MARYLAND DEPARTMENT OF NATURAL RESOURCES

SECTION 106
AMBIENT WATER QUALITY MONITORING
(CORE/TREND MONITORING)

QUALITY ASSURANCE PROJECT PLAN

May 24, 2019

MARYLAND DEPARTMENT OF NATURAL RESOURCES
RESOURCE ASSESSMENT SERVICE
MONITORING AND NON-TIDAL ASSESSMENT DIVISION

Maryland Department of Natural Resources
Section 106 - Ambient Water Quality Monitoring
(Core/Trend Monitoring)
Quality Assurance Project Plan
July 1, 2019 – June 30, 2020

Prepared by: Kristen Heyer
Maryland Department of Natural Resources
Monitoring and Non-tidal Assessment Division
Annapolis Field Office
1919 Lincoln Drive
Annapolis, MD 21401
Phone: 410-990-4600
Fax: 410-263-2468
kristen.heyer@maryland.gov

Signature: _____ Date:_____
Bruce Michael
Principal Investigator and Quality Assurance Officer
Maryland Department of Natural Resources

Signature: _____ Date:_____
Peter Tango
Project Officer
Chesapeake Bay Program Office, U.S. Geological Survey

Signature: _____ Date:_____
Carin Bisland
Acting Quality Assurance Officer
Chesapeake Bay Program Office, U. S. Environmental Protection Agency

LIST OF PREPARERS

Editors:

Kristen Heyer, Environmental Program Manager, Monitoring and Non-Tidal Assessment Division, Resource Assessment Service, Maryland Department of Natural Resources, 1919 Lincoln Drive, Annapolis, Maryland 21401

Tomas Ivasauskas, Monitoring and Non-Tidal Assessment Division, Resource Assessment Service, Maryland Department of Natural Resources, 580 Taylor Avenue, C-2, Annapolis, Maryland 21401

Contributors:

Laura Fabian, Monitoring and Non-Tidal Assessment Division, Resource Assessment Service, Maryland Department of Natural Resources, 1919 Lincoln Drive, Annapolis, Maryland 21401

Christine King, Monitoring and Non-Tidal Assessment Division, Resource Assessment Service, Maryland Department of Natural Resources, 73 Brant Road, Swanton, Maryland 21561

Renee Karrh, Tidewater Ecosystem Assessment Division, Resource Assessment Service, Maryland Department of Natural Resources, 580 Taylor Avenue, C-2, Annapolis, Maryland 21401

Ben Cole, Tidewater Ecosystem Assessment Division, Resource Assessment Service, Maryland Department of Natural Resources, 580 Taylor Avenue, D-2, Annapolis, Maryland 21401

Shahla Ameli, Supervisor, Division of Environmental Sciences, Maryland Department of Health, Laboratories Administration, 1770 Ashland Avenue, Baltimore, Maryland 21205

Jerome Frank, Manager, Nutrient Analytical Services Laboratory, University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, 146 Williams Street, PO Box 38 Solomons, MD 20688

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ACRONYMS AND ABBREVIATIONS

C	Carbon
CBP	EPA's Chesapeake Bay Program
CBPO	EPA's Chesapeake Bay Program Office
CBL	University of Maryland's Chesapeake Biological Laboratory
cm	Centimeter
CSSP	Coordinated Split Sample Program
DIWG	Data Integrity Workgroup (a workgroup of the Chesapeake Bay Program, formerly AMQAW)
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EPA	U.S. Environmental Protection Agency
g	Gram
H_2O	Dihydrogen oxide (water)
L	Liter
m	Meter
MDE	Maryland Department of the Environment
MDH	Maryland Department of Health
MDNR	Maryland Department of Natural Resources
min.	Minute
mg	Milligram
ml	Milliliter
mm	Millimeter
N	Nitrogen
NIST	National Institute of Science and Technology
NO_2	Nitrite
$NO_{2,3}$	Nitrate + nitrite
NO_3	Nitrate
P	Phosphorus
PC	Particulate carbon
PN	Particulate nitrogen
PO_4	Phosphate
PP	Particulate phosphorus
QAO	Quality Assurance Officer (unless otherwise noted, this refers to the DNR QAO)
QAPP	Quality Assurance Project Plan
RP	Replicate
TDN	Total dissolved nitrogen
TDP	Total dissolved phosphorus
TSS	Total suspended solids
USGS	U.S. Geological Survey
°C	Degrees Celsius

DISTRIBUTION LIST

Durga Ghosh, USGS/CBP
Peter Tango, USGS/CBP
Carin Bisland, EPA/CBP
Bruce Michael, MDNR
Kristen Heyer, MDNR
Laura Fabian, MDNR
Tomas Ivasauskas, MDNR
Renee Karrh, MDNR
Mark Trice, MDNR
Matt Rowe, MDE
Scott Stranko, MDNR
Shahla Ameli, MDH
Scott Phillips, USGS
Jerry Frank, UMCES/ CBL/NASL

PROJECT MANAGEMENT

A1 Project/Task Organization

This section lists the individuals responsible for the major aspects of Maryland's Ambient Water Quality Monitoring Program. The flow of project tasks is indicated in Figure 1.

Director and Principal Investigator: Bruce Michael, Resource Assessment Service, MDNR. 410-260-8627, bruce.michael@maryland.gov

Responsibilities: The director and principal investigator is responsible for overseeing the administrative aspects of the program including fiscal management, coordination among other DNR managers and coordination with cooperating agencies and institutions. This individual is also responsible for the technical design, management and data analysis of the program.

Quality Assurance Officer: Bruce Michael, Resource Assessment Service, MDNR. 410-260-8627, bruce.michael@maryland.gov

Responsibilities: The quality assurance officer is responsible for documenting and assuring the completion of field, laboratory and data management procedures that comprise this study.

Field Sampling Operations: Kristen Heyer, Monitoring and Non-tidal Assessment Division, MDNR. 410-990-4600, kristen.heyer@maryland.gov and Laura Fabian, Monitoring and Non-tidal Assessment Division, MDNR. 410-990-4524, laura.fabian@maryland.gov

Responsibilities: These individuals are responsible for administration of the field sampling activities including sample collection, sample storage and sample delivery to laboratories.

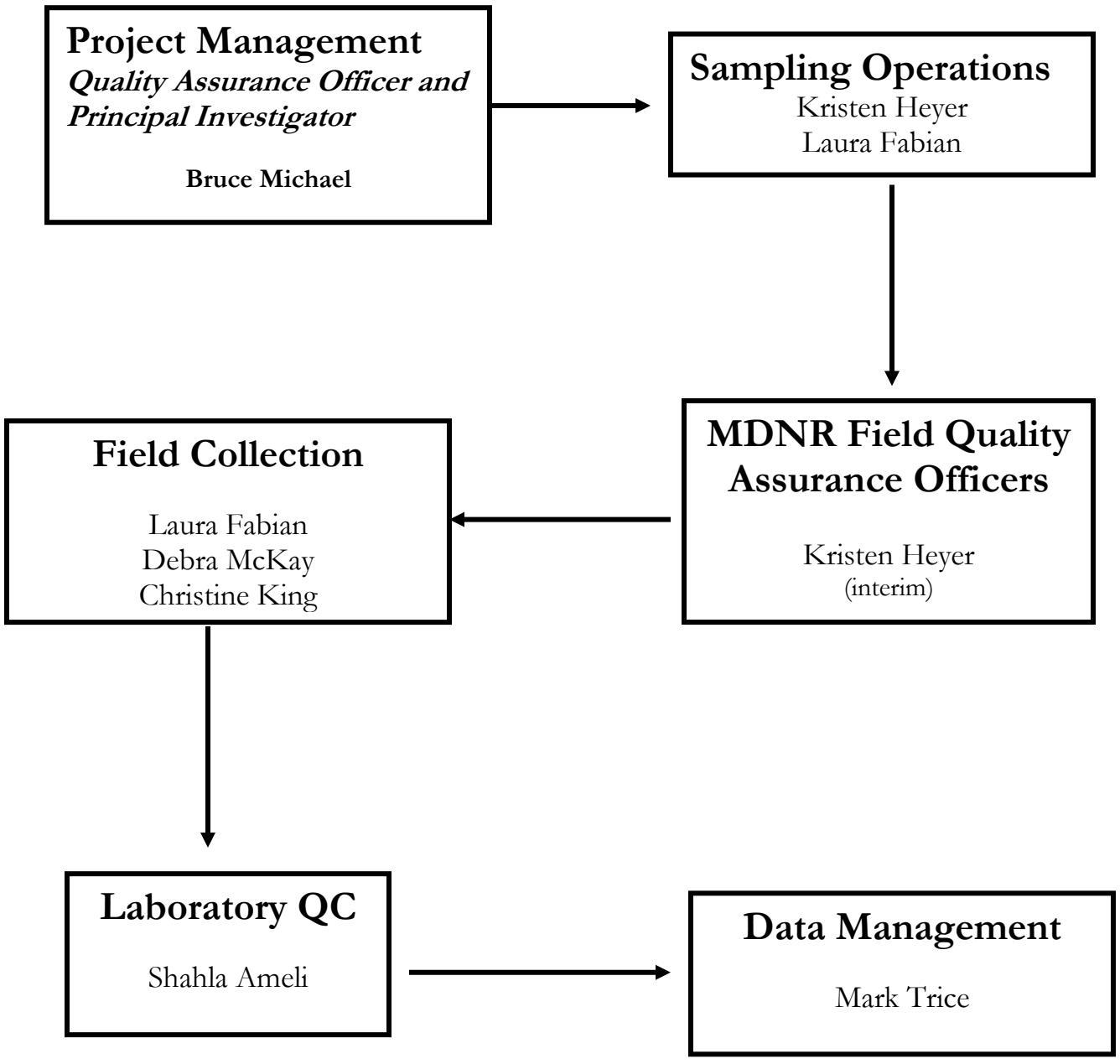
Laboratory Analyses/Water Column Chemistry: Shahla Ameli, Supervisor, Division of Environmental Sciences, MDH. 443-681-3855, shahla.ameli@maryland.gov

Responsibilities: This person oversees the laboratory that does all of the nutrient analysis and water chemistry for the Ambient Water Quality Monitoring Program.

Data Management; Mark Trice, Program Chief of Water Quality Informatics, Tidal Ecosystem Assessment, MDNR. 410-260-8649, mark.trice@maryland.gov

Responsibilities: This person is responsible for overseeing the management of field and laboratory data collected under this program; managing historical field and laboratory data collected under this program; and maintaining existing data management software.

Figure 1. Project Organization for Maryland's 106 Ambient Water Quality Monitoring Program



A2 Problem Definition/Background

Maryland DNR's Ambient Water Quality Monitoring Program (Core/Trend Monitoring) is part of a nationwide ambient monitoring effort designed to measure progress towards achieving EPA's national water quality goals. This program was initiated in 1974 to meet an EPA-mandated monitoring requirement for the State of Maryland to collect data that can be used to detect status and trends in the quality of the State's waters. To detect status and trends in Maryland's waters, ambient fixed stations were located in water use areas, problem areas, land use areas and in areas where future development may impact water quality. As of 1 July 1995, responsibility for Maryland's Ambient Water Quality Monitoring Program was transferred from the Maryland Department of the Environment to the Maryland Department of Natural Resources.

A3 Project/Task Description

Maryland's portion of this national ambient monitoring effort includes 37 Core stations located in non-tidal and tidal freshwater and estuarine areas and 25 Trend stations located on larger, non-tidal streams and rivers (4th order and larger). A map of station locations is presented in Figure 2 and a description of each station is presented in Table 1. The 62 stations that comprise this monitoring program are sampled monthly, year-round, for physical and chemical parameters.

A3.1 Core Stations

Core Station selection was based upon EPA's Basic Water Monitoring Program (BWMP) manual (EPA 1976). The selection of stations for the Core network was guided primarily by the need to assess conditions in water use areas. These included recreational areas, surface water supply areas, land use areas and potential areas of development. Since these data are used in the national assessment program, both impacted and non-impacted areas were included in the network to ensure that the evaluation would not be biased. Sampling stations were also selected to be representative of various regions in Maryland. Where consistent with the aforementioned criteria, stations were located to maintain continuity with existing sites. Overall, the locations of the 37 stations cover the major freshwater rivers of the State that flow into the Chesapeake Bay and also bracket major population centers.

Water quality data are collected monthly from each of the 37 Core stations located throughout the State. Surface samples are collected at 29 freshwater Core stations, but at various depths at the 8 estuarine stations. Nine sampling stations in the western part of the State (located in the Youghiogheny, North Branch Potomac, or Upper Potomac River) are sampled for additional water quality parameters (sulfate and chloride) to monitor for the impacts of acid mine-drainage. The sulfate and chloride sampling is described in the scope of work for the 106 supplemental memorandum of understanding between MDE and DNR. For logistical reasons, samples for the 8 estuarine Core stations are collected during sampling for other Maryland DNR monitoring programs. Samples for stations CB2.1, CB3.3C, and CB5.1 are collected during Bay Mainstem cruises, while ET5.2, XGG 8251, WT5.1, RET2.4 and TF2.3 are collected during tributary sampling (see Figure 2, estuarine Core stations are presented in orange). Sampling protocols for these 8 stations are outlined in this Quality Assurance Project Plan (QAPP). Sample analysis is conducted by the University of Maryland, Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory

(NASL). The analytical methods utilized by NASL are detailed in Appendix VII of the Quality Assurance Project Plan for Maryland DNR's Chesapeake Bay Water Quality Monitoring Program – Chemical and Physical Properties Component. The latest version of this Plan is available at:
http://mddnr.chesapeakebay.net/eyesonthebay/documents/MdDNR_MTQAPP2018.pdf.

Thirteen stations (identified with an asterisk in Table 1) are also sampled separately as part of the Chesapeake Bay Program's Non-tidal Network (Section 117e). For additional information on this monitoring program, please see Maryland DNR's Non-Tidal Network, Quality Assurance Project Plan. The latest version of this QAPP is available at:
http://mddnr.chesapeakebay.net/eyesonthebay/documents/NTN_QAPP1920.pdf

A3.2 Trend Stations

In addition to the Core stations, water samples are also collected monthly, year-round, at an additional 25 freshwater Trend stations. These stations are sampled to enhance the amount of water quality information available for status and trends assessments in the freshwater portions of Maryland's larger streams and rivers.

Both Core and Trend water quality samples are collected and analyzed in the exact same manner and follow the same quality assurance and quality control protocols. Parameters and analytical methods are listed in Table 2.

A3.3 Schedule of Tasks and Projects

Maryland DNR's Ambient Water Quality Monitoring Program was initiated in its present form. Since this is an ongoing effort, the schedule and time required to complete each of the tasks (from sampling to data verification) associated with monthly collections are presented below.

Schedule for Monthly Sampling and Data Processing

Task	Time Required	Cumulative Time
Sample Collection	20 person days	3 weeks
Laboratory Analysis	1 week	4 weeks
Data Verification	1 week	5 weeks
Data Keypunching	1 week	6 weeks
Final Data Verification	2 weeks	8 weeks

Data analysis to determine status and trends in water quality are calculated after the data has undergone the quality assurance process. Status and trends are calculated annually. Appendix I provides a complete description on how status and trends are calculated. Results will continue to be provided on the Internet at (http://www.dnr.maryland.gov/streams/) as well as provided directly to Tributary Strategy workgroups. The primary reporting mechanism for this program is the State's integrated 305b/303d report. This report provides a comprehensive assessment of Maryland's waters incorporating MDNR's Ambient Water Quality Monitoring Program results with other intensive or routine water quality surveys within Maryland. The integrated report is submitted to EPA's regional office for

review and approval. Draft and final versions are posted on Maryland Department of the Environment's website (www.mde.state.md.us) for access by federal, state, and local agencies as well as other officials and the public.

A4 Quality Objectives and Criteria

Maryland DNR's Ambient Water Quality Monitoring Program is designed to provide laboratory and field data that will help the State of Maryland to detect status and trends in the quality of its waters. Assessments of the quality of the data collected through this program can be expressed in terms of representativeness, comparability, completeness, accuracy and precision.

