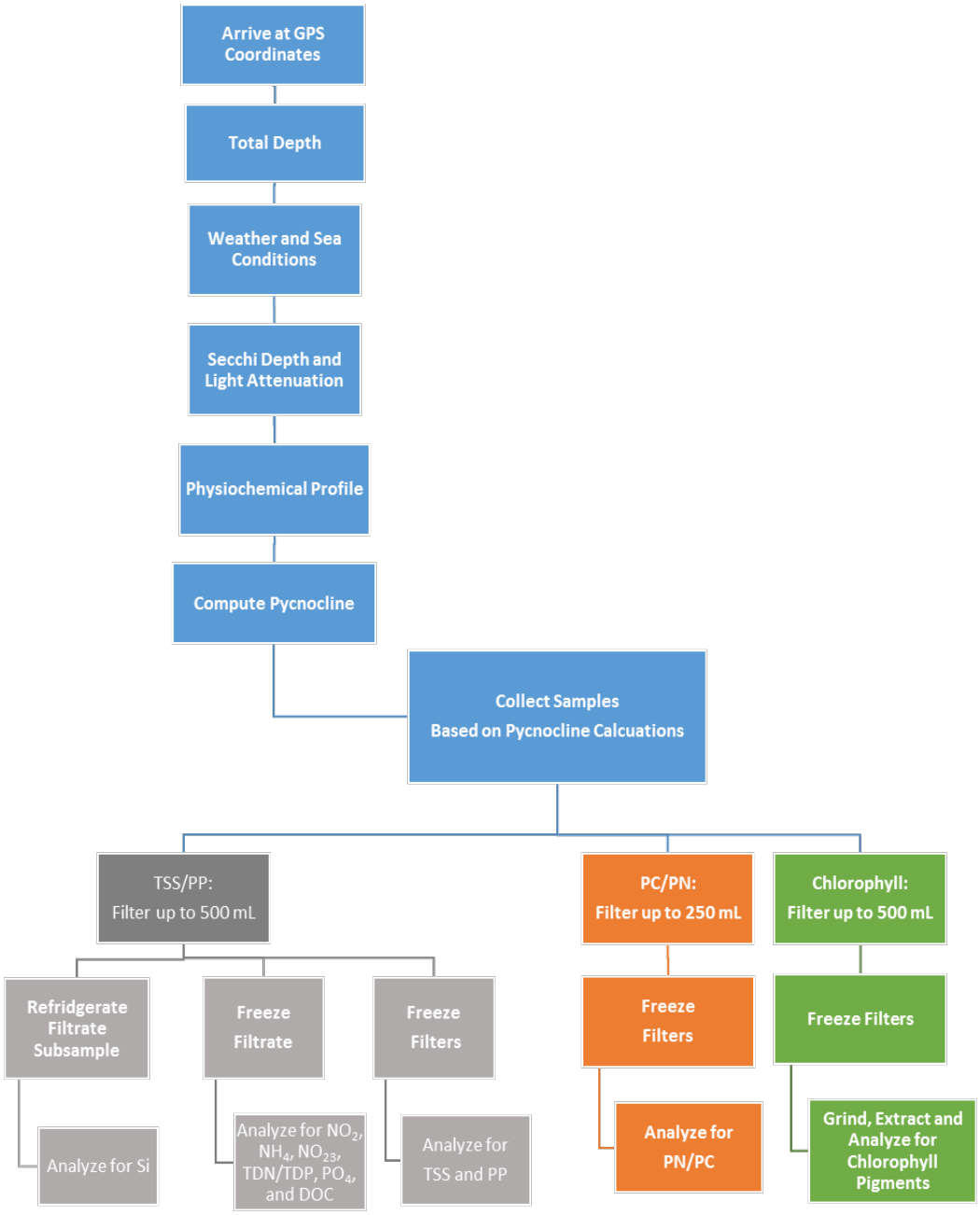
5.6.5. Remove the filter with residue with a forceps, transfer to a labeled sample container and place on ice or in freezer for short-term storage and transport.

5.7. **Particulate Phosphorus:**

5.7.1. Filter a 50-1000 mL sample under vacuum through a pre-rinsed 47 mm GF/F filter. Rinse the residue on the filter three times with ~10 mL DI water to remove dissolved solids.

5.7.2. Remove the filter with a forceps, transfer to a labeled sample container and place on ice or in a freezer for short-term storage and transport.

Figure 4.2 Field Measurements and Sample Processing



6. Quality Control Samples

6.1. **Field-Filtered Blank** – A field filtered blank is an equivalent aliquot of reagent water that is processed and preserved exactly as are samples. If the concentration of an analyte exceeds the lowest standard in the calibration curve, field and/or laboratory contamination should be suspected and corrective action initiated. (See Chapter 2 for corrective actions.)

6.1.1. Mainstem cruises: One field-filtered blank is recommended each day of the cruise; however, the required minimum is one for each 3-day cruise.

6.1.2. Tributary cruises: One field-filtered blank is required per month, or alternatively, one filtered equipment blank per month.

6.2. **Replicate Sample (S1, S2 & S3)** – A replicate sample is a second (or third) discrete sample taken in quick succession, at the same location and depth as the first samples. Data from replicate samples may be used to estimate overall precision or to deduce sampling precision.

6.3. **Field Split (FS1, FS2 & FS3)** – One sample is collected and two (or 3) homogeneous subsamples are processed identically for all parameters.

6.3.1. Mainstem Monitoring: Collect a field split or a replicate sample once for every 20 samples.

6.3.2. Tidal Tributary Monitoring: Each sampling group should collect field splits once per month, from the surface and bottom depths of the station.

6.4. **Sampling Equipment Blanks** (Mainstem and Tributary)

6.4.1. Equipment blanks indicate the effectiveness of the sampling equipment cleaning procedure. The equipment blank is processed in the office laboratory after the equipment has been cleaned.

6.4.2. An equipment blank is required once per year or whenever new equipment is used for the first time.

6.4.3. The equipment blanks consist of ASTM Type II water that has been passed sequentially through each component of the sample processing and collection equipment, e.g., submersible pump and hose, Rosette bottles, sampling containers, churn splitter, filtration unit, etc.

6.4.4. An analysis of the unfiltered reagent-grade water used to prepare the blanks maybe helpful in interpreting the results if contamination is found.

6.4.5. If the concentration of the equipment blank exceeds the lowest analytical standard in the calibration curve, prepare blanks of just the sampling equipment to isolate the cause of the contamination.

7. Documentation and Records

Field Processing Sheets and Laboratory Forms must have sufficient detail to allow for a complete historical reconstruction of the conditions and processes used to generate data points. A unique sample number or ID must be assigned to each sample collected and/or processed. See Chapter 2, Section B for additional document control protocols.

8. Decontamination

Filtration Units: At the end of each sampling day, clean the filtration units, flasks and graduated cylinders with a non-phosphate detergent, rinse with tap water then a final DI water rinse. For low level nutrient sampling, i.e., mainstem stations, rinse with 5-10% HCl between the tap and DI water rinses. If unable to use HCl during a multi-day cruise, soak or fill filtration equipment overnight in DI water.

Section D. References

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

[a]Method 440.0 Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis.

[b]Method 446.0 *In Vitro* Determination of Chlorophylls *a, b,* c_1+c_2 and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry.

Wasmund, N., Topp, I., Schories, D. (2006). *Optimising the Storage and Extraction of Chlorophyll Samples*, OCEANOLOGIA, 48 (1), 2006. pp. 125-144.

Zimmerman, C. and Keefe, C. (1997). *Immediate Filtration Processing of Water Samples to Separate Particulate and Dissolved Nutrient Parameters: What is the Critical Time Interval between Sample Collection and Filtration?* Report from the University of Maryland System C.E.E.S. Chesapeake Biological Laboratory, submitted to the Maryland Department of Natural Resources.

Salley, B. (1995). *A Comparison of Preservation Techniques for Dissolved Nutrient Analyses*, Special Report in Applied Marine Sciences and Ocean Engineering, Number 333. Virginia Institute of Marine Sciences.

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ANNAPOLIS, MARYLAND 21403

NOV 20 1986

Mr. Robert Siegfried
VA Water Control Board
2111 N. Hamilton Street
P.O. Box 11143
Richmond, Virginia 23230

Dear Mr. Siegfried:

At the October 22, 1986 meeting, the Monitoring Subcommittee approved a set of recommendations on acceptance of the use of freezing as an alternative method of sample preservation. The recommendations described here are based on the comparison study completed by the Virginia Institute of Marine Science. The recommendations for remaining parameters which were included under this study supported by the Chesapeake Biological Laboratory (particulate analyses) and the (carbon analyses) Old Dominion University will be presented to the Monitoring Subcommittee for approval at the November 25 meeting.

The use of freezing as an alternative method of sample preservation for the following parameters are now approved as an acceptable protocol for use in the Chesapeake Bay Monitoring Program.

Orthophosphate	Nitrite
Total Dissolved Phosphorus	Nitrate-Nitrite
Total Phosphorus	Total Kjeldahl Nitrogen
Ammonia	

For water column samples to be analyzed for the above stated parameters, the accepted protocol will be freezing at a temperature at -20 degree celsius for a maximum period of 28 days.

The results of the comparison study demonstrated that there were no significant measurable differences between the preservation treatments (freezing and EPA accepted preservation methods) for suspended solids. However, given the EPA accepted 7-day holding time, the Monitoring Subcommittee did not feel it would be necessary to freeze samples to be analyzed for suspended solids was not approved by the Monitoring Subcommittee.

The comparison study results and the literature have established that freezing as a preservation technique causes measureable difference in analyzed silica levels. The use of freezing as a preservation technique for the stabilization of water column samples to be analyzed for silica was not approved by the Monitoring Subcommittee.

Also at the October 22 meeting, the Monitoring Subcommittee approved the recommendations for the acceptance of the use of an alternate filter type. Specifically, the Subcommittee approved the use of glass filters rated at 0.7 microns nominal pore size in addition to the currently accepted membrane filters rated at 0.45 microns nominal pore size.

The filter study supported by the EPA Central Regional Laboratory and the Maryland Office of Environmental Programs demonstrated that for the parameters measured, there are no meaningful detectable differences between water filtered through either of the filters. Due to the number of non-detectable values for several of the parameters, a power analysis will be run on the entire data set by Dr. Magnien to determine the variability which would of been detectable if present.

This additional statistical analysis was requested to confirm the conclusions of the study.

Sincerely,

Richard Batiuk
Monitoring Coordinator

Bettina Fletcher
Chesapeake Bay Program
Quality Assurance Officer

cc: R. Alden
S. Sokolowski
B. Neilson
B. Salley

SPECIFIC RECOMMENDATIONS FOR THE ACCEPTANCE OF FREEZE STABILIZATION AS A TECHNIQUE FOR PRESERVATION FOR NUTRIENT ANALYSIS ON WATER SAMPLES

Orthophosphate

Water column samples to be analysed for orthophosphate can be frozen at -20 degrees celcius for a maximum period of 28 days as an alternative method of preservation.

Total Dissolved Phosphorus

Water column samples to be analyses for orthophosphate can be frozen at -20 degrees celcius for a maximum period of 28 days as an alternative method of preservation.

Total Phosphorus

Water column samples to be analysed for total phosphorus can be frozen at -20 degrees celcius for a maximum period of 28 days as an alternative method of preservation.

Ammonia

Water column samples to be analysed for ammonia can be frozen at -20 degrees celcius for a maximum period of 28 days as an alternative method of preservation.

Nitrite

Water column samples to be analysed for nitrite can be frozen at -20 degrees celcius for a maximum period of 28 days as an alternative method of preservation.

Nitrate-Nitrite

Water column samples to be analysed for nitrate-nitrite can be frozen at -20 degrees celcius for a maximum period of 28 days as an alternative method of preservation.

Total Kjeldahl Nitrogen

Water column samples to be analysed for total kjeldahl nitrogen can be frozen at -20 degrees celcius for a maximum period of 28 days as an alternative method of preservation.

Suspended Solids

The results of the comparison study demonstrated that there were no significant measureable differences between the preservation treatments for suspended solids. However, given the accepted 7 day holding time, freezing as a preservation technique is not recommended.

Silica

The comparison study results and the literature have established that freezing as a preservation technique causes measureable differences in analysed silica levels. Water column samples for analysis of silica will not be preserved by freezing for any period of time.

CHAPTER 5. NONTIDAL WATER QUALITY MONITORING

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Section A. Project Description

1. Management Objectives

Chesapeake Bay Program partners are implementing management actions through the Chesapeake Bay TMDL process to reduce nutrient and sediment pollution entering the Chesapeake Bay watershed. The Chesapeake Bay Nontidal Water Quality Network (NTN) was developed in 2004, to estimate nutrient and sediment loadings discharged and trends in loads from each of the 36 tributary strategy basins. In 2009, the objectives were modified to measuring the effectiveness of best management practices at multiple scales.

The NTN includes the nine Chesapeake Bay River Input Monitoring (RIM) stations which require additional parameters for the Chesapeake Bay Estuarine Model. Further information about the RIM monitoring program may be found at the website http://cbrim.er.usgs.gov/loadhighlights.html.

Loadings and trend data, along with data sets on nutrient and sediment sources, Best Management Practices (BMPs), land-use changes and watershed characteristics may be used to assess the factors which affect local stream and river nutrient and sediment concentrations, flow and the resultant loads to downstream waters. The observed concentration trends and calculated loadings data will help the tributary strategy teams to:

1.1. Assess progress toward meeting nutrient and sediment cap load allocations;

1.2. Evaluate the effectiveness of state tributary strategy implementation to improve water-quality of local streams; and

1.3. Determine if tributary strategy implementation in the watersheds will result in achievement of water-quality standards in the Bay.

2. Monitoring and Data Quality Objectives

2.1. The monitoring objectives of the Chesapeake Bay Nontidal Watershed Water-Quality Network are to:

2.1.1. Compute annual loadings of total nitrogen, phosphorus and suspended sediment from tributary strategy basins;

2.1.2. Assess the status and trends of nutrient and sediment concentrations and loads at each station;

2.1.3. Compare concentration data and loadings estimates among rivers; and

2.1.4. Improve calibration and verification of partners' watershed models.

2.2. The data quality objectives for computing loadings and trends in loadings are to: a) accurately sample stream conditions under the range of flow conditions during a given year and b) obtain a sample that is representative of the stream.

2.2.1. The sampling design is a combination of fixed-interval and storm event samples to capture the hydrologic and seasonal variability of nutrient and suspended-sediment

concentrations. A minimum of 10 years of data are required to calculate trends in loadings for the Estimator model. Annual loadings will be estimated for stations having 5 or more years of data. Section 4 below provides additional detail on the sampling design.

2.2.2. Isokinetic, depth- integrated samples are collected at equal-width increments across a stream channel and then composited to obtain samples that are representative of in-stream nitrogen, phosphorus and sediment concentrations.

2.2.3. Sampling procedures for the NTN are based on methods in the USGS National Field Manual for the Collection of Water-Quality Data. For greater detail, see http://water.usgs.gov/owq/FieldManual/chapter4/pdf/Chap4_v2.pdf.

3. Participating State and Federal Agencies

Sampling is conducted by five State agencies, four USGS Water Science Centers and the Susquehanna River Basin Commission as indicated below. Samples are analyzed by State and USGS laboratories.

State	Sampling Agencies
Delaware	Delaware Department of Natural Resources and Environmental Control
Maryland	Maryland Department of Natural Resources
	USGS Maryland-DC-Delaware WSC
New York	New York State Environmental Protection
	Susquehanna River Basin Commission
Pennsylvania	USGS Pennsylvania Water Science Center
	Susquehanna River Basin Commission
Virginia	USGS Virginia and West Virginia Water Science Center Virginia Department of Environmental Quality
West Virginia	USGS Virginia and WV Water Science Center WV Department of Environmental Protection

4. Sampling Design

4.1. Parameters:

TABLE 5.1 Parameters for Chesapeake Bay Nontidal Water Quality Network

Required NTN Parameters	Additional RIM Parameters (Recommended for NTN)
Total Nitrogen, as N (TDN + PN) or (TKN + NO23) or (TN)	Total Dissolved Nitrogen (TDN)
Ammonium, as N (dissolved*) (NH4F)	Particulate Nitrogen (PN)
Nitrate + Nitrite, as N (dissolved*) (NO23F)	Total Dissolved Phosphorus (TDP)
Total Phosphorus, as P (TP) or (TDP + PP)	Particulate Phosphorus (PP)
Phosphate, as P (dissolved*) (PO4F)	Particulate Carbon (PC), or TOC
Total Suspended Solids (TSS) and/or SSC	Dissolved Organic Carbon (DOC)
Suspended Sediment Concentration (SSC)	Fixed Suspended Solids (FSS)
SSC-Sand & SSC-Fines (1 storm/quarter)	Chlorophyll-*a* (corrected) (CHLA)
Field Parameters: Dissolved Oxygen, Temperature, pH, Specific Conductance	
* *Dissolved fraction is preferred but whole water is acceptable*	

4.2. **Site Specifications:** There are two types of NTN sites – Primary and Supplemental. As of Water Year 2016, there are 111 Primary NTN stations and 12 Supplemental NTN stations. Supplemental stations are also called secondary stations.

4.2.1. **Primary sites** are characterized as having:

4.2.1.1. The sampling locations within one mile of a continuous stream-flow gage so that the water-quality and discharge information are comparable;

4.2.1.2. Twenty water chemistry samples collected per year over a range of flow conditions (12 monthly routine + 8 storm flow);

4.2.1.3. Total nitrogen, total phosphorous, ammonium, nitrate, phosphate and total suspended solids analyses;

4.2.1.4. Storm samples must also include analyses of suspended sediment concentrations, and each quarter, a sand/fine particle size analysis; and

4.2.1.5. Equal-width increment (EWI), isokinetic, depth-integrated sampling techniques to obtain representative samples.

4.2.2. **Supplemental stations** do not have storm sampling, but follow primary station criteria such as:

4.2.2.1. Sites are associated with a stream-flow gage to allow computation of loadings trends;

4.2.2.2. Samples must be collected at least monthly;

4.2.2.3. Parameters shall include total nitrogen, total phosphorous and total suspended solids; and

4.2.2.4. Use of isokinetic, depth-integrated sampling techniques to obtain representative samples.

4.3. Routine Sampling Frequency

4.3.1. Primary and supplemental stations are sampled once per month on a predetermined schedule. These fixed-interval samples provide samples from a random, unbiased selection of flow conditions.

4.3.2. If high discharge occurs during routine monthly sampling, collect the samples on the scheduled date using procedures for storm event sampling, and including a SSC sample (primary stations only). These samples are to be counted as routine, monthly samples and designated as event type "Routine, Storm Impacted". **A routine storm-impacted event has a rising discharge (cfs) of at least twice that of the pre-storm, average daily discharge.**

4.4. Storm Sampling Frequency

4.4.1. Eight storm-event samples are required per year. Sampling of larger storms near the peak discharge is preferred to ensure that higher loadings are represented. The collection of at least one storm event per quarter to capture seasonal effects is desired but has less priority than larger storms. **In dry years, smaller discharges of at least twice that of the pre-storm discharge may be sampled.**

4.4.2. Samples may be collected at any point in the hydrograph, i.e., rising or falling limb, or at peak discharge.

4.4.3. Two samplings are permitted during a single storm event. However, samples must be collected on different days so that two estimates of daily load can be calculated. This practice also applies to taking a storm sample after the collection of a routine storm-impacted sample.

4.4.4. SSC samples are collected each storm sampling day, with a sand/fine particle size analysis each quarter.

4.4.5. Monitor the hydrological conditions leading up to, and predicted for the storm, including:

4.4.5.1. **Rainfall,** e.g., total rainfall over last 24-48 hours, rainfall intensity, current radar and forecast for the next few days;

4.4.5.2. **Current Hydrology,** e.g., stage, rising or falling limb, shape of the storm hydrograph and upstream river conditions; and

4.4.5.3. **Previous Hydrology,** i.e., the size of the storm relative to discharge over the last 6 months.

4.5. **Composite Samples**

4.5.1. The number of depth-integrated, equal-width interval (EWI) samples varies per station and waterway width. Table 5.3 in Section B.3 describes the minimum number of verticals to be composited at primary stations, for both routine and storm event sampling.

4.5.2. Verify stream mixing prior to taking field measurements and collecting samples. Take in-stream measurements of specific conductance, turbidity, and temperature and verify that the sampling points across the channel will be representative of the vertical and horizontal water-quality conditions within the cross-section. Add sampling increments as necessary.

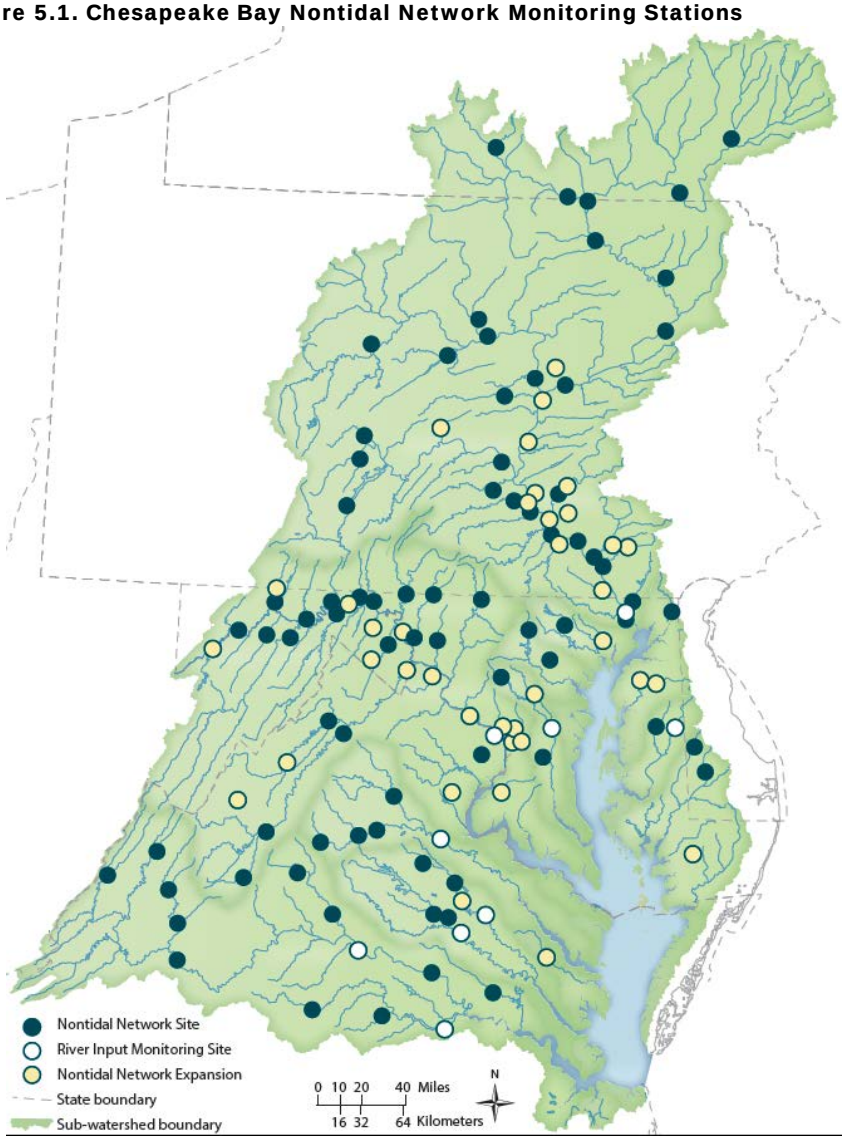
4.5.3. Periodically check the variability of suspended sediment concentration among the EWI increments by submitting relatively high concentration, discrete samples to the sediment laboratory.

5. Sampling Stations and Locations

5.1. Figure 5.1, Chesapeake Bay Nontidal Network Monitoring Stations, shows the locations of the sampling stations relative to state and sub-watershed boundaries.

5.2. Appendix 5-B, *CBP Nontidal Network Water Quality Stations, Locations and Streamflow Gages*, provides a description of each monitoring station, including the nearby USGS stream gage and coordinates of latitude and longitude

Figure 5.1. Chesapeake Bay Nontidal Network Monitoring Stations



Section B Sampling Procedures

1. Equipment

All sample collection and processing equipment should be kept in clean plastic bags or bins to minimize contamination.

1.1. **Samplers***

1.1.1. *DH-81*: A hand-held, depth-integrating suspended sediment and water quality sampler. The DH-81 samplers consist of a 1-L sample bottle, a D-77 sample cap that holds the nozzle, and a DH-81A adapter that snaps over the cap and to which a wading rod is attached. Make sure to collect an isokinetic sample if the discharge velocity is 1.5 ft/s or more. A 5/16-inch nozzle will be sufficient for most discharges over 1.5 ft/s that can be safely waded. If the discharge velocity is < 1.5 ft/s, a DH-81 may be used without a nozzle.

1.1.2. *DH-95:* A hand-line suspended sediment and water-quality sampler, for sampling depths ≤ 15 ft. The DH-95 weighs 29 lbs and is designed to be used with a 1-L bottle. If the discharge velocity is 1.5 ft/s or more, use the appropriate size nozzle and collect a depth integrated, isokinetic sample.

1.1.3. *WBH-96:* A weighted bottle sampler may be used to collect samples where discharge velocities are less than 1.5 ft/s.

1.1.4. *D-95*: This sampler is very heavy so must be suspended from a bridge using a cable, reel and bridge board or crane. The D-95 sampler may be used to a maximum depth of 15 ft. To collect an isokinetic sample, use a 5/16-inch nozzle and make sure the discharge velocity is 1.5 ft/s or more. If the discharge velocity exceeds 3 ft/s and the depth is > 15 feet, use a D-2 bag sampler equipped with a 1/4 or 5/16-inch nozzle.

*For additional information on samplers see: USGS TWRI, Book 3, Chapter C-2 (http://pubs.usgs.gov/twri/twri3-c2/)

1.2. **Churn Splitter:** A pre-cleaned 8 or 14-liter churn splitter is recommended. Store and transport clean churns in plastic bags to prevent contamination.

1.3. **1-liter Sampler Bottles**: A narrow-mouth 1-liter bottle is used with the WBH-96; a wide-mouth 1-liter bottle is used with the DH-81 and DH-95 samplers.

1.4. **Sample/lab bottles:** Pre-cleaned polyethylene bottles, as specified by the analytical laboratory. Ensure that sample bottles are in a clean state or have been thoroughly cleaned before reuse.

1.5. **Preservatives as required by the laboratory, e.g., nitric acid (HNO3), sulfuric acid (H2SO4), magnesium carbonate (MgCO3), etc.**

1.6. **Filtration apparatus**

1.6.1. For dissolved parameters, capsule filters with a pore size of 0.45 µm are required for USGS staff. State agencies have demonstrated that 0.7 µm GF/F filters produce equivalent results.

1.6.2. For particulate carbon and particulate nitrogen, use only GF/F filters that have been pre-combusted at 500°C for 1.5 hours.

1.6.3. Filter supports may be capsule or a tower unit with a fritted base.

1.7. **Vacuum pump and Masterflex tubing;**

1.8. **Water-quality meters for DO, temperature, pH, conductivity and turbidity;**

1.9. **Field forms and ballpoint or permanent-ink pens (no gel pens);**

1.10. **Field folders containing the following:**

- Site-specific sampling procedures;
- Table of velocities vs. stage height, to determine type of sample (isokinetic or non-isokinetic) and nozzle size;
- Directions to both the sampling location and gaging station;
- Job Hazard Analysis; and
- Traffic Safety Plans.

1.11. **Cleaning supplies** (tap water, deionized water, non-phosphate detergent solution, 5% HCl acid rinse, and baking soda to neutralize the used acid-rinsed water);

1.12. **Disposable, powderless gloves**; and

1.13. **Safety equipment:** Safety vests, flotation devices (pfd, float coat), hip-boots, chest waders, and traffic safety cones.

2. Sample Collection – Primary Stations

2.1. The procedures that follow are used for the collection of routine, storm event and routine, storm-impacted sample types.

2.2. Determine the number of depth-integrated, equal width increments (EWIs) that will result in a representative sample at each site. The number of EWIs samples is based on the width of the stream channel and how well the water is mixed vertically and horizontally across the channel.

2.2.1. Table 5.3, Minimum Number of Depth-Integrated, EWI Samples at Primary Stations, gives the minimum number of verticals to be composited at primary stations. Increase the number of increments and recalculate the width of each increment if the in-stream measurements show variability, or if the turbidity varies across the channel or from surface to bottom.

2.2.2. Measure the increments according to the procedure in USGS National Field Manual (NFM) for the Collection of Water-Quality Data, Chapter A4, Collection of Water Samples (p.45-53) http://water.usgs.gov/owq/FieldManual/chapter4/pdf/Chap4_v2.pdf.

2.2.3. Take the in-stream measurements **first** (Section 5.C below) to ensure that the EWIs adequately represent the vertical and horizontal water-quality conditions within the cross-section.

2.2.4. Obstructions such as bridge trusses, abutments, debris, fallen trees, etc., may cause variations in flow and water quality conditions. Document any deviations from the standard sampling protocols.

2.3. On each sampling day, record the stream discharge or stage height from the gaging station or from real-time reports on the USGS website.

2.4. Select the appropriate sampling device based on flow conditions, safety, and the type of samples being collected. If the discharge exceeds 1.5 ft/s, depth-integrated, isokinetic samples must be collected at the mid-point of each EWI across the stream channel. This applies to both routine and storm event samples. See Table 5.2 for details.

2.5. Isokinetic, depth-integrating samplers such as the DH-81 and DH-95 are to be used when the velocity of the stream is 1.5 ft/s or greater. Each sampler/nozzle combination has an optimum transit rate for surface to bottom collection. Refer to the NFM (2006), Appendix A4 -Transit Rate and Volume Guidelines and Filling Times for Isokinetic Samplers.

2.6. If the average velocity of the stream is < 1.5 ft/s it may not be feasible to collect an isokinetic sample. In this case it is acceptable to obtain one or more depth-integrated grab samples. If the stream is at least 2-3 ft. deep, use a DH-81 or DH-95 without a nozzle, or a weighted bottle.

2.7. Depth integration: Pre-determine the vertical transit rate by starting at the increment with the largest discharge (depth x velocity) to find the maximum transit rate. Lower and raise the sampler at the same rate – if the sampler overflows, discard the sample and repeat the collection at a faster rate. If under-filled, discard and repeat at a slower rate until the appropriate volume is obtained.

2.8. Beginning at the first increment, lower the sampler at the pre-determined rate until a few inches from the bottom, then immediately raise it to the surface using the **same rate.** The designated “clean-hands” person removes the sample bottle and empties the contents into the churn splitter. The reel operator should not touch the sample bottles.

2.9. Sample the remaining intervals using the **same** transit rate as in the first increment, and then composite them in the churn splitter. (See Section 4 below.) The volume of sample may be less in the remaining verticals.

Table 5.2. Minimum Requirements for Nontidal Network Sample Types

Station Type	Stream Velocity	Sample Type	Sampler	Number of Samples
Primary	≥ 1.5 ft./s	EWI, Isokinetic Depth-integrated	**Hand Held**: DH-81 or DH-95, with nozzle. **Cable & Reel**: DH-95; or D-2 in channels ≥ 15 ft. deep.	See Table 5.3
	< 1.5 ft./s	EWI, Depth-integrated	DH-81 or DH-95 ***without nozzle***, or Weighted Bottle (WBH-96)	See Table 5.3
Supplemental	≥ 1.5 ft./s	Isokinetic, Depth-integrated	DH-81 or DH-95, with nozzle.	1 centroid, or Table 5.3
	< 1.5 ft./s	Depth-integrated	DH-81 or DH-95 ***without nozzle***, or Weighted Bottle (WBH-96)	1 centroid, or Table 5.3

Table 5.3. Minimum Number of Depth-Integrated, EWI Samples at Primary Stations

Width of Waterway (ft.)	Minimum # of verticals*
0-25	**1**
25-100	**3**
100-250	**5**
250-500	**7**
> 500	**9**

*Routine and storm event sampling

3. Sample Collection – Secondary Stations

3.1. It is recommended that secondary stations be sampled according to primary station procedures.

3.2. If it is not possible to collect the number of verticals in Table 5.3, at least one depth-integrated sample is to be collected at the centroid of flow. This is the point where half of the discharge is to the left, and half is to the right.

3.3. Isokinetic samplers are recommended for average velocities ≥1.5 ft/s to produce more representative suspended sediment and total phosphorus data.

4. Compositing

4.1. Empty the EWI subsamples into a pre-cleaned churn splitter. Collect sufficient subsample volumes (e.g., at least 5.25 liters for an 8-liter churn). If the volume of sample in the churn is insufficient, collect a second, identical set of EWI subsamples across the channel.

4.2. Follow the procedures below to fill sample bottles. (Adapted from the USGS National Field Manual, Chapter 5. http://water.usgs.gov/owq/FieldManual/chapter5/pdf/chap5.pdf)

4.3. Take precautions to avoid sample contamination. Prepare a clean work area at the site or in the van for processing samples. Designate a "clean hands" person to churn and handle the sample bottles or if alone, use "clean hands/dirty hands" handling techniques. The use of disposable, powderless gloves is highly recommended while dispensing and filtering the samples.

4.4. Churn the composite sample at a uniform rate by raising and lowering the disk inside the churn splitter with smooth, even strokes.

4.5. When churning, the disk should touch the bottom on every stroke, and the stroke length should be as long as possible without breaking water surface. Do not break the surface of the water.

4.6. The churning rate should be about 9 inches per second (in/s). Inadequate churning can result in withdrawal of misrepresentative whole-water or suspended-material samples.

4.7. Pre-mix the composite sample by churning for about 10 strokes to uniformly disperse suspended material before subsampling.

4.8. Continue churning while subsampling. Dispense whole water samples first, in the following order:

4.8.1. Suspended Sediment and TSS

4.8.2. Particulate Nitrogen and Carbon

4.8.3. Chlorophyll

4.8.4. Whole water samples

4.8.5. Dissolved parameters to be filtered on site

4.9. Do not interrupt the churning/subsampling process, if possible. If an interruption occurs, reestablish the churning rate and remix the sample by churning ten strokes before resuming subsampling.

4.10. As the volume of composite sample in the churn decreases, adjust the stroke length to maintain a churning rate of about 9 in/s and avoid breaking the surface of the water being sampled.

5. Sample Processing and Preservation

5.1. **Whole water samples**

5.1.1. SSC samples from the churn splitter may be held at room temperature for 120 days. Ship the samples prior to September 1st so that the results are available for water-year based data analyses.

5.1.2. If the laboratory filters samples for dissolved and particulate parameters, place whole-water nutrient and TSS samples in a cooler on ice (≤ 6°C) and omit the following processing steps.

5.2. Particulate Samples

5.2.1. Total suspended solids (TSS): Churn the sample and fill a To-Deliver (TD) graduated cylinder to a known volume (50-1000 mL) and filter the aliquot through a pre-rinsed, tared, 47mm diameter GF/F filter. Rinse the filter and residue three times with DI water, allowing the suction to dry the residue after the final rinse. If the filtrate is to be used for dissolved parameters, dispense the filtrate in the filtration flask into sample bottles before rinsing the filter and residue. Place the TSS filter in a plastic case or foil pouch and immediately put in a cooler on ice (≤ 6°C).

5.2.2. Particulate carbon and particulate nitrogen: Churn the sample and fill a To-Deliver (TD) graduated cylinder to a known volume (25-500 mL) of sample and filter through a GF/F filter. Keep the vacuum at or below 10 in. Hg (5 psi) while filtering. Do not rinse the filter. Place the filter into a plastic case or foil pouch and immediately put in a cooler on ice (≤ 6°C)

5.2.3. Chlorophyll:

5.2.3.1. Immediately after collecting the sample, churn and fill a TD graduated cylinder to a known volume and filter the sample aliquot through a glass fiber filter to concentrate the algae. Use sufficient sample (100-1500 mL) to produce a green color on the filter pad. To avoid cell damage and loss of contents during filtration, do not exceed a vacuum of 10 in. Hg (≤ 5 psi), or a filtration duration greater than 10 minutes. Do not suck the filter dry with the vacuum; instead slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter. Add 1 mL of saturated $MgCO_3$ solution during the last few seconds of filtering.

5.2.3.2. Remove the filter from the base with a forceps, fold once with the particulate matter inside, lightly blot the filter with a Kimwipe™ to remove excess moisture and place it in a foil pouch or a plastic case (if transparent, wrap in aluminum foil). Store the filter at -20°C (-4°F) or colder. Short-term storage on ice is acceptable but filter pads should be frozen as soon as possible.

5.2.3.3. Chlorophyll samples that cannot be filtered immediately after collection may be chilled (but not frozen) at ≤ 6°C in the dark for up to 2 hours before the plankton are concentrated. However, any delay is strongly discouraged due to the possible growth or lysis of the phytoplankton cells.

5.3. Field-Filtered Samples

Dissolved ammonium, nitrite, nitrate + nitrite, orthophosphate, nitrogen, phosphorus, and organic carbon:

Samples are filtered in the field using a vacuum or a peristaltic pump and a pre-cleaned length of Masterflex tubing. Most sampling groups use Gelman® 0.45 µm pore size capsule filters, although 0.7µm pore GF/F filters are also acceptable. Hand pumps and syringe filters are also acceptable.

5.3.1. Samples for dissolved constituents should be filtered in the field, or before the end of the sampling day. Use a new filter for each sample and rinse the filter just prior to filtering with deionized water (DI) and sample water.

5.3.2. Rinse capsule filters with two liters (≈ ½ gallon) of DI water. Shake to remove excess DI and then rinse with ≈100 mL of sample water. This technique prevents clogging of the filter as would a 100% sample rinse.

5.3.3. Collect sufficient sample filtrate to rinse and fill necessary bottles. If required by the laboratory, add acid preservative to the samples for ammonium, nitrate + nitrite and DOC analyses. Place the samples on ice (≤ 6°C).

Use a new filter for each sample and rinse with DI water and sample just prior to filtering.

5.4. **Labeling**

5.4.1. Follow the sampling agency's protocol for labeling sample bottles.

5.4.2. Sediment bottles for the USGS Sediment Laboratory in Kentucky must be labeled with the following:

5.4.2.1. Collection site numbers

5.4.2.2. Date and Time

5.4.2.3. Discrete or composite sample (if composite, by transect or time

5.4.2.4. Constituents: SSC and/or particle size. (Both analyses can be done from the same bottle.)

5.5. **Sample Handling and Transportation**

Deliver nutrient and TSS samples and/or processed filters to the laboratory as soon as possible, preferably at the end of the day, or ship them on ice for next morning delivery. If the samples must be held overnight, refrigerate whole water samples at ≤ 6°C and freeze chlorophyll and particulate filters at -20°C (-4°F) or colder.

6. Field Blanks

6.1. Definition and Purpose

6.1.1. A NTN field blank (FB) is an aliquot of deionized water, free of the analytes of interest, which is collected in the field using the same collection equipment and methods as the environmental sample. The FB is treated as a sample in all respects, including exposure to sampling site conditions, sample collection, processing, filtration, preservation, storage and all analytical procedures. Field blank results are

used to evaluate the extent or lack of positive bias in the associated WQ data. Because a field blank is treated exactly like an environmental sample at the laboratory, it includes any contamination introduced during laboratory handling and analysis.

6.1.2. A field blank is collected in the field and used to demonstrate that: (1) the equipment has been adequately cleaned to remove contamination introduced by samples obtained at previous sites; (2) sample collection and processing have not resulted in contamination; and (3) sample handling and transport have not introduced contamination. (USGS 1997.) Quality-Control Design for Surface Water Sampling in the National Water-Quality Assessment Program

6.2. Field blanks are required for laboratory parameters only and include all nitrogen, phosphorus, carbon, TSS and sediment species routinely requested by each Data Collector. Field blank data for state-required parameters such as alkalinity, chloride, sulfate, etc., may be submitted and made available through CEDR.

6.3. Site Selection and Frequency for FBs (to obtain a representative bias estimate)

6.3.1. Each sample collection group listed in Appendix A, Table 5-A.2, is to collect FBs throughout the year, in proportion to the number of NTN stations that are routinely sampled**. A minimum of one blank per station per year is required.** If a sampling group has fewer than 4 stations, the collection of quarterly blanks is recommended.

6.3.2. Collect the field blanks during storm and routinely-scheduled events throughout the year to ensure that a variety of flow conditions and seasonal variability of concentrations are well represented. Appendix 5-A describes an unbiased randomized procedure to obtain FBs representative of the variations in these conditions throughout the year and from year to year.

6.4. Preparation of Field Blanks

FBs are to be prepared at the sampling site **prior to** collecting the water-quality samples. They are created by pouring blank water into the sample collection bottle and transferring it through the nozzle into the churn splitter. This process is repeated the same number of times routinely required to obtain the associated WQ sample at that particular NTN Monitoring site. The composite sample is then sub-sampled, processed, preserved and handled exactly the same as done for the WQ samples.

6.5. Reporting Field Blank Results

6.5.1. Laboratories are to quantify and report all field blank results above the method detection limit (MDL) to ensure that low-level contamination is not a significant contributor to low-level WQ concentrations just above the reporting limit.

6.5.2. For FBs >MDL, data collectors and/or agency staff will investigate potential sources of contamination and assess the significance of the contamination. Corrective action is required for significant problems, especially for chronic detections above a reporting limit.

6.5.3. Agencies will assign all FB detections ≥ MDL with one of the Problem Codes cited below.

- **UB** – Concentration of field blank reflects initial or isolated occurrence of contamination; source of contamination under investigation.

- **BB** – Spurious or persistent contamination which appears to affect the field blanks only. Contamination is related to the manner, equipment or supplies used to obtain the blank, such as contaminated source water.

- **CB** – Spurious or persistent contamination, which appears to reflect the manner, equipment or supplies used to obtain blanks AND associated water quality samples.

The use of these problem codes informs end-users of FB data of the implications of the contaminated FB. It also permits aggregation (normalization) of FB results beyond the individual Data Collector level. For further information, see the report by the USGS NAWQA monitoring program[3].

6.6. Definition of Biased WQ Data

DUET has initially adopted a decision criterion that if a FB concentration is ≥ 10% of the associated WQ concentration, the latter is considered biased and electronically assigns the Problem Code "BM" to the water-quality data collected on that day.

7. Field duplicates

7.1. Definition and Purpose

7.1.1. A field duplicate sample set consists of two samples collected and processed so that the samples are considered to be essentially identical in composition. The purpose of collecting field duplicate samples is twofold: 1) to estimate the reproducibility of water-quality sample measurements and 2) to provide water-quality data from those samples.

7.1.2. Field duplicate (split) samples are usually taken in the field from a single container (e.g., churn) which contains a composite stream sample. Field duplicate samples (FS1 & FS2) provide a measure of the variability introduced during sample processing and laboratory analysis.

7.1.3. Alternatively, collectors may choose to prepare **concurrent** duplicates, which consist of concurrently collected EWI samples that are poured into two separate churns and then processed as individual samples. These duplicate samples (S1 & S2) include the added variability of filling the sampler bottle with water.

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3 *Quality of Nutrient Data from Streams and Ground Water Sampled During Water Years 1992–2001,* by David K. Mueller and Cindy J. Titus. URL: http://pubs.usgs.gov/sir/2005/5106/pdf/sir2005-5106.pdf

Note: Sequentially collected and processed duplicates are unacceptable.

7.2. Site Selection and Frequency (For representative precision estimates)

7.2.1. Each sample collection group is to prepare field duplicate samples throughout the year that are representative of the WQ samples being collected, through randomized site selection, and with stratification across flow conditions.

7.2.2. The minimum number of field duplicates is 2 pairs of duplicate samples per station per year, which is 10% of the samples. The number of field duplicates for supplemental sites is one per station per year since fewer samples are collected.

7.2.3. Sample collection groups, or "data collectors" in Table 5-A.2, with more than 12 stations may limit the number of pairs of duplicate samples to 24 per year (i.e., 2 pairs per month), making sure that all stations have at least one duplicate pair per year.

7.2.4. Appendix 5-A describes a randomized procedure to obtain duplicate samples representative of the variations in water quality conditions throughout the year and from year to year.

7.3. Preparing Duplicate Samples

7.3.1. A split duplicate (FS1/FS2) sample pair is prepared by dividing a single volume of water from one churn into two sets of samples. Mix and dispense the particulate and whole water duplicates first, then the dissolved parameter FS1/FS2 sets last. (See Fig. A)

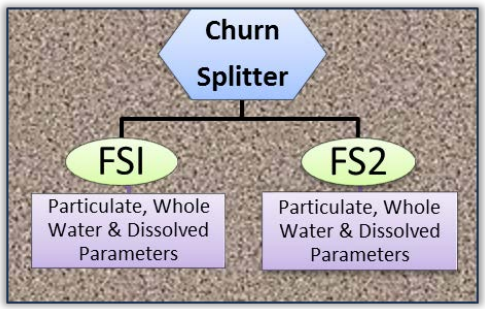

Figure A. Field Split Duplicates

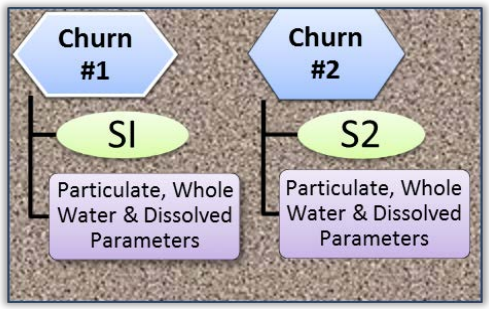

Figure B. Concurrent Field Duplicates

7.3.2. A concurrent field duplicate pair is prepared by collecting a second aliquot of sample immediately after the first at each equal-width increment (EWI). Place two or more aliquots from a single EWI into two separate churn splitters. Process the water from each churn to prepare a pair of concurrent duplicate samples. Label and report concurrent duplicates as sample types S1 and S2. (See Fig. B)

7.4. Reporting Field Duplicate Results

7.4.1. It's important for laboratories to report analytical results for field duplicates to at least 3 significant figures to obtain the most accurate estimates of precision[4]. Low concentrations near the MDL are typically reported to one or two significant figures but in the case of field duplicate samples, request that the lab submit unrounded, low-concentration data to at least 3 figures. For example, the value 0.005 has only one significant figure and the raw, unrounded version of this value is needed.

7.4.2. Another exception for field duplicate data involves those rare cases where one or both field duplicates fails a consistency check (e.g., TDP > TP). Data collection groups should evaluate both field duplicates for consistency and assign an appropriate problem code for failures, however, unlike normal data, failed duplicate results must be reported along with the problem code "NQ". This practice is necessary to obtain representative QC data, even if the sample data would normally be censored.

7.4.3. After the precision calculations are completed, DUET will censor the "NQ" coded data prior to uploading to CIMS. Similarly, the unrounded low concentration data will be rounded to the appropriate decimal place prior to the upload. All original duplicate sample data, including the unrounded and uncensored results, will be archived and made available through a request to the CBP NTN Project Data manager.

7.5. Precision Problem Codes

DUET will calculate the Relative Percent Difference (RPD) using field duplicates to assess a combined field and laboratory precision. If RPD values exceed 30% for particulate parameters (i.e., PN, PP, PC, TSS, SSC and Chlorophyll), or 20% for dissolved and total N, P, or C parameters, *and* both reported values are above the reporting limit, the Precision Problem Code "HI" will be added to the CIMS database. These control limits are subject to change once sufficient duplicate data are generated from which to establish different precision objectives.

8. Documentation and Records

Field sheets, calibration records, log books and laboratory forms must be maintained to permit a complete historical reconstruction of the data back to the calibration standards, sample volumes and preservatives used. A unique sample number or ID must be assigned to each sample processed. See Chapter 2.4 for additional document control protocols.

9. Decontamination

Cleaning procedures are detailed in the USGS NFM, *Chapter 3.2.1 – Cleaning of Equipment Used to Sample for Inorganic Constituents*. Two deviations from the National Field Manual are permitted:

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4 *Review of Trace Element Blank and Replicate Data Collected in Ground and Surface Water for the National Water-Quality Assessment Program, 1991–2002,* by Lori E. Apodaca, David K. Mueller, and Michael T. Koterba. URL: http://pubs.usgs.gov/sir/2006/5093/sir_2006-5093.pdf

1) the time for soaking the churn splitter in detergent solution may be less than 30 minutes, and 2) the use of 5% HCl for rinsing equipment is optional.

9.1. Start the cleaning procedures as early as possible after processing. Soak the churn splitter as close to 30 minutes as feasible given the time constraints of the day. Do preservation, paperwork, and packing of samples in the interim.

9.2. If cleaning the equipment for reuse in the field (e.g., sampler, churn splitter, filtration units), it is recommended that a 5-gallon polyethylene container for each solution below be transported in the vehicle.

9.2.1. Detergent water (0.1- 2% percent v/v ratio of Liquinox: tap water)

9.2.2. Tap water

9.2.3. 5% Hydrochloric Acid, ACS grade (this rinse is optional)

9.2.4. Deionized Water

Section C. Field Measurements

1. Field Measurement Procedures

1.1. *Parameters: In-situ* field measurements shall be collected for pH, dissolved oxygen, temperature, and specific conductance (at 25°C).

1.2. *Equipment:* A multi-parameter instrument or a combination of meters that can provide these same measurements.

1.3. Calibration

1.3.1. All probes must be calibrated according to the manufacturers' recommended methods. Field staff must document calibration, maintenance and repair information for each instrument and sensor in logbooks using permanent ink.

1.3.2. **Specific Conductance sensor:** The conductivity sensor must be calibrated against a reference solution, according to manufacturer's specifications. As a minimum, conductivity should be verified before and after each sampling date using standards that bracket the expected seasonal range.

1.3.3. **pH sensor:** The pH sensor should be calibrated at the beginning of every sampling event using two standard solutions of pH 4, pH 7 or pH 10 buffer solutions. The standards should bracket the expected pH of the streams. Follow the manufacturer's instructions for cleaning and storing the pH probe. If the post calibration drift is ± 0.2 pH units or more, censor all pH data back to the last calibration with the problem code "V".

1.3.4. **Dissolved oxygen (DO) sensor:** The DO sensor must be fully calibrated at the beginning and end of each multiple-day cruise according to manufacturer's specifications. DO sensors may be calibrated against water-saturated air or air-saturated water.

Check the DO calibration at the beginning of each sampling day. If daily checks drift by ± 0.3 mg DO/L or more, the sensor must be serviced and recalibrated before using again. If post-calibration drift is ≥ 0.5 mg/l, censor all DO data back to the last calibration with the problem code "V".

1.3.5. **Temperature sensor:** Check the agreement of the thermistor reading at least once a year against a NIST certified thermometer over a range of temperatures.

1.4. Procedure

1.4.1. Temperature and DO measurements **must** be collected in-situ at the center of each width increment from which samples are collected. Do not take measurements on a discrete sample and avoid taking measurements near the stream banks, or in sections with turbulence or high velocities.

1.4.2. Lower the sensors below the surface and allow at least one minute to equilibrate. When stable, record the values on the field data sheet or data logger. Move to the next EWI in the cross-section and repeat the procedure.

1.4.2.1. Record all readings and report the median temperature and median DO values within the cross-section.

1.4.3. Specific Conductance should be measured in-situ at the center of each EWI increment. (If this is not feasible, measure the conductivity of a composite sample dispensed from the churn splitter. Do not take conductivity and pH measurements on the same discrete sample because the pH electrode solution may contaminate the sample and affect the specific conductance.)

1.4.3.1. The use of a temperature-compensating instrument is recommended so that manual temperature corrections are unnecessary.

1.4.3.2. Lower the conductivity sensor below the surface and gently move it up and down to remove trapped air bubbles. Continue moving until the meter display stabilizes. Record the value on the field data sheet and then move to the next EWI in the cross section and repeat the procedure above.

1.4.3.3. Record all readings and report the median specific conductance and pH values to three or more significant figures.

For more detailed guidance, consult the U.S. Geological Survey procedures in the *National field manual for the collection of water-quality data*. (http://pubs.water.usgs.gov/twri9A)

Section D. Nontidal WQ Laboratory Methods

Analytical methods for Chesapeake Bay nontidal samples are fully described in Chapter 6, Section D. Table 5.4 below lists the method used for each parameter and a reference to the Chesapeake Bay Program (CBP) method. CBP analytical methods contain specifications for the analysis of tidal and nontidal samples and are written in Chapter 6 of *Methods and Quality Assurance for Chesapeake Bay Program Water Quality Monitoring Programs* (this document).

Table 5.4. Laboratory Methods for Chesapeake Bay Nontidal Water Quality Network

Parameter	Method	Chapter 6 Section
Ammonium, as N (dissolved) (NH4F)	EPA 350.1	**D.2**
Nitrate + Nitrite, as N (dissolved) (NO23F)	EPA 353.2	**D.5**
Total Dissolved Nitrogen (TDN), and Total Nitrogen, as N (TN)	Standard Methods, Method 4500-N C-2011, or 4500-P J. (alkaline persulfate digestion)	**D.1 and D.5**
Particulate Nitrogen (PN)	EPA 440.0	**D.9**
Total Phosphorus, as P (TP)	EPA 365.1 or 365.4	**D.8**
Total Dissolved Phosphorus (TDP)	Alkaline persulfate (SM 4500-P J-2011) or acid digestion, then EPA 365.1	**D.1 and D.8**
Ortho Phosphate, as P (dissolved) (PO4F)	EPA 365.1	**D.8**
Particulate Phosphorus (PP)	Combustion & acid extraction, then EPA 365.1	**D.10**
Total Organic Carbon (TOC)	Standard Methods, Method 5310 B-2011	**D.7**
Dissolved Organic Carbon (DOC)	Standard Methods, Method 5310 B-2011	**D.7**
Particulate Carbon (PC)	EPA 440.0	**D.9**
Suspended Sediment Concentration (SSC)	ASTM 3977-97, Method B (filtration)	**None**
Suspended Sediment, Coarse (> 62µm) (SSC_SAND)	ASTM 3977-97, Method C (wet sieve)	**None**
Suspended Sediment, Fine (< 62µm) (SSC_FINE)	ASTM 3977-97, Method C	**None**
Total Suspended Solids (TSS)	Standard Methods, Method 2540 D-2011, or USGS I-3765	**D.11**
Fixed Suspended Solids (FSS)	Standard Methods, Method 2540 E-2011, or USGS I-3766	**D.12**
Chlorophyll-*a* (CHLA)	SM, Method 10200 H	**D.3**

Section E. References

Chesapeake Bay Program. 2004. *Establishing a Chesapeake Bay Nontidal Watershed Water-Quality Network*, Sept. 2004. Prepared by the Chesapeake Bay Program Nontidal Water Quality Monitoring Workgroup. http://archive.chesapeakebay.net/pubs/subcommittee/msc/ntwqwg/Nontidal_Monitoring_Report.pdf

U.S. Geological Survey, variously dated. *National field manual for the collection of water-quality data*. U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chaps. A1-A9, available online at http://pubs.water.usgs.gov/twri9A.

U.S. Geological Survey. 1999. *Field methods for the measurement of fluvial sediment.* U.S. Geological Survey Techniques of Water-Resources Investigations, book 3, chapter C2, available online at http://pubs.usgs.gov/twri/twri3-c2/.

U.S. Geological Survey. 2005. *A guide to the proper selection and use of federally approved sediment and water-quality samplers.* USGS Open-File Report 2005-1087, available online at http://pubs.usgs.gov/of/2005/1087/pdf/OFR_2005-1087.pdf

APPENDIX 5-A. An Annual Randomized and Stratified Procedure to Obtain Field QC Samples Representative of Varying Environmental, Weather and Flow Conditions among Nontidal Network Monitoring Sites

Background and Perspective on QC Sampling Design and Process

The QC sampling design and process described below provides a completely randomized design which over time will provide representative QC samples that cover a data collector's stations every water year (WY), at the frequency required for Field Blanks (FBs) and Duplicate Samples (DSs), and for both non-storm and storm or storm-impacted flows during different seasons (just not at the same station). However, if this design process is repeated every new WY, it also will provide QC data for every station that accounts for Seasonal x WY variations in the environmental conditions at each station.