A4.1 Representativeness

Representativeness is the degree to which the sample data represent the actual conditions or concentrations present in the sampled population or area. Representativeness can be affected by experimental design and sample collection and handling. The experimental design (described in section A6) for this monitoring program requires monthly sampling (12 collections/year) for physical and chemical parameters on a pre-determined date, which is adequate for capturing long-term annual trends in concentration (Alden et al. 1994).

A4.2 Comparability

Comparability refers to the confidence with which one data set can be compared with another. Comparability must be ensured so that the results for one station are of comparable quality to other stations. In addition, the data generated by Maryland's Ambient Water Quality Monitoring Program must also be of comparable quality to the data generated by other states and laboratories. Comparability among data sets is assured through the use of consistent field methods and protocols, participation in the Data Integrity Workgroup and the use of field splits and blind audit samples. Comparability of monitoring data is achieved as a result of quality assurance procedures at each phase of data gathering and processing. It includes representative sampling and sample handling procedures, uniform laboratory methods and validation of laboratory data and procedures for reduction, validation and reporting of environmental data.

A4.3 Completeness

Completeness is a measure of the amount of valid data obtained compared to the amount that was expected under normal conditions. Completeness is a condition to be achieved in order to meet the data requirements of the program. Factors that can affect completeness include problems encountered by the field crews such as adverse weather conditions or equipment failures and laboratory-related issues such as sample preservation, exceeding holding times and accidents. To ensure that data are of the quality required to aid and support management decisions, Maryland's Ambient Water Quality Monitoring Program strives to provide monitoring data of known and consistent quality by generally following the guidelines outlined in Section E of the Recommended Guidelines for Sampling and Analysis in the Chesapeake Bay Monitoring Program, August 1996 (EPA 1996). These guidelines recommend precision goals of field and laboratory measurements of < 20 percent

of the coefficient of variation; accuracy goals within 80 to 120 percent, and the completeness goals of 90 percent.

A4.4 Accuracy

The accuracy (closeness to the true value) of the collected data is controlled and assured by the proper use, calibration, and maintenance of both field and laboratory equipment for the measurement of physical and chemical parameters. All instruments are identified by a unique number, used as an index for documentation of calibration, repair, and preventative maintenance. Multi-parameter field instruments are calibrated prior to field sampling to ensure accuracy. Where possible, standards used for calibration purposes are validated against a primary standard such as those available from the National Institute of Science and Technology (NIST). Daily quality control checks (including the running of blanks and standards) are used to control and assure laboratory accuracy.

Accuracy of laboratory results is also assessed through MDNR's participation in the Chesapeake Bay Coordinated Split Sample Program (CSSP), a split sampling program in which nine laboratories involved in Chesapeake Bay monitoring analyze quarterly, coordinated split samples. CSSP was established in June 1989 to establish a measure of comparability between sampling and analytical operations for water quality monitoring throughout the Chesapeake Bay and its tributaries. MDNR follows the protocols in the Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines (EPA 1991) and its revisions. Split samples are collected quarterly. Results are analyzed by appropriate statistical methods to determine if results differ significantly among laboratories. When a difference occurs, discussion begins regarding techniques and potential methods changes to resolve discrepancies and identify potential problems. Additionally, MDH participates biannually in the USGS reference sample program and permits USGS to release the results to the Chesapeake Bay Program Quality Assurance Officer. Laboratory accuracy is 90-110% recovery.

A4.5 Precision

Precision (repeatability) measures the closeness of values for a parameter within a data set. Quality control samples along with appropriate statistical techniques are used to ensure precision in the production of laboratory data. Precision of the chemical analytical methods is determined and documented from duplicate analyses. MDH performs precision calculations for laboratory duplicates. Every tenth sample is analyzed in duplicate. The acceptable value for the relative percent difference (RPD) is +/- 10%. If the calculated RPD does not fall within the acceptable range, the corresponding analysis is repeated. Precision of the entire measurement system for laboratory-analyzed parameters, including field processing, storage, and chemical analysis, can be assessed from duplicate field samples. Maryland DNR data analysts responsible for quality assurance checks examine field duplicate data. Based on preliminary analysis, there can be relatively large differences in measured values for certain water quality parameters. Maryland DNR's data management section is currently devising more robust procedures to address this issue in a statistically quantifiable manner.

A5 Special Training/Certification

Maryland DNR field personnel are required to demonstrate proficiency in all aspects of sample collection to the Chesapeake Bay Program Quality Assurance Coordinator, who conducts annual site visits to assure the continued proficiency of field personnel and adherence to the procedures specified in the Department's SOP. Training for field personnel who collect samples for the Ambient Water Quality Monitoring Program is provided by experienced field staff and the field Quality Assurance Officers. All training and procedures adhere to the Standard Operating Procedures developed for the Ambient Water Quality Monitoring Program.

Like DNR field personnel, analysts working for MDH are required to demonstrate proficiency in laboratory procedures. New analysts are trained by an experienced analyst on the laboratory procedures he/she will be assigned to perform. Training is documented using the Division's training forms and signed by the Supervisor, Quality Assurance Officer and the Division Chief.

A6 Documentation and Records

Documenting sampling events is an important component of the Ambient Water Quality Monitoring Program. Field crews document all data obtained in the field on field sheets. Examples of the field sheets are provided in the Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program (Appendix II). Since the data generated by this program are not used for legal purposes, a formal chain-of-custody sheet is not required. Field sheets and any information regarding a specific problem and/or event during a sampling run, as well as modifications to the sampling program are maintained by MDNR field office staff.

A water quality monitoring field sheet is completed on arrival at each station. This form is used to record field measurements and other data pertinent to the collection of samples, including sample type and date and time of collection. An instrument specific calibration logbook is also used to record instrument calibration data, notes on instrument testing, and notes on instrument performance, problems and repairs.

Critical project personnel receive copies of the QAPP (please see distribution list on page vii). The QAPP and all associated SOPs referenced herein will be updated annually by June 30th. Any modifications to the QAPP will be reviewed and approved by personnel conducting the sampling, the principal investigators and MDNR's Quality Assurance Officer. Once approved, the final version will be made available to all interested parties by placing downloadable copies on the CBP's and MDNR's websites. Project reporting to management will be accomplished by quarterly progress reports of activities. Electronic summaries of provisional instantaneous water quality data will be provided on an annual basis. The final data set is stored in a local designated DNR database directory for data user access. Data requests should be directed to Mark Trice, Program Chief of Water Quality Informatics (410-260-8630). Data are also entered into the CIMS database and are available to the public via the Bay Program web site at http://www.chesapeakebay.net/data.

Figure 2. Maryland Department of Natural Resource's Ambient Water Quality Monitoring Stations

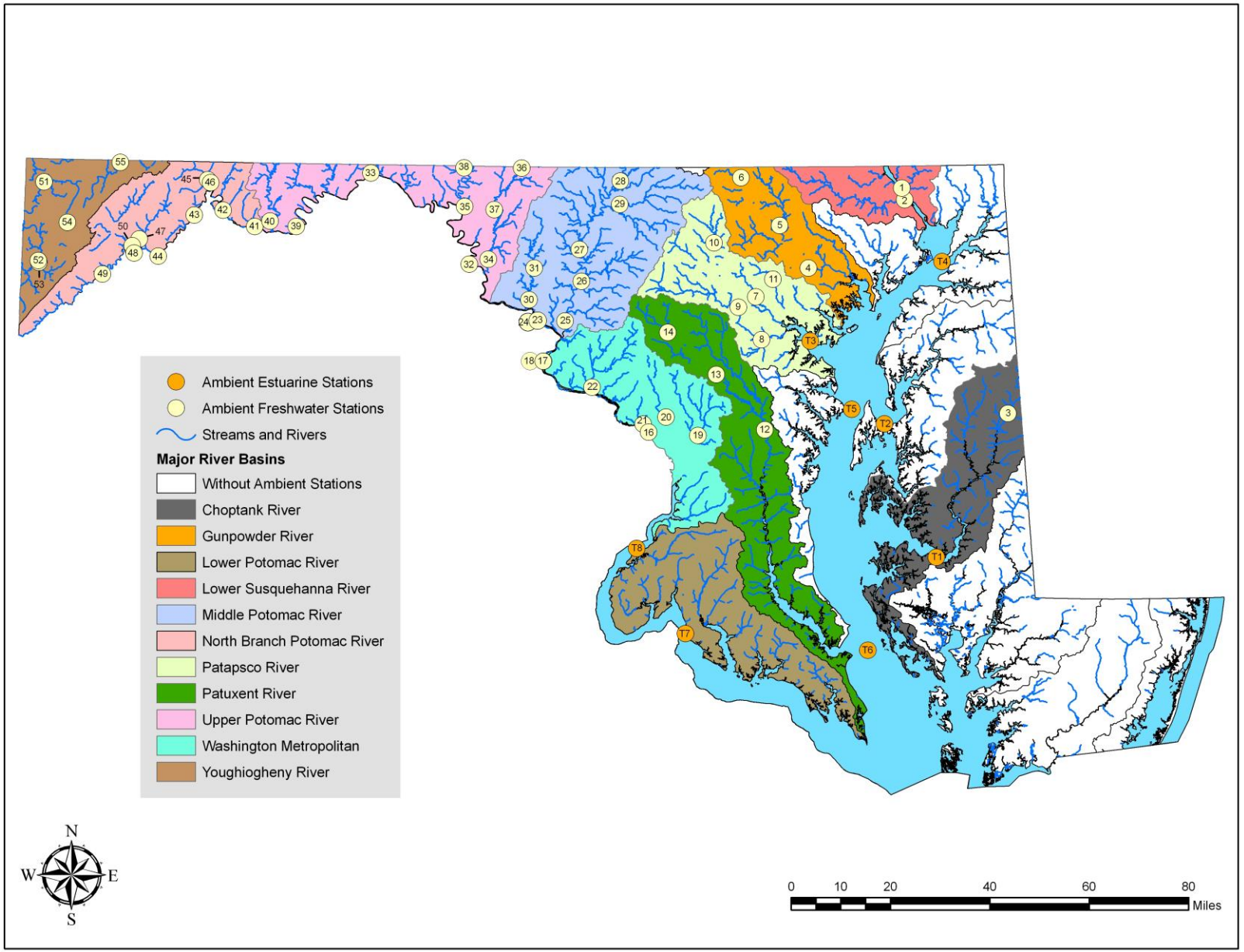


Table 1. Maryland Department of Natural Resource's Ambient Water Quality Monitoring Station Information.

Map #	Station I.D.	Stream Name	River Mile	Longitude (NAD 83)	Latitude (NAD 83)	Description and Site Type (Core or Trend)
02-12-02 SUSQUEHANNA RIVER BASIN						
1	CB1.0 (SUS 0109)	Susquehanna River	10.90	076 10.5023788	39 39.3729986	Below Conowingo Dam at boat ramp - gaging station USGS-01578310 - C
2	DER 0015*	Deer Creek	1.50	076 09.8863318	39 37.4085651	Bridge on Stafford Bridge Road – USGS-01580520- Tr
02-13-04 CHOPTANK RIVER BASIN						
T1	ET5.2 (XEH 4766)	Choptank River		076 03.5202530	38 34.8394323	At drawspan on U.S. Rt. 50 bridge - C
3	ET5.0 (CHO 0626)	Choptank River	62.60	075 47.1864631	38 59.8311087	At Red Bridges near Sewell Mills USGS-01491000 - C
02-13-05 CHESTER RIVER BASIN						
T2	XGG 8251	Kent Island Narrows		076 14.8401240	38 58.2675736	At drawspan on MD Route 18 bridge - C
02-13-08 GUNPOWDER RIVER BASIN						
4	GUN 0125	Gunpowder Falls	12.50	076 31.7336277	39 25.5375149	At bridge on Cromwell Bridge Road - C
5	GUN 0258*	Gunpowder Falls	25.80	076 38.1520258	39 33.0386351	End of Glencoe Road at old bridge crossing USGS – 01582500 - C
6	GUN 0476	Gunpowder Falls	47.60	076 46.8285205	39 41.3615564	Bridge at Gunpowder Road –USGS-01581810- C
02-13-09 PATAPSCO RIVER BASIN						
7	GWN 0115*	Gwynns Falls	11.50	076 43.5833003	39 20.5671785	At bridge on Essex Road in Villa Nova near gaging station USGS-01589300 - C
8	PAT 0176	Patapsco River	17.60	076 42.3202382	39 13.0687759	At bridge on Washington Boulevard (U.S. Rt. 1) - C

Table 1. Maryland DNR's Ambient Water Quality Monitoring Sampling Locations (cont.)

Map #	Station I.D.	Stream Name	River Mile	Longitude (NAD 83)	Latitude (NAD 83)	Description and Site Type (Core or Trend)
02-13-09 PATAPSCO RIVER BASIN (cont.)						
9	PAT 0285	Patapsco River	28.50	076 47.5345192	39 18.7467204	At bridge on Md. Rt. 99 near Hollofield gage USGS-01589000 - Tr
10	NPA 0165*	North Branch Patapsco River	16.50	076 52.9250807	39 28.9671330	Wire pedestrian bridge in industrial plant near gage USGS-01586000 - C
11	JON 0184	Jones Falls	10.8	076 39.68155	39 23.0730508	Bridge on Falls Road (Md. Rt. 25) near Sorrento gage– USGS-01589440 - C
T3	WT5.1 (XIE 2885)	Patapsco River	5.31	076 31.3521434	39 12.7856735	At buoy 5M, Hawkins Point - C
02-13-11 PATUXENT RIVER BASIN						
12	TF1.0 (PXT 0603)	Patuxent River	61.58	076 41.6465749	38 57.3343692	At bridge on U.S. Route 50 USGS-01594440 - C
13	PXT 0809	Patuxent River	81.91	076 52.4958913	39 07.0081428	At the gaging station just below Rocky Gorge Dam USGS-01592500 - C
14	PXT 0972*	Patuxent River	102.22	077 03.3713472	39 14.3584868	At bridge on Md. Route 97 near Unity gage USGS-01591000 - C
02-13-99 CHESAPEAKE BAY MAINSTEM						
T4	CB2.1 (XJH 6680)	Chesapeake Bay		076 01.5594740	39 26.4894865	200 yds. northeast of buoy RBA, mid-bay, south of Turkey Point, 15' depth - C
T5	CB3.3C (XHF 1373)	Chesapeake Bay		076 22.1808	39 00.84772	2100 yds., NE of Sandy Point, 55' depth - C
T6	CB5.1 (XCG 8613)	Chesapeake Bay	94	076 18.6833820	38 18.6510555	Off Patuxent River near mid-channel - depth 55 - 100' - C
02-14-01 LOWER POTOMAC RIVER BASIN						
T7	RET2.4 [a] (XDC 1706)	Potomac River		076 59.4376865	38 21.7559638	In mid-channel at Morgantown Bridge (U.S. Route 301), 58' depth - C
T8	TF2.3 [a] (XEA 6596)	Potomac River		077 10.4383095	38 31.8040859	Buoy N54 off Indian Head, 44' depth - C

Table 1. Maryland DNR's Ambient Water Quality Monitoring Sampling Locations (cont.)