The QC sampling design and process for each WY must be done twice—once for FB collection and again for DS collection. We do not want to make the collection of FBs and DSs totally dependent upon one another for pragmatic reasons. It also is much easier to design their collection independently. That said, both types of QC samples could be collected during the same sample visit to a station when the independent designs indicate both types of QC samples are to be collected for similar flow conditions during a similar time period and field crews aren't heavily pressed to also sample many other stations as well.

The QC sampling design and process described below is meant to provide a common starting point for all NTN sampling groups to determine QC sampling for a given WY. As the WY actually unfolds, logic and common sense and safety issues will influence whether or not one can collect the targeted QC sampling of flow conditions at a given station during the specified time period that this design initially provides. For example, if the design indicates DSs are to be collected at three specific NTN Stations sometime during the months of October through December, and Hurricane Charley hits, does the field crew give up sampling at ten of their NTN Stations in order to collect WQ samples at only 7 of these stations and the DSs as dictated by the design at 3 of those 7 stations? Common sense would say no. But the field crew might take DSs at one of these stations because the water quality at that Station under such storm conditions is extremely important (for example, that particular Station among all others is a huge contributor to nutrients and suspended sediment at high flows).

As another example, what happens if for the same design results above, the region is locked into a severe drought during this time period? Does the field crew not take any DSs at these three stations because there aren't any storms or storm impacted flows? Again, common sense would dictate that one obtain the QC data for non-storm impacted flows because that in the end was the extended flow condition at each of these stations during the period QC samples were to be taken at these stations. However, one also might spread out the DS sample collection at these three stations during this three-month period on the off chance that a storm could occur in the latter part of the period, rather than take all three DS at these three stations in the last month of the period.

The QC sampling design and process described below also uses some very simple tools and processes to randomize the QC sampling design. Other truly randomizing tools and techniques no doubt exist and can be used. However, the four-step sequence used makes it simple to carry out the design.

Randomized Design and Design Process for NTN Quality-Control Sampling

The process described below is for Field Blank (FB) collection for one WY. It is simply repeated for Duplicate Sample (DS) collection for that WY taking into account that twice as many DSs than FBs are to be collected at a NTN station each WY. The process for FBs and DSs would be repeated each WY before sample collection began for that WY. Repeating these processes prior to the upcoming WY is essential to ensuring sampling is representative of varying environmental, weather and flow conditions that can occur at each station within a WY from WY to WY.

For each Data Provider x Data Collector combination in Table 5-A.1 for which your agency collects samples:

1. **Determine the Number of FBs to collect:** Equals the number of NTN stations being monitored that WY by the NTN Data Collector; given one FB is to be collected per station per WY. Be sure to annually update to reflect actual stations in that group for the upcoming new WY.

2. **Determine Order of FB Collection at those Stations**: Place an identical type, but station ID labeled, marker for each of the N stations in a bag; shake and select one marker from the bag without replacement; repeat process until all N markers have been withdrawn. List each marker station ID in the order in which it was withdrawn. The order of stations selection is the order in which FBs are collected at those stations during the WY.

3. **Determine EVENT_TYPEs for which FBs will be Collected:** For **1st** station in ordered list, flip a fair coin to determine whether the FB will be collected during a routine (R or RSI) Event_Type or a storm (S) Event_Type. Assign all other "odd" numbered stations in the ordered list the same Event_Type as that determined for the 1st Station; assign all remaining and "even" numbered stations on the ordered list the other Event_Type.

4. **Determine who collects FBs at Stations where more than one Agency group collects data:** At shared monitoring stations, FB collection is logically distributed among data collectors on the FB Event Types (e.g., USGS-VAWSC collects storm FBs, VADEQ Regional Offices collect routine event types at their shared stations) and or their intra-annual periods of monitoring (e.g., NYSDEC collects FBs for only routine events for half the WY they conduct monitoring; SRBC collects FBs for routine events during the other half of the WY they conduct monitoring; in addition, SRBC collects FBs for all storms throughout the WY).

5. **Determine when FBs actually are collected during the WY:** Using the table below, and given the total number of FBs to be collected (N) in the WY, determine the type and number of FB collection periods, over which the N FBs will be distributed in accordance with the order in which they were listed. This timing schedule effectively provides for the uniform distribution of FB collection throughout the WY across environmental conditions (possible weather x flow (Event_Types) x station setting).

6. **Final Adjustments to FB collection:** Use of a fixed protocol requiring one FB be collected at each NTN station every WY doesn't provide representative FB data throughout the WY if N is very small (2 stations). It also could adversely affect WQ sampling at many stations during storm or storm-impacted conditions because of the required high frequency of FB collection each WY when N is very large (12 or more stations).

Table 5-A.1
Scheduling Nontidal Network (NTN) field blank (FB) or duplicate sample (DS) collection for a Water Year

Number of FBs or Duplicate Pairs per WY	Schedule for Field Blank (FB) or Duplicate Sample (DS) collection
1	Not applicable at present
2	Semiannual: Collect one FB (DS) in each of two sequential 6-month periods of WY
3	Triennially: Collect one FB (DS) in each three sequential 4-month periods of WY
4	Quarterly: Collect one FB (DS) in each of four sequential 3-month periods in the WY
5	Quarterly: Collect one FB (DS) in each of four sequential 3-month periods in the WY, randomly select one 3-month period in which 5th FB (DS) is collected
6	Triennially: Collect two FBs (DSs) in each of three sequential 4-month periods in WY
7	Triennially: Collect two FBs (DSs) in each of three sequential 4-month periods, randomly selected one 3-month period in which the 7th FB is collected
8	Quarterly: Collect two FBs (DSs) in each of four sequential 3-month periods of WY
9	Triennially: Collect three FBs (DSs) in each of three sequential 4-month periods of WY
10	Triennially: Collect three FBs (DSs) in each of three 4 month-periods, randomly selected one three-month period when 10 FB (DS) is collected
11	Quarterly: Collect three FBs (DSs) in three 3-month periods, randomly selecting one 3-month period in WY when only two FBs (DSs) are collected
12	Quarterly: Collect three FBs (DSs) in each of four sequential 3-month periods month in WY
13	Quarterly: Collect three FBs (DSs) in each of four 3-month periods month in WY, randomly select one 3-month period when 13th FB (DS) is collected
14	Triennially: Collect five FBs (DSs) in each of two 4-month periods in WY, randomly select one 4-month period when 14th FB is collected
15	Triennially: Collect five FBs (DSs) in each of three sequential 4-month periods in WY
16	Quarterly: Collect four FBs (DSs) in each of four sequential 3-month periods of the WY
17	Quarterly: Collecting four FBs (DSs) in each of four sequential 3-month periods, randomly selected one 3-month period in which 17th FB is collected
18	Triennially: Collect six FBs (DSs) in each of three 4-month periods of WY
19	Quarterly: Collect five FBs (DSs) in each of three 3-month periods, randomly selected one 3-month period in which 4 FBs (DSs) are collected
20	Quarterly: Collect five FBs (DSs) in each sequential 3-month period of WY
21	Triennially: Collect seven FBs (DSs) in each sequential 4-month period of WY
22	Triennially: Collect seven FBs (DSs) in each sequential 4-month period of WY, randomly selected one 4-month period in which 22nd FB (DS) is collected
23	Quarterly: Collect six FBs (DSs) in each of four 3-month periods, randomly select one 3-month period in which five FBs (DSs) are collected
24	Quarterly: Collect six FBs (DSs) in each of four sequential 3-month periods of WY

Table 5-A.2
Minimum Number of Field Blank and Duplicate Samples for CBP Nontidal Network Collection Groups (Based on Water Year 2015 Monitoring Stations)

Data Provider	Data Collector	Event Type	Monitoring Location Name **(WY 2015)**	Field Blank & Duplicate Samples **(minimum per year)**
DEDNREC	DEDNREC	R, RSI, S, ONS or OS	304191, 302031.	2 Field Blanks 4 Field Duplicates
USGSWV	USGSWV	R, RSI, S, ONS or OS	01595300, 01614000, 01636500, 01604500, 01608500, 01611500, 01613030, 01616400, 01616500, 01618100.	10 Field Blanks 20 Field Duplicates
SRBC (NY)	SRBC	R, RSI, S, ONS or OS	01502500, 01503000, 01529500.	3 Field Blanks 6 Field Duplicates
SRBC	SRBC or NYSDEC	R, RSI, S, ONS or OS	01515000, 01531000	2 Field Blanks 4 Field Duplicates
MDDNR	MDDNR	R, RSI, S, ONS or OS	TUK0181, BEL0053, DER0015, GUN0258, NPA0165, GWN0115, PXT0972, TF1.2, GEO0009, WIL0013, ANT0047, CAC0148, MON0546, LXT0200, MGN0062, NWA0016, WCK0001, MKB0016, CON0180, LIC0042, TOC0037, ANT0366, SID0015, TOW0030.	24 Field Blanks 24 Field Duplicates
USGSMD	USGSMD (Maryland RIM)	R, RSI, S, ONS or OS	01491000, 01578310, 01594440, 01646580.	4 Field Blanks 8 Field Duplicates
USGSMD	USGSMD	R, RSI, S, ONS or OS	01648010, 01651770, 01651800, 01493112, 01581752, 01658000.	6 Field Blanks 12 Field Duplicates
PADEP	SRBC	R, RSI, S, ONS or OS	WQN0201, WQN0214, WQN0273, WQN0301, WQN0305, WQN0401, WQN0204, WQN0210, WQN0223, WQN0229, WQN0243, WQN0263, WQN0271, WQN0272, WQN0302, WQN0404, WQN0445, WQN0448, WQN0226, WQN0281, WQN0282.	21 Field Blanks 24 Field Duplicates
PADEP	USGSPA	R, RSI, S, ONS or OS	WQN0202, WQN0203, WQN0212, WQN0217, WQN0317, WQN0410, WQN0224, WQN0259, WQN0269, WQN0278, WQN0280, WQN0284, WQN0285, WQN0286, WQN0462.	15 Field Blanks 24 Field Duplicates
VADEQ	USGSVA (Virginia RIM+)	R, RSI, S, ONS or OS	TF5.0A, TF4.0P, TF5.0J, TF3.0, TF4.0M, 2-JMS113.20	6 Field Blanks 12 Field Duplicates
VADEQ	USGSVA	R, RSI, S, ONS or OS	1BNFS010.34, 1BSMT004.60, 1BSSF003.56, 2-CHK035.26, 2-JMS113.20, 3-RAP030.21, 8-NAR005.42, BMDD005.81, 1ADIF000.86, 7-DRN010.48, 1ASOQ006.73, 8-PCT000.76, 1AACO014.57.	13 Field Blanks 24 Field Duplicates
VADEQ	VADEQ/SCRO or USGSVA	R, RSI, S, ONS or OS	2-JMS279.41, 2-APP110.93.	2 Field Blanks 4 Field Duplicates
VADEQ	VADEQ/NRO or USGSVA	R, RSI, S, ONS or OS	3-RPP147.49, 8-MPN094.94.	2 Field Blanks 4 Field Duplicates
VADEQ	VADEQ/VRO or USGSVA	R, RSI, S, ONS or OS	2-RVN015.97, 1BSSF100.10.	2 Field Blanks 4 Field Duplicates
VADEQ	VADEQ/NRO (Secondary)	R, RSI, or ONS	3-RAP066.54, 3-ROB001.90, 8-POR008.97, 1ACAX004.57, 1ACAX004.57.	5 blanks, 5 duplicates (quarterly)
VADEQ	VADEQ/VRO (Secondary)	R, RSI, or ONS	1BSTH027.85, 2-BCC004.71, 2-BLP000.79, 2-CFP004.67, 2-MCM005.12, 2-MRY014.78.	6 blanks, 6 duplicates
VADEQ	VADEQ/PRO (Secondary)	R, RSI or ONS	8-LTL009.54, 2-DPC005.20.	2 blanks, 2 duplicates (quarterly)

Appendix 5-B CBP Nontidal Network Water Quality Stations, Locations and Streamflow Gages (123 stations)

FIPS State Code	USGS Stream Gage	Location Name	Monitoring Location Description	Latitude Measure	Long. Measure	Data Collector
DC	01648010	01648010	Rock Creek at Joyce Road at Washington, DC	38.9602	-77.0421	USGSMD
DC	01651770	01651770	Hickey Run at New York Avenue at Washington, DC	38.9171	-76.9693	USGSMD
DC	01651800	01651800	Watts Branch at Washington, DC	38.9011	-76.9422	USGSMD
DE	01488500	304191	Marshyhope Creek near Adamsville, DE	38.8497	-75.6733	DEDNREC
DE	01487000	302031	Nanticoke River near Bridgeville, DE	38.7292	-75.5614	DEDNREC
MD	01619500	ANT0047	Antietam Creek near Sharpsburg, MD	39.4504	-77.7317	MDDNR
MD	01495000	BEL0053	Big Elk Creek at Elk Mills, MD	39.6571	-75.8224	MDDNR
MD	01619000	ANT0366	Antietam Creek near Waynesboro, PA	39.7169	-77.6078	MDDNR
MD	01637500	CAC0148	Catoctin Creek near Middletown, MD	39.4258	-77.5590	MDDNR
MD	01614500	CON0180	Conococheague Creek at Fairview, MD	39.7158	-77.8245	MDDNR
MD	01580520	DER0015	Deer Creek near Darlington, MD	39.6235	-76.1648	MDDNR
MD	01599000	GEO0009	Georges Creek at Franklin, MD	39.4936	-79.0447	MDDNR
MD	01582500	GUN0258	Gunpowder Falls at Glencoe, MD	39.5506	-76.6359	MDDNR
MD	01589300	GWN0115	Gwynns Falls at Villa Nova, MD	39.3428	-76.7264	MDDNR
MD	01613525	LIC0042	Licking Creek at Pectonville, MD	39.6777	-78.0365	MDDNR
MD	01593500	LXT0200	Little Patuxent River at Guilford, MD	39.1678	-76.8513	MDDNR
MD	01493500	MGN0062	Morgan Creek near Kennedyville, MD	39.2800	-76.0146	MDDNR
MD	01486000	MKB0016	Manokin Branch near Princess Anne, MD	38.2139	-75.6714	MDDNR
MD	01639000	MON0546	Monocacy River at Bridgeport, MD	39.6965	-77.2395	MDDNR
MD	01586000	NPA0165	North Branch Patapsco River at Cedarhurst, MD	39.5011	-76.8835	MDDNR
MD	01651000	NWA0016	NW Branch Anacostia River near Hyattsville, MD	38.9523	-76.9661	MDDNR
MD	01591000	PXT0972	Patuxent River near Unity, MD	39.2393	-77.0562	MDDNR
MD	01610155	SID0015	Sideling Hill Creek near Bellegrove, MD	39.6495	-78.3441	MDDNR
MD	01594526	TF1.2	Western Branch at Upper Marlboro, MD	38.8143	-76.7509	MDDNR
MD	01613095	TOC0037	Tonoloway Creek near Hancock, MD	39.7064	-78.1528	MDDNR
MD	01609000	TOW0030	Town Creek near Oldtown, MD	39.5532	-78.5550	MDDNR
MD	01491500	TUK0181	Tuckahoe Creek near Ruthsburg, MD	38.9671	-75.9431	MDDNR
MD	0158175320	WCK0001	Wheel Creek near Abingdon, MD	39.4817	-76.3405	MDDNR
MD	01601500	WIL0013	Wills Creek near Cumberland, MD	39.6619	-78.7803	MDDNR
MD	01578475	WQN0263	Octoraro Creek near Richardsmere, MD	39.6903	-76.1281	SRBC
MD	01493112	01493112	Chesterville Branch near Crumpton, MD	39.2571	-75.9401	USGSMD
MD	01581752	01581752	Plumtree Run near Bel Air, MD	39.4964	-76.3478	USGSMD
MD	01658000	01658000	Mattawoman Creek near Pomonkey, MD	38.5961	-77.0560	USGSMD
MD	01491000	01491000	Choptank River near Greensboro, MD (RIM)	38.9972	-75.7861	USGSMD

FIPS State Code	USGS Stream Gage	Location Name	Monitoring Location Description	Latitude Measure	Long. Measure	Data Collector
MD	01578310	01578310	Susquehanna River at Conowingo, MD (RIM)	39.6587	-76.1741	USGSMD
MD	01594440	01594440	Patuxent River near Bowie, MD (RIM)	38.9558	-76.6933	USGSMD
MD	01646580	01646580	Potomac River at Chain Bridge, Washington, DC (RIM)	38.9296	-77.1169	USGSMD
NY	01502500	01502500	Unadilla River at Rockdale, NY	42.3778	-75.4064	SRBC
NY	01503000	01503000	Susquehanna River at Conklin, NY	42.0353	-75.8033	SRBC
NY	01529500	01529500	Cohocton River near Campbell, NY	42.2525	-77.2169	SRBC
NY	01515000	01515000	Susquehanna River near Waverly, NY	41.9856	-76.5017	SRBC, NYSDEC
NY	01531000	01531000	Chemung River at Chemung, NY	42.0022	-76.6350	SRBC, NYSDEC
PA	01576000	WQN0201	Susquehanna River at Marietta, PA	40.0544	-76.5311	SRBC
PA	01576787	WQN0204	Pequea Creek at Martic Forge, PA	39.9058	-76.3284	SRBC
PA	01574000	WQN0210	West Conewago Creek near Manchester, PA	40.0822	-76.7203	SRBC
PA	01567000	WQN0214	Juniata River at Newport, PA	40.4783	-77.1295	SRBC
PA	01562000	WQN0223	Raystown Branch Juniata River at Saxton, PA	40.2158	-78.2656	SRBC
PA	01555500	WQN0226	East Mahantango Creek near Dalmatia, PA	40.6111	-76.9122	SRBC
PA	01555000	WQN0229	Penns Creek at Penns Creek, PA	40.8667	-77.0486	SRBC
PA	01576754	WQN0273	Conestoga River at Conestoga, PA	39.9464	-76.3681	SRBC
PA	01568000	WQN0243	Sherman Creek at Shermans Dale, PA	40.3233	-77.1692	SRBC
PA	01570000	WQN0271	Conodoguinet Creek near Hogestown, PA	40.2522	-77.0214	SRBC
PA	01573560	WQN0272	Swatara Creek near Hershey, PA	40.2983	-76.6681	SRBC
PA	01571000	WQN0281	Paxton Creek near Penbrook, PA	40.3083	-76.8500	SRBC
PA	01565000	WQN082	Kishacoquillas Creek at Reedsville, PA	40.6547	-77.5833	SRBC
PA	01540500	WQN0301	Susquehanna River at Danville, PA	40.9581	-76.6195	SRBC
PA	01536500	WQN0302	Susquehanna River at Wilkes-Barre, PA	41.2508	-75.8811	SRBC
PA	01531500	WQN0305	Susquehanna River at Towanda, PA	41.7653	-76.4411	SRBC
PA	01553500	WQN0401	West Branch Susquehanna River at Lewisburg, PA	40.9681	-76.8736	SRBC
PA	01542500	WQN0404	West Branch Susquehanna River at Karthaus, PA	41.1175	-78.1092	SRBC
PA	01548005	WQN0445	Bald Eagle Creek near Beech Creek Station, PA	41.0801	-77.5475	SRBC
PA	01549760	WQN0448	West Branch Susquehanna R. at Jersey Shore, PA	41.2023	-77.2521	SRBC
PA	01570500	WQN0202	Susquehanna River at Harrisburg, PA	40.2547	-76.8864	USGSPA
PA	01554000	WQN0203	Susquehanna River at Sunbury, PA	40.8344	-76.8269	USGSPA
PA	01571500	WQN0212	Yellow Breeches Creek near Camp Hill, PA	40.2247	-76.8983	USGSPA
PA	01558000	WQN0217	Little Juniata River at Spruce Creek, PA	40.6126	-78.1406	USGSPA
PA	01556000	WQN0224	Frankstown Branch Juniata R. at Williamsburg, PA	40.4631	-78.1997	USGSPA
PA	01577500	WQN0259	Muddy Creek at Castle Fin, PA	39.7725	-76.3161	USGSPA
PA	01573710	WQN0269	Conewago Creek near Falmouth, PA	40.1511	-76.6900	USGSPA
PA	01573695	WQN0278	Conewago Creek near Bellaire, PA	40.1953	-76.5678	USGSPA
PA	015765195	WQN0280	Big Spring Run near Mylin Corners, PA	39.9959	-76.2640	USGSPA

FIPS State Code	USGS Stream Gage	Location Name	Monitoring Location Description	Latitude Measure	Long. Measure	Data Collector
PA	01576767	WQN0284	Pequea Creek near Ronks, PA	40.0091	-76.1618	USGSPA
PA	01573160	WQN0285	Quittapahilla Creek near Bellegrove	40.3426	-76.5627	USGSPA
PA	01575585	WQN0286	Codorus Creek near Pleasureville, PA	40.0186	-76.6933	USGSPA
PA	01534000	WQN0317	Tunkhannock Creek near Tunkhannock, PA	41.5573	-75.8944	USGSPA
PA	01549700	WQN0410	Pine Creek below Little Pine Cr. near Waterville, PA	41.2831	-77.3222	USGSPA
PA	01553850	WQN0462	Chillisquaque Creek near Potts Grove, PA	40.9744	-76.8000	USGSPA
VA	01654000	1AACO014.57	Accotink Creek near Annandale, VA	38.8113	-77.2302	USGSVA
VA	01646000	1ADIF000.86	Difficult Run near Great Falls, VA	38.9758	-77.2461	USGSVA
VA	01658500	1ASOQ006.73	S F Quantico Creek near Independent Hill, VA	38.5872	-77.4289	USGSVA
VA	01621050	1BMDD005.81	Muddy Creek at Mount Clinton, VA	38.4867	-78.9606	USGSVA
VA	01634000	1BNFS010.34	N F Shenandoah River near Strasburg, VA	38.9768	-78.3367	USGSVA
VA	01632900	1BSMT004.60	Smith Creek near New Market, VA	38.6935	-78.6428	USGSVA
VA	01631000	1BSSF003.56	S F Shenandoah River at Front Royal, VA	38.9137	-78.2098	USGSVA
VA	02042500	2-CHK035.26	Chickahominy River near Providence Forge, VA	37.4358	-77.0608	USGSVA
VA	02037500	2-JMS113.20	James River near Richmond, VA	37.5631	-77.5472	USGSVA
VA	01667500	3-RAP030.21	Rapidan River near Culpeper, VA	38.3590	-77.9733	USGSVA
VA	01669520	7-DRN010.48	Dragon Swamp at Mascot, VA	37.6336	-76.6967	USGSVA
VA	01671020	8-NAR005.42	North Anna River at Hart Corner near Doswell, VA	37.8501	-77.4278	USGSVA
VA	01674182	8-PCT000.76	Polecat Creek at Route 301 near Penola, VA	37.9603	-77.3436	USGSVA
VA	01668000	TF3.0	Rappahannock River near Fredericksburg, VA (RIM)	38.3224	-77.5178	USGSVA
VA	01674500	TF4.0M	Mattaponi River near Beulahville, VA (RIM)	37.8843	-77.1630	USGSVA
VA	01673000	TF4.0P	Pamunkey River near Hanover, VA (RIM)	37.7679	-77.3319	USGSVA
VA	02041650	TF5.0A	Appomattox River at Matoaca, VA (RIM)	37.2250	-77.4756	USGSVA
VA	02035000	TF5.0J	James River at Cartersville, VA (RIM)	37.6711	-78.0858	USGSVA
VA	01664000	3-RPP147.49	Rappahannock River at Remington, VA	38.5289	-77.8203	VADEQ/NRO, USGSVA
VA	01674000	8-MPN094.94	Mattaponi River near Bowling Green, VA	38.0618	-77.3860	VADEQ/NRO, USGSVA
VA	02039500	2-APP110.93	Appomattox River at Farmville, VA	37.3074	-78.3890	VADEQ/SCRO, USGSVA

FIPS State Code	USGS Stream Gage	Location Name	Monitoring Location Description	Latitude Measure	Long. Measure	Data Collector
VA	02024752	2-JMS279.41	James River At Blue Ridge Pkwy near Big Island, VA	37.5555	-79.3672	VADEQ/SCRO, USGSVA
VA	01628500	1BSSF100.10	South Fork Shenandoah River near Lynnwood, VA	38.3129	-78.7700	VADEQ/VRO, USGSVA
VA	02034000	2-RVN015.97	Rivanna River at Palmyra, VA	37.8579	-78.2658	VADEQ/VRO, USGSVA
VA	01665500	3-RAP066.54	Rapidan River near Ruckersville, VA (secondary)	38.2799	-78.3408	VADEQ/NRO
VA	01666500	3-ROB001.90	Robinson River near Locust Dale, VA (secondary)	38.3251	-78.0953	VADEQ/NRO
VA	01673800	8-POR008.97	Po River near Spotsylvania, VA (secondary)	38.1712	-77.5950	VADEQ/NRO
VA	01638480	1ACAX004.57 (CVA0046)	Catoctin Creek at Taylorstown, VA (secondary)	39.2548	-77.5764	VADEQ/NRO
VA	02041000	2-DPC005.20	Deep Creek near Mannboro, VA (secondary)	37.2840	-77.8686	VADEQ/PRO
VA	01671100	8-LTL009.54	Little River near Doswell, VA (secondary)	37.8729	-77.5133	VADEQ/PRO
VA	01626000	1BSTH027.85	South River near Waynesboro, VA (secondary)	38.0574	-78.9078	VADEQ/VRO
VA	02011500	2-BCC004.71	Back Creek near Mountain Grove, VA (secondary)	38.0696	-79.8970	VADEQ/VRO
VA	02015700	2-BLP000.79	Bullpasture River at Williamsville, VA (secondary)	38.1953	-79.5706	VADEQ/VRO
VA	02020500	2-CFP004.67	Calfpasture River above Mill Creek at Goshen, VA (secondary)	37.9874	-79.4942	VADEQ/VRO
VA	02024000	2-MRY014.78	Maury River near Buena Vista, VA (secondary)	37.7522	-79.3919	VADEQ/VRO
VA	02031000	2-MCM005.12	Mechums River near White Hall, VA (secondary)	38.1027	-78.5929	VADEQ/VRO
WV	01595300	01595300	Abram Creek at Oakmont, WV	39.3672	-79.1782	USGSWV
WV	01604500	01604500	Patterson Creek near Headsville, WV	39.4431	-78.8222	USGSWV
WV	01608500	01608500	South Branch Potomac River near Springfield, WV	39.4469	-78.6544	USGSWV
WV	01611500	01611500	Cacapon River near Great Cacapon, WV	39.5822	-78.3100	USGSWV
WV	01613030	01613030	Warm Springs Run near Berkeley Springs, WV	39.6405	-78.2185	USGSWV
WV	01614000	01614000	Back Creek near Jones Springs, WV	39.5118	-78.0365	USGSWV
WV	01616400	01616400	Mill Creek at Bunker Hill, WV	39.3344	-78.0534	USGSWV
WV	01616500	01616500	Opequon Creek near Martinsburg, WV	39.4236	-77.9389	USGSWV
WV	01618100	01618100	Rockymarsh Run at Scrabble, WV	39.4831	-77.8318	USGSWV
WV	01636500	01636500	Shenandoah River at Millville, WV	39.2819	-77.7894	USGSWV

CHAPTER 6. ANALYTICAL METHODS & QUALITY CONTROL

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Section A. Introduction

1. Scope

1.1. Chapter 6 contains analytical methods and QC protocols for laboratories that provide data for the Chesapeake Bay Program (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 2, Section 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 2, Section 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 laboratory 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. The majority of the laboratories providing data to the CBP are certified under an accrediting body. Many requirements and recommendations in this document are consistent with the related standards of the NELAC Institute (TNI) or the International Standards Organization. However, there are some certification requirements not included in this document.

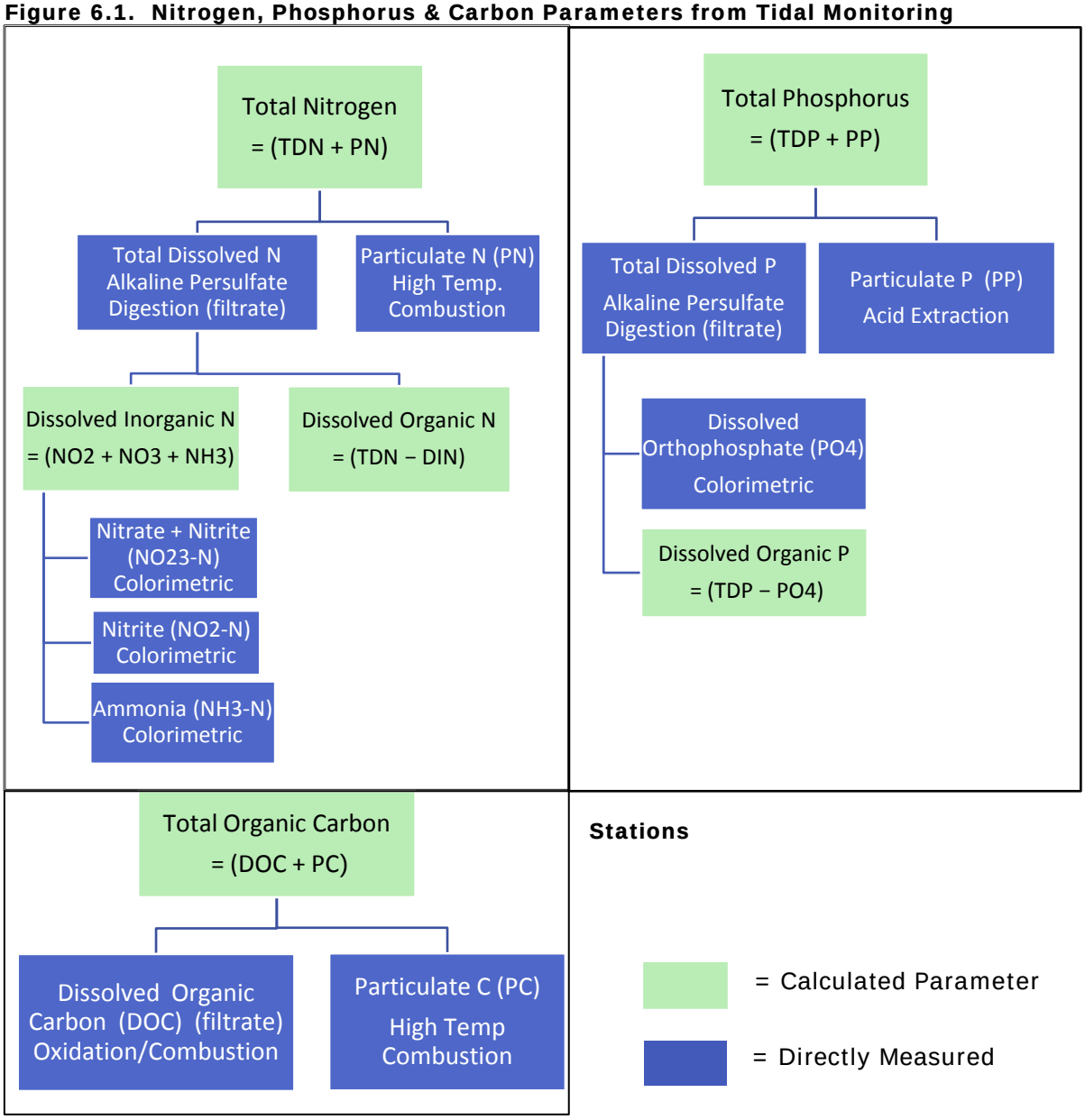
3. Parameters and Method Schemes

3.1. Tidal Analytical Methods – The methods focus on the directly-measured parameters in Figure 6.1.

3.2. Nontidal Analytical Methods – In most cases, the same method may be used for Tidal and Nontidal samples. A few nontidal agencies collect whole water samples and analyze them directly for TN, TP and/or TOC. In these cases the method for the total dissolved parameter is used on a whole water sample.

3.3. See Section D for the analytical method specifications for the parameters listed in Table 6.1

Figure 6.1. Nitrogen, Phosphorus & Carbon Parameters from Tidal Monitoring



Section B. Definitions and Terms

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

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

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.

Calibration Verification Standard (ICV) - a quality control sample used to verify the initial instrument calibration. Traceability shall be to a national standard when commercially available. Material for the ICV must be obtained from a different manufacturer or separate lot as that used to prepare the calibration standards.

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).

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.

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.

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

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.

Laboratory Control Sample[1] **(LCS)** – A 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.

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.

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 reliably detect in their facility. Also called the Method Detection Limit (MDL).

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).

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).

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.

Method Blank – See Laboratory Reagent Blank.

Proficiency Test (PT) Sample[a] – 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.

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.

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.

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.

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[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

1.1. 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 6.1 below. Deviations from these requirements are allowed if comparability data on record show equivalent results.

Table 6.1: PRESERVATION and HOLDING TIMES for TIDAL and NONTIDAL PARAMETERS

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

Parameter	Tidal WQ[a]		Nontidal WQ[b]	
	Preservation	Maximum Holding Time	Preservation	Maximum Holding Time
Suspended Sediment Concentration	Not Applicable	–	Cool & Dark	120 days
Silicates	Cool, ≤ 6°C	28 days	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, and H_2SO_4 to pH<2	28 days

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 would require pH adjustment prior to 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 6.1 unless the laboratory has demonstrated that an alternative preservation method yields equivalent results. Deviations from these conditions shall be approved by the CBP Data Integrity Workgroup 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. **Analytical balance**

4.1.1. The analytical balance is the most important piece of equipment in an analytical laboratory. A balance with a sensitivity of at least 0.1 milligram is required for weighing analytical standards. Reagents may not need this low sensitivity.

4.1.2. 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.

4.1.3. 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.

4.1.4. 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 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 500 - 675 **g**. The relationship between RPM and g is as follows:

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

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 relative centrifugal force (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.

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 each working thermometer with an identification number and record the dates of calibration, calibration temperatures and correction factors in a log book.

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 limit for which quantitative data are reported without qualification.

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 or reporting limit).

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 consistent with that required in the method. If the initial calibration or verification results are outside established acceptance criteria, corrective actions must be performed and all associated samples reanalyzed. If 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 PQL or reporting limit shall be reported as having less certainty and through the use of the appropriate qualifier, i.e.,

- Report values below the MDL with the MDL value in the results field and a "< " in the qualifier field.
- Report actual values ≥ MDL and < PQL (or RL) with a "G" in the qualifier field.
- Report actual values < MDL in the BMDL Table.

5.4. Continuing Calibration Verification

5.4.1. A continuing calibration verification standard (CCV) 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 concentration should be a mid-range sample 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.

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

5.4.4. The CCV standard result must be within 90-110% of the known or certified concentration.

5.4.5. If the CCV recovery exceeds these control limits, identify the source of the problem and resolve the problem before continuing analyses. Reanalyze all samples and CCV back to the last acceptable CCV or ICV. If the CCV recovery is within 90-110% of expected value, report the new values.

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 TNI requirement for accredited 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 is ≥ PQL, reporting limit, or a more stringent criterion, 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 problem code.

6.2. Laboratory Replicates

6.2.1. Laboratory replicate analyses provide a measure of laboratory precision. At least one duplicate should be analyzed for every 20 CBP samples. (A field duplicate counts as a sample.)

6.2.2. 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.

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

The following equation is used to calculate RPD:

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

where: A1 = Sample result

A2 = Duplicate sample result (or matrix spike duplicate)

6.2.4. Table 2.3, *Quality Objectives for Laboratory Analyses,* lists the maximum acceptable RPDs for each parameter. In general, the acceptable RPDs for dissolved parameters are ≤ 20%, and ≤ 30% RPD for particulate parameters.

6.2.5. When a sample exceeds the RPD listed in Table 2.3, analyze another sample and report the new results. If the RPD still exceeds the criterion, report the data with the appropriate problem code.

6.2.6. 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

6.3. Matrix Spike (not required for chlorophyll, PN, PC, PIC or TSS)

6.3.1. 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.

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

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

6.3.4. 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.

6.3.5. 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.

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

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

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

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

where:

SSR = Spike sample result

SR = Sample result

SA = Spike added

6.3.9. If the spike recovery is outside the range designated in Table 6.2, the spike analysis is repeated after checking for obvious sources of error. At a minimum, this involves an immediate repeat of the instrumental analysis. (Note: EPA methods require another spike of the same sample.) If the result is still beyond acceptance limits and the analytical process employed a digestion step, the matrix spike should be re-digested and reanalyzed.

6.3.10. 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 at least 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. The laboratory may use the upper and lower control chart limits as acceptance criteria for duplicate and spiked samples. Alternatively, the precision and accuracy objectives listed in Chapter 2, Table 2.3. Quality Objectives for Laboratory Analyses, may be used.

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.

8. Method Detection Limits

8.1. The method detection limit (MDL) is the minimum concentration of a substance that can be reported with 99% confidence that the concentration can be distinguished from a blank. The determination of a realistic detection limit is significant when studying trends in natural systems containing very low concentrations of the analytes of interest.

8.2. MDLs must be determined prior to reporting data from a method. After an initial determination, MDLs are then verified or re-determined annually or if there is a significant change in the operating parameters of the method or instrumentation. Other factors which may require an MDL study include new matrix, change of analyst, or change in operating range.

8.3. The procedure for determining the MDL will be dictated by the method and/or the accreditation requirements for the laboratory. The accepted procedure for performing the MDL is outlined in 40 CFR 136 Appendix B and is the method stated in several EPA procedures. This method is subject to update any time the Federal Register is updated so it is important to use the most recent accepted version.

8.3.1. An alternative method for determining the MDL has been published by the EPA and listed as, Definition and Procedure for the Determination of the Method Detection Limit, Revision 2. The document number is “EPA 821-R-16-006”.

8.3.2. It is expected that laboratories performing analysis for the CBP will use the most recent accepted version of the MDL procedure in the Federal Register.

8.4. The MDL may be determined using spiked reagent water or an environmental sample representative of the samples normally analyzed by the lab. A suggested procedure for analytes that cannot be spiked, such as particulate nitrogen and carbon, is presented in section 8.5.

8.4.1. The analyte concentration range used for the MDL determination will be based on the method used to perform the study as described in section 8.4.

8.4.1.1. Environmental samples may be used to perform the study and can be diluted or fortified into the proper range for the study.

8.4.2. The analysis must include all steps performed for the method and use the same calibration curve that will be used for routine samples.

8.4.3. A minimum of seven replicates should be used for the determination and the individual measurements spread over multiple runs and days to account for variation from run to run.

8.4.4. Use the appropriate T statistic, based on the number of replicates and n-1 degrees of freedom, for the final calculation as dictated in the procedure used (see Table below).

8.4.5. The data points used for the determination should be a continuous set and a justification for the removal of any point must be kept on file. Examples for deleting a point may be assignable cause, such as the solution being made at the wrong concentration, or a statistical test showing the point as an outlier to the population.

8.5. Analytes that cannot be spiked such as particulate nitrogen and carbon, particulate phosphorus and TSS are not covered by the procedure listed in 40 CFR 136 Appendix B. It is the policy of the CBP that laboratories design a procedure with which they can assess standard deviation on a uniform sample near the lower end of the range of analysis. The standard deviation can then be used to determine the MDL. Additionally, the laboratory should assess the calculated MDL against the blank contribution from the filter media. If the calculated MDL is lower than the blank MDL determination then the process for determining the MDL should be investigated. The quoted MDL should not be lower than the value of blanks for any test to avoid false positives. It may be more appropriate to use the blank procedure in the reference in 8.3.1 if the blanks are higher than the MDL. The following procedure is an example.

8.5.1. Combine previously analyzed samples, preferably using equal volumes, from several sampling locations. Determine the concentration of the combined sample. Alternatively a very low concentration sample for the analytes of interest may be used if it meets the criteria of 8.5.2.

8.5.2. If necessary, dilute the sample with reagent water to bring the analyte concentration to a level of approximately 1 to 20 times the theoretical or current MDL. Make sure the final diluted volume is adequate for at least 8 filtrations.

8.5.2.1. Filter a portion and analyze to verify the correct range.

8.5.3. If the range is correct then proceed with filtering an additional seven aliquots for the analyte of interest.

8.5.4. Analyze seven of the aliquots in the same manner as routine samples and distribute among several runs to account for run variance.

8.5.5. 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

8.5.6. This process will provide a precision statement for reproducibility at the lower end of the analytical range but may not correlate with the lowest amount of analyte that can be seen by a particular instrument or method.

8.5.7. A table of MDL and PQL or reporting limit 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. To determine if an MDL is statistically different from the existing MDL, multiply the existing MDL by 0.5 and then by 2.0. This forms the upper and lower limits of the 95% confidence interval. A new calculated MDL within that interval is considered to be within the same population and would not require the MDL to be changed.

9. Practical Quantitation Limits

9.1. The practical quantitation limit (PQL) is approximately 3.18 (MDL); or:

PQL = 10 (S), where S = the standard deviation of the replicate analyses as described in subsection C.8.5.5 above.

9.2. The lowest calibration standard concentration must be at or below the PQL or reporting limit. (See C.5.2.4.)

9.3. Analytical results between the MDL and PQL or reporting limit shall be quantified. Report the numerical value and qualify with the appropriate qualifier code, i.e., “G”.

Student’s t Values at the 99 Percent Confidence Level

Number of replicates	Degrees of freedom (n-1)	$t_{(n-1,\ 1-\alpha=.99)}$
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 6.2. 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)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, 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)	≤ PQL or reporting limit (RL)	Reanalyze another aliquot of blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	Method dependent, typically at beginning and end of preparation batch.	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	Analyte-specific See Table 2.3	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory Duplicate Sample	At least 1 per 20 samples	Analyte-specific See Table 2.3	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.
MDL and PQL verification spike	Every 3 months, two low-level spikes in separate batches. (EPA: At MDL spike conc.)	Detected ≥ MDL and ≤ PQL (or RL)	If the verification spike is not detected, repeat with a higher concentration spike.

References

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

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

U.S. EPA (2016). Definition and Procedure for the Determination of the Method Detection Limit. Revision 2. EPA 821-R-16-006, December 2016. (Also in 40 CFR [part] 136.)

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

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

Macdonald, R.W. and McLaughlin, F.A. 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.

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

SECTION D.1

ALKALINE PERSULFATE DIGESTION FOR NITROGEN & PHOSPHORUS, TOTAL and DISSOLVED

CEDR Method Codes:

- **TDN L01 (Total Dissolved Nitrogen)**
- **TN L01 (Total Nitrogen)**
- **TDP L01 (Total Dissolved Phosphorus)**

1. Scope and Application

1.1. This method describes the digestion procedure for the determination of total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) in fresh and estuarine surface waters by the alkaline persulfate oxidation technique. The method is suitable for the determination of total nitrogen (TN) and total phosphorus (TP) with necessary precautions to ensure that particulates are fully digested.

1.2. Typical analytical ranges in estuarine samples are 0.05 to 5.0 mg-N/L for total nitrogen and 0.005 to 0.50 mg-P/L for total phosphorus. Analytical ranges may be extended by digesting and analyzing a diluted sample. A higher calibration range for nitrogen is permitted for filtered samples only (i.e., TDN).

2. Summary of Method

2.1. The persulfate oxidation technique for nitrogen and phosphorus in water is performed under heated alkaline conditions, where all organic and inorganic forms of nitrogen are oxidized to nitrate. As the reaction proceeds, NaOH is consumed and the pH drops to < 2.2, which allows the oxidation of all phosphorus compounds to orthophosphate.

2.2. An aliquot of digested sample is analyzed for nitrate and orthophosphate using an automated colorimetric method (Chapter 6, Sections D.5 & D.8) to produce total nitrogen and total phosphorus concentrations.

3. Interferences

3.1. Some particulate nitrogen compounds in unfiltered samples may be resistant to alkaline persulfate digestion (i.e., refractory N) and yield low total nitrogen results.

3.2. Samples preserved with acid will result in low recoveries of nitrogen unless the pH is neutralized to the same pH as the reagents2011 (USGS 2003).

3.3. Organic carbon reacts with the persulfate oxidation reagent to form carbon dioxide. Concentrations over 150 mg-C/L may deplete the persulfate before all nitrogen compounds are oxidized and cause a low bias for total nitrogen values.

4. Apparatus and Materials

4.1. Autoclave or pressure cooker capable of maintaining 100-121 °C for 30-55 minutes.

4.2. Glass Digestion Tubes: 30 mL culture tubes with polypropylene liner-less screw-caps or 40 mL vials with Teflon-lined screw-caps. New tubes should be conditioned prior to their first use by filling with persulfate oxidizing reagent and autoclaving at 100-121 °C for 30 minutes.

5. Reagents and Standards

5.1. Reagent Water: Nitrogen-free reagent water.

5.2. Borate Buffer Solution: Add approximately 800 mL of reagent water to a two-liter volumetric flask. Quantitatively transfer boric acid (H_3BO_3) and low-nitrogen (< 0.001% N) sodium hydroxide (NaOH) to the flask. Allow the solution to cool and dilute to 2.0 liters with reagent water. This solution is stable for two months at room temperature.

5.3. Persulfate Oxidizing Reagent: Add 400 mL of reagent water to a one-liter volumetric flask. Quantitatively transfer and dissolve low-nitrogen NaOH and low-nitrogen potassium persulfate ($K_2S_2O_8$ with < 0.001% N) in the flask. Dilute to one-liter with reagent water and store in a glass reagent bottle. Prepare this solution just before use.

5.4. Instrument Wash Water: Prepare auto-analyzer wash water by mixing oxidizing reagent and reagent water in a flask in a 2:1 (v/v) ratio, the same proportion as that added to the digestion tubes. Cover with foil and autoclave the solution for at least 30 min. at 100-121°C; cool and add borate buffer (or 3N NaOH if following SM 4500-P J).

5.5. Calibration Standards: A laboratory may purchase or prepare stock and working standards. The initial calibration verification standard must be purchased or made from a second source.

5.5.1. Potassium nitrate (KNO_3) for nitrogen (Section 6.D.5): Use primary standard-grade KNO3 that has been oven-dried and desiccated. The primary stock standard is stable for up to 6 months if stored at 4°C.

5.5.2. Potassium dihydrogen phosphate (KH_2PO_4) for phosphorus (Section 6.D.8): Use anhydrous, primary standard grade KH_2PO_4 that has been oven-dried and desiccated. The primary stock solution is stable for 6 months if stored at 4°C.

5.5.3. Prepare a series of working standards just prior to digesting each analytical batch by diluting suitable volumes with reagent or ASW water.

5.5.4. Standards should bracket the expected concentrations of samples. Analytical ranges may be extended by digesting and analyzing a diluted sample. However, for total nitrogen determinations in unfiltered samples, the upper calibration range should be limited to 5 mg N/L to ensure that the oxidizing reagent is not consumed prior to reducing organic nitrogen.

5.5.5. When analyzing estuarine samples of known salinity it is permissible to dilute the working standards and instrument wash water with artificial sea water to match the salinity of the samples. Salinity matching is unnecessary if using a flow

injection analyzer or if background correction is built into the instrument.

5.5.6. When analyzing samples of varying salinities, it is recommended that the standard curve be prepared in reagent water and Refractive Index corrections be made to the sample concentrations. Refractive Index correction is unnecessary if using a flow injection analyzer or if background correction is built into the instrument.

5.5.7. Digestion Check Standards: A laboratory must analyze one nitrogen and one phosphorus digestion check standard to demonstrate that all compounds containing N and P were completely digested. Standards may be purchased or prepared in the laboratory. Some laboratories use certified reference materials to check the calibration as well as the completeness of digestion. Listed below are various digestion check standards cited in the reference methods.

5.5.7.1. Nitrogen Digestion Check Standards

- Glutamic Acid ($C_5H_9NO_4$), Stock and Working Standards – Stock solutions are stable for ten months when preserved with chloroform and stored at 4°C. Prepare the working digestion check standard the day of digestion, diluting to volume with wash water solution.
- Nicotinic Acid p-touluenesulfonate ($C_{13}H_{13}NO_5S$) – See Standard Methods 4500-P J (APHA 2011).
- Glycine ($C_2H_5NO_2$) – Stable for 6 months at 4°C. (USGS 2003)

5.5.7.2. Phosphorus Digestion Check Standards

- Glycerophosphate (B-glycerophosphoric acid-disodium salt-5-hydrate – C3H7O6PNa2.5H2O) stock solutions are stable for 10 months when preserved with chloroform and stored at 4°C. Prepare the working digestion check standard the day of digestion.
- Adenosine triphosphate (C10H16N5O13P3) – See Standard Methods 4500-P J (APHA 2012a).

5. Procedure

5.1. Preparation of Analytical Batch

5.1.1. Prepare a series of standard solutions covering the analytical range by diluting either the stock or standard solutions.

5.1.2. Add 10.0 mL of sample (or a smaller aliquot diluted to 10.0 mL) to a clean glass test tube or vial.

Take care to obtain a representative subsample. Use a wide-bore pipette for taking aliquots of whole-water samples. A magnetic stirrer may be necessary to keep the particles in suspension if the sample has high suspended solids or sand particles.

5.1.3. Pipette 10.0 mL of each calibration standard, ICV, CCV, digestion check standard, etc., into a test tube or vial.

5.1.4. Prepare method blanks by adding 10 mL of reagent water to test tubes or vials.

5.1.5. Dispense 5 mL of the persulfate oxidizing reagent to each tube. Immediately cap tightly to prevent volatilization, and then invert twice to mix.

5.1.6. Prepare a flask of instrument wash water (mix reagent water and persulfate oxidizing agent in a 2:1 (v/v) ratio) and cover with aluminum foil.

5.2. Digestion

5.2.1. Heat the analytical batch at 100-121°C for 30 - 55 minutes in an autoclave or pressure cooker. If only determining total nitrogen, heating at 100-110°C for at least 30 minutes is permitted (APHA 2012b). No volatilization of ammonia occurs after autoclaving.

5.2.2. Remove tubes from autoclave and cool to room temperature. Digested samples can be held at room temperature or refrigerated for up to one month before analysis.

5.2.3. Prior to analysis, add 1 mL of buffer (or 3N NaOH) solution to each tube and mix.

5.2.4. Add buffer or (3N NaOH) to the digested wash water, depending on the reagent used.

5.2.5. Remove any suspended particles remaining in digests by decantation or filtration prior to colorimetric analyses.

5.3. Analysis – See procedures for Nitrate + Nitrite (Section 6.D.5) and Orthophosphate (Section 6.D.8)

5.4. Calibration and Data Reduction

5.4.1. Prepare a standard curve by plotting the instrument responses of the digested calibration standards against their known concentrations. Designate a digested method blank as having a concentration of 0.0 mg N/L and 0.0 mg P/L.

Do not select a curve-fitting function that forces a zero y-intercept. The calibration curve will have a positive y-intercept that approximates the baseline-corrected absorbance of the digestion blank.

6. Quality Control

6.1. Filtered samples with results greater than the highest calibration standard must be diluted to a concentration within the calibration range and re-analyzed. Whole water samples with results greater than 5.0 mg N/L must be diluted and re-digested prior to re-analyzing.

6.2. Digestion check standards must achieve a 90-110% recovery of the known concentration.

6.3. Method blanks: see Chapter 6, Section C.

6.4. Matrix spike samples: see Chapter 6, Section C.

6.5. Laboratory duplicates: see Chapter 6, Section C.

6.6. Laboratory control samples: see Chapter 6, Section C.

6.7. Method detection limits (MDL): Method detection limits should be established as specified in Chapter 6, Section C.

References

APHA 2012a. Standard Methods for the Examination of Water and Wastewater, 22nd ed., *Method 4500-P J-2011, Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus* (2012).

APHA 2012b. Standard Methods for the Examination of Water and Wastewater, 22nd ed., *Nitrogen Method 4500-N C-2011, Persulfate Method* (2012).

EPA 1987. *Nitrogen and Phosphorus Determinations in Estuarine Waters: A Comparison of Methods Used in Chesapeake Bay Monitoring.* U.S. EPA CBP/TRS 7/87, Aug. 1987. (Also Ref. No. UMCES 87-19 CBL.)

USGS 2003. USGS WRIR 03–4174: Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water. Methods I-2650-03 (total dissolved nitrogen and total dissolved phosphorus) and I-4650-03 (total nitrogen and total phosphorus).

Valderrama, J.C., 1981. The Simultaneous Analysis of Total Nitrogen and Total Phosphorus in Natural Waters: Marine Chemistry, v. 21, p. 109–122.

SECTION D.2

AMMONIA NITROGEN

CEDR Method Code:

- **NH4F L01**

1. Scope and Application

1.1. This method describes the determination of low-level ammonia nitrogen concentrations in filtered samples taken from fresh and estuarine surface waters.

1.2. The reaction chemistry described may be used with auto-analyzer instruments with segmented flow, flow injection or discrete mixing apparatus. The analytical range is determined by the instrument, its configuration and the standard curve that is constructed.

1.3. Typical MDLs and reporting limits are 0.005 and 0.01 mg/L, respectively.

2. Summary of Method

2.1. The procedure is based on EPA Method 350.1, without the sample distillation requirement. The chemistry is based on the Berthelot reaction, where alkaline phenol and sodium hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration present. The blue color formed is intensified by the use of sodium nitroprusside and measured spectrophotometrically at a wavelength of 630-660 nm.

2.2. The method measures both ammonia (NH_3) and ammonium ions (NH_4^+) and results are reported as mg NH_4-N/L. Figure 1 shows how the percentage of each species is dependent on pH.

2.3. Tidal monitoring program samples are filtered in the field through 0.7 µm glass fiber filters. Nontidal monitoring program samples are filtered in the field through 0.45 µm capsule filters, with some exceptions.

3. Interferences

3.1. Sampling procedures are susceptible to contamination from airborne and surface sources such as fumes, dust, detergents and fingerprints. Use clean techniques during sampling and analysis. It is recommended to wear gloves when filtering and handling samples.

3.2. Color development is pH dependent – sample pH should be between 4 and 10 so that the buffer can adjust for variances in sample pH.

3.3. Turbidity can bias the results through the absorption and scattering of light. A second filtration may be necessary to remove this effect.

3.4. Refractive Index interferences should be corrected for when analyzing estuarine/coastal samples. This can be achieved by using dual-beam background correction at a different wavelength, increasing the flow rate, or by matching the salinity of the calibration standards, rinse and blank waters to the salinity of the samples.

3.5. High concentrations of calcium and magnesium can cause a precipitate to form and result in spikes in absorption spectra. The use of a buffer containing sodium citrate, tartrate or EDTA can mitigate the calcium interference. For water samples very high in magnesium, such as seawater, the use of a sodium citrate buffer is recommended.

4. Apparatus and Materials

4.1. Continuous-flow automated analytical system equipped with an auto sampler, manifold, proportioning pump, tubing heater, colorimeter, photomultiplier, detector (λ = 630-660 nm), and a computer-based data system. Flow injection and discrete spectrophotometric instrumentation are considered equivalent to continuous-flow systems when using the same reaction chemistry. Changing the buffer to mitigate interferences is not considered a reaction chemistry change.

4.2. Nitrogen-free glassware: All glassware used in the determination must be low in residual ammonia to avoid sample and reagent contamination. Washing glassware with 10-50% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use nitrogen-free detergents instead of, or before acid rinsing. The glassware cleaning procedure will be considered sufficient if all quality control samples are within the expected ranges.

5. Reagents and Standards

5.1. Stock reagent solutions: The prescribed recipe for these reagents is generally instrument dependent and may change according to the concentration of the samples being analyzed. The chemicals needed for reagents and standards are listed below but not in specific amounts. For continuous flow analyzers, a surfactant such as Brij™ may be added to one or more reagents.