Map #	Station I.D.	Stream Name	River Mile	Longitude (NAD 83)	Latitude (NAD 83)	Description and Site Type (Core or Trend)
02-14-02 WASHINGTON METROPOLITAN AREA						
16	POT 1184 [a]	Potomac River	118.40	077 07.6400929	38 56.8928182	At gaging station just above Little Falls Dam USGS-01646500 - C
17	POT 1471 [a]	Potomac River	147.10	077 31.2750641	39 09.2651668	At Eastern Terminus off Whites Ferry - C
18	POT 1472 [a]	Potomac River	147.0	077 31.3390209	39 09.3307768	At Western Terminus of Whites Ferry - Tr
19	ANA 0082 [a]	Anacostia River	8.20	076 56.6068030	38 56.3360716	At boat ramp in Bladensburg Waterfront Park - C
20	RCM 0111 [a]	Rock Creek	11.10	077 03.7817405	38 59.5812919	At bridge on Md. Route 410 - Tr
21	CJB 0005 [a]	Cabin John Branch	0.50	077 08.9301668	38 58.4069338	At bridge on MacArthur Boulevard - Tr
22	SEN 0008 [a]	Seneca Creek	0.80	077 20.3781583	39 04.7749739	At bridge on Md. Route 112 - Tr
02-14-05 UPPER POTOMAC BASIN						
23	POT 1595 [a]	Potomac River	159.50	077 32.6203211	39 16.4085768	At boat ramp just downstream of MD side of U.S. Rt. 15 near Pt. of Rocks USGS-01638500 – Tr
24	POT 1596 [a]	Potomac River	159.55	077 32.8740048	39 163250283	At boat ramp just upstream of VA side of U.S. Rt. 15 near Pt. of Rocks - Tr
25	MON 0020 [a]	Monocacy River	2.00	077 26.4946321	39 16.3025469	Bridge on MD 28 - C
26	MON 0155 [a]	Monocacy River	15.50	077 22.8656221	39 23.2669471	Pine Cliff Park ramp upstream of bridge - C
27	MON 0269 [a]	Monocacy River	26.90	077 23.3631412	39 28.8165566	Bridge on Biggs Ford Rd. - C
28	MON 0528* [a]	Monocacy River	52.80	077 14.0929806	39 40.7500155	At bridge on MD 140, near gage house in Bridgeport– USGS – 1639000 - C
29	BPC 0035 [a]	Big Pipe Creek	3.50	077 14.2924934	39 36.7306812	Bridge on Md. Rt. 194 USGS gaging station USGS – 1639500 - Tr

Table 1. Maryland DNR's Ambient Water Quality Monitoring Sampling Locations (cont.)

Map #	Station I.D.	Stream Name	River Mile	Longitude (NAD 83)	Latitude (NAD 83)	Description and Site Type (Core or Trend)
02-14-05 UPPER POTOMAC BASIN (cont.)						
30	CAC 0031 [a]	Catoctin Creek	3.10	077 34.8107379	39 19.9069327	Right bank, just upstream of bridge on Md. Route 464 - Tr
31	CAC 0148* [a]	Catoctin Creek	14.80	077 33.5401108	39 25.5468858	Right bank just downstream ofbridge on Md. Route 17, at gaging station USGS-01637500 - Tr
32	POT 1830 [a]	Potomac River	183.00	077 48.1594887	39 26.1046394	At boat ramp WV side below bridge on Md. Rt. 34, near discontinued gage USGS-01618000 (discontinued 2004) - C
33	POT 2386	Potomac River	238.60	078 10.5781510	39 41.4671425	At rocky outcropping downstream of boat ramp @ C&O park, near gaging station 0.5 mile below bridge on U.S. Rt. 522 USGS-0161300 - C
34	ANT 0044* [a]	Antietam Creek	4.40	077 43.8991688	39 27.0219634	Downstream of Sharpsburg gage house, streamside – USGS-01619500 - Tr
35	CON 0005	Conococheague Creek	0.50	077 49.2963323	39 36.1943845	Md. 68 bridge - C
36	ANT 0366*	Antietam Creek	20.30	077 36.4935486	39 42.9592863	At bridge on Millers Church Road adjacent to gaging station at Rocky Forge – USGS – 01619000 - Tr
37	ANT 0203	Antietam Creek	20.30	077 42.6475848	39 35.6775584	At bridge on Poffenberger Rd. near Funkstown - C
38	CON 0180	Conococheague Creek	18.00	077 49.5032338	39 42.9627173	At gaging station on Wishard Rd. USGS-01614500 - Tr
39	POT 2766	Potomac River	276.60	078 27.2695565	39 32.3189316	At bridge on Md. Rt. 51 near Paw Paw, W. Va. USGS-01610000 - Tr
40	TOW 0030* [+]	Town Creek	3.00	078 33.2032866	39 33.1821660	At gage on Pack Horse Road USGS-01609000 - C
02-14-10 NORTH BRANCH POTOMAC RIVER BASIN						
41	NBP 0023 [+]	North Branch Potomac	2.30	079 39.3300605	37 58.4618290	Toll bridge at Oldtown - Tr
42	NBP 0103 [+]	North Branch Potomac	10.30	078 43.8873501	39 34.9607011	Boat ramp off Rt. 51 in Spring Gap - C

Table 1. Maryland DNR's Ambient Water Quality Monitoring Sampling Locations (cont.)

Map #	Station I.D.	Stream Name	River Mile	Longitude (NAD 83)	Latitude (NAD 83)	Description and Site Type (Core or Trend)
02-14-10 NORTH BRANCH POTOMAC RIVER BASIN (cont.)						
43	NBP 0326	North Branch Potomac	32.60	078 50.3348823	39 34.0064182	Bank sample upstream MD route 936 bridge at Pinto- USGS:01600000 (discontinued) - C
44	NBP 0461 +	North Branch Potomac	46.10	078 58.3048527	39 26.6943955	Bank sample under U.S. Route 220 bridge- Tr
45	BDK 0000	Braddock Run	0.01	078 47.4487205	39 40.2286587	Old Mt. Savage Road bridge - Tr
46	WIL 0013*	Wills Creek	1.38	078 46.8174564	39 39.7110428	Locust Grove Road bridge crossing near gaging station USGS-01601500 - Tr
47	GEO 0009* +	Georges Creek	0.90	079 02.6819423	39 29.6183080	Victory bridge in Westernport next to Town Hall - near USGS – 01599000 - C
48	NBP 0534 +	North Branch Potomac	53.48	079 04.0814362	39 28.7536221	North Branch at Bloomington just upstream of confluence with Savage River USGS-01596000 - C
49	NBP 0689	North Branch Potomac	68.90	079 10.7614696	39 23.3607386	Rt. 38 bridge over North Branch USGS – 01595500 - C
50	SAV 0000	Savage River	0.02	079 04.0838436	39 28.8359583	Savage River at Md. 135 - Tr
05-02-02 YOUGHIOGHENY RIVER BASIN						
51	YOU 0925 +	Youghiogheny River	94.00	079 24.5074447	39 39.1739972	Bridge Crossing in Friendsville on Main street near USGS – 03076500 - Tr
52	YOU 1139	Youghiogheny River	115.91	079 25.3143438	39 25.4158776	Liberty Street/Herrington Manor Bridge crossing near USGS – 03075500 - Tr
53	LYO 0004 +	Little Youghiogheny R.	0.38	079 25.1550365	39 25.1060846	Bridge Crossing at Oakland/Rosedale Rd. - Tr
54	CCR 0001 +	Cherry Creek	0.15	079 18.9509986	39 32.2335135	Bridge Crossing on State Park Road –USGS-03075905- Tr
55	CAS 0479	Casselman River	47.92	079 08.1846184	39 42.1242778	Casselman River where crossed by River Road at USGS – 03078000 - Tr

* Ambient Water Quality Monitoring Stations that are also sampled separately as part of the Chesapeake Bay Program's Non-tidal Network – 117(e). For additional information on this monitoring program, please see Maryland DNR's Non-tidal Network, Quality Assurance Project Plan, 2014.

+ Western Maryland stations sampled for additional water quality parameters - sulfate and chloride (106 supplemental)

[a] Potomac River Basin Ambient Water Quality Monitoring Stations sampled for an additional water quality parameter – 5-day biochemical oxygen demand

( ) = Original Station Name

Table 2. Water Quality Parameters, Methods, Preservation/Holding Times and Method Detection Limits for MDNR's Ambient Water Quality Monitoring Program.

Parameter (units)	Method/Reference	Condition/ Holding Time	Method Detection Limits
Field (In situ)			
Temperature (°C)	N.B.S. calibrated EPA 1979 #170	N/A- *in situ*	0.1°C
Dissolved Oxygen (mg/L)	Luminescent method ASTM-D888-09 (C)	N/A- *in situ*	0.2 mg/L
pH	Glass Probe EPA 1979 #50	N/A- *in situ*	0.1 unit
Specific Conductance (umhos/cm)	Conductivity Bridge APHA #205	N/A- *in situ*	5% of calibration standard
Secchi Disc (cm) *estuarine stations*	20 cm Black/White	N/A- *in situ*	0.1 meter
Laboratory			
Dissolved Organic Carbon (mg/L)	Standard Method 5310B	Frozen, 28 days/ 4 °C, 48 hrs	0.0517 mg/L
Particulate Carbon (mg/L)	Exeter Analytical Model CE-440 Elemental analyzer	Frozen 28 days	0.032 mg/L
Ammonium (mg/L)	EPA Method 350.1	Frozen, 28 days/ 4 °C, 48 hrs.	0.0016 mg/L
Particulate Nitrogen (mg/L)	Exeter Analytical Model CE-440 Elemental analyzer	Frozen 28 days	0.006 mg/L
Total Dissolved Nitrogen (mg/L)	EPA Method 353.2	Frozen, 28 days/ 4 °C, 48 hrs.	0.0223 mg/L
Nitrate + Nitrite (mg/L)	EPA Method 353.2	Frozen, 28 days/ 4 °C, 48 hrs.	0.0052 mg/L
Nitrite (mg/L)	EPA Method 353.2	Frozen, 28 days/ 4 °C, 48 hrs.	0.0005 mg/L
Orthophosphate (mg/L)	EPA Method 365.1	Frozen, 28 days/ 4 °C, 48 hrs.	0.0005 mg/L
Particulate Phosphorus (mg/L)	EPA Method 365.1	Frozen 28 days	0.0005 mg/L
Total Dissolved Phosphorus (mg/L)	EPA Method 365.1	Frozen, 28 days/ 4 °C, 48 hrs.	0.0054 mg/L
Biochemical Oxygen Demand (BOD)	Standard Method 5210B	4 °C 48 hrs.	NA
Total Suspended Solids (mg/L)	Standard Method 2540D	4 °C 7 days	0.7 mg/L
Turbidity (NTU)	EPA Method 180.1	4 °C 48 hrs.	0.1 NTU
Chlorophyll "a" (µg/L)	Standard Method 10200 H EPA Method 446.0	Frozen 28 days	0.62 µg/L
Phaeophytin "a" (µg/L)	Standard Method 10200 H EPA Method 446.0	Frozen 28 days	0.74 µg/L
Chloride (mg/L)	Standard Method 4110B	4 °C 28 days	0.08 mg/L
Sulfate (mg/L)	Standard Method 4110B	4 °C 28 days	0.09 mg/L
Alkalinity, Total (mg/L)	Standard Method 2320B	4 °C 14 days	1 mg/L

MEASUREMENT/DATA ACQUISITION

B1 Program Design

Table 1 on pages 9-13 provides the station location descriptions for Maryland DNR's Ambient Water Quality Monitoring Program. Water quality data are collected at select stations in tributaries of the Choptank, Gunpowder, Patapsco, Patuxent, Susquehanna, Potomac and Youghiogheny Rivers. The selection of stations for this monitoring program was guided primarily by the need to assess conditions in water use areas. These included recreational areas, surface water supply areas, land use areas and potential areas of development. All ambient stations are sampled monthly (12 collections/year) on a pre-determined date. This sampling design allows the collection of data over a wide range of stream/river flows and provides adequate data for capturing long-term annual trends.

B2 Sampling Methods

Maryland DNR staff obtain field data and water quality samples (grab samples) from all stations by land (freshwater stations) or boat (8 estuarine stations). Bucket sampling is used on all land runs to collect samples from bridges, weirs, and stream banks. Bridge sampling is the preferred sample method and is used whenever possible. For the tidal stations, a submersible pump is used to collect a surface sample (at 0.5 m depth) and several other samples at various depths, from a boat. Appendix II provides the Standard Operating Procedures for Maryland DNR's Ambient Water Quality Monitoring Program (referred to as #PR-03: Maryland Core/Trend Monitoring Program).

B2.1 Field Measurements

Maryland DNR personnel obtain field data with the use of multi-parameter instruments. The operation and calibration protocols are outlined in Section 6.0 in MDNR's Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program (Appendix II). The instruments are maintained according to the operating manuals provided by the manufacturers for each instrument. Field measurements include dissolved oxygen, temperature, specific conductance and pH. These parameters are currently measured with instruments manufactured by Hydrolab or YSI. At the estuarine stations, secchi depth is measured with a 20 centimeter black and white secchi disc. Table 2 provides the methods and detection limits for the field measurements.

B2.2 Water Quality Samples

Grab samples for each station are collected by MDNR personnel utilizing methods dependent on the physical conditions of the station location. Estuarine stations (8) are sampled by boat, whereas the land run stations are sampled from a bridge, weir, or streamside. At each land run station, a surface grab sample is collected, if possible, at mid-channel. For a complete description of the collection methods utilized under each condition please refer to Section 8.0 in MDNR's Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program (Appendix II). Both whole water and filtered water samples for each freshwater station are provided to the Maryland Department of Health

(MDH), Environmental Chemistry Division for analysis. A complete list of the physical and analytical parameters obtained, holding times, methods, and method detection limits is provided in Table 2 (page 15).

B3 Sample Handling and Custody

Laboratory samples are placed on ice (i.e., stored at 4°C) in a large cooler and transported by Maryland DNR field personnel to Annapolis. Iced samples are then shipped overnight directly to MDH. Frozen samples are placed in a freezer in Annapolis and delivered weekly to MDH. Table 2 provides the holding times for each water quality parameter. For a complete description of sample handling and the procedures utilized to label and track all samples, please see Sections 9 and 10 in MDNR's Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program (Appendix II). Because the data generated by MDNR's Ambient Water Quality Monitoring Program are not used for legal purposes, formal chain-of-custody procedures are not required.

B4 Analytical Methods

Table 2 provides a list of analytical methods for all water quality parameters utilized by MDNR's Ambient Water Quality Monitoring Program. All analysis (except chlorophyll "a" and phaeophytin "a") is conducted by the State of Maryland, MDH Laboratories. The Standard Operating Procedures for all water quality parameters utilized by MDH are detailed in Appendix III. For each water quality parameter, scope of application, methods, equipment and supplies, reagents and standards, sample collection, quality control, procedures, data analysis and calculations, and data management are detailed. Starting in January 2009, chlorophyll "a" and phaeophytin "a" is analyzed by the University of Maryland, Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory (NASL). These parameters were originally measured by MDH. The analytical methods utilized by NASL are identical to the ones that were utilized by MDH and are detailed in Appendix VIII of the Quality Assurance Project Plan for Maryland DNR's Chesapeake Bay Water Quality Monitoring Program – Chemical and Physical Properties Component. The latest version of this Plan is available at:
http://mddnr.chesapeakebay.net/eyesonthebay/documents/MdDNR_MTQAPP2018.pdf

B5 Quality Control

The data collected as part of the Ambient Water Quality Monitoring Program are used in making management decisions regarding Chesapeake Bay water quality as described in section A5. DNR follows specific procedures to ensure that the design is properly implemented and that monitoring measurements are made and managed with sufficient accuracy, precision, and detection limits. Section 11.0 in Appendix II provides a list of quality control and quality assurance procedures that are utilized for this monitoring program. General discussions of quality assurance and quality control aspects associated with accuracy, precision, and audits are provided in the subsections below.

B5.1 Accuracy

The accuracy (closeness to the true value) of the collected data is controlled and assured by the proper use, calibration, and maintenance of both field and laboratory equipment for the measurement of physical and chemical parameters. All instruments are identified by a unique number, used as an index for documentation of calibration, repair, and

preventive maintenance. Daily quality control checks (including the running of blanks and standards) are used to control and assure laboratory accuracy. Accuracy of laboratory results is also assessed through DNR's participation in the Chesapeake Bay Coordinated Split Sample Program (CSSP), a split sampling program in which nine laboratories involved in Chesapeake Bay monitoring analyze the coordinated split samples.

B5.2 Precision

Precision of the chemical analytical methods is determined and documented from duplicate analyses. Every tenth sample is analyzed in duplicate at MDH. Precision of the entire measurement system for laboratory-analyzed parameters, including field processing, storage, and chemical analysis, can be assessed from duplicate field samples. Duplicate field samples are routinely collected approximately every 10 to 20 samples.

B5.3 Audits

Performance audits for chemical analyses are based on the results of samples distributed by the EPA Chesapeake Bay Program Blind Audit Program. These samples must fall within the 95% confidence interval for acceptance. If results fall outside this range, corrective actions for each parameter and measurement are taken. The DNR Quality Assurance Officer communicates on a weekly basis with the field program staff and confers with the laboratory quality assurance officers to ensure that all aspects of the program are being conducted properly.

B6 Instrument/Equipment Testing, Inspection, and Maintenance

Field crews carry two calibrated multi-parameter instruments in case of failure. The meter in use is compared to the reserve meter any time (a) the field scientist recording measurements observes values outside the "typically expected range"; (b) the meter generates variable or erratic values; or, (c) the meter in use displays an error message. If the meters do not agree within acceptable limits, the reserve meter is used. This is noted on the field data sheet (the field sheet is illustrated in MDNR's Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program (Appendix II)). All equipment repairs are handled by the Field Quality Assurance Officer for this monitoring program. All parts are ordered directly from the manufacturer. If the repairs cannot be performed by the Field Quality Assurance Officer, the instrument is sent to the manufacturer for repairs.

Laboratory instrument standard operating procedures include preventive maintenance procedures as well as performance checks and calibration procedures. Appropriate maintenance is scheduled based on the results of performance checks or after a specified number of hours of operation. Instrument preventive maintenance, repairs, and analytical corrective actions are documented on laboratory notebooks or on MDH's Analytical Corrective Actions form (Appendix IV).

B7 Instrument/Equipment Calibration and Frequency

As mentioned previously, Section 6.0 in MDNR's Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program provides detailed information regarding multiparameter instrument (Hydrolab or YSI) calibration. The following information provides general procedures that should be followed while utilizing multiparameter instruments for this monitoring program.

Calibration and Frequency

A. Set up a calibration logbook for each unit, with make, model, and serial number and purchase date. Assign a letter for MDNR use as required.

B. Calibrate meters on Friday for use the next week. After one to four days of field use, post calibrate equipment to determine if parameters have drifted.

C. Specific conductance calibration shall be made using standards generated by the field office from dry KCl and deionized water. Standards used are 292, 718, 2767, 6668, 12950, and 24820 microsiemens/cm (microsiemens=microS=ΦS); or 0.002, 0.005, 0.02, 0.05, 0.1, and 0.2 molar KCl, respectively. (At 25 EC microsiemens/cm = micromhos/cm.)

D. A pH calibration shall be made using premixed standards of color-coded pH 4.00, pH 7.00 and pH 10.00 purchased from Fisher Scientific. Standards are specifically labeled (contain expiration dates) and color coded - red for pH 4.00, yellow for pH 7.00 and blue for pH 10.00.

E. The dissolved oxygen calibration method is dependent on the type of dissolved oxygen probe used on the instrument. If the instrument is outfitted with a standard Clark cell, the dissolved oxygen calibration shall be done on the common standard of water-saturated air. After correcting for the barometric pressure and temperature, the oxygen content of water-saturated air can be checked against standard DO tables. The DO membrane is also visually checked every time the meter is pre- or post-calibrated. If the membrane appears damaged, the meter is posted as is. Then the membrane and electrolyte are replaced and the meter is calibrated after 24 hours. If the instrument is outfitted with an optical probe (LDO for Hydrolab; ROX for YSI) then the standard of air-saturated water is used. After correcting for the barometric pressure and temperature, the oxygen content of the air-saturated water can be checked against standard DO tables. The cap (LDO) or membrane (ROX) should also be visually checked every time the meter is pre- or post-calibrated. As of March 2015, all sondes were outfitted with optical probes.

F. Record all pre-calibration, post-calibration, and maintenance procedures in the log book, including any values (e.g. barometric pressure, temperature) that are used in the calibration procedures. An example of the equipment calibration log is included.

G. Record any unusual circumstances that may affect the instrument readings in the logbook.

B8 Inspection/Acceptance of Supplies and Consumables

From 1974 to September 2007, the deionized water used at the MDNR Annapolis field office was generated from Annapolis City water passed through a non-pressurized Barnstead cartridge system equipped with two Ultrapure mixed bed cartridges and one organic removal cartridge. This produced ASTM Type III water. Starting on September 10, 2007, the MDNR field office switched to a system that produces ASTM Type II water. The deionized water is generated from tap water using a Thermo Scientific Barnstead DIamond TII RO/DI system with a GE SmartWater external pre-filter. The RO/ DI system is linked to a Thermo Scientific Barnstead DIamond TII 60L storage reservoir. The system uses a thin film composite reverse osmosis membrane with pretreatment to produce RO water. This water is then put through a two-stage deionization process combined with UV oxidation and a 0.2 micron final filter. The reagent grade water provided by this system exceeds ASTM Type II and NCCLS/CAP Type I standards. All manufacturer recommendations are followed regarding cartridge replacement and system sanitation (Refer *Apr 17, 2008, Revision 14, QAPP: Chemical & Physical Property Component Page VI-3* to Thermo Scientific. 2007. Barnstead DIamond TII Type II Water System Operation Manual and Barnstead DIamond TII Type II Storage Reservoir Operation Manual). The GE SmartWater pre-filter was placed inline to improve the integrity of feed-water going into the Barnstead DIamond System. The pre-filter is changed at least every three (3) months or more frequently during periods of heavy use. A log is kept at the front of the DI System Manual to document all changes and updates made to the system.

The Maryland Department of Health and Mental Hygiene produces deionized water by utilizing a water system provided, serviced and set up by Millipore. In this system, tap water is passed through a 1 micron filter, a carbon tank, and two mix bed ion exchange resin columns. The water is then subjected to UV oxidation and passed through a 0.2 micron filter. Appendix III (DHMH's Standard Operating Procedures for Water Quality Parameters) lists all supplies and consumables utilized by the analytical laboratory for sample analysis.

B9 Non-direct Measurements

No data are needed for implementation or decision-making that are obtained from non-direct measurement sources.

B10 Data Management

Data collection for the Ambient Water Quality Monitoring Program begins when measurements from field recording instruments are entered onto field data sheets. A section on the field sheet is used to document any problems encountered in the field that might affect the field parameters or the samples brought back for the laboratory analysis. A senior field scientist ensures that all measurements are taken and recorded properly. After field personnel have completed data sheets for a given calendar month, they make a photocopy of the sheets to keep in the Field Office, and send the original field sheets to data management staff at the MDNR Tawes State Office Building in Annapolis. The Field Office also generates a Cross Reference Sheet for each set of field sheets, which is sent to the DNR data management personnel along with the field data sheets. The Cross Reference Sheet provides the data management personnel with the documentation to determine what field and laboratory results to expect. The Cross Reference Sheet is illustrated in

Appendix B contained in MDNR's Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program (Appendix II).

Laboratory analysis sheets are also initiated in the field (laboratory sheet is illustrated in Appendix D MDNR's Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program (Appendix II)). These laboratory sheets list each parameter requested for analysis and include basic information about the sample, such as station, date, time, and depth. The sheets serve as sample transfer sheets, traveling with the samples to the Maryland Department of Health laboratory (MDH) for analysis. Both the sheets and the samples are logged in at the laboratory.

The laboratory analyst reviews the data and, if the data exceed their control limits, the entire run is re-analyzed. Re-analysis can occur for any number of reasons, such as, a poor r-squared on the standard curve, the wrong set of pump tubes (which would provide abnormally low peaks), or high blank values (in the case of DOC). Once laboratory staff has completed the laboratory sheets, they are sent to the DNR data management at the Tawes Building.

Data review and verification are conducted at four levels by DNR data management personnel. At the first level, DNR data management personnel review cross reference sheets and field data sheets: (1) comparing field sheets to cross reference sheets to ensure that all field sheets have been received; (2) reviewing all field sheets to check that they are filled out completely and legibly, and; (3) sending the sheets to a data entry service for keypunch. At the data entry service, the field sheet data are double-entered to minimize errors at the keypunch stage. The entered field data are sent back to DNR as an electronic file for further processing.

At the second level, a Data Processing Technician generates reports and plots for data verification using the Water Quality Import v3 software. The WQ Import v3 software was designed in late 1998 and completely developed in 2000 in Microsoft Access. The WQ Import v3 software is used to conduct data management activities, such as performing an initial data check, conducting major key field checks, performing a parameter range check (including measured and calculated parameters), conducting combination checks for specific parameters, generating an error report and verification plots, generating a "data verified list," reforming data, creating a database, and submitting data.

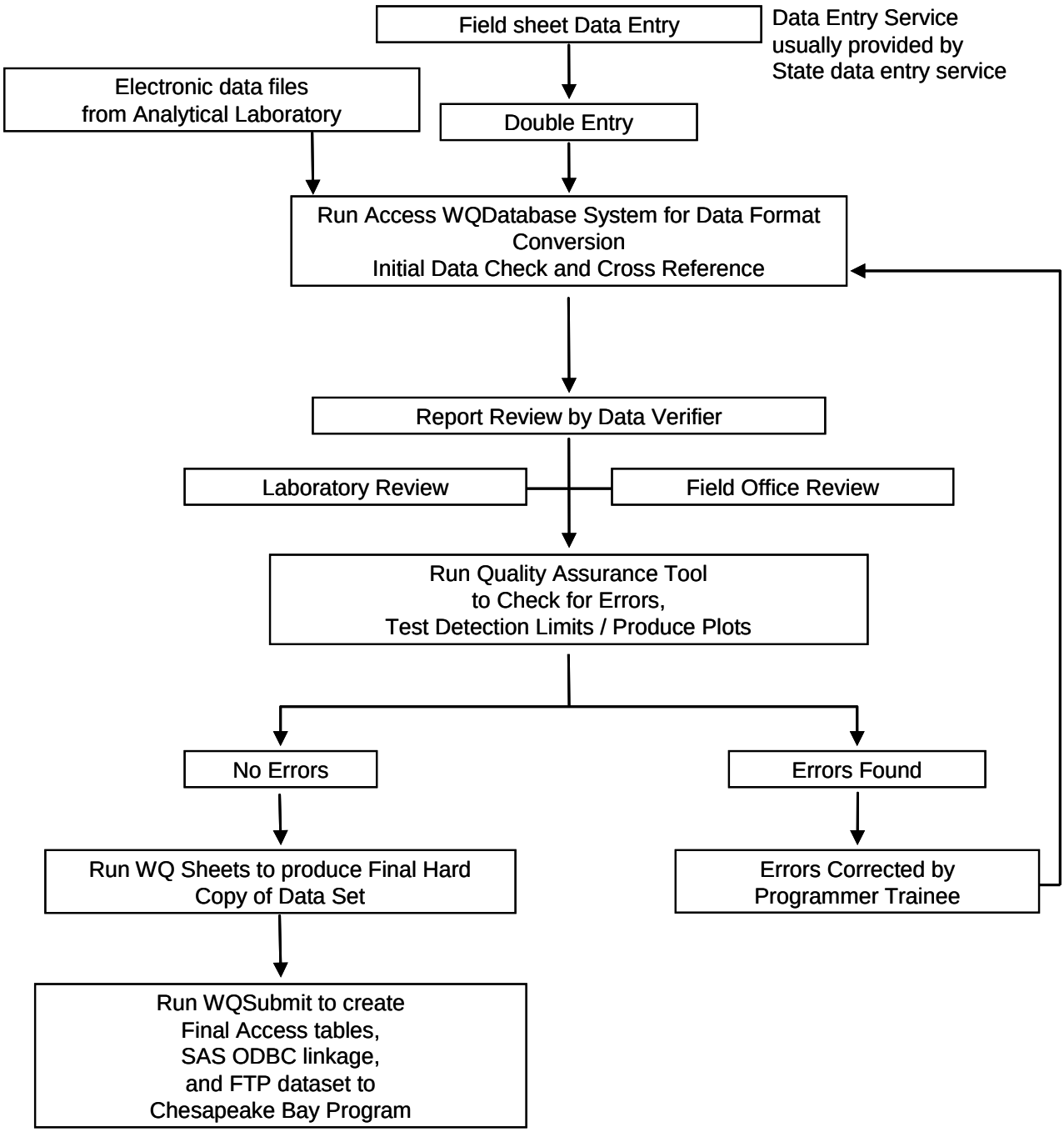
Third, system printouts or PDF files of each data set are sent to a biologist and the Quality Assurance Officer for verification and editing. The Quality Assurance Officer and DNR biologists ensure that measured or calculated information for all types of data are correct and that the codes associated with parameters are properly established. In addition, the Quality Assurance Officer identifies data problems, provides data correction instructions, and coordinates data correction activities. Possible errors are identified, and sent to the laboratory or field office for verification or verified over the phone. Any necessary corrections are written on an edit form, which is given to a Data Processing Technician. The technician makes changes to correct the electronic data set, re-runs the verification programs, and updates the verification reports and plots. This procedure is repeated until a clean data set is produced.

The fourth step is for data management staff to ensure that the overall data verification processes are completed, all data errors are corrected, and that the finalized data sets are created and formatted to be consistent with historical data sets.

The final data set combining the field and laboratory data is created as an Access "MDB file" after the completion of data verification processes. This final data set is stored in a local designated DNR database directory for data user access. Data requests should be directed to Mark Trice, Program Chief of Water Quality Informatics (410-260-8630). A formatted submission data set and associated data documentation is also transferred to the Chesapeake Bay Program Data Center on a monthly basis.

Files submitted by DNR are further screened by the Bay Program's Quality Assurance Tool (QAT). The QAT generates a Chesapeake Information Management System (CIMS) Water Quality Data Quality Assurance Report for each file submitted. The Report identifies fatal errors for records that are incompatible with the CIMS database and prevents files that have fatal errors from being entered into CIMS. Nonfatal errors, such as those with values falling out of a historical range are reviewed and accepted or rejected. Once data are entered into the CIMS database, they are available to the public via the Bay Program datahub at: www.chesapeakebay.net. The data management process is diagramed in Figure 3.

Figure 3. Data Management Flow Chart: Data Entry through Production of Final Master Data Set



ASSESSMENT AND OVERSIGHT

Program and performance audits verify that procedures specified in the Project Plan are being followed throughout the entire MDNR Ambient Water Quality Monitoring Program. These audits ensure the integrity of the reported data so they can be used for their intended purpose.

C1 Assessments and Response Action

C1.1 Field Activities

If a station or specific sample cannot be collected, it is noted on the cross-reference sheet. Specific problems associated with field collection of a site are also noted on the field sheet. Conditions that may affect data results are included in the comments section of the field sheet so that they are available to each data analyst. If post calibration results are outside acceptable limits, the individual calibrating notifies the Field QA Officer who decides if data should be deleted or flagged. If split sample results suggest that there is a problem with the data, the issue is thoroughly discussed by laboratory and field representatives at DIWG and possible solutions are offered. The Field QA Officer regularly reviews Equipment Log Books to ensure that all staff is following QC procedures. Standard maintenance recommended by Hydrolab and YSI is performed at twelve-week intervals. All serious Quality Control issues are reported directly to the Field Office Project Chief. Audits of the field staff are conducted as needed.

C1.2 Laboratory Activities

Corrective actions are initiated by the analyst, with the input of the Lead Scientist of the Laboratory Section, if necessary. The Lead Scientist and the Supervisor review corrective actions. A copy of the completed form is submitted to the division QA officer, and the original is kept in the laboratory. The MDH Division of Environmental Sciences is audited approximately every three years by EPA Region III or Chesapeake Bay Program Office staff.

C1.3 Data Management Activities

The Data Input Editor is the first line of defense for data correction. Maryland DNR data management personnel review all incoming data and compare the data to the cross-reference file. Data management personnel verify the submitted data and apply corrections to the physical datasheet if errors are identified. During the data-import process, a Data Processing Technician makes all corrections to the data and key fields as they are imported into the WQ Database System. The Data Processing Technician assists where needed in constructing better tools to edit and apply to large quantities of data corrections if necessary. Documenting the correction is handled within WQ Maintenance process. If the correction is fairly generic, edits to the changes are logged. There is no formal documentation for editing data sheets. These tasks are considered extreme and performed only when confirmed by field office or laboratory personnel.

C2 Reports to Management

Maryland DNR provides four quarterly progress reports (January, April, July and November) that indicate the number of Ambient Water Quality Stations that were sampled and an update on the status of water clarity assessments, efforts to acquire and assess data for the Integrated Report and a copy of the State 305(b) update. Status reports also provide explanations, if needed, for why accomplishments fell short of the projections. In addition, any changes to the Quality Assurance Project Plan or the SOPs referenced herein are documented and approval is required for all of those involved in project management (i.e., the individuals responsible for the major aspects of this monitoring program).