5.1.1. **Buffer solution:** This reagent is used to ensure that all samples and standards are analyzed at the same pH. The U.S. EPA allows the use of several different buffers depending on interferences. Three common buffers are: sodium potassium tartrate ($KNaC_4H_4O_6$), sodium potassium tartrate + sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), and disodium EDTA ($C_{10}H_{14}N_2Na_2O_8$).

5.1.2. **Alkaline Phenol solution:** Liquid or crystalline phenol is combined with sodium hydroxide under a ventilation hood. The use of crystalline phenol is preferred since a preservative such as oxalic acid is added to liquid phenol by the manufacturer. Prepare this reagent weekly in an amber glass bottle and store in a refrigerator at ≤ 6°C.

5.1.3. **Sodium Nitroprusside:** Nitroprusside is a catalyst for color formation. Sodium nitroferricyanide ($Na_2Fe(CN)_5NO \cdot 2H_2O$) is dissolved in reagent water. Its shelf life is 6 months when protected from atmospheric contamination.

5.1.4. **Sodium hypochlorite solution:** Dissolve a proper portion, according to method, of a hypochlorite solution with approximately 5% free chlorine into reagent water. Prepare this reagent weekly and refrigerate when not in use.

5.2. Calibration Standards: Laboratories may purchase or prepare stock and working standards. The calibration verification standard must be purchased or made from a second source, and be traceable to a national standard.

5.2.1. Anhydrous ammonium chloride (NH_4Cl): Primary standard-grade NH_4Cl is dried to remove moisture and cooled in a desiccator. Weigh desired amounts on an analytical balance and dissolve in ammonia-free reagent water. This solution is stable for up to 6 months when refrigerated at ≤ 6°C.

5.2.2. Prepare a series of working standards each analysis day by diluting suitable volumes of stock solutions with reagent or ASW water. Prepare working standards daily that bracket the expected concentration of the samples.

5.3. Reagent water, ammonia-free: see Chapter 6, Section C.4.2

5.4. Artificial Sea Water (ASW): see Chapter 6, Section C.4.3.

5.4.1. ASW may be used instead of reagent water to match the salinity of the standards to the salinity of the samples being analyzed. If precipitation occurs, eliminate the magnesium sulfate in the ASW.

5.4.2. When analyzing samples of varying salinities, it may be necessary to prepare standards in a series of salinities to quantify the "salt error", i.e., the shift in the colorimetric response of ammonia due to the change in the ionic strength of the solution. Salinity matching is unnecessary if using a flow injection analyzer or if background correction is built into the instrument.

6. Sample Handling

6.1. Samples should be analyzed as quickly as possible. If samples will be analyzed within 48 hours of collection, keep refrigerated at ≤ 6°C.

6.2. If samples will not be analyzed within 48 hours of collection, freeze and store them at -20°C or less for a maximum of 28 days.

7. Procedure

7.1. Calibration: Set up calibration standards to establish a curve that brackets the expected concentration of samples. See Chapter 6, Section C.5 for additional requirements.

7.2. Sample analysis

7.2.1. If samples have been frozen, allow to thaw.

7.2.2. Allow the instrument to warm up sufficiently to obtain a steady instrument state, ready to collect data. Use a sampling rate which ensures reliable results.

7.2.3. Analytical sequence: The samples and associated QC samples and standards should be run according to the following sequence.

7.2.3.1. Three or more calibration standards per order of magnitude, within the linear range of the instrument;

7.2.3.1.1. One calibration standard with zero analyte concentration to estimate the y-intercept.

7.2.3.1.2. The lowest standard must have a concentration ≤ PQL, or ≤ reporting limit.

7.2.3.2. Initial calibration verification (ICV) standard, traceable to a national standard;

7.2.3.3. Reagent/Method blank;

7.2.3.4. Twenty CBP samples;

7.2.3.5. One matrix spike sample and one duplicate sample;

7.2.3.6. Method blank or laboratory reagent blank (LRB).

7.2.3.7. One continuing calibration verification standard (CCV) per order of magnitude.

7.2.3.8. Repeat steps 7.2.3.4 through 7.2.3.7 until all samples are analyzed (or QC samples indicate that the system is out of control and recalibration is necessary).

7.2.4. If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected in continuous flow instruments. If the low concentration peak is not clearly defined, it is recommended to reanalyze that sample at the end of the sample run.

7.3. Calculations

7.3.1. Prepare a calibration curve by plotting instrument response against standard concentrations. Compute sample concentration by comparing sample response with the standard curve. Multiply concentration by the appropriate dilution factor.

7.3.2. Report results in units of mg NH_4-N/L.

8. Quality Control

8.1. This method should be used by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

8.2. **Method detection limits (MDL)**: Method detection limits should be established using the procedures in Chapter 6, Section C.8.

8.3. **Calibration**

8.3.1. Linear calibration range: Calibration standards should bracket the range of CBP samples.

8.3.2. Correlation coefficient (r): The correlation coefficient must be 0.995 or better for the calibration curve to be used.

8.4. **Method blank:** see Chapter 6, Section C.6.1.

8.5. **Matrix spike sample**: see Chapter 6, Section C.6.3.

8.6. **Laboratory duplicate:** see Chapter 6, Section C.6.2.

8.7. Certified reference material (CRM): The laboratory must analyze an ammonia CRM to verify the accuracy of the initial calibration. Alternatively, a material from a second-source or lot that is traceable to a national standard may be used.

Table 6.D.2. Frequency of Routine Calibration, Blank and QC Samples for ammonia nitrogen

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Action
Instrument Calibration	Each analysis day	Using all standards in curve, r ≥ 0.995. Linear at PQL or RL	Repeat full calibration.
Initial Calibration Verification (ICV)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, 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)	≤ PQL or reporting limit (RL)	Reanalyze another aliquot of blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	At the end of each preparation batch (≤ 20 samples)	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	80-120% recovery	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory Duplicate Sample	At least 1 per 20 samples	20% RPD[1]	Analyze another sample aliquot. Qualify the sample result if still exceeds 20%.

Laboratories may establish less stringent RPD criteria for duplicate samples near the reporting limit.

References

U.S. Environmental Protection Agency. 2012. 40 CFR Part 136 – Guidelines Establishing Test Procedures for the Analysis of Pollutants under the Clean Water Act; Analysis and Sampling Procedures. Federal Register/Volume 77 / No. 97/ May 18, 2012/ Rules and Regulations

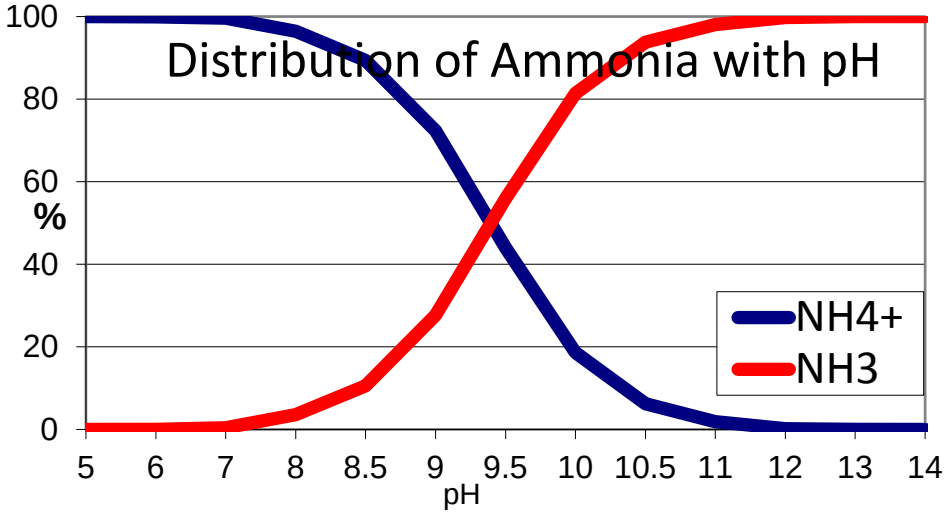
U.S. EPA 1993. "Methods for the Determination of Inorganic Substances in Environmental Samples,'' NERL–CI, EPA/600/R–93/100, August, 1993. Method 350.1, Rev. 2.0, ammonia (as N) - Automated, spectrophotometric.

Fishman, M.J. and Friedman, L.C. 1985. Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments. TWRI/USGS; Book 5; Chapter A1; Denver, CO. Method I-2523-85.

American Public Health Association. 2012. "Standard Methods for the Examination of Water and Wastewater", Method 4500-NH_3 G-2011, Ammonia Nitrogen by the Automated Phenate Method or Method 4500-NH_3 H-2011, Flow Injection Analysis.

Solorzano, L. 1969. "Determination of Ammonia in Natural Water by the Phenylhypochlorite Method for the determination of Ammonia in Sea Water". Deep Sea Research, 18:531-532

Figure 1. Distribution of Ammonia with pH. At pH 9.25, both ammonia and ammonium ions are present in a 1:1 ratio. As pH levels decrease, the fraction of ammonium ions (NH_4^+) increase. As pH levels increase above 9.5, the ammonia (NH_3) increases.



Section D.3

Chlorophyll and Pheophytin

CEDR Method Codes:

- **CHLA L01**
- **PHEO L01**

1. Scope and Application

1.1. This spectrophotometric method is used in the determination of chlorophyll *a*, *b*, and *c*, and pheophytin *a*. This method can be used to estimate phytoplankton biomass.

2. Summary of Method

2.1. Algal cells are filtered on a glass fiber and ground in aqueous acetone to extract the pigments. The extract is analyzed using a spectrophotometer to measure the absorbances at the specified wavelengths.

3. Apparatus and Materials

3.1. Glass fiber filters: Whatman GF/F or equivalent, 0.7µm nominal pore size.

3.2. Centrifuge. (A centrifuge capable of cooling to 4°C is recommended.)

3.3. Scanning spectrophotometer: Visible, multi-wavelength, with a bandpass not to exceed 2 nm. Glass cells for the spectrophotometer can be 1, 2, 5 or 10 cm. in length. If using multiple cells, they must be matched.

3.4. Tissue Grinder - Teflon pestle with attached stainless steel rod, or a sonicator. Sonication may be used if demonstrated to be equivalent.

3.5. Filtration apparatus with porous disk base.

3.6. Solvent-resistant glass-fiber syringe filter (optional).

4. Reagents

4.1. Aqueous acetone (C_3H_6O), 90%: add 1 part water to 9 parts of reagent grade acetone (v/v), made within 48 hours of time of use.

Note: Incorrect preparation of this solution may cause erroneous 750 nm readings.

4.2. Hydrochloric acid (1N), HCl.

4.3. Magnesium carbonate suspension, (10 mg/L $MgCO_3$): add 1 g finely powdered magnesium carbonate to 100 mL reagent grade water.

4.4. Reagent Grade Water: See Chapter 6, Section 4.2.

5. Sample Processing

5.1. Samples are filtered in the field according to procedures in Chapter 4, Section C.5.3, which are repeated below:

5.2. Immediately after collecting the sample, filter a known volume of sample water (measured with a graduated cylinder) through a glass fiber filter to concentrate the algae. Use sufficient sample (100-1500 mL) to produce a green color on the filter pad. To avoid cell damage and loss of contents during filtration, do not exceed a vacuum of 10 in. Hg (≤5 psi), or a filtration duration greater than 10 minutes. If sampling non-saline water (< 0.5 ppt salinity), add 1mL of saturated MgCO3 solution during the last few seconds of filtering. Do not suck the filter dry with the vacuum; instead slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.

5.3. Remove the filter from the base with forceps, fold once with the particulate matter inside, lightly blot the filter with a tissue to remove excess moisture and place it in a foil pouch or other suitable container that is protected from light, and store the filter at ≤ -20°C. Processed filters may be stored for 2-4 hours on ice before storing at ≤ -20 °C.

5.4. Samples that cannot be filtered immediately after collection may be chilled (≤ 6°C) and held in the dark for up to 2 hours before the plankton are concentrated, however, any delay is strongly discouraged.

5.5. Store the residue on the filter in the dark at ≤ -20 °C for up to 28 days before extracting and analyzing the pigments. Alternatively, perform the grinding procedure and store the extracts at ≤ -20 °C. Some studies have reported less pigment degradation in frozen extracts than in frozen filters. (Wasmund 2006).

6. Grinding Procedure

6.1. Remove frozen filters from the freezer but keep them in the dark. Keep workspace lighting to a minimum. Place filter into a centrifuge tube and add 2-3 ml of 90% acetone using a volumetric pipet.

6.2. Insert pestle into centrifuge tube and turn on grinder. Alternatively, the cells may be sonicated.

6.3. Grind filter for approximately 1 to 2 minutes being sure there are no discernible pieces remaining. If the tube gets warm from the friction of grinding, place the tube in a beaker of ice while grinding.

6.4. Pull pestle from vessel, rinse with 90% acetone if necessary while adding an exact volume with a volumetric pipet. Record the total volume of acetone added for grinding and extraction.

6.5. Cap the centrifuge tube and shake vigorously before steeping overnight in a ≤ 6°C refrigerator.

6.6. Centrifuge the extract prior to spectrophotometric analysis. If the centrifuge has a temperature control, cool the unit to ≤ 6°C; centrifuge samples for approximately 15 minutes at 675 g (EPA 1997) or 20 minutes at 500 g (APHA 2012).

The relationship between RPM and g is as follows: $\mathbf{g} = RCF = (1.118 \times 10^{-5}) R \cdot S^2$,

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 (RPMs). Values of relative centrifugal force (RCF) in units of times gravity (× g) for common micro-centrifuge rotor radii are found in standard conversion tables. A calculator is available online at http://www.endmemo.com/bio/grpm.php.

6.7. Keep centrifuged samples cool and protected from light. The centrifuged extract can be decanted and stored at ≤ -20 °C for up to 28 days after sample collection. If this time is exceeded, report the values with a problem code.

7. Instrument Optimization

7.1. Allow the instrument to warm up prior to use. Daily calibration of the spectrophotometer with known standards is not required.

7.2. Absorbance responses, i.e., optical densities, for samples should be between 0.1 and 1.0 absorbance units to ensure a linear response. Alternatively, the instrument's actual linear range may be demonstrated using a series of diluted samples. A higher absorbance response can be obtained by using a longer path length cell, a smaller extract volume, or a larger sample size.

7.3. Check the accuracy of the wavelength readings using a standard reference material such as holmium oxide (NIST SRM 930e) or didymium filter at least quarterly.

8. Procedure

8.1. Use a 90% acetone solution to zero the instrument at each of the wavelengths 750 nm, 665 nm, 664 nm, 647 nm and 630 nm. (See section 9.3.1 below for checks on instrument performance). If using a dual beam instrument leave one of the cuvettes in the reference cell.

8.2. Carefully pour or dispense the supernatant of the extracted sample into the cuvette. If the initial absorbance reading at 750 nm exceeds 0.007, recentrifuge or filter the extract through a solvent- resistant glass fiber syringe filter to remove turbidity interference.

If recentrifugation or filtration does not remove a 750 nm turbidity interference, continue to measure the absorbances at the rest of the wavelengths and write in the comment section that the sample had been recentrifuged and/or filtered.

8.3. Scan the range of wavelengths from high to low, and record the absorbances at the following wavelengths: *750 nm, 664 nm, 647 nm, 630 nm*.

8.4. After the 630 nm reading is taken, add the volume of 1N HCL that results in a final normality of 0.02N in the cuvette. Mix well.

Example: 5 mL cuvette:
mL 1N HCL = (0.02N)(cuvette volume (mL)).
mL 1N HCL = (0.02N)(5mL) = 0.1mL
0.1mL (~20 drops/mL) ≈ 2 drops of 1N HCl

8.5. Ninety seconds after acidification and mixing, record sample absorbances at *750 nm* and *665 nm*.

9. Quality Control

9.1. Samples should have an optical density (OD) ratio of OD 664_b (before acidification) to OD 665_a (after acidification) between 1.0 and 1.7. Ratios outside of this range may be caused by interfering pigments or in low-level samples, from variability near detection levels. Ratios near 1.7 are considered to have no pheophytin and to be in excellent physiological condition.

9.2. CRMs are not required for chlorophyll analysis but are recommended as availability and costs improve.

9.3. It is especially important to maintain the spectrophotometers in peak operating condition. This should be confirmed by the following guidelines:

9.3.1. The holmium oxide or didymium absorption spectrum needs to be checked quarterly or when problems are suspected. Details are not provided here since this and subsequent evaluation should be performed only by or under direct supervision of experienced personnel.

9.3.2. In lieu of a CRM, periodically evaluate the calibration curves of spectrophotometer analyses for other parameters for which there are reliable standards. If significant slope deviation or consistent unidirectional slope change over time is noted, an alternate spectrophotometer should be used until the problem is corrected by an authorized repair person.

9.4. Method detection limits (MDL): The method detection limit should be established in natural water using the guidelines in Chapter 6, Section C.8 of this manual.

9.5. Method blank: See Chapter 6, Subsection C.6.1 of this manual.

9.6. Laboratory duplicate: See Chapter 6, Subsection C.6.2 of this manual.

10. Calculation and Reporting

10.1. The Chesapeake Bay Program staff use Lorenzen's pheopigment-corrected equations to calculate chlorophyll *a* and pheophytin *a*. The 750_b nm and 750_a OD values are subtracted from the readings before (OD 664 nm) and after acidification (OD 665 nm). Lorenzen's equations are:

$$\textbf{Chlorophyll } \boldsymbol{a}, \mu g/L = \frac{26.7\ (664_b - 665_a) \times V_1}{V_2 \times L}$$

$$\textbf{Pheophytin } \boldsymbol{a}, \mu g/L = \frac{26.7\ [1.7\ (665_a) - 664_b] \times V_1}{V_2 \times L}$$

where:

V_1 = volume of extract, mL

V_2 = volume of sample, L

L = light path length or width of cuvette, cm, and

664_b, 665_a = optical densities of extracts before and after acidification, respectively.

10.2. Laboratories report all optical densities, volumes and light path lengths so that chlorophylls *a, b* and *c* may be calculated by the trichromatic method.

References

American Public Health Association, 2012. Standard Methods for the Examination of Water and Wastewater, 22th Edition, Method 10200H-2011. Chlorophyll.

EPA, 1997. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices - 2nd Edition, Method 446.0. EPA/600/R-97/072.

Parsons, T., Maita, Y., and Lalli, C. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis, Pergamon Press, pp. 101-112.

Wasmond, N., Topp, I., and Schories, D. 2006. *Optimising the storage and extraction of chlorophyll samples. OCEANOLOGIA, 48 (1), 2006. pp. 125–144.*

Section D.4

Dissolved Organic Matter Absorption Coefficient (CDOM *a*) at 440nm and Slope CDOM *a*

1. Scope and Application

1.1. A spectrophotometric method is used to determine the spectral absorption coefficient of dissolved organic matter (CDOM) in water. This parameter is also called Gelbstoff, or yellow matter.

CDOM absorption is one of several optical properties that contribute to the attenuation of light in a column of natural water. CDOM absorption is related to light attenuation as follows.

Attenuation = Absorption (a) + Scattering (b), and,

Absorption (*a*) $= a_{particulate} + a_{dissolved} + a_{water}$, *where*

$a_{dissolved}$ = ***CDOM a (440)***

The absorption coefficient is measured at 440 nm and expressed per meter (m-1) to be consistent with the unit for photosynthetic light attenuation (Kd).

1.2. The Chesapeake Bay Program uses CDOM results for SAV research and modeling. CDOM may also be used for ocean color satellite investigations, however, in clear waters it is recommended that the optical density of the reference cell water be measured relative to air.

2. Summary of Method

2.1. Water samples are collected and filtered through a 0.22µm polycarbonate filter to remove particulates. Samples may be pre-filtered in the field through a 0.7µm GFF and held up to 24 hours prior to filtration through the 0.22µm filters. A filtered deionized (DI) water blank is prepared in the laboratory each analysis day.

2.2. The absorption spectra of the sample and blank are recorded from 380 to 750 nm using a scanning dual beam spectrophotometer, using filtered DI in the reference cell.* The absorption coefficient is calculated as the difference between the optical density of the sample and the optical density of the filtered DI (instrument) blank at 440 nm, per meter of light path.

*This deviates from the NASA procedure in which *unfiltered* DI is used in the reference cell. Chesapeake Bay laboratories will verify that the use of a filtered DI reference is preferred because it removes all particulates and does not introduce light absorbing matter.

2.3. All equipment utilized to prepare CDOM samples must minimize contamination by organic or other colored material. Samples must be protected from photo-degradation during preparation, storage and measurement.

3. Apparatus and Materials

3.1. **PVC gloves** are recommended during filtration especially if field blanks show contamination.

3.2. **Filtration apparatus:** Plastic or glass filtration apparatus may be used provided that the units are equipped with mesh filter supports made either of stainless steel or plastic, but not with ground glass frits. (Glass frits tend to become clogged over time, and may cause uneven distribution on the filter, reduce the rate of filtration and may contaminate the sample filtrate.) A pre-rinsed glass fiber (GF/F or GF/C) backing filter may be used underneath the polycarbonate filter if problems are encountered with filter leaking or tearing with the mesh filter supports.

3.3. **Vacuum pump and gage capable of maintaining a vacuum** < 10 psi.

3.4. **Pre-filters**: Pre-rinsed Whatman GF/F or equivalent, 0.7µm nominal pore size are permitted when samples appear optically thick.

3.5. **Filters:** 0.22 µm polycarbonate (Nuclepore, or Osmonics, Inc. Poretics)

3.6. **Sample bottles:**

3.6.1. Pre-filtered samples and blanks: ≥125 mL brown HDPE bottles

3.6.2. 0.22 µm filtered samples and blanks: ≥ 60 mL **clear** borosilicate Qorpak™ bottles with polyethylene lined caps. (cone shaped seal to remove headspace – these are called Poly-Seal in the Fisher catalog)

3.7. **Spectrophotometer:** Visible, multi-wavelength, dual-beam scanning spectrophotometer with a bandpass ≤ 2 nm. The instrument should automatically correct for baseline drift and be equipped with computerized recording software. Baseline noise should be <0.001 OD units.

3.8. **Cuvettes:** Matched 10 cm and 5 cm quartz cells. (If possible, 10 cm should be used, especially for southern mainstem Bay samples.)

3.9. **Hydrochloric acid (10%):** for cleaning Qorpak™ bottles and filtration apparatus.

3.10. **Ethanol:** for cleaning cuvettes.

3.11. **DI Water:** ASTM Type II, or equivalent. For filter rinsing and blanks, keep DI water in acid-washed glass bottles (lab) or brown HDPE bottles (field). Refill bottles daily with fresh DI water to minimize contamination. Fresh DI is unnecessary as long as daily field blanks consistently show no contamination (< 0.005 CDOM_440).

4. Preparation of Equipment and Bottles

4.1. All filtration equipment and sample bottles are to be thoroughly cleaned in advance to remove organic contaminants.

4.1.1. Was plastic sample bottles and caps in dilute detergent water, rinse with tap water then DI water. After drying, cap bottles to keep clean.

4.1.2. Wash Qorpak™ bottles, filtration units and blank water bottles in dilute detergent water, rinse with tap water, then 10% HCl. Finally, rinse with DI water and dry. Cover with caps or aluminum foil to keep clean.

4.2. Clean the submersible pump and hose (or Kemmerer) as necessary to remove microbial slime and other organic contaminants. Equipment blanks are recommended at least once a month to demonstrate that the equipment is sufficiently decontaminated.

4.3. Label the brown HDPE sample bottles and include station and collection date. Label at least one FIELD BLANK bottle for each collection day.

5. Sample Collection and Field Filtration

5.1. Using a submersible pump or a depth sampler, collect one surface sample at each station from the shallowest depth normally sampled (0.5 meter in MD, 1 m in VA). Fill a clean plastic container directly from the hose. Keep container in subdued light as much as possible.

5.2. Set up the vacuum filter apparatus with a 0.7µm GFF pad on the support base. Attach the funnel and apply a vacuum of < 10 psi.

5.3. Filter 30 to 50 mL of sample for a sample rinse and discard filtrate. Filter 75 to 100 mL of sample, taking care not to suck the filter dry with the vacuum. Slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter.

5.4. Rinse a labeled brown HDPE bottle with filtrate then fill the bottle to the top. Discard the remaining filtrate.

5.5. Place field filtered samples in a refrigerator or cooler at 0 to 4°C. These may be stored up to 24 hours before filtering through the 0.22µm filter.

5.6. Repeat steps b) through e) for each sample. For a field filter blank, filter DI water exactly the same as samples.

6. Filtration through 0.22µm filter.

6.1. Set up the vacuum filter apparatus with a 0.22µm filter pad on the support base. Attach the funnel and apply a vacuum of 10 psi.

6.2. Beginning with the FIELD BLANK samples, filter ~ 30 mL of sample for a sample rinse.

6.3. Filter 75 to100 mL of sample, taking care not to suck the filter dry with the vacuum. More than one 0.22µm filter may be used per sample if clogging occurs. Pour the filtrate into a clear Qorpak™ bottle. Take care to leave as little airspace as possible.

6.4. If analyzing the samples within 4 hours, store the bottles in the dark at room temperature. For samples that will be run later, store in the dark at 4 ± 1°C for up to 28 days before analysis. Some studies indicate degradation after a day so the less time in storage the better.

7. Optical Density Measurements (NASA Protocol (Mitchell et al., 2000))

7.1. Warm the refrigerated samples to room temperature before beginning optical density measurements. If it is practical to do so, control the samples and the reference water to equal temperatures during the spectrophotometer measurements. Absorption by water is strongly temperature dependent at red and near infrared wavelengths.

Using the steps in 6) above, filter DI water drawn directly from the water preparation system through the 0.22µm filter. Use of DI water stored in plastic containers is not recommended. This filtered DI water is used in the reference cell and also for the instrument blank. Store filtered DI water in clear glass bottles.

7.2. Allow the spectrophotometer to warm up for 30 min. Confirm that the optical windows of the spectrophotometer are clean. If necessary, clean them with purified water and ethanol, sequentially, and dry them thoroughly with lint-free laboratory tissues.

7.3. Between use, store the cuvettes filled with DI water. For analysis, discard the storage water and rinse cuvettes inside and outside twice with 10% HCl, twice with ethanol, then with a generous amount of DI water. After the cuvettes have been cleaned, use laboratory tissues to handle them. Avoid contacting the cuvettes with bare-hands, and do not contaminate their optical windows by touching them.

7.4. Fill both cuvettes with the filtered DI water.

7.5. Carefully dry the cuvettes. Bulk dry with paper towels, but dry the quartz optical windows with lint-free laboratory wipes only (*e.g.* Kimwipes™).

7.6. Inspect the cuvettes to ensure that they are clean. Make sure there are no bubbles, floating dust, or contaminants on the optical windows or in suspension. Looking through the cuvette against a black background can usually identify any problems in the samples. Repeat cleaning and drying procedures as needed to obtain a clean sample.

7.7. **Instrument baseline correction**: Put reference and sample cuvettes filled with freshly filtered DI water into the spectrophotometer. Scan wavelengths 750 to 380 nm as a baseline and set the instrument to autocorrect with this scan. Save the digital baseline spectrum and note any anomalies in the instrument logbook.

7.8. **Instrument blank scan (ODb):** Run a second scan with the cuvettes still in the instrument. These readings comprise the instrument blank (OD_b) used in the calculations.

7.9. **Sample scan (ODs):** Remove the sample cuvette and discard the liquid. Rinse the inside of the cuvette three times with 5 to 10 mL of the next sample (or blank) to be measured. If sample volume is limited, several vigorously shaken small sample rinses are recommended. Dry the exterior of the sample cuvette carefully, and inspect it as described above to ensure a clean sample. Place the sample cuvette in the spectrophotometer and scan from 750 to 380nm.

If a sample has absorption (OD) > 3.0, reanalyze the sample using smaller cuvettes.