DATA REVIEW AND USABILITY

D1 Data Review, Verification, and Validation

Field: Described in C1.1 above.

Laboratory: The MDH Environmental Sciences Division uses data review checklists for data validation (example provided in Figure 4). Appendix III provides copies of each data review checklist for all water quality parameters.

Data Management: The data-management group validates key fields. The key fields ensure that the data are accurate and will not be lost or duplicated within the system. After the key fields are validated, the values are plotted to discover any anomalies. The scientists/project managers review the reports and determine if any additional edits are needed to data values. These edits are reported to the Data Processing Technician who makes the changes (Figure 3).

D2 Verification Validation Methods

Maryland DNR data management personnel conduct data review and verification at four levels: At the first level, DNR data management personnel review cross reference sheets and field data sheets: (1) comparing field sheets to cross reference sheets to ensure that all field sheets have been received; (2) reviewing all field sheets to check that they are filled out completely and legibly, and; (3) sending the sheets to a data entry service for keypunch. At the data entry service, the field sheet data are double-entered to minimize errors at the keypunch stage. The entered field data are sent back to DNR as an electronic file on a diskette for further processing.

At the second level, a Data Processing Technician generates reports and plots for data verification using the Water Quality Import v3 software.

At the third level, system printouts of each data set are sent to a biologist and the Quality Assurance Officer for verification and editing. The Quality Assurance Officer and DNR biologists ensure that measured or calculated information for all types of data are correct and that the codes associated with parameters are properly established.

At the fourth level, data management staff ensure that the overall data verification processes are completed, all data errors are corrected, and that the finalized data sets are created and are formatted to be consistent with historical data sets. The final data set combining the field and laboratory data is created as an Access database file after completion of data verification processes. This final data set is stored in a local designated DNR database directory for data user access. A formatted submission data set and associated data documentation is also transferred to the Chesapeake Bay Program Data Center on a monthly basis.

D3 Reconciliation with User Requirements

The data generated by Maryland DNR's Ambient Water Quality Monitoring Program are utilized to calculate relative status and long-term linear and non-linear trends (Appendix I describes methods). These calculations are performed for MDNR under contract by a statistical consultant.

The experimental design of this program requires monthly collections of water quality data (i.e., 12 collections per year) which are adequate for capturing long-term annual trends (Alden et al., 1994). As a result, the data generated by this program directly meet the objectives for which it is collected.

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Quality Assurance Project Plan for the Maryland Department of Natural Resources Non-tidal Network Program for the period October 1, 2019 – September 30, 2020. 2019. Maryland Department of Natural Resources, Resource Assessment Service, Annapolis, MD

Appendix I: Methods for Calculating Status and Trends at Maryland DNR's Ambient Water Quality Monitoring Stations

Status and trend analyses are performed at all CORE/Trend monitoring stations for nutrients (nitrogen and phosphorus), field-measured parameters such as conductivity, pH, and water temperature, and total suspended solids, total alkalinity, and total organic carbon. Trends in chlorophyll and sulfate are also assessed at stations where those parameters are measured.

Water quality status is a measure of the current water quality condition compared to some benchmark. Trends are measured to assess changes in water quality over time. For the CORE/Trend stations increasing trends are generally associated with degrading water quality and decreasing trends are generally associated with improving water quality conditions. Linear and non-linear trend analyses are performed on "long-term" data (1986 to present) and "short-term" data (1995 to present). Trends for both long- and short-term periods are deemed significant if the probability value (p-value) is less than or equal to 0.01, a level which was chosen to reduce the Type I error rate (i.e., reduce the chance of stating that there is a trend when in fact there is no trend).

Relative Status

In the absence of water quality reference levels or restoration targets, members of the Data Analysis Workgroup (DAWG), which was a workgroup of the U.S. Environmental Protection Agency Chesapeake Bay Program Monitoring Subcommittee, developed a method of comparing stations in similar salinity zones (Aden and Perry, 1997). The first version of the relative status method was developed and implemented for the 1997 Re-evaluation effort of the Chesapeake Bay Program to assess progress in meeting nutrient reduction goals established in 1987.

The relative status method compares the median of the most recent three years of data for a specific parameter at a particular station to all stations in the same salinity regime using "cut-points" in a benchmark data set. The most recent three years of data are used in the status data set to even out year-to-year climatic variation. The benchmark data set consists of water quality data that were collected between January 1985 and December 1990 to establish a Bay-wide baseline against which to score water quality at each station. Data in the benchmark data set were partitioned using a beta cumulative distribution function to establish the "cut-points" for individual parameters in a particular salinity zone; in this case non-tidal, fresh water systems.

The status data set is scored using the logistic probability integral transform, and the score is adjusted based on sample size to account for the inter-dependence of observations. The lack of independence in observations at a site tends to result in too many observations in the ends of the distribution, i.e., in the "good" and "poor" categories. The adjustment results in a more even distribution of scores. Both the benchmark and status data sets are log transformed prior to analysis to meet the distributional assumptions of the procedure.

It is important to note that this is a relative ranking procedure. Just because a parameter at a station is scored as "good" does not mean that water quality is acceptable. It is only a relative indication that the station scored better than other stations rated as "fair" or "poor" based on a comparison to cut-points in the benchmark data set for similar stations.

Linear Trend Analysis

A combination of statistical procedures is used to calculate the significance of linear trends and the percent change in a water quality parameter over time. First, the seasonal Kendall test, which is a generalization of the Mann-Kendall test, is used to assess the presence of a trend in time in seasonal data (Hirsh, et al., 1982). The seasonal Kendall test was proposed by Hirsh for use in

data with 12 seasons (months). Basically, the Mann-Kendall "S" statistic and its variance VAR(S) are computed for each month (season) for data collected over a number of years. The seasonal statistics are then summed over the years and an overall Z statistic is then calculated, which is compared to standard normal tables to assess significance (Gilbert, 1987). The seasonal Kendall test is particularly useful for water quality trend assessment because it is non-parametric (i.e., does not assume any particular distribution in the data), and is not affected by missing, tied, or below detection limit values.

The magnitude of change in the data over time, for trends that are significant, is calculated using the seasonal Kendall slope estimator, which is a generalization of Sen's estimator of slope (Sen, 1968). The seasonal Kendall slope estimator is calculated from the median of all possible differences in the data. The magnitude of change is expressed as the percent change since the beginning of the period of record. Percent change is calculated by multiplying the Sen's slope estimator times the number of years of the study period, and dividing by the initial median:

$$\text{Percent change} = [(\text{slope}*\text{nyrs})/\text{base median}] * 100$$

The base median is calculated from the first two years of data. For most stations that period is January 1986 to December 1987.

Non-linear Trend Analysis

As the monitoring timeline has lengthened, non-linear trend analyses have been added. The utility of identifying non-linear trends was investigated by Alden (Alden et al., 2000). Non-linear trends provide a picture of how a parameter has changed within the period of evaluation and can serve as early warning signals for managers. These analyses identify whether a trend is primarily linear (generally unidirectional), U-shaped (decreasing early in the time series, increasing later in the time series), or the reverse (inverse U-shaped). They can also determine where the critical point (change in direction of trend), if any, is located (either within or outside the period of evaluation).

Non-linear trends are assessed using a general linear model with a linear TIME term and a quadratic ($TIME^2$) term. Data are log-transformed prior to the analysis in an effort to conform to the distributional requirements of the general linear model. A MONTH term is included in the model to account for seasonal effects. The TIME and $TIME^2$ terms are first standardized to a mean of zero to ensure that the TIME and $TIME^2$ terms are orthogonal (uncorrelated), thus reducing the chances of misinterpretation of correlated regression terms (E. Perry, personal communication).

The log of the water quality parameter in question is then regressed on TIME and $TIME^2$ using SAS® PROC GLM (SAS, 1989) with the following statements:

```
PROC GLM;
  CLASS MONTH;
    MODEL log(WQ variable) = TIME TIME2 MONTH;
```

References

Alden, R.W. III and E.S. Perry. 1997. *Presenting Measurements of Status*. A "white paper" written for and presented to the Chesapeake Bay Program Data Analysis Workgroup. 15 pp.

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Gilbert, R.O. 1987. *Statistical methods for environmental pollution monitoring*. Van Nostrand Reinhold: New York. 253 pp.

Hirsh, R.M., J.R. Slack, and R.A. Smith. 1982. *Techniques of trend analysis for monthly water quality data*. Water Resources Research 18(1): 107-121.

SAS Institute Inc., SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC: SAS Institute Inc., 1989. 846 pp.

Sen, P.K. 1968. Estimates of the regression coefficient based on Kendall's tau. Journal of the American Statistical Association 63: 1379-1389.

Appendix II: Maryland Department of Natural Resources: Standard Operating Procedures #PR-03: Maryland Core/Trend Monitoring Program

Standard Operating Procedure # PR-03
MARYLAND CORE/ TREND MONITORING PROGRAM

Prepared by: Laura Fabian

Approved by: Kristen Heyer

Table of Contents

1.0 Scope and Application

1.1 This Standard Operating Procedure is applicable to the collection of water quality samples for the Maryland Core/ Trend Program (Section 106).

1.2 The water samples are collected for physical and chemical analysis.

2.0 Summary of Method

2.1 Stations sampled for the Core/ Trend Monitoring Program are mostly sampled by land. A few Core/Trend stations are sampled in conjunction with other programs: Chesapeake Bay Mainstem (refer to SOP# PR-01); Chesapeake Bay Tributary (refer to SOP# PR-02); and Potomac River Program (refer to SOP# PR-05). These samples are collected at depth, aboard a research vessel and follow the respective program Standard Operating Procedure.

2.2 Whole water samples are collected at every station for the Core/ Trend Program. The following equipment are used for collection of water samples depending on the station description and conditions:

- Bucket
- Submersible pump

2.3 The whole water samples are collected and analyzed for both physical and chemical properties; whole water nutrients and dissolved fractions.

3.0 Health and Safety Warnings

3.1 When sampling from boats and piers, wear appropriate safety gear and follow appropriate safety procedures for working around the water and under slippery conditions.

3.2 When sampling from road bridges, wear appropriate safety gear and follow appropriate safety procedures for working around high speed traffic.

3.3 When sampling along the stream bank, care should be exercised in areas with slippery and uneven terrain.

3.4 Proper methods for lifting and moving equipment and samples should be exercised to avoid bodily injury.

3.5 Any water sample collected could contain a potentially harmful algae species. If the presence of a toxic species is suspected, protective equipment **must** be used. Protective gear must include gloves and raingear. Respirators and goggles must be used when sampling a bloom suspected of containing algae species that can produce toxic aerosols.

4.0 Interferences

4.1 Contamination of samples can be minimized or eliminated by following the procedure for cleaning of sampling equipment. Equipment is washed on a regular basis to include acid rinsing. Refer to SOP # MC-01: *Cleaning and decontamination of sampling equipment*.

4.2 Improper sample collection can be avoided by following the guidelines in this SOP and taking care not to disturb the substrate of the area being sampling.

4.3 Care should be exercised to avoid collecting debris in the sample when it is present in the body of water being sampled.

4.4 Inaccessibility to sampling stations may occur due road and/or bridge construction, maintenance or closure. Inaccessibility can also be caused by boat ramp closure, homeowner denial of access to the site and overgrowth of brush.

4.5 Weather conditions may interfere with the collection of samples. High winds and ice cover are examples of interferences that may occur. Sample collection should be rescheduled if possible.

4.6 Insufficient water volume would interfere with the collection of grab samples. This problem could manifest itself as an inaccessibility problem (i.e. there is not enough water to reach the station) or there is not sufficient volume to grab a sample.

4.7 Malfunctioning sampling equipment, i.e. submersible pumps, etc. will interfere with the collection of grab samples. Malfunctioning or missing filtration equipment, i.e. electric vacuum pumps or filter funnels, will interfere with the generation of particulate and dissolved samples. Having spare filtration equipment and/ or a hand pump could solve this problem. If too many samples need to be processed by hand, the whole water sample used for filtering can be preserved in ice and filtered as soon as you return to the office. Be sure to note the time the sample was filtered on the volume sheet.

4.8 Contamination can occur from dirt and debris near the processing area. This can be minimized by maintaining clean vehicles, vessels and lab areas. Filtration equipment is placed on clean lab towels during processing to further maintain a clean working environment. The boat and/ or vehicle engines should not be running while processing samples if the fumes could accumulate in the processing area.

4.9 Insufficient lab supplies would also interfere with collection of particulate and dissolved nutrient samples. This problem can be solved by maintaining a back-up supply of necessary items.

5.0 Equipment and Supplies

5.1 A bucket is typically the equipment used for collection of water samples for the Core/ Trend Program. For bucket sampling, we use a square 3 gallon bucket (Rubbermaid, model #2964). The bucket is then tied to a line long enough to reach the water surface. The bucket should be cleaned periodically (refer to SOP # MC-01: *Cleaning and decontamination of sampling equipment*). Each van is equipped with its own sampling buckets. Bucket sampling will be described in this SOP.

5.2 A submersible pump is used for Core/ Trend stations that are sampled in conjunction with another program, i.e. Chesapeake Mainstem or Potomac. Submersible pump sampling uses either a well pump (Dayton, ½ HP, 230 V, model # D10KS05221) or a bilge pump (Rule, 2000 gph, 12 V, model # 10). Refer to the Standard Operating Procedure for the Field Collection of Grab Water Samples (SOP # SC-01) for sample collection details.

5.3 The whole water samples collected for the Water Quality Monitoring Program are collected in new HDPE (plastic jug) bottles. These may include, but are not limited to, half- gallon (2 qt. bottles), quart, 16 oz. and 8 oz. bottles. Ice-filled coolers labeled for courier delivery to the Baltimore Lab are necessary for transporting the whole water samples.

5.4 Any or all of the following equipment is used to collect and record data on the field sheets:

- pencils, pens & sharpies
- watch, clock or instrument that displays the current time
- thermometer (readings in Celsius)
- water quality instrument (Hydrolab or YSI) with stirrer and/ or probe guard
- calculator (for boat stations sampled where there is a pycnocline)
- compass & secchi disk (for tidal stations)

5.5 Any or all of the following equipment is used for collection of particulate and dissolved samples.

- 25mm filter funnel, 200ml; polysulfone (Pall Corp.# 4203)
- 47mm filter funnel, 300 ml; magnetic (Pall Corp.# 4242)
- Filter funnel manifold; polyurethane (Pall Corp.# 4205) with trap
- 47mm filter funnel & base; Millipore
- Filter flasks, 1000ml/ 500 ml (Fisher# 10-181F; 10-180E)
- Graduated Cylinders; 10ml, 50ml, 100ml, 250ml
- Forceps
- DI squirt bottles
- Adjustable vacuum pump (115V AC or 12 V DC Air Cadet) with pressure gage and trap

5.6 Any or all of the following supplies are used for collection of particulate and dissolved samples. When sampling Core/ Trend stations that are sampled in conjunction with another program, i.e. Chesapeake Mainstem or Potomac additional supplies may also be used. Refer to the Standard Operating Procedure for the Field Filtration for Particulate and Dissolved Nutrient Constituents (SOP # SC-03) for full filtration details for other programs within the Water Quality Monitoring Program.

- Pads
    - CHLA & PP: 47mm GF/F Whatman glass fiber filter (#1825-047, Fisher # 09-874-71); pore size 0.7 μm.
    - PC/ PN: 25mm GF/F Whatman glass fiber filter, pre-combusted at 490°C; pore size 0.7 μm. Direct from lab.
- 8 oz. or 16 oz. HDPE bottles
- DI water
- Sample water
- Freezer or cooler with ice for sample storage and/ or transport

6.0 Instrument Calibration

These procedures refer to Hydrolab Series 5 and Yellow Springs Instrument (YSI) Series 6 instruments. Detailed calibration procedures are performed as described in their respective operating manuals.