7.10. Save the records for the raw and corrected absorption spectra in computer files.

7.11. Change the reference cuvette water about every 15 minutes to ensure that the water in both cuvettes are the same temperature. Also, another instrument blank should be run with the fresh DI water.

8. Quality Control Checks

8.1. Holmium Oxide check on spectrophotometer – Quarterly

8.2. Field Blanks – Collect at least one field filter blank each sampling day as a check for field contamination.

8.3. Field Duplicates – Collect duplicate CDOM samples at the same frequency and locations as water quality duplicates.

8.4. Blank scan requirements

8.5. The r-squared value for the slope of the ln CDOM *a* regression must be greater than or equal to 0.95.

9. Calculations and Reporting

9.1. Prior to calculating the CDOM absorption coefficient and slope, be sure that the OD spectra for both samples and filtered instrument blanks have been automatically corrected for the baseline spectrum.

9.2. **Null point correction:** To correct for any residual scattering, the OD at a long wavelength at which CDOM does not absorb is subtracted from the sample and blank spectra. Choose the null point that the final absorption spectrum asymptotically approaches zero in the 600-700 nm range. It appears that an average of OD from 700-710 nm may be appropriate for most Bay Program samples. It may need to be lowered (no lower than 590 nm) for more dilute samples or raised (no greater than 740 nm) for more concentrated samples. A graph and instructions will be built into templates distributed to the labs. It is this correction that makes temperature control of the reference water critical.

9.3. Calculate the CDOM absorption coefficient **CDOM *a*** at each wavelength according to the equation:

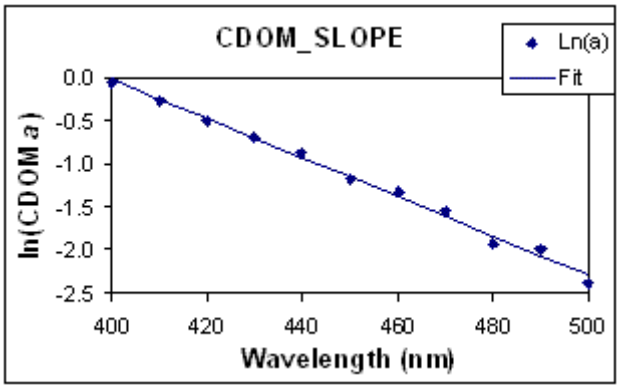
$$\textbf{CDOM } \boldsymbol{a}\ (m^{-1}) = \frac{2.303\ [OD_s\ (\lambda) - OD_b(\lambda)]}{l} \qquad \text{where,}$$

OD_s = optical density of the sample at a discrete wavelength
OD_b = optical density of the filtered instrument blank at same wavelength
l = cuvette pathlength in meters (5 cm = 0.05 m)

Select the **CDOM *a*** calculated at 440 nm, and report as parameter CDOM_440 (m^{-1}).

9.4. **Slope of CDOM absorption coefficient:** The slope reported (CDOM_SLOPE (nm^{-1})) is the slope of the regression line fitted to the natural log of CDOM *a* from 400-500 nm (dependent variable) versus wavelength (independent variable), multiplied by -1 (to make it a positive number). Occasionally in relatively clear water, there may be zero or

negative absorbances (due to noise) at wavelengths less than 500 nm. If this occurs, adjust the x and y ranges to shorter wavelengths to obtain a satisfactory regression slope. See figure below. The r-squared value for this regression must be greater than or equal to 0.95.



Reference

Mitchell B.G., Bricaud A., Carder K., Cleveland J., et al., 2000, *Determination of spectral absorption coefficients of particles, dissolved material and phytoplankton for discrete water samples*, [in:] *Ocean Optics Protocols for Satellite Ocean Color Sensor Validation. Revision 2*, G. S. Fargion & J.L.Mueller (eds.), NASA/TM–2000–209966, Greenbelt, Maryland, Goddard Space Flight Centre, 125–153.

Section D.5.

Nitrate + Nitrite Nitrogen

CEDR Method Code:

- **NO23F L01**

1. Scope and Application

1.1. This method describes the determination of combined nitrate + nitrite (NO23F) concentrations in filtered samples collected from fresh and estuarine surface waters. Samples may be filtered through 0.45 µm membrane filters or 0.7 µm GFF filters.

1.2. The analytical range is dependent on the instrument used, its configuration and the standard curve that is prepared. Tidal laboratory MDLs are about 0.002 mg N/L and the nontidal laboratory MDLs are about 0.005 mg N/L.

2. Summary of Method

2.1. This method is based on EPA Methods 353.2 and 353.4, where filtered samples are passed through a copper-coated cadmium reduction column and buffered to reduce nitrate to nitrite. The nitrite is reacted with sulfanilamide and coupled with N-(1-naphthyl)ethylenediamine dihydrochloride to form a magenta-colored azo dye. The intensity of the color produced is proportional to the nitrite concentration present in the sample and is measured spectrophotometrically at a wavelength in the range of 520 - 550 nm.

2.2. The reaction chemistry described may be used with auto-analyzer instruments with segmented flow, flow injection, or discrete mixing apparatus.

2.3. Combined nitrate + nitrite (NO23) concentrations are reported to the Chesapeake Bay Program as mg N/L. Nitrate (NO3) concentrations may be calculated by subtracting nitrite (NO2) from a separate determination (Section D.6) of NO23, i.e., NO3 = NO23 – NO2.

3. Interferences

3.1. Sample turbidity should be removed by filtration prior to analysis.

3.2. Salinity may cause a small negative error due to differences in the refractive index of saline and reagent water in segmented-flow instruments.

3.3. Hydrogen sulfide > 0.1 mg/L can precipitate on the cadmium column and reduce reduction efficiency.

3.4. The efficiency of the cadmium reduction column may be affected by iron, copper and other heavy metals at concentrations > 1 mg/L. Phosphate at concentrations > 0.1 mg/L may do this as well.

4. Apparatus and Materials

4.1. An automated analytical system equipped with an auto sampler, manifold, proportioning pump, colorimeter, photomultiplier and a computer-based data system. Gas-segmented continuous-flow, flow injection and discrete mixing analyzers are considered equivalent when using the same reaction chemistry. Manual spectrophotometers may be accepted on a case-by-case basis.

4.2. Nitrogen-free glassware: All glassware used in the determination must be low in residual nitrogen to avoid sample/reagent contamination. Washing glassware with 10-50% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use nitrogen-free detergents instead of, or before acid rinsing. A laboratory's glassware cleaning method will be considered sufficient if all quality control samples are within the expected ranges.

5. Reagents and Standards

5.1. Stock reagent solutions: The chemical reagents needed for the reaction are listed below. The quantities and concentrations of the reagents are generally instrument-specific, and may change due to the concentration of the samples being analyzed. If a continuous flow analyzer is being utilized an appropriate surfactant like Brij may be added to one or more of the reagents.

5.1.1. **Ammonium Chloride (NH_4Cl) Reagent**: This reagent is used as a buffer solution. It may not be needed for discrete instrument analyses. Adjust the pH of the NH_4Cl solution to approximately pH 9.1 using sodium hydroxide or ammonium hydroxide. (Sodium hydroxide is recommended if conducting ammonia analyses at the same time.)

5.1.2. **Imidazole ($C_3H_4N_2$) Buffer**: This buffer solution is specified in EPA Method 354.4 and replaces the ammonium chloride reagent. Imidazole and a small amount of hydrochloric acid (HCl) are used to make a buffer with an approximate pH of 7.8.

5.1.3. **Color Reagent:** Phosphoric acid (H_3PO_4), sulfanilamide ($C_6H_8N_2O_2S$)and N-(1 napthyl)ethylenediamine dihydrochloride ($C_{12}H_{14}N_2$) is added to reagent water while stirring. HCl or another acid may be substituted if recommended by the instrument manufacturer.

5.2. Calibration Standards: Laboratories may purchase or prepare stock and working standards. If standards are prepared in the laboratory, a purchased CRM or second source standard should be used to verify the initial calibration.

5.2.1. **Stock Nitrate Solution:** Primary standard-grade Potassium Nitrate (KNO_3) that has been dried and desiccated to remove moisture. Store the solution in a polyethylene or glass bottle and refrigerate for up to 6 months. Store commercially prepared standards according to the supplier's instructions.

5.2.2. Prepare a series of working standards by diluting suitable volumes of stock solution with reagent water or Artificial Sea Water (ASW).

5.2.2.1. Prepare working standards daily with three or more standards per order of magnitude, and an additional zero standard.

5.2.2.2. Calibration standards should bracket the expected concentrations of the samples or dilution and reanalysis will be necessary.

5.2.3. **Nitrite Solution:** Standard-grade potassium nitrite (KNO_2) or sodium nitrite ($NaNO_2$) to check the cadmium column reduction. See Section D.6 for preparation.

5.2.4. When analyzing samples of varying salinities using a segmented-flow analyzer, it may be necessary to prepare the calibration standards in reagent water and correct for refractive index differences (EPA 1997). This is unnecessary if background correction is built into the instrument.

5.3. Reagent water: See Chapter 6, Section 4.2.

5.4. Artificial Sea Water (ASW): See Chapter 6, Section 4.3. ASW may be used instead of reagent water to match standards to the salinity of the samples being analyzed. If precipitation occurs eliminate the magnesium sulfate in the ASW.

6. Sample Handling

6.1. Samples must be analyzed as quickly as possible. If the samples are to be analyzed within 48 hours of collection, keep refrigerated at ≤ 6°C.

6.2. If samples will not be analyzed within 48 hours of collection, freeze and store them at -20°C or less for a maximum of 28 days.

7. Procedure

7.1. Calibration: Set up calibration standards to establish a curve that brackets the expected concentration of samples. See Section 6.C.5 for additional calibration requirements.

7.2. Sample analysis

7.2.1. If samples have not been freshly collected and are frozen, thaw the samples.

7.2.2. Allow the instrument to warm up sufficiently to obtain a steady instrument state, ready to collect data. Use a sampling rate which ensures reliable results.

7.2.3. Analytical sequence: The samples and associated QC samples are typically run according to the following sequence.

7.2.3.1. Three or more calibration standards per order of magnitude, within the linear range of the instrument, and in order of decreasing concentration.

7.2.3.1.1. An additional calibration standard with zero analyte concentration to estimate the y-intercept and,

7.2.3.1.2. The lowest standard must have a concentration ≤ PQL or reporting limit.

7.2.3.2. KNO_2 (or $NaNO_2$) standard to check the nitrate reduction efficiency.

7.2.3.3. Initial calibration verification (ICV) standard, traceable to a national standard;

7.2.3.4. Reagent/method blank;

7.2.3.5. Twenty CBP samples;

7.2.3.6. One matrix spike sample and one duplicate sample;

7.2.3.7. Reagent/method blank; and,

7.2.3.8. One mid-range continuing calibration verification standard (CCV), per range.

7.2.3.9. Repeat steps 7.2.3.5 through 7.2.3.8 until all samples are analyzed. If the QC samples indicate that the system is out of control, recalibration is necessary.

7.2.4. If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected *in continuous flow instruments*. If this low-concentration peak is not clearly defined, it is recommended to reanalyze that sample at the end of the sample run.

7.3. Calculations

7.3.1. Calculate the nitrate reduction efficiency using the absorbances of KNO_2 (or $NaNO_2$) and KNO_3 standards of the same concentration according to the equation:

$$\text{Reduction Efficiency } \% = \frac{Absorbance\ of\ Nitrate}{Absorbance\ of\ Nitrite} \times 100$$

7.3.2. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply concentration by the appropriate dilution factor.

7.3.3. If applicable, correct for refractive index error and then correct for salt error following the procedures in EPA Method 353.4, Sections 12.2 and 12.3.

7.3.4. Report results in mg N/L.

8. Quality Control

8.1. This method should be used by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

8.2. Method detection limit (MDL): Establish the MDL using the procedure in 40 CFR Part 136. (See Chapter 6, Section C.8.)

8.3. Calibration Checks

8.3.1. The correlation coefficient must be 0.995 or better for the calibration curve to be used.

8.3.2. Results of the initial and continuing calibration verification samples must be within 10% of their expected values.

8.4. Nitrate Reduction Efficiency: The ratio of KNO_2 (or $NaNO_2$) and KNO_3 standards of the same concentration must be ≥ 0.9 (90%).

8.5. Method Blank: see Chapter 6, Section C.6.1.

8.6. Matrix spike sample: see Chapter 6, Section C.6.3.

8.7. Laboratory duplicate: see Chapter 6, Section C.6.2.

8.8. Certified reference material (CRM): Analyze a nitrate CRM to verify the accuracy of the initial calibration. Alternatively, a material from a second source or lot that is traceable to a national standard may be used.

8.9. Table 6.D.5 below summarizes the frequencies for calibration and quality control samples and the associated acceptance criteria.

Table 6.D.5 Frequency of Routine Calibration, Blank and QC Samples for Nitrate + Nitrite

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Actions
Instrument Calibration	Each analysis day	Using all standards in curve, r ≥ 0.995. Linear at PQL or RL	Repeat full calibration.
Initial Calibration Verification (ICV)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, 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)	≤ PQL or reporting limit (RL)	Reanalyze another aliquot of blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	Beginning and end of preparation batch	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	80 – 120% recovery	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory Duplicate Sample	At least 1 per 20 samples	20% RPD[1]	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.
Nitrate Reduction Efficiency	Each analysis day	≥ 90%	Reactivate or replace reduction column

[1] Laboratories may establish less stringent RPD criteria for duplicate samples near the reporting limit.

References

EPA 1993. "Methods for the Determination of Inorganic Substances in Environmental Samples," NERL–CI, EPA/600/R–93/100, August, 1993. Method 353.2, Rev. 2.0, Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry.

EPA 1997. "Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices - 2nd Edition", EPA EPA/600/R-97/072. Method 353.4, Rev. 2.0, Determination of nitrate and nitrite in estuarine and coastal waters by gas segmented continuous flow colorimetric analysis.

Fishman, M.J., ed., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory – Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93-125, 217 p. Method ID: I-2545-90

Section D.6.

Nitrite Nitrogen

CEDR Method Code:

- **NO2F L01**

1. Scope and Application

1.1. This method describes the determination of low-level nitrite (NO2F) concentrations in filtered samples taken from fresh and estuarine surface waters. Samples may be filtered through 0.45 μm membrane filters or 0.7 μm GFF filters.

1.2. The analytical range is determined by the instrument used, its configuration and the standard curve that is prepared. Chesapeake Bay tidal waters can range as low as 0.001 to 0.040 mg N/L for samples near the Bay mouth, to as high as 0.03 – 0.30 mg N/L in low oxygen waters.

1.3. The reaction chemistry described may be used with auto-analyzer instruments with segmented flow, flow injection, or discrete mixing apparatus.

2. Summary of Method

2.1. This method is based on EPA Methods 353.2 and 353.4, where filtered samples are analyzed by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)ethylenediamine dihydrochloride to form a magenta-colored azo dye. The intensity of the color produced is proportional to the nitrite concentration present in the sample and is measured spectrophotometrically at a wavelength of 520 or 540 nm.

2.2. The reaction chemistry described may be used with auto-analyzer instruments with segmented flow, flow injection, or discrete mixing apparatus. The method is identical to the determination of Nitrate + Nitrite (NO23F), without the use of a copper-cadmium reduction step.

2.3. Nitrite (NO2F) concentrations are reported to the Chesapeake Bay Program as mg N/L.

3. Interferences

3.1. Sample turbidity should be removed by filtration prior to analysis.

3.2. Salinity may cause a small negative error due to differences in the refractive index of saline and reagent water in segmented-flow instruments.

3.3. Hydrogen sulfide, metals and phosphate do not affect the results of nitrite since the cadmium reduction column is excluded.

4. Apparatus and Materials

4.1. An automated analytical system equipped with an auto sampler, manifold, proportioning pump, colorimeter, photomultiplier and a computer-based data system. Gas-segmented continuous-flow, flow injection and discrete mixing analyzers are considered equivalent when using the same reaction chemistry. Manual spectrophotometers may be accepted on a case-by-case basis.

4.2. Nitrogen-free glassware: All glassware used in the determination must be low in residual nitrite to avoid sample/reagent contamination. Washing glassware with 10-50% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use nitrogen-free detergents instead of, or before acid rinsing. A laboratory's glassware cleaning method will be considered sufficient if all quality control samples are within the expected ranges.

5. Reagents and Standards

5.1. Stock reagent solutions: The chemical reagents needed for the reaction are listed below. The quantities and concentrations of the reagents are generally instrument-specific, and may change due to the concentration of the samples being analyzed. If a continuous flow analyzer is being utilized an appropriate surfactant like Brij may be added to one or more of the reagents.

5.1.1. **Ammonium Chloride (NH_4Cl) Reagent**: This reagent is used as a buffer solution. It may not be needed for discrete instrument analyses. Adjust the pH of the NH_4Cl solution to approximately pH 9.1 using sodium hydroxide or ammonium hydroxide. (Sodium hydroxide is recommended if conducting ammonia analyses at the same time as nitrite.)

If using this same reagent for nitrate analysis, EDTA may be added to prevent interferences from metal constituents.

5.1.2. **Imidazole ($C_3H_4N_2$) Buffer**: This buffer solution is specified in EPA Method 354.4 and replaces the ammonium chloride reagent. Imidazole and a small amount of hydrochloric acid (HCl) are used for a buffer with an approximate pH of 7.8.

5.1.3. **Color Reagent:** Phosphoric acid (H_3PO_4), sulfanilamide ($C_6H_8N_2O_2S$)and N-(1 napthyl)ethylenediamine dihydrochloride ($C_{12}H_{14}N_2$) is added to reagent water while stirring. HCl or another acid may be substituted if recommended by the instrument manufacturer.

5.2. Calibration Standards: Laboratories may purchase or prepare stock and working standards. If standards are prepared in the laboratory, a purchased standard or second source should be used as the calibration check standard.

5.2.1. **Stock Nitrite Solution**: Primary standard-grade potassium nitrite (KNO_2) or sodium nitrite ($NaNO_2$), preserved with 2 mL of chloroform. The solution is stable for 3-6 months if kept refrigerated or protected from light. Store commercially prepared

standards according to the supplier's instructions.

5.2.2. Prepare a series of working standards by diluting suitable volumes of stock solution with reagent water or Artificial Sea Water (ASW).

5.2.2.1. Prepare working standards daily with three or more standards per order of magnitude, and an optional zero standard.

5.2.2.2. Calibration standards should bracket the expected concentrations of the samples or dilution and reanalysis will be necessary.

5.2.3. When analyzing samples of varying salinities using a segmented-flow analyzer, it is recommended that the calibration standards be prepared in reagent water and corrections for refractive index differences be made to the sample concentrations determined (EPA 1997). This is unnecessary if background correction is built into the instrument.

5.3. Reagent water: See Chapter 6, Section 4.2.

5.4. Artificial Sea Water (ASW): See Chapter 6, Section 4.3. ASW may be used instead of reagent water to match standards to the salinity of the samples being analyzed. If precipitation occurs eliminate the magnesium sulfate in the ASW.

6. Sample Handling

6.1. Samples must be analyzed as quickly as possible. If the samples are to be analyzed within 48 hours of collection, keep refrigerated at ≤ 6°C.

6.2. If samples will not be analyzed within 48 hours of collection, freeze and store them at -20°C or less for a maximum of 28 days.

7. Procedure

7.1. Calibration: Set up calibration standards to establish a curve that brackets the expected concentration of samples. See Chapter 6, Section 6.C.5 for additional calibration requirements.

7.2. Sample analysis

7.2.1. If samples have not been freshly collected and are frozen, thaw the samples.

7.2.1.1. Allow the instrument to warm up sufficiently to obtain a steady instrument state, ready to collect data. Use a sampling rate which ensures reliable results.

7.2.1.2. Analytical sequence: The samples and associated QC samples are typically run according to the following sequence.

7.2.1.3. Three or more calibration standards per order of magnitude, within the linear range of the instrument, and in order of decreasing concentration.

7.2.1.3.1. An additional calibration standard with zero analyte concentration to estimate the y-intercept and,

7.2.1.3.2. The lowest standard must have a concentration ≤ PQL or reporting limit.

7.2.1.4. Initial calibration verification (ICV) standard, traceable to a national standard;

7.2.1.5. Reagent/method blank;

7.2.1.6. Twenty CBP samples;

7.2.1.7. One matrix spike sample and one duplicate sample;

7.2.1.8. Reagent/method blank; and,

7.2.1.9. One mid-range continuing calibration verification standard (CCV), per range.

7.2.1.10. Repeat steps 7.2.1.6 through 7.2.1.9 until all samples are analyzed. If the QC samples indicate that the system is out of control, recalibration is necessary.

7.2.2. If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected *in continuous flow instruments*. If this low-concentration peak is not clearly defined, it is recommended to reanalyze that sample at the end of the sample run.

7.3. Calculations

7.3.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply concentration by the appropriate dilution factor.

7.3.2. If applicable, correct for refractive index error and then correct for salt error following the procedures in EPA Method 353.4, Sections 12.2 and 12.3.

7.3.3. Report results in mg N/L.

8. Quality Control

8.1. This method should be used by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

8.2. Method detection limit (MDL): Establish the MDL using the procedure in 40 CFR Part 136. (See Chapter 6, Section C.8.)

8.3. Calibration Checks

8.3.1. The correlation coefficient must be 0.995 or better for the calibration curve to be used.

8.3.2. Results of the initial and continuing calibration verification samples must be within 10% of their expected values.

8.4. Method Blank: see Chapter 6, Section C.6.1.

8.5. Matrix spike sample: see Chapter 6, Section C.6.3.

8.6. Laboratory duplicate: see Chapter 6, Section C.6.2.

8.7. Certified reference material (CRM): The laboratory must analyze a nitrite CRM to verify the accuracy of the initial calibration. Alternatively, a material from a second source or lot that is traceable to a national standard may be used.

8.8. Table 6.D.6 below summarizes the frequencies for calibration and quality control samples and the associated acceptance criteria.

Table 6.D.6 Frequency of Routine Calibration, Blank and QC Samples for Nitrite

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Actions
Instrument Calibration	Each analysis day	Using all standards in curve, r ≥ 0.995. Linear at PQL or RL	Repeat full calibration.
Initial Calibration Verification (ICV)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, 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)	≤ PQL or reporting limit (RL)	Reanalyze another aliquot of blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	Beginning and end of preparation batch	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	80 – 120% recovery	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory Duplicate Sample	At least 1 per 20 samples	20% RPD[1]	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.

[1] Laboratories may establish less stringent RPD criteria for duplicate samples near the reporting limit.

References

EPA 1993. "Methods for the Determination of Inorganic Substances in Environmental Samples,'' NERL–CI, EPA/600/R–93/100, August, 1993. Method 353.2, Rev. 2.0, Nitrite (as N) - Automated, spectrophotometric, "bypass" cadmium reduction.

EPA 1997. "Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices - 2nd Edition", EPA EPA/600/R-97/072. Method 353.4, Rev. 2.0, Determination of nitrate and nitrite in estuarine and coastal waters by gas segmented continuous flow colorimetric analysis.

Fishman, M.J., ed., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory – Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93-125, 217 p. Method ID: I-2545-90

Section D.7

Organic Carbon, Total and Dissolved

CEDR Method Code:

- **DOC L01**

1. Scope and Application

1.1. This method describes the determination of dissolved organic carbon (DOC) in filtered samples taken from fresh, estuarine and/or coastal waters. DOC by this method is considered equivalent to dissolved nonpurgeable organic carbon (NPOC).

1.2. For CBP tidal monitoring programs, DOC is defined as the fraction of total organic carbon (TOC) that passes through a 0.7 µm pore size glass fiber filter.

1.3. For CBP nontidal monitoring programs, most agencies filter DOC samples through a 0.45 µm pore size filters.

1.4. A typical range for carbon analysis is 0.5 to 100 mg C/L.

2. Summary of Method

2.1. This method is based on Standard Methods 5310 B-2011, High-Temperature Combustion. It involves the complete oxidation of nonpurgeable organic compounds to carbon dioxide (CO2), followed by non-dispersive infrared detection (NDIR) of the CO2, which is directly proportional to the concentration of non-volatile DOC in the sample.

2.2. Filtered samples for DOC are pretreated with acid and sparged with ultrapure carrier-grade air to purge off inorganic carbon. A portion of the purged sample is injected into a heated reaction chamber packed with a catalyst, combusted at a high temperature to produce carbon dioxide (CO2) gas, which is then carried by ultrapure air to the NDIR detector.

2.3. Total organic carbon (TOC) is calculated by adding results of DOC (in mg C/L) to results of particulate organic carbon from the analysis of particulates retained on a glass fiber filter. (See Section D.9, Particulate Nitrogen and Particulate Carbon.)

2.4. Direct analyses of unfiltered samples for TOC by this or other methods may be accepted on a case-by-case basis.

3. Interferences

3.1. Carbonates and bicarbonates interfere with the determination of organic carbon by increasing the concentration of CO_2 detected. These impurities are removed by lowering the pH of the sample to less than 2, followed by sparging with ultra-pure, CO_2-free gas for a predetermined time.

3.2. Preventing a rise in temperature can minimize the loss of semi-volatile organic substances during acidification and sparging.

4. Apparatus and Materials

4.1. A total organic carbon analyzer capable of maintaining an optimum combustion temperature for the oxidation of carbon.

4.2. A completely automated auto-sampler capable of acidifying and sparging samples is recommended. Purged samples have the potential to absorb CO_2 from the air so immediate analysis is important.

4.3. A computer with software for running the instrument, processing the signal from the infrared detector and for storing data.

4.4. All reusable labware used in the determination must be sufficiently clean. A laboratory's labware cleaning method will be considered sufficient if all quality control samples are within the expected ranges.

5. Reagents and Standards

5.1. The chemicals needed for the method are listed below. The concentrations are not specified since the amount of each is dependent on the instrument and the concentrations of the samples being analyzed.

5.2. Carrier and purging gases: Purified oxygen or air, as specified by the instrument manufacturer.

5.3. Calibration standards: Laboratories may purchase or prepare stock and working standards. The initial calibration verification standard (ICV) must be a CRM or a standard made from a second source that is traceable to a national standard.

5.3.1. Stock organic carbon standard solution: Prepare the stock solution by dissolving anhydrous, primary-standard-grade potassium hydrogen phthalate ($C_8H_5KO_4$) in reagent water. Preserve with acid to pH ≤ 2 and store at ≤ 6 °C. Alternatively, purchase and use commercially prepared standards and store them according to the supplier's instructions.

5.3.2. Inorganic carbon removal check standard: Standard-grade anhydrous sodium carbonate (Na_2CO_3), anhydrous sodium bicarbonate ($NaHCO_3$) or other inorganic carbon compounds to check the efficiency of inorganic carbon removal during the sparging step. Although the stock solution can be stored at ≤ 6 °C for up to 4 months, smaller batches of working solution must be used within 1-2 days.

5.3.3. Prepare a series of standards by diluting suitable volumes of stock solutions with reagent water. Prepare working standards each analysis day, or acidify and chill at ≤ 6°C for up to one month. Standards should bracket the expected concentration of the samples.

5.4. Acid: Use the instrument manufacturer's specifications for the type and concentration of acid.

6. Sample Handling

6.1. DOC samples must be filtered through a 0.7 µm GF/F or a 0.45 µm filter. Samples should be stored in borosilicate glass or Teflon containers.

6.2. Non-acidified samples should be stored at ≤ 4°C and analyzed within 48 hours. Alternatively, samples may be frozen at ≤ -20°C and analyzed within 28 days. They can also be acidified to a pH of ≤ 2, and refrigerated at ≤ 6°C for a maximum of 28 days.

7. Procedure

7.1. Follow instrument manufacturer's instructions for setting up and operating the analyzer. Allow sufficient time for the combustion temperature to stabilize before using the instrument.

7.2. If samples have not been freshly collected and are frozen, thaw prior to analysis.

7.3. Prepare calibration standards to establish a curve that brackets the expected concentration of samples.

7.4. Sample analysis

7.4.1. Allow the instrument to warm up sufficiently to obtain a steady instrument state.

7.4.2. Analytical sequence: The samples and associated QC samples are typically run according to the following sequence.

7.4.2.1. Three or more calibration standards per order of magnitude, within the linear range of the instrument, and in order of decreasing concentration.

7.4.2.1.1. An additional calibration standard with zero analyte concentration to estimate the y-intercept and

7.4.2.1.2. The lowest standard must have a concentration ≤ PQL or reporting limit.

7.4.2.2. Initial calibration verification (ICV) standard;

7.4.2.3. Reagent/method blank;

7.4.2.4. Twenty CBP samples;

7.4.2.5. One matrix spike sample and one duplicate sample;

7.4.2.6. Reagent/Method blank; and,

7.4.2.7. One mid-range continuing calibration verification standard (CCV), per range.

7.4.2.8. Repeat steps 7.4.2.4 through 7.4.2.7 until all samples are analyzed. If the QC samples indicate that the system is out of control, recalibration is necessary.

7.4.2.9. Samples above the highest calibration standard may be diluted to fall within the range of the calibration curve. See Chapter 6, Section C.5 for additional calibration requirements.

8. Calculations

8.1. Calculate organic carbon concentrations from the linear regression obtained from the standard curve in which the concentrations of the standards are entered as the independent variable (x-axis) and the corresponding response is the dependent variable (y-axis). Sample concentration is calculated using the equation:

$$mg\ DOC/L = \frac{Area - y\ intercept}{slope\ of\ the\ regression\ line}$$

8.2. Calculate the relative percentage difference (RPD) for sample duplicates as follows:

$$\%\ RPD = \frac{difference\ between\ duplicates}{average\ of\ duplicates}\ x\ 100$$

8.3. Report results in units of mg C/L.

9. Quality Control

9.1. This method should be performed by analysts experienced in the use of high-temperature combustion analyzers. Analyst training and/or a demonstration of capability should be documented.

9.2. Inorganic carbon removal check: Periodically monitor the inorganic carbon removal efficiency of the instrument's purging apparatus. Consistently high standard DOC values, and rising blank values may be indicative of inefficient purging, and would necessitate running the inorganic carbon check standard. The standard must always be run after routine servicing of the instrument, and when maintenance involves instrument components related to inorganic carbon removal.

9.3. Method detection limits (MDL): Method detection limits should be established using the guidelines in Chapter 6, Section C.8.

9.4. Reference material: The laboratory must prepare a calibration curve each analysis day and verify the curve with a certified reference material (CRM) or standard from a second source that is traceable to a national standard.

9.5. A summary of the required quality control samples are listed in Table 6.D.7 below.

Table 6.D.7. Frequency of Routine Calibration, Blank and QC Samples for organic carbon

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Actions
Instrument Calibration	Each analysis day	Using all standards in curve, r ≥ 0.995. Linear at PQL or RL	Repeat full calibration.
Initial Calibration Verification (ICV)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, 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)	≤ PQL or reporting limit (RL)	Reanalyze another aliquot of blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	Beginning and end of preparation batch	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	80 – 120% recovery	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory Duplicate Sample	At least 1 per 20 samples	20% RPD[1]	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.

[1] Laboratories may establish less stringent RPD criteria for duplicate samples near the reporting limit.

References

APHA 2014. *Standard Methods for the Examination of Water and Wastewater*. 2014. Method 5310B-2011, High Temperature Combustion Method.

Sugimura, Y. and Suzuki, Y. 1988. A high temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater aby direct injection of a liquid sample. Mar. Chem. 24:105-131.

SECTION D.8

ORTHOPHOSPHATE, TOTAL AND DISSOLVED

CEDR Method Codes:

- **PO4F L01**
- **PO4W L01**

1. Scope and Application

1.1. This method describes the determination of low-level orthophosphate concentrations in filtered or unfiltered samples taken from fresh and estuarine surface waters.

1.2. This method should be performed by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

1.3. The reaction chemistry described may be used with auto-analyzer instruments with segmented flow, flow injection, or discrete mixing apparatus. The analytical range is determined by the instrument used, its configuration and the standard curve that is prepared.

2. Summary of Method

2.1. Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration in the sample and is measured spectrophotometrically at a wavelength of 660 or 880 nm.

2.2. The procedures conform to EPA Methods 365.5 for estuarine samples and Method 365.1 for fresh water samples. The Chesapeake Bay Program permits use of 0.7 μm filters.

3. Interferences

3.1. Color development is pH dependent and it is recommended that samples be in the pH range of 4 to 8.

3.2. Turbidity can bias the results through the absorption or scattering of light. A second filtration may be necessary in the determination of dissolved phosphorus (PO4F) to remove this effect.

3.3. Refractive Index interferences should be corrected for when analyzing estuarine/coastal samples (EPA 1997). This can be performed by using dual-beam background correction at a different wavelength, increased flow rate (FIA), or by matching the salinity of the calibration standards and rinse/blank water to the salinity of the samples.

3.4. High concentrations of ferric iron (Fe^{+3}) can cause precipitation and loss of orthophosphate.

4. Apparatus and Materials

4.1. Continuous flow automated analytical system equipped with an auto sampler, manifold, proportioning pump, colorimeter, detector (λ = 660 or 880 nm) and a computer-based data system. Flow-injection and discrete spectrophotometric instrumentation are considered equivalent to continuous-flow systems when using the same reaction chemistry.

4.2. Phosphorus-free glassware: All glassware used in the determination must be low in residual phosphate to avoid sample/reagent contamination. Washing with 10-50% HCl and thoroughly rinsing with reagent water has been found to be effective. Some laboratories use phosphorus-free detergents instead of, or before acid rinsing. The glassware cleaning procedure will be considered sufficient if all quality control samples are within the expected ranges.

5. Reagents and Standards

5.1. Stock reagent solutions: The specific recipe for these reagents is generally instrument dependent, and may change due to the concentration of the samples being analyzed. In this SOP the chemicals needed for the reaction will be listed, but not the specific amounts. For continuous flow analyzers, a surfactant such as FFD-6 or SDS may be added to one or more reagents.

5.1.1. Color reagent solution: Combine proper portions of the following and dilute with reagent water.

5.1.1.1. Ammonium molybdate tetrahydrate ($(NH_4)6Mo_7O_{24}\cdot 4H_2O$),

5.1.1.2. Antimony potassium tartrate ($K(SbO)C_4H_4O_6\cdot \frac{1}{2}H_2O$ or equivalent), and

5.1.1.3. Sulfuric acid (H_2SO_4).

5.1.2. Ascorbic Acid ($C_6H_8O_6$) solution: Dissolve the proper amount of ascorbic acid granules or crystals in reagent water. It can be used as a separate reagent or combined with the color reagent (1) in proper proportion to make a single reagent test. When combined it is only good for one day.

5.2. Calibration Standards: Laboratories may purchase or prepare stock and working standards. The initial calibration verification standard (ICV) must be purchased or made from a second source.

5.2.1. Potassium phosphate monobasic (KH_2PO_4): Primary standard-grade KH_2PO_4 (pre-dried for at least 1 hr. at 105°C) and then dissolved in reagent water. This solution is stable for up to 6 months when refrigerated at ≤ 6°C.

5.2.2. Prepare a series of standards by diluting suitable volumes of stock solutions with reagent or Artificial Sea Water. Prepare working standards daily, with three or

more standards per decade and an additional zero standard. Standards should bracket the expected concentrations of the samples or dilution and reanalysis will be necessary.

5.3. Reagent water: see Chapter 6, Section C.4.2.

5.4. Artificial Sea Water (ASW): see Chapter 6, Section C.4.3.

5.4.1. ASW may be used instead of reagent water to match the salinity of the standards to the salinity of the samples being analyzed. If precipitation occurs, eliminate the magnesium sulfate in the ASW.

5.4.2. When analyzing samples of varying salinities, it may be necessary to prepare standards in a series of salinities to quantify the "salt error", i.e., the shift in the colorimetric response of phosphate due to the change in the ionic strength of the solution. Salinity matching is unnecessary if using a flow injection analyzer or if background correction is built into the instrument.

6. Sample Handling

6.1. Samples must be analyzed as quickly as possible. If the samples are to be analyzed within 48 hours of collection, keep refrigerated at ≤ 6°C.

6.2. If samples will not be analyzed within 48 hours of collection, freeze and store them at -20°C or less for a maximum of 28 days.

7. Procedure

7.1. Prepare calibration standards to establish a curve that brackets the expected concentration of samples. Samples above the highest calibration standard may be diluted to fall within the calibration curve. See Chapter 6, Section C.5 for additional calibration requirements.

7.2. Sample analysis

7.2.1. If samples have not been freshly collected and are frozen, thaw the samples to room temperature.

7.2.2. Allow the instrument to warm up sufficiently to obtain a steady instrument state, ready to collect data. Use a sampling rate which ensures reliable results.

7.2.3. Analytical sequence: The samples and associated QC samples are typically run according to the following sequence.

7.2.3.1. Three or more calibration standards per order of magnitude, within the linear range of the instrument, and in order of decreasing concentration.

7.2.3.1.1. An additional calibration standard with zero analyte concentration to estimate the y-intercept and

7.2.3.1.2. The lowest standard must have a concentration ≤ PQL or reporting limit.

7.2.4. Initial calibration verification (ICV) standard, traceable to a national standard;

7.2.5. Reagent/method blank;

7.2.6. Ten to twenty CBP samples;

7.2.7. One matrix spike sample and one duplicate sample;

7.2.8. One mid-range continuing calibration verification standard (CCV) per order of magnitude;

7.2.9. Method blank.

7.2.10. Repeat steps 7.2.6 through 7.2.9 until all samples are analyzed or QC samples indicate that the system is out of control and recalibration is necessary.

7.2.11. Reagent/method blank

7.2.12. CCV standard(s)

7.3. If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected in continuous flow instruments. If the low concentration peak is not clearly defined, it is recommended to reanalyze that sample at the end of the sample run.

7.4. Calculations

7.4.1. Orthophosphate concentrations are calculated from the linear regression obtained from the standard curve in which the concentrations of the standards are entered as the independent variable and their corresponding response is the dependent variable.

7.4.2. Results shall be reported in mg PO_4-P /L.

8. Quality Control

8.1. Method detection limit (MDL): Establish the MDL using the procedure in 40 CFR Part 136. (see Chapter 6, Section C.8).

8.2. Calibration Checks

8.2.1. The correlation coefficient must be 0.995 or better for the calibration curve to be used.

8.2.2. Results of the initial and continuing calibration verification samples must be within 10% of their expected values.

8.3. Method Blank: see Chapter 6, Section C.6.1.

8.4. Matrix spike sample: see Chapter 6, Section C.6.3.

8.5. Laboratory duplicate: see Chapter 6, Section C.6.2.

8.6. Reference materials: The laboratory must analyze a certified reference material or other second -source performance check with each run. Generally, the ICV satisfies this requirement.

Summary of acceptance limits and corrective actions for Orthophosphate QC samples

INDICATOR	ACCEPTANCE/ ACTION LIMITS	ACTION	FREQUENCY (BATCH)
Correlation Coefficient (r)	≥ 0.995	If < 0.995, evaluate data points of the calibration curve. If any data point is outside established limits, reject as outlier.	1 per batch if acceptable.
ICV	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.
Method Blank/ Laboratory Reagent Blank (LRB)	≤ PQL or reporting limit	If the LRB exceeds the PQL, results are suspect. Rerun the LRB. If the concentration still exceeds the quantitation limit, reject or qualify the data, or raise the quantitation limit.	Following the ICV, after every 20 samples and at the end of the run.
CCV	± 10%	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 20 samples and at end of batch
Laboratory Duplicate	± 20%	If the RPD fails to meet the acceptance limits, the samples should be reanalyzed. If the RPD again fails to meet the acceptance limits, the sample must be reported with a qualifier identifying the sample analysis result as not having acceptable RPD for duplicate analysis.	After every 20 samples.
Laboratory Fortified Matrix Spike Sample	± 20%	If the recovery of any analyte falls outside the designated acceptance limits and the QCS is in control, the recovery problem is judged matrix induced. Repeat the LFM and if the sample results are again outside the acceptable recovery range, the sample should be reported with a "matrix induced bias" qualifier.	After every 20 samples
MDL and PQL Verification Spikes	Detected ≥ MDL and ≤ PQL	If the spike is not detected, repeat with a higher concentration spike.	Two quarterly low-level spikes, run in separate batches. (EPA: at MDL spike conc.)

References

EPA 1993. "Methods for the Determination of Inorganic Substances in Environmental Samples,'' NERL–CI, EPA/600/R–93/100, August, 1993. Method 365.1, Rev. 2.0, orthophosphate (as P) - Automated, spectrophotometric.

Fishman, M.J., ed., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory – Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93-125, 217 p. Method ID: I-2523-85

American Public Health Association. 2012. "Standard Methods for the Examination of Water and Wastewater", Method 4500-P F -2011, Automated Ascorbic Acid Reduction Method. Also 4500-P G. Flow Injection Analysis for Orthophosphate.

MacDonald, R.W. and McLaughlin, F.A. 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.

Section D.9

Particulate Carbon and Particulate Nitrogen

CEDR Method Codes:

- **PN L01**
- **PC L01**

1. Scope and Application

1.1. Elemental analysis is used to determine total particulate carbon (PC) and total particulate nitrogen (PN) in estuarine and surface waters. The method measures all carbon and nitrogen compounds irrespective of source (inorganic or organic).

1.2. This procedure is used for the analysis of Chesapeake Bay Program (CBP) tidal and nontidal water quality samples. Measurements of PC and PN by this direct method were shown to be more accurate than low-level results calculated from the difference between whole-water and filtered-water concentrations (D'Elia, 1997).

1.3. This procedure conforms to EPA Method 440.0 (Zimmerman, et. al 1997). The MDL is dependent on the volume of sample filtered and the weight of the particulate matter. In a 250 mL sample, CBP laboratories routinely calculate MDLs of 0.1 mg C/L and 0.02 mg N/L respectively.

2. Summary of Method

2.1. A known volume of sample is filtered through a glass fiber filter to collect suspended particulate matter. The filter is dried and then placed in a combustion chamber where the carbon and nitrogen compounds are oxidized. Helium gas carries the oxidation products through a reduction tube where nitrogen oxides are converted to N2 and carbon oxides are converted to CO2. These gases are separated through a series of traps or columns and then are detected by thermal conductivity.

3. Interferences

3.1. There are no known interferences for the determination of particulate carbon and nitrogen in estuarine/coastal water.

3.2. The presence of volatile organic compounds, as well as contaminated laboratory surfaces, finger-prints, detergents and dust necessitates the utilization of clean techniques. The use of gloves and metal forceps is recommended in all parts of this procedure to prevent contamination.

4. Apparatus and Materials

4.1. Elemental combustion analyzer for the determination of carbon and nitrogen

4.2. Clean metal forceps.

4.3. Glass fiber filter manifold

4.4. Graduated cylinder (various volumes)

4.5. Glass transfer pipettes.

4.6. Desiccator.

4.7. Glass fiber filters: 25 mm diameter, with a nominal pore size of 0.7 µm.

4.8. Muffle furnace capable of 550 ± 50 °C

4.9. Analytical microbalance.

4.10. Freezer capable of ≤ -20°C

4.11. Pre-muffled instrument sample containers, or "cups".

5. Reagents and Standards

5.1. Calibration Standards – Use a solid material recommended by the manufacturer such as:

- Acetanilide (C_8H_9NO) (99.9% purity),
- Chloramine-T (N-chloro-p-toluene sulfonamide sodium salt, $C_7H_7ClNO_2S.Na$), or
- Aspartic acid ($C_SH_7NO_4$).

Storing the standard in a desiccator is recommended.

5.2. External Reference Materials - The CRM can be any assayed and certified sediment or solid material which is obtained from an external source. The materials below are currently used by many CBP laboratories.

5.2.1. NIST RM 8704 Buffalo River Sediment is certified for carbon content (3.351% carbon).

5.2.2. NIST RM 2781 Domestic sludge is certified for nitrogen content (4.78% nitrogen).

5.3. Nitrogen-free, reagent-grade DI water.

6. Filter Preparation and Sample Collection

6.1. Filter Preparation (prior to field collection)

6.2. Separate new glass fiber filters and spread out on a tray or ceramic dishes.

6.3. Place filters in muffle furnace for 15 minutes or more at 550 ± 50°C. Increase muffling time if the blanks contain nitrogen and carbon – e.g., more than 10% of sample concentration.

6.4. Remove the filters from muffle furnace and cover with aluminum foil until cool.

6.5. Store muffled filters in a clean desiccator or a sealed container until ready for use.

7. Sample Collection

7.1. Samples must be filtered as soon as possible after collection, preferably in the field. If filtering is delayed, keep water samples refrigerated or in a cooler on ice.

7.2. Use a forceps to transfer a pre-combusted 0.7 µm glass fiber filter onto the base of a vacuum filtration apparatus. Concentrate the suspended matter on the filter pad by pouring a known volume of water through the filter under vacuum pressure of ≤ 10 in. Hg. (< 5 psi).

7.3. Do not rinse filter with DI water.

7.4. Remove the filter with forceps. Carefully fold in half and place into a labeled filter container.

7.5. Freeze filters at ≤ −20°C and analyze for PN and PC within 28 days.

8. Sample preparation

8.1. Remove filters from the freezer and place in labeled containers suitable for drying.

8.2. Transfer to a drying oven at 50-105°C until dry. Remove, allow to cool and then desiccate until ready for analysis.

8.3. Clean the metal forceps and preparation area using reagent water and a Kimwipe™. Never use acid on metal.

8.4. Using metal forceps, place a clean cup into filter loading device. Transfer the glass fiber filter (sample) from the sample container to the cup, and then pack and seal it.

8.5. Samples may be prepared ahead of time and placed in a clean desiccator until analysis.

9. Procedure

9.1. **Standards and Calibration**

9.1.1. Always work on a clean surface whenever handling standards or samples. If weighing standards into metal cups, handle them with clean metal forceps.

9.1.2. Calibrate the electronic microbalance at the proper range each day prior to weighing any standards.

9.2. **Calibration**

9.2.1. For PN: Acetanilide, chloramine-T, aspartic acid or other suitable standard should be used to calibrate the analyzer. The final weights of standard should be between 0.05 and 2.0 mg.

9.2.2. For PC: Acetanilide or another suitable primary standard should be used to calibrate the analyzer. The final weight of standard should be between 0.05 and 2.0 mg.

9.2.3. Using metal forceps to handle the cups, weigh a clean cup (see cleaning procedure, 1.7.1) on a calibrated microbalance, and tare the balance to eliminate the weight of the cup from the weight measurement.

9.2.4. Using a clean metal spatula, place approximately 0.05 to 2.0 mg of standard into the cup. Use forceps (2 pairs) to seal the cup by pinching the top closed.

9.2.5. Record the weight of the standard. Place the cup inside the holder and put in proper location of the sample carousel.

9.2.6. Purge the instrument prior to calibrating by running three empty holders through the system. The system is now ready for a blank followed by the number of standards necessary for the instrument used.

9.3. **Sample Analysis**

9.3.1. Analytical Batch

9.3.1.1. Calibration Standards: Follow the manufacturer's calibration instructions for the number of nitrogen and carbon standards. A minimum of one standard is required per day, per parameter. Determine the response factors if single-point calibrations.

9.3.1.2. Initial Calibration Verification (ICV) – External nitrogen and carbon CRMs.

9.3.1.3. Method Blank – Pre-combusted blank filter.

9.3.1.4. Twenty samples.

9.3.1.5. Laboratory Duplicate Sample.

9.3.1.6. Method Blank – Pre-combusted blank filter.

9.3.1.7. Continuing calibration verification (CCV) – PN and PC calibration standards.

9.3.1.8. Repeat steps 9.3.1.4 through 9.3.1.7 until all samples are prepared.

9.3.2. Place auto sampler tray into the instrument, with the empty cups, blanks and standards in the front of tray. Run the nitrogen and carbon CRMs prior to running the samples.

9.3.3. If the calibration and CRM samples are acceptable then allow the instrument to continue to run samples.

9.3.4. Run a CCV after every 20 samples to confirm that the instrument is still within calibration.

9.3.5. Divide the results for particulate carbon or nitrogen (in micrograms), by the volume of sample in milliliters that was filtered through the glass fiber filter. The result will then be in μg/mL which is equivalent to mg/L.

10. Quality Control

10.1. This method should be performed by analysts experienced in the theory and application of elemental analysis. Analyst training and a demonstration of capability must be documented.

10.2. Method detection limits (MDLs): Method detection limits should be established using the procedures in Chapter 6, Section C.8. Since PN and PC cannot be spiked, utilize seven aliquots of a low concentration sample (i.e., 1-20 times the estimated MDL). If there are no low concentration samples available, dilute an aliquot or composite samples that have been run previously. The final concentration should be approximately between the detection limit and the reporting limit.

10.3. Calibration:

10.3.1. If the instrument uses a multipoint calibration curve, then make the curve high enough that no samples will exceed it.

10.3.2. If a single point calibration is used then run a QC sample that exceeds the range of samples being tested to prove performance.

10.3.3. For multipoint curves the correlation coefficient must be 0.995 or better.

10.4. Method Blank: A pre-combusted (muffled) filter, from the same lot as that used to filter the particulates is recommended. See Chapter 6, Section C.6.1.

10.5. Laboratory duplicate: See Chapter 6, Section C.6.2.

10.6. Reference materials: See Section 1.5.2 above. The recovery of the CRM material should be tracked and monitored for performance. The acceptance criteria are 90-110% recovery of the certified nitrogen and carbon content.

10.7. The frequency, acceptance criteria and corrective actions for PN and PC are summarized in Table 6.D.9.

Table 6.D.9. Frequency of Calibration, Blank and QC Samples for Particulate Nitrogen and Carbon

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Action
Instrument Calibration	Each analysis day	90-110% recovery of CRM	Repeat calibration.
Initial Calibration Verification - *External QCS or 2nd source of primary standard*	After calibration standards, 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)	≤ PQL or reporting limit	Reanalyze another aliquot of filter/blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	Beginning and end of preparation batch.	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or QCS.
Matrix Spike Sample	Not Applicable	Not Applicable	Not Applicable
Laboratory or Field Duplicate Sample	At least 1 per 20 samples	≤ 30% RPD	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.

References

D'Elia, C.F., Magnien, R.E., Zimmermann, C.F., Vass, P.A., Kaumeyer, N.L., Keefe, C.W., Shaw, D.V., Wood, K.V. 1987. Nitrogen and phosphorus determinations in estuarine waters: A comparison of methods used in Chesapeake Bay monitoring. University of Maryland Center for Environmental and Estuarine Studies, publication number UMCEES 87-19 CBL, p 26.

Zimmerman, C.F., Keefe, C.W., and Bashe, J. Method 440.0: Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-15/009, 1997.

Section D.10

Particulate Phosphorus and Particulate Inorganic Phosphorus

CEDR Method Codes:

- **PP L01**
- **PIP L01**

1. Scope and Application

1.1. This method describes the preparation of water samples for the determinations of: a) total particulate phosphorus (PP) and b) particulate inorganic phosphorus (PIP) from fresh, estuarine and/or coastal waters.

1.2. A typical MDL in a 250 mL sample is 0.003 mg PP/L. The analytical range is up to 4.0 mg P/L.

1.3. Measurements of PP by this direct method were shown to be more accurate at low levels than PP results calculated from the difference between whole-water phosphorus and filtered-water phosphorus measurements. (D'Elia, et. al, 1987)

2. Summary of Methods

2.1. Particulate Phosphorus (PP): Particulates in a known volume of sample are concentrated on a 0.7 µm glass fiber filter. The filters are combusted at 550°C to oxidize organic and inorganic phosphorus compounds to orthophosphate and then are extracted in dilute HCl.

2.2. Particulate Inorganic Phosphorus (PIP): Particulates in a known volume of sample are concentrated on a 0.7 µm glass fiber filter. The filters are dried and then are extracted in dilute HCl.

2.3. Dissolved orthophosphate in the extract is analyzed according to Section D.8 of this chapter (automated ascorbic acid, molybdenum blue method).

3. Interferences

3.1. Turbidity in the sample extracts can cause interferences. Remove by filtration if necessary.

3.2. High iron concentrations can lead to precipitation and loss of phosphorus in samples.

4. Apparatus and Materials

4.1. Filtering Apparatus – Vacuum filtration is recommended.

4.2. Glass fiber filters, 47 or 25 mm, 0.7 □m pore size.

4.3. Sample container for filters with particulates, labeled with sample identifier and volume filtered.

4.4. Drying oven.

4.5. Muffle furnace capable of maintaining temperatures of 550 ± 50°C.

4.6. Glass or plastic tubes with screw caps.

4.7. Labware that is low in residual phosphate to avoid contamination. Washing reusable labware with 5-10% HCl (v/v) and rinsing with reagent grade water is an effective practice. A laboratory's glassware cleaning method will be considered sufficient if all quality control samples are within the expected ranges.

5. Reagents and Standards

5.1. Extraction Solution: 1N Hydrochloric Acid (HCl)

5.2. Calibration standards: Laboratories may purchase or prepare stock and working standards. The calibration verification standard must be purchased or made from a second source.

5.2.1. Stock Phosphate Standard Solution: Dry anhydrous potassium dihydrogen phosphate (KH_2PO_4) in drying oven and desiccate. Weigh on an analytical balance and dissolve in reagent water containing 1 mL chloroform that serves as a preservative. The stock standard solution is stable for six months when refrigerated at ≤ 6 °C.

5.2.2. Prepare a series of standards by diluting suitable volumes of stock solution with 1N HCl or reagent water to match the acidity of the extract solution. Prepare working standards daily. Standards should bracket the expected concentration of the samples.

6. Sample Handling

6.1. Samples must be filtered as soon as possible after collection, preferably in the field. If filtering is delayed, keep water samples refrigerated or in a cooler on ice.

6.2. Use a forceps to transfer a 0.7 µm glass fiber filter onto the base of a vacuum filtration apparatus. Concentrate particulates on the filter pad by pouring a known volume of water through the filter under vacuum pressure. Rinse particulates with distilled water and continue the suction until dry.

6.3. Remove filter with forceps, carefully fold in half and place into a labeled filter container.

6.4. Freeze filters at ≤ −20°C, extract and analyze the particulates within 28 days.

6.5. Prepare a second filter if both PP and PIP will be analyzed.

7. Sample Preparation Procedure

7.1. If filters are frozen, handle carefully to make sure that particulates don't adhere to container.

7.2. PP Combustion - Place filters in a clean numbered container (e.g. crucible or aluminum pan) and heat in a muffle furnace at 550 ± 50°C for ≥ 90 minutes. Remove and allow to cool.

It is permissible to extract the filters and particulates from completed Fixed Suspended Solids (FSS) analyses without re-combusting them.

7.3. PIP Sample Drying - Place the filters in a clean numbered container and dry in a drying oven at ≤ 105°C. Remove and allow to cool.

It is permissible to extract filters and particulates from completed Total Suspended Solids (TSS) analyses without re-drying them.

8. Extraction Procedure

8.1. Place the sample and blank filters in labeled screw cap tubes.

8.2. Add 10 mL of 1N HCl to each tube and ensure the filters are submerged and saturated in the HCl.

8.3. Allow the extraction to proceed for at least 24 hours, during which the tubes may be gently shaken.

8.4. Transfer the supernatant to auto analyzer cups and analyze according to the method in Chapter 6, Section D.8, Orthophosphate. The supernatant may be diluted and/or filtered prior to analysis.

8.5. Preparation of Analytical Batch

8.5.1. Calibration Standards – Use the 1N HCl extraction solution as the zero calibration standard.

8.5.2. Initial Calibration Verification (ICV) – Aqueous CRM or second source LCS, traceable to a national standard.

8.5.3. Method Blank – Blank filter extract

8.5.4. Twenty samples

8.5.5. Matrix Spike Sample

8.5.6. Laboratory Duplicate Sample

8.5.7. Method Blank – Blank filter extract

8.5.8. Continuing calibration verification (CCV) – Mid-range calibration standard

8.5.9. Repeat steps 8.5.4 through 8.5.8 until all samples are prepared.

9. Calculations

Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample extract concentration by comparing sample response with the standard curve. Multiply concentration by the appropriate dilution factor.