NOTE:

In March 2015 all remaining Series 4a instrument equipped with Standard Clark Polarographic Dissolved Oxygen Sensors were replaced with Series 5 instruments equipped with optical dissolved oxygen sensors (Luminescent Dissolved Oxygen Sensor - LDO). Calibration logs for each instrument will list specific replacement dates. Sensors for temperature, specific conductance, pH and depth are identical for Series 4a and 5 instruments.

Beginning in February 2009, YSI Series 6 instruments were added to the field instrument inventory. YSI instruments are equipped with optical dissolved oxygen sensors (Reliable Oxygen Sensor - ROX). YSI temperature, specific conductance, pH and depth sensors are different than their respective Hydrolab sensors, but perform similarly.

Both the Hydrolab and YSI optical dissolved oxygen sensors use similar luminescent technology to measure dissolved oxygen.

6.1 Hydrolab Series 5 Calibration

1. Temperature – temperature is measured with a stainless steel thermistor. Calibration is not required because it is factory set and not user adjustable. During periodic routine servicing, temperature is checked at one temperature against a standard mercury thermometer. The multiprobe is returned to the manufacturer if test results are not within 0.2°C of standard thermometer.

2. Dissolved Oxygen – dissolved oxygen is measured with an optical probe (LDO) using a 100% saturation protocol in air saturated water. Calibration dissolved oxygen concentration is determined from a table of saturation concentrations in mg/L as calculated from the instrument temperature and local barometric pressure measured with a Standard Fortin Mercury Barometer.

3. Specific Conductance – conductivity is measured with a probe having two opposing graphite electrodes oriented horizontally inside a vertical plastic channel. The conductivity reading is corrected to standard temperature (25 °C). The probe is calibrated with a standard potassium chloride solution using a 2 point linear protocol. These standard solutions are made in house. The zero point is calibrated in air with the probe dry. The slope is calibrated with a standard potassium chloride solution. The slope standard is selected so that field measurements fall between zero and the specific conductance of this standard, but as close to the specific conductance of this standard as possible.

4. pH – pH is measured with a two probe system (*in situ* pH and reference probes). The *in situ* pH probe is a standard silver/silver chloride glass probe and the reference probe is a pellet of silver inside a hollow plastic sleeve containing 4 M potassium chloride solution saturated with silver chloride. This sleeve has a porous Teflon™ junction at one end to connect this probe to the environment. The pH system is calibrated with standard pH buffers using a two point linear protocol. The zero point is first calibrated with a pH 7.00 standard buffer.

The slope is calibrated with either pH 4.00 or 10.00 standard buffer. The slope buffer is selected depending on pH measurements anticipated during field deployment. The pH value of each buffer is adjusted for instrument temperature before calibration.

6.2 YSI Series 6 Instrument Calibration

1. Temperature – temperature is measured with a stainless steel thermistor. Calibration is not required because it is factory set and not user adjustable. During periodic routine servicing, temperature is checked at one temperature against a standard mercury thermometer. The multiprobe is returned to the manufacturer if test results are not within 0.2°C of standard thermometer.

2. Dissolved Oxygen – dissolved oxygen is measured with an optical probe (ROX) using a 100% saturation protocol in air saturated water. Check and calibrate, if necessary, the YSI 650 MDS display unit barometer to local barometric pressure in mm Hg as measured from the Standard Fortin Mercury Barometer. Calibration dissolved oxygen concentration is determined from a table of saturation concentrations in mg/L as calculated from the instrument temperature and instrument barometric pressure.

3. Specific Conductance –The conductivity reading is corrected to standard temperature (25 °C). The probe is calibrated with a standard potassium chloride solution using a 2 point linear protocol. These standard solutions are made in house. The zero point is factory calibrated and cannot be adjusted by the user. The slope is calibrated with a standard potassium chloride solution. The slope standard is selected so that field measurements fall between zero and the specific conductance of this standard, but as close to the specific conductance of this standard as possible.

5. pH – pH is measured with a combined probe system (*in situ* pH and reference probes in one). The pH system is calibrated with standard pH buffers using a two point linear protocol. The zero point is first calibrated with a pH 7.00 standard buffer. The slope is calibrated with either pH 4.00 or 10.00 standard buffer. The slope buffer is selected depending on pH measurements anticipated during field deployment. The pH value of each buffer is adjusted for instrument temperature before calibration.

6.3 Frequency of Calibration

All instruments are calibrated prior to field use. Calibration is typically completed the Friday before the sampling week. If office time is available on a closer day preceding the run, then the calibration will be completed at that time.

- If large adjustments to the DO value were necessary during calibration, the DO should be checked prior to taking the instrument in the field.
- Instrument calibrations may be rechecked in the calibration lab any time the field readings seem suspect.
- Instruments are post-calibrated (checked) after use. Typically the post-calibration occurs on the Friday after the sampling week. After post-calibration, the instrument is ready to be calibrated for field sampling the following week.

7.0 Preparation for Sampling

1. When preparing for a Core/ Trend sampling run the first step is to get the field pack for the specific run you are doing. The packs are on the bottom shelf in the back garage prep area. The field packs contain all information to complete the run: field sheets, chemistry sheets, volume sheets, directions to all of the stations with maps, list for samples being collected, foils squares, baggies, PC/PN pads, lab towel, and extra sheets. When the run is complete, everything (except for the volume & chemistry sheets which accompany the samples) should be returned to the field pack and turned in to Laura Fabian.
2. Ensure that the field van has necessary supplies for safe collection of the sample. Each van should have buckets with line, orange cones and safety vests.
3. Ensure that the field van has the necessary supplies for processing the sample. Make sure there are enough quart, two quart and 16 oz. bottles for the whole and filtered samples. Pre-labeling bottles prior to sampling will help eliminate errors. Check for a WORKING vacuum pump and appropriate filtration supplies: forceps, $MgCO_3$, Whatman pads.
4. Just prior to leaving for the run, the following equipment should be loaded into the van: fully charged & calibrated meters (294 µs/cm for specific conductance); filter unit, courier cooler big enough for all of the sample bottles, another small cooler for bringing pads back to the office after delivery to the courier and ice.

8.0 Sample Collection

8.1 Bucket Sampling

Bucket sampling is used on all land runs, including Core/ Trend sampling. Bucket samples are taken from bridges, weirs and stream banks. However, bridge sampling is the preferred sample method and should be used whenever possible. Below, the steps to be taken for each type of bucket sampling are listed.

8.1.1 Bridge Sampling

NOTE: When sampling from bridges, always make sure you have the appropriate lengths of rope before leaving for your run. Once you arrive at the station:

1. Select the appropriate length of rope for the bridge from which you will be sampling. You may need to tie 2 or more ropes together to reach the water surface at some stations. Secure the rope to the bucket, making sure that it will not come loose while retrieving the sample. Most sample buckets already have the ropes tied on to ensure that the bucket "hangs" properly.
2. Sample on the upstream side of the bridge (if possible) and as close to the center of the stream/river as possible, where the majority of flow is located.
3. Lower the bucket to the water.
4. Tip the bucket and fill with enough water to rinse the bucket (at least a few inches). Depending on the height of the bridge, you may want to shake the rope to expel the rinse water from the bucket, or pull the bucket back up to dump the rinse water out of the bucket. Follow this procedure 3 times; making sure the bucket is properly rinsed.
5. Fill the bucket as full as possible.
6. Pull the bucket back up, making sure the rope does not rub against the side of the bridge. This can sometimes cause dirt, rust, paint, etc. to fall into the sample.
7. Carry the bucket back to the van.

8.1.2 Weir Sampling

NOTE: You may want to wear waders, waterproof boots or water shoes when sampling from weirs or stream banks. Depending on the depth of the water, you may need to walk

into the stream to collect a sample.

1. Select the shortest length of rope, and secure to the bucket.
2. Depending on the water level, you may be able to walk out onto the weir to get closer to the center of the stream. If the water level is too high, sample from the stream bank close the weir.
3. Always sample just upstream of the weir. Holding onto the rope, throw or drop the bucket into the water and fill with enough water to rinse without disturbing or touching the bottom of the stream with the bucket.
4. Dump the rinse water downstream of the sampling area.
5. Repeat steps 3 & 4 two more times.
6. Once the bucket is properly rinsed, throw or drop the bucket into the water and fill to the lip of the bucket.
7. Pull the bucket from the water, and carry it back to the van.

8.1.3 Stream Bank Sampling

1. Select the shortest length of rope, and secure to the bucket..
2. Try to get as close to the center of the stream as possible when it is safe to do so (using rocks, etc.). If it is shallow near the sample area, do your best to get a sample without touching or disturbing the bottom of the stream with the bucket. At times, you may have to walk out farther into the stream.
3. Holding the rope, throw or drop the bucket into the water and fill with enough water to rinse.
4. Dump the rinse water downstream of the sampling area.
5. Repeat steps 3 & 4 two more times.
6. Once the bucket is properly rinsed, throw or drop the bucket into the water and fill to the lip of the bucket, or as full as you can get in low flow conditions.
7. Pull the bucket from the water, and carry the sample back to the van.

8.2 Recording the physical data

After the bucket has been collected, the next step is to take readings of physical parameters and record them on the field sheet.

8.2.1 Completing the Field Sheet

For complete details, see Standard Operating Procedure # DR-01: *Recording Physical Data on Field Sheets*. For an example Core/ Trend field sheet, see Appendix IV.

1. The start date is recorded as YYMMDD (2 digit year, 2 digit month, 2 digit day). Do not record an end date, it is assumed that it is a one day sampling period.
2. The start and end times are recorded in military time (4 digits) using the current time either EDT or EST. The start time is when you start sampling the station (collecting the bucket) and the end time is when you finish with the readings and whole water sample collection.
3. The two digit number of samples reflects the number of water samples collected at individual, discrete depths. It also denotes if there was a duplicate sample taken.
4. The total depth is only recorded on tidal stations. This is the depth, in meters, to the bottom.
5. Air temperature is recorded in Celsius to the nearest 0.5 degree. The thermometer should be hung out when you arrive on station to allow it to equilibrate to the current temperature. The thermometer should be placed approximately 3-4 feet from the

ground and in the shade. Hanging the thermometer on the van in times of weather extremes may skew the reading.

6. Weather codes recorded on the field sheet are as follows:

10- no precipitation	11- drizzle
12- rain	13-heavy rain
14- squally	15- frozen precipitation

Yesterday's weather code is recorded as the predominate weather the day prior to sampling for the station being sampled.
Today's weather code is the weather conditions at or near the time of sampling. If a weather condition had occurred that will affect the water quality sample, but does not represent today or yesterday's weather it can be noted in the comment section of the field sheet: e.g. 2 feet of snow covering the ground or hurricane 2 days ago.

7. Percent cloud cover is reported as a value from 0% to 100%. Numbers are three digits and are right justified on the field sheet: e.g. 005 would be 5% cloud cover. Thin clouds and haziness may be noted in the comment section of the field sheet.
8. Wind direction & velocity, wave height, secchi, tide state, pycnocline limits are only collected at tidal stations.
9. The flow value is recorded by Laura Fabian after the sampling run if the station is associated with a USGS Gaging Station. The flow value box is 8 boxes long. The basis, flow value, exponent, & G/L boxes are included.
   a. The basis number refers to whether a flow value is measured or estimated. If the flow value was measured then a 1 goes in the basis box. A 2 is used when the flow value was estimated.
   b. The flow value is a five digit number in cfs (cubic feet per second). If the value given less than five digits then zeros are added following the value to fill all boxes. For example, if the flow value is 261 cfs, then you would enter 26100.
   c. The exponent box denotes the placement of the decimal point for the flow value in the preceding boxes. Following the example above of a flow value of 26100 (for 261 cfs), the exponent would be 3. This exponent denotes moving the decimal three place from the left, i.e. 261.00 giving the flow value as 261 cfs (the original value).
   d. The G/L box is for noting if a value is greater or less than the reported value.
   e. For most sampling, the flow values are currently taken from the USGS real-time data website, http://waterdata.usgs.gov/md/nwis/current?type=flow . These values are recorded in 15 minute increments. The increment that most closely matches the sampling time is entered on the field sheet. Note that all data on the website are timed in Eastern Standard Time all year long.
10. The equipment & probe numbers correspond to the number or letter associated with the water quality instrument used to record data. All instruments have an individual letter associated with them. The instrument is recorded as 9 and then its assigned letter, i.e. 9L. Indicate the D.O. method being used.
11. The scientist and senior scientist sign-offs are places for the sampling team to initial, denoting the fact that they collected and verified the data. The spaces are three boxes long. All letters are left justified. If someone only has 2 initials, then they would be placed in the first 2 boxes and the third is left blank.
12. Any comments pertinent to the station or collection of data or samples should be placed in the comments section of the field sheet.

8.2.2 Recording Instrument Readings

1. Remove the storage cup from the sonde.

2. Inspect all probes for before installing probe guard.
3. Install the probe guard.
4. Swirl the meter in the bucket until the readings stabilize.

- Record the water temperature is degrees Celsius. The temperature should be recorded to the nearest tenth.
- Record the pH to the nearest tenth. Place a zero in the hundredths place following the reading.
- Record the dissolved oxygen in mg/L to the nearest tenth. Place a zero in the hundredths place following the reading. The G/L box is available for instances when the DO is reaching its upper or lower limit.
- Record the specific conductance in µS/cm to three significant figures.
- Salinity is recorded in ppt to the nearest tenth.

8.3 Collecting the whole water sample

1. Whole water samples for the Core/ Trend program are collected directly from the bucket. Whole water samples from tidal stations are collected according to the specific SOP for the program being sampled. The sample will either be a one quart or a two quart sample that is sent to MDH for analysis. One quart is collected for all stations. A 2-quart whole water bottle is collected for stations sampling for BOD (Potomac core stations).
2. Bottles sent to MDH for analysis must have CORE, whole water, the station name, date and sample number, e.g. C-12, on the bottle. Bottles must be labeled legibly with a permanent marker (sharpie). Add layer code if multiple depths are being collected at a single station (tidal stations only). Do not write on the caps of bottles being sent to the MDH lab.
3. Stir the water in the sampling bucket (without introducing bubbles) to create a homogeneous sample. Rinse the pre-labeled collection bottle(s) and cap(s) with the sample water 3 times. Fill the bottle(s) to the shoulder and cap tightly.
4. Place the bottle in ice in the sample cooler.
5. The following parameters are analyzed from the whole water bottle: total suspended solids, total alkalinity and turbidity. A 2-quart bottle is collected to analyze for 5-day BOD on the Potomac core runs (Monocacy, Mid-Potomac & Lower Potomac runs).

8.4 Collecting the filtered and particulate samples

8.4.1 Follow the above instructions for filling a bottle for filtration of the sample. Usually a 2-quart bottle is used.

8.4.2 Chlorophyll (CHLA)

Chlorophyll samples are not collected for the Western Maryland Core.

1. For each sample, clean a 47mm bell with deionized (DI) water. Set up unit for filtering. Be sure that there is a trap in line between the manifold and the vacuum source.
2. Place a Whatman 47mm GF/F glass fiber filter pad on the filter frit. Always use clean forceps when handling the filter pads.
3. Mix sample thoroughly by agitating and shaking the sample bottle vigorously, then rinse graduated cylinder three times with sample.
4. Agitate the sample again before measuring in the graduated cylinder. Fill graduated cylinder with sample and filter desired volume through filtration unit. Be sure to use a graduate that is close to the volume being filtered (ex: if you are only filtering 80 ml of

sample use a 100 ml graduate). Keep the vacuum pressure below 10 inches of Hg (around 8" Hg is good).

5. Filter sufficient volume of sample (20 - 2000 ml) to leave **noticeable color** on the filter pad.
6. Record the total volume filtered on the foil square.
7. Agitate the squirt bottle of $MgCO_3$, as it settles rapidly. Add approximately 1 ml of $MgCO_3$ suspension (1.0 g $MgCO_3$ in 100 ml of DI water) to the last 50 ml of sample in the filtration bell.

NOTE: Samples for dissolved parameters are not to be collected from this filtrate.

8. The pad should be removed as soon as the sample is completely filtered. The pad should not be left on the frit under vacuum. If you are unable to remove it immediately, be sure to release the vacuum to avoid damaging the sample.
9. Using forceps (1 or 2 pair), fold filter in half with sample inside and remove filter pad.
10. Place pad in pre-marked foil square, and carefully fold foil square in thirds, horizontally. Then fold the ends in to seal the filter inside. Be sure forceps do not touch sample residue on the filter pads, because the sample will adhere to the forceps.
11. Be sure that foil square is marked with the date, station, volume of sample filtered, and sample number.
12. Place foil packet into the labeled zip-lock plastic bag and place in the sample cooler on ice.

NOTE: The filter pads for chlorophyll analysis should not be exposed to direct sunlight. Store as soon as possible.

8.4.3 Particulate Carbon/ Particulate Nitrogen (PC/PN)

1. For each sample, clean two 25 mm filter bells with deionized (DI) water.
2. Place a pre-combusted 25 mm GF/F filter (direct from the lab) on each filter frit. Always use clean forceps when handling the filter pads.
3. Mix sample thoroughly by agitating and shaking the sample bottle vigorously, then rinse graduated cylinder three times with sample.
4. Agitate the sample again before measuring in the graduated cylinder. Fill graduated cylinder with sample and filter desired volume through filtration unit.
5. Filter 10-800 ml through each filter. Filter enough sample to leave **noticeable color** on the filter pad.
6. Make sure filter is sucked dry and the **same volume is filtered for both pads**.
7. Record the volume filtered (total volume through one pad - do not add the volumes for the 2 pads together) on the foil square.

NOTE: Samples for dissolved parameters are not to be collected from this filtrate.

8. Using forceps, fold each filter in half.
9. Place both filters in a foil square labeled with date, sample number, station, PC/PN, and volume filtered. Be sure that the pads are not overlapping in the foil square to keep them from freezing together. Fold foil square as described above.
10. Place the folded foil in the labeled zip-lock bag and place in the sample cooler

8.4.4 Particulate Phosphorus (PP)

6. Follow steps above setting up and rinsing two 47 mm filter bells and flasks. The filters used are two Whatman 47 mm GF/F filters (same pads we use for chlorophyll).
7. Filter 50 ml of sample through each filter pad. If 50ml will not go through one pad then filter a smaller volume through multiple pads to attain 50ml of filtrate.
8. Use the filtrate as an equipment rinse and discard.

9. Then filter enough additional (another 20 - 950 ml) to leave **noticeable color** on the filter pad.
10. Record the **total** volume filtered through one pad being sure to add the 50 ml rinse water (total volume through one pad – do not add the volumes for the 2 pads together) on the foil square.
11. **Use this filtrate to fill up the filtrate bottle for the dissolved parameter analysis.**
12. After collecting filtrate, make sure filter is sucked dry.
13. Rinse the filter pad using at least three - 10 ml rinses of DI water, sucking the pad dry after each rinse.

NOTE: If the volume filtered through one pad is ≤ 100 ml, you may want to collect all filtrate in one flask. To do this, filter 50ml to rinse one flask. Collect the remaining volume being filtered in this rinsed flask. Move the filter bell with the unused pad atop the flask containing the filtrate from the first pad (collection flask). Filter and collect the entire volume through the second pad. You may use the second flask as the "pad rinse flask". Place the bell with the already filtered sample pad atop this second flask. Use deionized water to rinse each sample pad 3 times, sucking dry after each rinse.

14. Using forceps, fold each filter in half.
15. Place both filters in a foil square labeled with station, date, PP, sample number, and volume filtered (this is the total volume of sample through each pad, including the initial 50 ml rinse). Be sure that the pads are not overlapping in the foil square to keep them from freezing together.
16. Fold the foil square as described above. Place foil square in labeled zip-lock bag and place in the sample cooler on ice until you return to the field office.

8.4.5 Filtrate collection (for the dissolved parameters TDN, TDP, NH_4, NO_2+ NO_3, NO_2, PO_4 & DOC)

1. A bottle of filtrate is collected for submission to the MDH lab for the analysis of dissolved parameters. A 16 oz. bottle is typically used for this purpose. The bottle should be labeled with the station name, date, sample number, e.g. C-12, "CORE" and "filtrate". Bottles must be labeled legibly with a permanent marker (sharpie). Do not write on the caps of bottles going to the MDH lab.
2. The filtrate should be collected from the PP filtration described in 7.4.4 above.
3. Rinse the labeled bottle and cap 3 times with filtrate.
4. Fill the bottle at least ¾ full with filtrate. Do not fill the bottle above the shoulder.
5. Place the bottle in ice in the sample cooler.

8.4.6 Filtrate collection for the collection of Cl & SO_4

1. Filtrate is collected at a subset of stations for the analysis of chloride and sulfate.
2. The filtrate should be collected from the PP filtration described in 7.4.4 above.
3. Rinse 1 labeled 60ml bottle and cap 3 times with filtrate.
4. Fill the bottle up to the shoulder/ neck and cap.
5. Place the bottles in a rack in the sample cooler. Place them in the refrigerator at the Lake Management Office when you return. The Western MD samples will be mailed to CBL once the monthly sampling is completed.

8.5 Completing the Laboratory Sheets

8.5.1 MDH Chemistry Sheet

17. The chemistry sheets should already be labeled with the station numbers. There should be a separate sheet for each station. The type of sample (whole, filtered, etc.) and bottle number should also be pre-filled in. See Appendix V for an example.
18. The following items will need to be filled in:
    - Collector (use last names)
    - Date
    - Start time (current time)
    - Salinity (Fill in the salinity for the tidal core stations)
    - Field scientist sign-off
    - Start depth (always 0.0m for bucket samples, but needs to be filled in at tidal core stations)

8.5.2 MDH volume Sheet

1. The MDH volume sheet should have the run name, station names, sample numbers, layer codes and depths all pre-filled in. Depths may need to be added on the volume sheets for the tidal core stations.
2. The following items will need to be filled in
    - Date
    - Time (start time in current military time-EST/EDT)
    - PP volume filtered, in ml
    - PC/PN volume filtered, in ml
    - Scientist sign-off

8.5.3 CBL Volume Sheet

1. The CBL volume sheet should have the run name, station names, sample numbers, layer codes and depths all pre-filled in. Depths may need to be added on the volume sheets for the tidal core stations.
2. The following items will need to be filled in
    - Date
    - Time (start time in current military time- EST/EDT)
    - CHLA volume filtered, in ml
    - Scientist sign-off
    - Salinity (stations for CL & SO_4 only)

9.0 Sample Handling and Preservation

19. All samples (whole water, filtrate & pads) must be iced immediately after collection.
20. The whole water and filtrate samples are typically sent to MDH via a courier. If courier service is used, drain all ice melt water from the cooler and repack, filling with ice up to the necks of the bottles before leaving the cooler at the courier. Be sure that the cooler reads "Baltimore Lab, Water Chemistry, 2nd Floor" and "Return to Anne Arundel Health Dept." All lab sheets must accompany the samples in the cooler. Place the completed sheets in the zip-lock bag provided in the field pack.
21. The pads collected should be kept on ice and placed in the freezer in the bin marked "DHMH" upon return to the Field Office. The volume sheet for the particulate samples should be folded and placed in one of the bags of pads. If you are delivering directly to the MDH lab then the pads may be given to the lab tech along with the whole water and filtrate bottles and they will

store them properly. Samples that have been stored frozen at the Field Office are delivered to MDH at the end of the sampling week. Use enough ice in the delivery cooler to ensure that the samples stay frozen.

22. The Western Maryland Core samples are delivered to the Western Maryland Regional Laboratory in Cumberland. The whole water and filtrate bottles are delivered to DHMH in Baltimore via courier. The filtered pads can be left in the freezer at the end of the day. The pads must be mailed to MDH in a frozen state. Dry ice is available for shipment (Wilson Supply, Inc. 15401 McMullen Hwy, Cumberland (301) 729-2515; acct # 7724).

10.0 Data and Records Management

10.1 All samples collected are labeled and recorded on the field sheet. The field sheets are reviewed for accuracy and completeness and then submitted to the Principal Investigator and Quality Assurance Officer (RAS/TEA). A progress report/ cross reference sheet accompanies the original field sheets. The progress report/ cross reference sheet tracks which samples were taken at each site. Any comments or additional samples are noted on this sheet. Notes about instrumentation problems, etc are included with the report. For more information refer to SOP # DR-05: *QA/QC and submission of field data*. An example of a cross reference sheet can be found in Appendix III. Copies of all field sheets, with their attached progress report/ cross reference sheets, are kept on file at the Field Office.

10.2 The whole water and filtered samples submitted to MDH also have laboratory sheets associated with them. The sheets are submitted with the samples at the time of delivery, by either direct delivery to DHMH or in the cooler via courier. The laboratory data are reported on the lab sheet and sent directly to TEA. No copies of the MDH lab sheets remain at the Field Office.

10.3 The filter pads have a volume sheet associated with them. There is one volume sheet for the particulate pads (PC/PN & PP) that are sent to MDH. The results for these parameters are submitted to TEA at the bottom of the whole and filtered water sample laboratory sheet. A second volume sheet accompanies the chlorophyll samples to CBL and the results are sent directly to TEA in a data report. No results or lab sheets are kept on file at the Field Office.

11.0 Quality Control and Quality Assurance

11.1 Samples are collected by properly trained staff to ensure continuity of high quality samples. Field staff must adhere to all Standard Operating Procedures.

11.2 Field duplicates (replicates) are collected every 20 samples to check for accuracy of field collection and preparation of the samples.

11.3 Quality control and quality assurance are maintained by proper cleaning and decontamination of sampling gear. Refer to SOP # MC-01: *Cleaning and decontamination of sampling equipment*.

11.4 Equipment blanks of deionized water are submitted monthly to catch any possible contamination. Refer to SOP MC-02: *Deionized water, blank sample checks*.

11.5 If contamination occurs, every effort is made to pinpoint the source of the contamination and eliminate it.

12.0 References

1. Maryland Department of Natural Resources. 2018. *Quality Assurance Project Plan. Chesapeake Bay Water Quality Monitoring Program- Chemical and Physical Properties Component, 2018-2019.*

2. Maryland Department of Natural Resources. May 2018. *Quality Assurance Project Plan. Section 106. Ambient Water Quality Monitoring (Core/ Trend Monitoring). July 1, 2018- June 30, 2019.*

3. Maryland Department of Natural Resources. *Standard Operating Procedures Manual.* Water Quality Monitoring Program. WQMP Field Office, 1919 Lincoln Drive, Annapolis, MD 21401.
    1. Standard Operating Procedure # SC-01: *Field Collection of Grab Water Samples*
    2. Standard Operating Procedure # SC-02: *Collection of Whole Water Samples*
    3. Standard Operating Procedure # SC-03: *Field Filtration for Particulate and Dissolved Nutrient Constituents*
    4. Standard Operating Procedure # SC-04: *Pycnocline Calculation*
    5. Standard Operating Procedure # SC-05: *Collection of Live Plankton Samples*
    6. Standard Operating Procedure # MC-01: *Cleaning and Decontamination of Sampling Equipment*
    7. Standard Operating Procedure # MC-02: *Deionized Water, Blank Sample Checks*
    8. Standard Operating Procedure # DR-05: *Quality Assurance/ Quality Control and Submission of field data*

Appendix I.: Station List for Core/ Trend

BALTIMORE CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1B	ELKRIDGE	HOWARD	PATAPSCO RIVER	PAT 0176	U.S. ROUTE 1
1D	HOLLOFIELD	HOWARD	PATAPSCO RIVER	PAT 0285	MD ROUTE 99
1B	VILLA NOVA	BALTIMORE	GWYNNS FALLS	GWN 0115	ESSEX ROAD
1B	FINKSBURG	CARROLL	N.BR.PATAPSCO	NPA 0165	MD ROUTE 91, GAGE
1B	SORRENTO	BALTIMORE	JONES FALLS	JON 0184	FALLS ROAD GAGE
1B	TOWSON	BALTIMORE	GUNPOWDER FALLS	GUN 0125	CROMWELL BRIDGE

SUSQUEHANNA CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1D	DARLINGTON	HARFORD	DEER CREEK	DER 0015	STAFFORD BRIDGE
1B	CONOWINGO	HARFORD	SUSQUEHANNA	CB1.0	BELOW CONOWINGO DAM,
1B	HOFFMANVILLE	BALTIMORE	GUNPOWDER FALLS	GUN 0476	GUNPOWDER ROAD
1B	GLENCOE	BALTIMORE	GUNPOWDER FALLS	GUN 0258	GLENCOE ROAD BRIDGE,GAGE

HAGERSTOWN CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1B	FUNKSTOWN	WASHINGTON	ANTIETAM CREEK	ANT 0203	POFFENBERGER ROAD
1D	LEITERSBURG	WASHINGTON	ANTIETAM CREEK	ANT 0366	MILLER CHURCH RD
1D	FAIRVIEW	WASHINGTON	CONOCOCHEAGUE	CON 0180	GAGE NEAR FAIRVIEW
1B	WILLIAMSPORT	WASHINGTON	CONOCOCHEAGUE	CON 0005	MD ROUTE 68 BRIDGE
1B	HANCOCK	WASHINGTON	POTOMAC RIVER	POT 2386	GAGE NEAR RT 522

MONOCACY CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1D	TANEYTOWN	CARROLL	BIG PIPE CREEK	BPC 0035	BRUCEVILLE GAGE
1B	EMITTSBURG	FREDERICK	MONOCACY RIVER	MON 0528	BRIDGEPORT BRIDGE, GAGE
1D	FREDERICK	FREDERICK	MONOCACY RIVER	MON 0269	BIGGS FORD ROAD
1D	MIDDLETOWN	FREDERICK	CATOCTIN CREEK	CAC 0148	MD ROUTE 17, GAGE
1B	SHEPHERDSTOWN	WASHINGTON	POTOMAC R.	POT 1830	SHEPHERDSTOWN W.V. WEST VIRGINIA
1D	SHARPSBURG	WASHINGTON	ANTIETAM CREEK	ANT 0044	BURNSIDE BRIDGE, gage

PATUXENT LAND CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1B	UNITY	HOWARD	PATUXENT RIVER	PXT 0972	MD ROUTE 97 GAGE
1B	LAUREL	ANNE ARUNDEL	PATUXENT RIVER	PXT 0809	BELOW ROCKY GORGE DAM

LOWER POTOMAC CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1D	CABIN JOHN	MONTGOMERY	CABIN JOHN BR.	CJB 0005	MACARTHUR BLVD.
1B	BROOKMONT	MONTGOMERY	POTOMAC RIVER	POT 1184	LITTLE FALLS GAGE
1D	CHEVY CHASE	MONTGOMERY	ROCK CREEK	RCM 0111	MD ROUTE 410
1B	BLADENSBURG	PR. GEORGES	ANACOSTIA R.	ANA 0082	BLADENSBURG ROAD

MID POTOMAC LAND CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1B	FREDERICK	FREDERICK	MONOCACY RIVER	MON 0155	PINE CLIFF PARK, REELS MILL ROAD
1D	BRUNSWICK	FREDERICK	CATOCTIN CREEK	CAC 0031	MD ROUTE 464,
1B	DICKERSON	FREDERICK	MONOCACY R.	MON 0020	ROUTE 28
1E	PT. OF ROCKS	FREDERICK	POTOMAC RIVER	POT 1595	EAST BANK ROUTE 15
1E	PT. OF ROCKS	FREDERICK	POTOMAC RIVER	POT 1596	WEST BANK ROUTE 15
1B	POOLESVILLE	MONTGOMERY	POTOMAC RIVER	POT 1472	WEST BANK WHITES
1B	POOLESVILLE	MONTGOMERY	POTOMAC RIVER	POT 1471	EAST BANK WHITES FERRY
1B	SENECA	MONTGOMERY	SENECA CREEK	SEN 0008	MD ROUTE 112