9.1. The actual concentration of particulate phosphorus in samples (mg/L) is determined using the following equation:

$$P\ concentration\ in\ sample\ (mg/L) = \frac{concentration\ in\ sample\ extract\ (mg/L)\ x\ 10\ mL}{sample\ volume\ used\ in\ filtrate\ (mL)}$$

9.2. Calculate the relative percentage difference (RPD) for sample duplicates as follows:

$$\%\ RPD = \frac{|difference\ between\ duplicates|}{average\ of\ duplicates}\ x\ 100$$

9.3. Results should be reported in units of mg P/L.

10. Quality Control

10.1. This method should be used by analysts experienced in the use of combusted, extractive particulate nutrient analyses. Analyst training and/or a demonstration of capability should be documented.

10.2. Method detection limit (MDL): Method detection limits should be established using the procedures in Chapter 6, Section C.8. Since PP and PIP cannot be spiked, utilize seven aliquots of a low concentration sample. If no low-concentration samples are available, dilute an aliquot or composite of samples that have been run previously. Dilute into a range that is somewhere between the detection limit and reporting limit.

10.3. Method (Filter) Blank: A dry filter from the same batch of filters as that used to filter particulates is recommended. Usually, field staff submit a dry pad from the filter lot used the day of sample processing.

10.4. Certified Reference Material (CRM): The laboratory must analyze a CRM with each analytical batch. Until a particulate phosphorus CRM is commercially available, prepare an aqueous CRM or laboratory control sample (LCS) in the acid matrix used for extractions.

10.5. The frequency, acceptance criteria and corrective actions for PP and PIP are summarized in Table D.10-1.

Table D.10-1 Frequency of Routine Calibration, Blank and QC Samples for PP and PIP

Control Sample	Frequency of Application	Acceptance Criteria	Corrective Action
Instrument Calibration	Each analysis day as specified in PO_4 method	Using all standards in curve, r ≥ 0.995. Linear at PQL or RL	Repeat full calibration.
Initial Calibration Verification (ICV)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, 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)	≤ PQL or reporting limit	Reanalyze another aliquot of filter/blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	Beginning and end of preparation batch.	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	80-120% recovery	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory or Field Duplicate Sample	At least 1 per 20 samples	≤ 30% RPD	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.

References

Aspila, K.I., Agemian, H., Chau, A.S.Y. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *Analyst* 101: 187-197.

D'Elia, C.F., Magnien, R.E., Zimmermann, C.F., Vass, P.A., Kaumeyer, N.L., Keefe, C.W., Shaw, D.V., Wood, K.V. 1987. Nitrogen and phosphorus determinations in estuarine waters: A comparison of methods used in Chesapeake Bay monitoring. University of Maryland Center for Environmental and Estuarine Studies, publication number UMCEES 87-19 CBL, p 26.

Keefe, C.W. 1994. The contribution of inorganic compounds to the particulate carbon, nitrogen, and phosphorus in suspended matter and surface sediments of Chesapeake Bay. Estuaries 17:122-130.

SECTION D.11

TOTAL SUSPENDED SOLIDS

CEDR Method Codes:

- **TSS L01 (Gravimetric; 103-105°C; Subsampled)**
- **TSS L02 (Gravimetric; 103-105°C; Entire Sample)**

1. Scope and Application

1.1. This method is applicable to the determination of non-filterable matter in drinking, surface, and saline waters. The reporting level is 5 mg/L, based on the requirement for 2.5 mg of residue on the filter and a typical sample volume of 500 mL.

2. Summary of Method

2.1. A well-mixed aliquot of sample is vacuum-filtered through a glass-fiber filter, and the residue retained on the filter is dried at 103–105°C. The increase in weight of the filter represents the total suspended solids.

2.2. The method may be modified to filter the entire contents of a water sample. Report these results with method code TSS L02.

3. Interferences

3.1. Samples high in filterable residue (dissolved solids), such as saline waters, may be subject to a positive interference. Take care in selecting filtration equipment and sample volumes to ensure that rinsing the filter and residue minimizes this potential interference.

3.2. Certain biological materials, such as algae, slimes, insects or other small crustaceans may be considered to be positive interferences for non-filterable matter. Modifications or adjustments may be needed to generate a representative subsample.

3.3. The filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results.

4. Apparatus and Materials

4.1. Glass fiber filter discs, without organic binder: Whatman® 47 mm diameter, 0.70 - 1.5µm pore size or equivalent must be used. Document the filter type and pore size in the field and/or laboratory SOP.

4.2. Vacuum filtering apparatus with reservoir and a coarse fritted disc as a filter support. Vacuum flasks with Gooch crucibles may be used for laboratory filtration.

4.3. Vacuum pump.

4.4. Drying oven capable of maintaining a temperature of 103-105°C.

4.5. Desiccator with color indicator.

4.6. Analytical balance capable of weighing to 0.1 mg.

4.7. Graduated cylinders, Class A, TC (to contain). Plastic graduated cylinders are permitted for field filtration.

4.8. Wide-bore pipets, Class A or B.

4.9. Wash bottle with deionized water.

5. Sample Handling

5.1. Non-representative particulates such as leaves, sticks, stones, and detritus may be excluded from the sample if it is determined that their inclusion is not desired in the final result.

5.2. Store aqueous samples in plastic or resistant-glass containers and refrigerate them at ≤ 6°C to minimize microbiological decomposition of solids. Begin analysis as soon as possible, preferably within 24 hours of sample collection. The maximum holding time for lab-filtered TSS samples is 7 days.

5.3. Field-filtered filter pads must be frozen at ≤ −20°C (≤ −4° Fahrenheit) by the end of the sampling day. The maximum holding time for frozen field-filtered TSS filters is 28 days from the day of collection.

6. Procedure

6.1. Preparation of filters (this step is omitted if using pre-prepared, pre-weighed filters)

6.1.1. Place the glass fiber filter or Gooch on the filter apparatus.

6.1.2. While vacuum is applied, wash each filter with three successive 20 mL volumes of deionized, distilled water. Allow the vacuum pump to run until the filters are "dry".

6.1.3. Remove the filter (and Gooch if used) from filter apparatus, place on tray and then dry in an oven at 103−105°C for at least one hour.
If fixed suspended solids will also be measured, ignite washed filters at 550ºC for 15 minutes in a muffle furnace instead of the drying oven.

6.1.4. Place dried filters in a desiccator and cool to room temperature. Remove filters one-by-one and weigh each on a calibrated analytical balance and record the weights.

6.1.5. Return the filters to the oven for one hour. Repeat the drying cycle until a constant weight is obtained, i.e., the second weight of each filter is within 10% of initial weight.

6.1.6. Record the second filter weight and store the filters in a desiccator until needed.

6.2. Sample analysis

6.2.1. Assemble the filtering apparatus and place a pre-weighed filter (and Gooch if used) wrinkle side up on the filtering apparatus. Wet the filter and begin suction.

6.2.2. For lab-filtered samples:

6.2.2.1. Choose a sample volume to yield between 2.5 and 200 mg of residue. If the volume filtered fails to meet the minimum yield, increase sample volume up to 1 L. If the filtration time exceeds 10 minutes discard the measured sample volume and filter and use another filter with a smaller volume size.

6.2.2.2. If samples contain sand or a large amount of suspended matter, use a magnetic stirrer and a wide-bore pipet to obtain a representative subsample.

6.2.3. For field-filtered samples, filter 500mL of sample. Reduce the volume as necessary to keep the filtration time under 10 minutes.

6.2.4. Shake the sample vigorously and quickly fill a graduated cylinder to the desired volume to prevent the solids from settling. Quantitatively transfer the subsample to the filtration apparatus and remove all traces of water by continuing to apply vacuum after sample has passed through. Record the volume of sample filtered.

6.2.5. With suction on, wash the filter, residue and wall of filter-funnel or crucible with three portions of deionized water, allowing complete drainage between washings. Remove all traces of water from the filter by continuing to apply vacuum pressure after water has passed through.

6.2.6. Field-filtration only: Carefully remove the filter (or Gooch) from the filter support using forceps. Place the filter into a sample container and place on ice or in freezer for storage and transport.

6.2.7. Place batch of filters in drying oven and dry for at least one hour at 103-105°C. Cool in a desiccator and weigh using an analytical balance. On 10% or more of filters[6], repeat the drying cycle until a constant weight is obtained, i.e., no more than 10% difference. Record final weights.

6.2.8. If conducting subsequent analyses for fixed suspended solids or particulate phosphorus, return the filters to the desiccator or use another means to prevent contamination.

6.2.9. Determine the concentration of Total Suspended Solids (TSS) in mg/L by calculating the amount of non-filterable residue as follows:

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6 Standard Methods requires that all of the filters be re-weighed to a constant weight, defined as no more than 4% difference or 0.5mg, whichever is less. The CBP requirement is less stringent.

$$mg\ TSS/L = \frac{(A-B)\times 1000}{sample\ volume,\ mL}$$

Where: A = weight of filter and dried residue (mg)

B = weight of filter (mg).

6.2.10. Report TSS concentrations as follows:

6.2.10.1. Concentrations less than 1,000 mg TSS/L - report 3 or more significant figures

6.2.10.2. Concentrations of 1,000 mg TSS/L and above - report only whole numbers with 3 or more significant figures.

7. Quality Control

7.1. Reporting Limit: The reporting limit is dependent on the maximum volume of sample filtered. For 500 mL of sample and ≥ 2.5 mg of residue on the filter pad, the reporting limit will be 5 mg/L. If the maximum sample volume is 1000 mL, the reporting limit will be 2.5 mg/L.

7.2. Laboratory Reagent Blank: At least one lab-filtered DI water blank per 20 CBP samples filtered by the lab. (The number of field-filtered blanks is dependent on the sampling program requirements.)

7.3. Laboratory duplicate: At least one duplicate per 20 CBP samples filtered by the lab.[7]

7.4. Reference materials: The laboratory must analyze a TSS certified reference material (CRM) at least quarterly.

7.5. Balance calibration: Check the calibration of the analytical balance each day of use with NIST-traceable weights that bracket the working range, e.g., a high and a low weight.

References

Standard Methods for the Examination of Water and Wastewater, Method 2540 D-2011, "Total Suspended Solids Dried at 103 – 105 ° C, 22nd Edition, pp 2-66, 2012.

U.S. Geological Survey, Techniques of Water-Resources Investigations of the United States Geological Survey. Chapter A1, Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5, Laboratory Analysis, 3rd Ed.; Method I-3765-85, p. 443, (1989).

[7] Standard Methods requires one duplicate for every 10 samples and duplicate results should agree within 5% of their average weight.

SECTION D.12

FIXED SUSPENDED SOLIDS

CEDR Method Codes:

- **FSS L01 (Gravimetric; 550°C; Subsampled)**
- **FSS L02 (Gravimetric; 550°C; Entire Sample)**

1. Scope and Application

This procedure is used to obtain the amount of fixed matter present in the solid fraction of total suspended solids. This procedure is applicable to the determination of fixed matter in drinking, ground, surface, and saline waters.

2. Summary of Method

The residue obtained from the determination of total suspended solids is ignited at 550 ± 50°C in a muffle furnace. The weight of the residue after ignition is reported as mg fixed suspended solids/L.

3. Interferences

3.1. The principal source of error in the determination is failure to obtain a representative sample.

3.2. The test is subject to many errors due to loss of water of crystallization, loss of volatile organic matter prior to combustion, incomplete oxidation of certain complex organics, and decomposition of mineral salts during combustion.

4. Apparatus and Materials

4.1. Filter pad from completed TSS analysis, with final weight of dried suspended residue recorded. See Section 6.D.11, Total Suspended Solids.

4.2. Muffle Furnace capable of maintaining a temperature of 550 ± 50°C.

4.3. Desiccator with calcium chloride desiccant and color-indicator.

4.4. Analytical balance capable of weighing to 0.1 mg.

5. Sample Handling

5.1. Use only TSS filters whose preparation included muffling at 550ºC for 15-20 minutes. Follow the sample handling and preparation procedures in Section 6.D.11, Total Suspended Solids.

5.2. Analyses must be completed within the holding times listed in Table 6.1 (7 days for whole-water chilled samples or 28 days for field-filtered, frozen filters).

5.3. Store weighed TSS filters with residue in a desiccator if muffling is delayed.

6. Procedure

6.1. Ignite the filter with residue from the suspended solids procedure at 550 ± 50°C for approximately 15 to 20 minutes in a muffle furnace to a constant weight.

6.2. Let the filter and nonvolatile residue partially cool in air until most of the heat has dissipated. Transfer to a desiccator, cool to room temperature and record the filter weight using an analytical balance. On 10% or more of filters[8], repeat the cycle of ignition, cooling and desiccating, until a constant weight is obtained (i.e., within 10% of initial weight). Record the final weight of each filter.

7. Calculations

7.1. Calculate the concentration of **fixed** suspended solids in mg/L using the following equation.

$$mg\ FSS / L = \frac{(B - C) \times 1000}{sample\ volume,\ mL}$$

Where: B = weight of filter and residue after ignition (mg)
C = weight of filter without residue (mg)

7.2. Calculate the concentration of **volatile** suspended solids in mg/L using the following equation.

$$mg\ VSS / L = \frac{(A - B) \times 1000}{sample\ volume,\ mL}$$

Where: A = weight of filter and dried residue before ignition (mg)
B = weight of filter and residue after ignition (mg)

8. Quality Control

8.1. Balance calibration: Check the calibration of the analytical balance each day of use with NIST-traceable weights that bracket the working range, e.g., a high and low weight

8 Standard Methods requires that all of the filters be re-weighed to a constant weight, defined as no more than 4% of the initial weight. The CBP requirement is less stringent.

8.2. Laboratory Reagent Blank: At least one lab-filtered DI water blank per 20 CBP samples filtered by the lab. (The number of field-filtered blanks is dependent on the sampling program requirements.)

8.3. Laboratory duplicate: At least one duplicate per 20 CBP samples filtered in the lab.[9]

8.4. Filter Blank: Periodically assess the quality of the filter lots by checking the weight loss of an ignited, plain filter.

8.5. Reporting Limit: The reporting limit is dependent on the maximum volume of sample filtered. For 500 mL of sample and ≥ 2.5 mg of residue on the filter pad, the reporting limit will be 5 mg/L. For a sample volume of 1000 mL, the reporting limit will be 2.5 mg/L.

9 Standard Methods requires a duplicate for every 10 samples and duplicate results should agree within 5% of their average weight.

References

Standard Methods for the Examination of Water and Wastewater, Method 2540 E-2011 , "Fixed and Volatile Solids Ignited at 550°C", 22nd Edition, pp 2-67, 2012.

U.S. Geological Survey, Techniques of Water-Resources Investigations of the United States Geological Survey. Chapter A1, Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5, Laboratory Analysis, 3rd Ed.; Solids, nonvolatile on ignition, suspended (parameter code 00540) Method I-3766-85, p. 457, (1989).

U.S. Geological Survey, Techniques of Water-Resources Investigations of the United States Geological Survey. Chapter A1, Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5, Laboratory Analysis, 3rd Ed.; Method I-3765-85, p. 443, (1989).

SECTION D.13

SILICA, DISSOLVED

CEDR Method Codes:

- **SIF L01**

1. Scope and Application

This method describes the determination of dissolved silicate, mainly in the form of silicic acid found in estuarine and/or coastal waters.

1.1. In Chesapeake Bay tidal waters, the applicable range can be as low as 0.001 to 0.004 mg Si/L for samples near the Bay mouth, to as high as 0.03 to 0.30 mg Si/L when very high nitrite samples are encountered.

1.2. The method detection limits (MDL) are determined on a yearly basis, and should be established using the guidelines in Chapter 6, Section C.8.

2. Summary of Method

2.1. Tidal samples are field-filtered through 0.7 µm glass fiber filters.

2.2. In this method the silicate in the samples reacts with ammonium molybdate under acidic conditions to form β-molybdosilicic acid. This complex is then reduced by ascorbic acid to form molybdenum blue that is measured at 660 nm. The color is proportional to the silicate concentrations present in the sample. The colorimetric procedure conforms to EPA Method 366.0 (1997).

3. Interferences

3.1. Sample turbidity should be removed by filtration prior to analysis.

3.2. Interference from phosphate may be suppressed by adding oxalic acid.

3.3. Hydrogen sulfide may be removed by either boiling prior to analysis, by oxidation with bromine or stripping with nitrogen gas after acidification.

3.4. Large amounts of iron and sulfide interfere with analysis.

3.5. The difference in refractive index of seawater and reagent water should be corrected for when analyzing estuarine/coastal samples. Alternatively, match the salinity of the calibration standards to the salinity of the samples.

4. Apparatus and Materials

4.1. Continuous flow automated analytical system equipped with an autosampler, manifold, proportioning pump, colorimeter, phototube or recorder or computer based data system.

4.2. Plastic containers and labware should be utilized for the analysis of silica. Any glassware used in the analysis must be low in silica to avoid sample reagent contamination. Washing with 10% HCl and thoroughly rinsing with reagent water has been found to be effective. A laboratory's glassware cleaning method will be considered sufficient if all quality control samples are within the expected ranges.

5. Reagents

5.1. Stock reagent solutions: The specific recipe for these reagents is generally instrument-dependent and may change due to the concentration of the samples being analyzed. This method lists the chemicals needed for the reaction but not the specific amount.

5.1.1. Ammonium Molybdate Solution: Prepare this reagent by dissolving ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$) in 0.05M H_2SO_4. Store the solution in a plastic container for up to three months at 4 ± 2°C.

5.1.2. Ascorbic Acid Solution - Dissolve ascorbic acid ($C_6H_8O_6$) in 200 mL of reagent water and acetone (C_3H_6O) and dilute in a volumetric flask with reagent water. Store in a plastic container. This solution is stable for one week if stored at 4 ± 2 °C. Discard the solution if it turns brown.

5.1.3. Oxalic Acid Solution - Dissolve oxalic acid ($C_2H_2O_4$) in approximately 800 mL of reagent water and dilute to 1000 mL with reagent water. Store in a plastic container. This solution is stable for approximately 3 months.

5.2. Calibration standards: Laboratories may purchase or prepare stock and working standards. The initial calibration verification standard must be purchased or made from a second source.

5.2.1. Stock Silicate Solution: Sodium hexafluorosilicate (Na_2SiF_6), dried to remove moisture. To prepare the stock solution, dissolve 0.6696 g in 1000 mL reagent water. The solution is stable for one year when stored at 4 ± 2°C.

If the stock standard solution is purchased, follow manufacturer's instructions for storage and shelf-life.

5.2.2. Prepare a series of standards by diluting suitable volumes of stock silicate solutions with reagent water or artificial seawater.

5.2.3. Salinity matching is unnecessary if using a flow injection analyzer or if background correction is built into the instrument. If need to match the salinity of the standards to the samples, select one of the options below.

5.2.3.1. When working with samples of known salinity it is recommended that the standard curve concentrations be prepared in artificial sea water diluted to that salinity and that the sampler wash solution also be artificial sea water diluted to that salinity.

5.2.3.2. When analyzing samples of varying salinities, it is recommended that the standard curve be prepared in reagent water and refractive index corrections be made to the sample concentrations.

5.2.4. Standards should bracket the expected concentration of the samples, and not exceed two orders of magnitude. At least five calibration standards with equal increments in concentration should be used to construct the calibration curve.

5.3. Reagent water: Refer to Chapter 6, Section 4.2.

5.4. Artificial seawater (ASW): Refer to Chapter 6, Section 4.3. This can be used for the matrix at an appropriate salinity for the samples being analyzed.

6. Sample Handling

6.1. Samples must be filtered using a 0.7 µm glass fiber filter as soon as possible after collection, preferably in the field. Transfer to HDPE sample bottles and cool to ≤ 6 °C.

6.2. Samples may be refrigerated at ≤ 6 °C for up to 28 days. Do not freeze samples.

7. Procedure

7.1. Prepare calibration standards to establish a curve that brackets the expected concentration of samples. Samples above the highest calibration standard may be diluted to fall within the calibration curve. See Chapter 6, Section C.5 for additional calibration requirements.

7.2. Sample analysis.

7.2.1. If samples have not been freshly collected and are frozen, thaw prior to analysis.

7.2.2. Allow the instrument to warm up sufficiently to obtain a steady instrument state, ready to collect data. Use a sampling rate which ensures reliable results.

7.2.3. Analytical sequence: The samples and associated QC samples are typically run according to the following sequence.

7.2.3.1. Five calibration standards within the linear range of the instrument, not to exceed two orders of magnitude. Set up and run standards in order of decreasing concentration.

7.2.3.1.1. An additional calibration standard with zero analyte concentration to estimate the y-intercept and

7.2.3.1.2. The lowest standard must have a concentration ≤ PQL or reporting limit.

7.2.3.2. Initial calibration verification (ICV) standard, traceable to a national standard;

7.2.3.3. Reagent/method blank;

7.2.3.4. Twenty CBP samples;

7.2.3.5. One matrix spike sample and one duplicate sample;

7.2.3.6. Reagent/Method blank; and,

7.2.3.7. One mid-range continuing calibration verification standard (CCV), per range.

7.2.3.8. Repeat steps 7.2.3.4 – 7.2.3.7 until all samples are analyzed. If the QC samples indicate that the system is out of control, recalibration is necessary.

7.3. If a low concentration sample peak follows a high concentration sample peak, a certain amount of carryover can be expected in continuous flow instruments. If this low concentration peak is not clearly defined it is recommended that the sample be reanalyzed at the end of the run.

8. Calculations

8.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply concentration by the appropriate dilution factor.

8.2. Refractive index correction for estuarine/coastal samples is optional. If performed, follow procedures described in EPA Method 366.0, Section 12.2.

8.3. Report results in units of mg Si/L.

Note: USGS method I-2700-85 says to report results as mg SiO_2/L. To convert and report data as mg Si/L, multiply mg SiO_2/L by 0.467.

9. Quality Control

9.1. This method should be used by analysts experienced in the use of colorimetric analyses, matrix interferences and procedures for their correction. Analyst training and/or a demonstration of capability should be documented.

9.2. Method detection limits (MDL): Method detection limits should be established using the procedures in Chapter 6, Section C.8.

9.3. Certified reference material (CRM): The laboratory must analyze a silicate CRM to verify the accuracy of the initial calibration. Alternatively, a material from a second source or lot that is traceable to a national standard may be used.

9.4. Additional quality control parameters are listed in the table below.

Table 6.D.13 Frequency of Routine Calibration, Blank and QC Samples for Silica

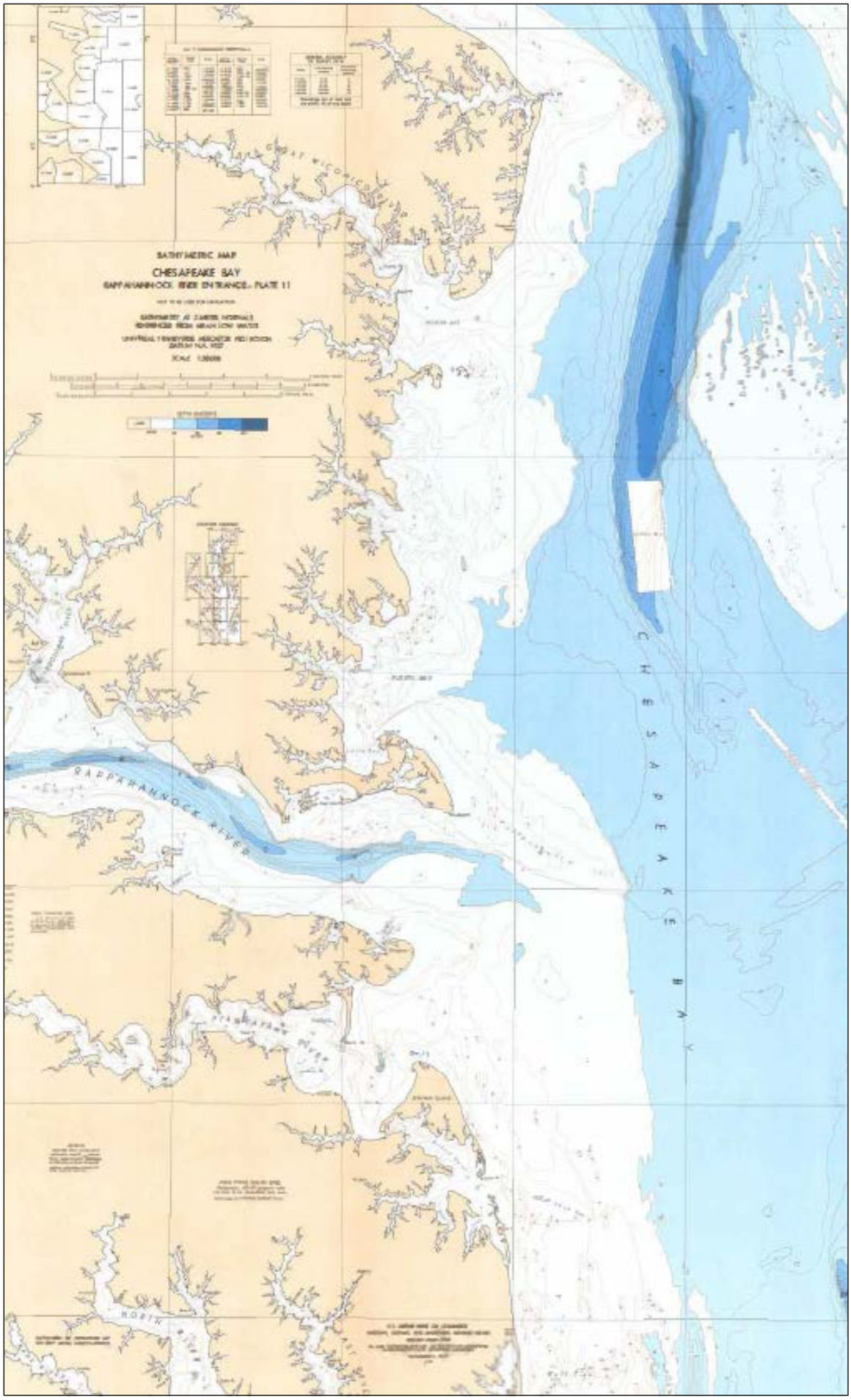
Control Sample	Frequency of Application	Acceptance Criteria	Corrective Actions
Instrument Calibration	Each analysis day	Using all standards in curve, r ≥ 0.995. Linear at PQL or RL	Repeat full calibration.
Initial Calibration Verification (ICV)– *2nd source or CRM, Traceable to a national standard*	After calibration standards, 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)	≤ PQL or reporting limit (RL)	Reanalyze another aliquot of blank solution. Investigate possible sources of contamination.
Continuing Calibration Verification (CCV)	Beginning and end of preparation batch	90-110% recovery of known concentration	Investigate problem; rerun all samples following the last in-control CCV or ICV.
Matrix Spike Sample	At least 1 per 20 samples	80 – 120% recovery	Spike another sample aliquot and analyze. If still exceeds control limits, suspect matrix interference and remove interference if possible.
Laboratory Duplicate Sample	At least 1 per 20 samples	20% RPD[1]	Analyze another sample aliquot. Qualify the sample result if still exceeds precision limits.

[1] Laboratories may establish less stringent RPD criteria for duplicate samples near the reporting limit.

References

Fishman, M. J. and Friedman, L., "Silica, colorimetric, molybdate blue, automated-segmented flow" in Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the U.S. Geological Survey Method I-2700-85. 1989.

Zhang, J. and Chi, J., "Determination of Dissolved Silicate in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis", in U.S. EPA Methods for Determination of Chemical Substances in Marine and Estuarine Matrices – 2nd Edition (EPA/600/R-97/072). Sep 1997, Method 366.0.


BATHYMETRIC MAP
CHESAPEAKE BAY
RAPPAHANNOCK RIVER ENTRANCE - PLATE 11
RAPPAHANNOCK RIVER
CHESAPEAKE BAY