WESTERN MARYLAND

DATA CODE	BOT #	NEAREST TOWN	COUNTY	CO. CODE	BODY OF WATER	STATION	STATION LOCATION
DAY 1							
1D	C-7	GRANTSVILLE	GARRETT	11	CASSELMANS R.	CAS0479	RIVER ROAD GAGE
1D	C-8	FRIENDSVILLE	GARRETT	11	YOUGHIOGHENY R.	YOU0925	FRIENDSVILLE
1D	C-9	DEEP CREEK	GARRETT	11	CHERRY CREEK	CCR0001	STATE PARK ROAD
1D	C-9 dup						
1D	C-10	OAKLAND	GARRETT	11	YOUGHIOGHENY R.	YOU1139	RT 20, GAGE
1D	C-11	OAKLAND	GARRETT	11	LITTLE YOUGHIOGHENY R	LYO0004	OAKLAND
1B	C-12	KITZMILLER	GARRETT	11	N. BR.POT. R.	NBP0689	MD 38, GAGE
1D	C-14	BLOOMINGTON	GARRETT	11	SAVAGE RIVER	SAV0000	Rt 135
1B	C-13	BLOOMINGTON	GARRETT	11	N. BR. POT. R.	NBP0534	BLOOMINGTON

1B	C-15	WESTERNPORT	ALLEGANY	01	GEORGES CREEK	GEO0009	WESTERNPORT, GAGE
1D	C-16	KEYSER WV	ALLEGANY	01	N.Br.Potomac R.	NBP0461	RT 220
1B	C-17	PINTO	ALLEGANY	01	N.Br.Potomac R.	NBP0326	RT. 956, GAGE

DAY 2

1D	C-5	CUMBERLAND	ALLEGANY	01	WILLS CREEK	WIL0013	LOCUST GROVE RD, GAGE
1D	C-6	CUMBERLAND	ALLEGANY	01	BRADDOCK RUN	BDK0000	OLD MT SAVAGE Rd
1B	C-1	CUMBERLAND	ALLEGANY	01	N.Br.Potomac R	NBP0103	SPRING GAP
1D	C-2	OLDTOWN	ALLEGANY	01	N.Br.Potomac R	.NBP0023	OLDTOWN
1D	C-4	PAW PAW WV	ALLEGANY	01	POTOMAC RIVER	POT2766	PAW PAW WV, Rt. 51,GAGE
1B	C-3	OLDTOWN	ALLEGANY	01	TOWN CREEK	TOW0030	PACK HORSE RD

PARAMETERS CHECKED ON THE ALLEGANY LAB SHEET ARE
ALKALINITY (TOTAL), SULFATE, DISSOLVED SOLIDS, TURBIDITY
PARAMETER ADDED TO THE SHEET IS: SUSPENDED SOLIDS.
IRON no longer sampled as of 6/30/02, bactis no longer sampled after 11/03, all whole water to WMRL and only 16 ounce filtrate to DHMH as of 10/05. **SUBMITTER IS 52**

POTOMAC BOAT CORE

DATA CODE	NEAREST TOWN	COUNTY	BODY OF WATER	STATION #	STATION LOCATION
1B	-----	CHARLES	POTOMAC RIVER	XDC 1706	MORGANTOWN BRIDGE
1E	-----	CHARLES	POTOMAC RIVER	XDA 1177	BUOY C19 OF MD POINT
1E	-----	CHARLES	POTOMAC RIVER	XDA 4238	BUOY 27 OFF SMITH POINT
1E	-----	CHARLES	POTOMAC RIVER	XEA 1840	BUOY 44 OFF POSSUM POINT
1E	-----	CHARLES	MATTAWOMAN CR.	MAT 0016	DAYMARKER 5 OFF SWEDAN PT.
1B	-----	CHARLES	POTOMAC RIVER	XEA 6596	BUOY N54 OFF INDIAN HEAD
1E	-----	PR. GEORGES	POTOMAC RIVER	XFB 1433	BUOY 67 OFF DOGUE CREEK
1E	-----	PR. GEORGES	POTOMAC RIVER	XFB 2470	BUOY 77 OFF PISCATAWAY CR.
1E	-----	PR. GEORGES	POTOMAC RIVER	XFB 1986	OFF FT. WASHINGTON MARINA
1E	INDIAN HEAD	CHARLES	MATTAWOMAN CR.	MAT 0078	MD ROUTE 225
1E	ACCOCEEK	PR. GEORGES	PISCATAWAY CR.	PIS 0033	MD ROUTE 210

Appendix II: Core/ Trend Program History

Core History

1995-1997 Bacteriological samples collected all core stations and Potomac Boat.

April 1997- XJH6680 (Turkey Pt) & XHF1373 (Sandy Pt) now being sampled on Main Bay. 15 ft plankton for XHF1373 now sampled @ 5 meters.

May 1998- Bacteriological Labs @ Frederick & Cheverly closed. Monocacy, Mid Potomac, Lower Potomac/ Patuxent and Potomac Boat runs bacti samples dropped.
Bactis still collected for Baltimore, Susquehanna, Hagerstown & Western MD.

April-Dec 1999 and April, May & Sept 2000- Only WMD core (no trend) stations sampled due to body shortage at field office.

October 2000- Extra bactis collected @ CCR0001, NBP0103, NBP0534 and NBP0689 for bucket vs. direct stream comparison.

Prior to June 2001 name change CHO0626 aka ET5.0 (Red Bridges

July 2002 Western MD samples no longer tested for iron. Bob retired.

November 2003-WMD bacteriological samples no longer submitted. Whole water to WMRL and only filtrate plus pc/pn/pp filter pads to DHMH as of October 2005. Prior to that we were submitting a second quart of water to DHMH that was acid fixed by WMRL and shipped to DHMH via courier.

2004-Remaining Core runs dropped bacteriological sampling.

October & November 2004- Laboratory comparison. Collected regular sampling and 16 ounce filtrate and pc/pn pads at all stations.

July 2005- Began pc/pn/pp & filtrated sampling for all core stations in addition to whole water and chlorophyll pads.

July 2006 PXT0603 (TF1.0). and Kent Narrows (XGG8251) were no longer submitted with the core paperwork. TF1.0 submitted with Patuxent Boat and XGG8251 submitted with Tributary data. Whole water to WMRL and only 16 ounce filtrate and pc/pn/pp pads to DHMH. Prior to

that we were submitting 2 quarts of whole water to DHMH one of which was acid fixed by WMRL and both were shipped to DHMH via courier.
2008 Nov/Dec- Extra TSS pads generated with a churn splitter for Monocacy, Susquehanna, Baltimore, Lower Potomac Patuxent and the 2nd day of Western MD core.

Jan 2009- Chlorophylls now being processed by CBL due to budget cuts and people shortages @ DHMH. Equipment used @ DHMH will be given to CBL to process samples.

XCF9575 is Cedar Point. I'm not sure when we added sampling the planktons on Main Bay. We always sampled the station. But you probably can check the Patuxent data set and see when it dropped off the sheets.

Summer2010- CAC0031 bridge removed and river appears stagnant during construction

September 2010-Western MD and Hagerstown Core runs transferred to Christine King' western MD regional sampling office. All data for these stations will be submitted by her to Tawes. Western MD stations consist of any station including and west of Hancock, MD.

October 2010-Christine King will now be submitting Red Bridges field sheet with the WMD & Hagerstown core sheets.

January 2011- CAC0031- New bridge up but still closed. Construction completion ETA is Spring 2011. River is backed up.

March 2011- CAC0031- new bridge open river flowing.

May 2011-Red Bridges now submitted with AFO sheets.

April 2011-Began collecting 2 AA vials for submission to CBL for chloride and sulfate. Salinities will be recorded on the field sheet for CBL processing of these samples.

September 2011- TS Lee?- Glencoe bridge under water for core sampling. Sampled from roadway. Station was also sampled for NTN.

October 2011- Extra bottles of filtrate collected in exchange for MDE $$ = ION sample for CBL/SERC-TF5.0, ANT0044, MON0528.

Sept 2012- MON0528 bridge replacement construction beginning.

October 2012- Extra bottles for Ion samples discontinued.

January 2013- Reduced stations sampled for CL & SU due to budget cuts.

January 2014- MON0528- New bridge complete and in use. Old bridge demolition underway. Demolition completed prior

Oct 2014-CL & SU sampling has been discontinued due to budget cuts from MDE.

Nov/2015-Sept/2017-collected 100 ml bottles for analysis of atmospheric Nitrate at Core stations that are also NTN stations. ANT0044, MON0528, CAC0148, GWN0115, GUN0258. These samples were frozen at the field office and delivered to the Appalachian Laboratory in Western MD.

Dec/2015-comparison sampling with U of DE @ DER0015 & CB1.0.

2017-future-- Restoration of Rocky Gorge Dam-construction had sometimes prevented sampling.

Actual gage sampling locations and additions.

#9 PAT0285- gage no longer active.
#11 JON0184-sample taken @ gage
#23 POT1595- MD side of Point of Rocks
#24 POT1596- VA side of Point of Rocks
#26 MON0155- Pine Cliff Park boat ramp up stream of bridge.
#29 BPC0035- gage not online, access limited.
#32 POT1830- gage no longer active
#38- CON0180- USGS gage & station is located on Wishard Road
#40-TOW0030- USGS gage & station is located on Pack Horse Road
#42- NBP0103- boat ramp off Rt. 51 in Spring Gap
#43 NBP0326- gage no longer active. No longer sampled @ old RR crossing bridge in ballistics plant.
#46- WIL0013- Locust Grove Road bridge crossing.
#47- GEO0009- Victory bridge in Westernport next to Town Hall
#49- NBP0689- January 2009 began sampling @ Rt. 38 bridge. Hillside next to gage is unstable and slippery.
#51- YOU0925- bridge crossing in Friendsville on Main Street.
#52- YOU1139- Liberty Street/ Herrington Manor bridge crossing.
#53-LYO0004- Bridge crossing @ Oakland/Rosedale Road.
#54-CCR0001- bridge crossing on State Park

Appendix III: Progress Report/ Cross Reference Sheet

Maryland Department of Natural Resources
Chesapeake Bay Water Quality Monitoring

Progress Report / Cross Reference Sheet - CORE

Month/ Year: January/ 2009 **Submitted by: Laura Fabian**

Station	Day	Sequence #	Depth (M)	Sample #	Lab (DHMH)	Chloro. (CBL)	Comments
ET5.0 Red Bridges		0901C01	0.0	ET5.0			
Patuxent Land Core							
PXT0809 Rocky Gorge	7	0901C02	0.0	C-34			
PXT0972 Unity	7	0901C03	0.0	C-35			
Susquehanna Core							
DER0015 Deer Creek	6	0901C04	0.0	C-24			
GUN0258 Glencoe	6	0901C05	0.0	C-27			
GUN0476 Above Prettyboy	6	0901C06	0.0	C-26			
CB1.0 Below Conowingo Dam	6	0901C07	0.0/1	C-25			
			0.0/2	C-25 dup			

Appendix IV: Field Sheet

R 1 **Project Name:** Core-Western MD

Submitter: AFO-Fabian

Maryland Department of Natural Resources

Field Sheet

Sequence Number C 3 8 (punch in 3-9 all cards)

Sampling Station Number	Start Date Year	Month	Day	Start time	End Date Year	Month	Day	End Time	Number of Samples	submitter code	Data Category Code	Total Depth M	Study Code
P O T 2 7 6 6									0 1	8 0	A A		0 4 0

R 2

Sample Method	+/-	Air Temp °C	Tide State	Weather code Yesterday	Weather code Today	Cloud Cover (%)	Wind Direction	Wind Velocity (Knots) Min.	Max.	Secchi (M)	Basis	Flow Value	Exp	G/L	Senior Scientist
1															

DO Method	Equip. Set Unit #	Probe Number	Flow Tide Unit #	Wave Height (M)	Upper (M) Pycnocline Limit	Lower (M) Pycnocline Limit	Scientist Sign Off	gage height
H	9	9						0

R 3 begin comments here

R 4 0

Rep No.	Depth M	Water Temp. °C	Field pH	Cor. Flag	D O mg/l G/L	Conductivity Micromhos/cm G/L	Salinity ppt	Rep Code	Sample Depth M	Layer Code	LAB LOGIN Bottle Number	other information
1	0 0		0	C	0			1	0 0	S	C-4	

Weather Codes

10 = none
11 = drizzle
12 = rain
13 = rain, heavy
14 = squally
15 = frozen precipitation

pc/pn volume _____ml

pp/tss volume _____ml

USGS # 01610000
Potomac River @ Paw Paw, WV

Date Entered _____

DNR 2/2008

Page 1 of 1

Appendix V: MDH Chemistry Sheet

R 5 study code: 0 4 Replicate:

Maryland Department of Natural Resources

Sequence Number

Survey: Core: Western MD

Collector: _____

Laboratory Analysis Sheet (Core/Trend Program)

AFO-410-990-4524/L. Fabian

Data Code 1D

Sample Station Number: P O T 2 7 6 6

Date (Year Month Day) | Submitter Code: 8 0

Start Depth M: 0.0 | End Depth M | Start Time

Bottle Numbers:

Type of Sample: Filtered (2-8 oz. bottles) | Salinity: ppt

C - 4 | C - 4 | 0

R 6

Sample Method: 1 | Data Category Code: A A | Sample Layer: S | Field Scientist Sign Off | Date received by Lab (Year Month Day) | Time Received by Lab | Batch Number | 0

R 7

Check test Required	Parameter Description / Units	Parameter Code	Method Code	Analysis Problem Code	G/L	Results Record Decimal in a Box	Percent Recovery	Standard Deviation	Number in Sample	Analyst Sign Off
X	TDN as N (F) mg/l	T D N								0
X	Ammonia as N (F) mg/l	N H 4								
X	$NO_2 + NO_3$ as N (F) mg/l	N O 2 3								
X	Nitrite as N (F) mg/l	N O 2								
X	PO_4 as P (F) mg/l	P O 4								
X	Total Dissolved P (F) mg/l	T D P								
X	Dissolved Organic C (F) mg/l	D O C								
	Sulfate as SO_4 mg/l	S O 4								
	Turbidity NTU (W)	T U R B								
	Total Alkalinity (W) mg/l	T A L K								
	Total Susp. Solids (W) mg/l	T S S								
	Dissolved Solids mg/l	D S O L								
X	Part. Phosphorus as P mg/l	P P								
X	Part. Carbon as C mg/l	P C								
X	Part. Nitrogen as N mg/l	P N								

R 8 | Date Reported (Year Month Day) | Final Lab sign off | QA/QC sign off | Transcriber sign off | 0

Date Entered: _____

Send Results To:

Bruce Michael
DNR D-2 Tawes Building
Annapolis, MD 21401
bmichael@dnr.state.md.us
410-260-8627

DNR 5/2005

Appendix VI: Particulate Sample Labels

DNR 3/ /2009 DHMH
CAS0479 S
C-7

PP_____ML

DNR 3/ /2009 DHMH
CAS0479 S
C-7

PC/PN_____ML

DNR 1/ /2009 Western MD
PC/PN Blank Core
Place 2 (if you have them left over) pads in foil & submit with rest of samples.

DNR 3/ /2009 DHMH
PAT0176 S
C-18

PP_____ML

DNR 3/ /2009 CBL
PAT0176 S
C-18

CHLA_____ML

DNR 3/ /2009 DHMH
PAT0176 S
C-18

PC/PN _____ML

Appendix VII: MDH Volume Sheet

Baltimore Core **DNR-MANTA**
DHMH

DATE_____ **SCIENTIST SIGNOFF_____**

STATION	SAMPLE #	LAYER CODE	DEPTH (M)	TIME (MLTY)	PP Vol. Filtered. (ml) Big pads	PC/PN Vol. Filt (ml) Little pads
PAT0176 Patapsco	**C-18**	**S**	**0.0**			
PAT0285 Patapsco	**C-19**	**S**	**0.0**			
GWN0115 Gwynns Falls	**C-20**	**S**	**0.0**			
NPA0165 N BR Patapsco	**C-21**	**S**	**0.0**			
JON0184 Jones Falls	**C-22**	**S**	**0.0**			
GUN0125 Gunpowder Falls	**C-23**	**S**	**0.0**			
Please note anything out of the ordinary- late filtering, reasons for not sampling, extreme weather-						

Appendix VIII: CBL Volume Sheet

Baltimore Core

DNR-MANTA CBL

DATE_____ SCIENTIST SIGNOFF_____

STATION	SAMPLE #	LAYER CODE	DEPTH (M)	TIME (MLTY)	Chlorophyll volume (ml)
PAT0176 Patapsco	C-18	S	0.0		
PAT0285 Patapsco	C-19	S	0.0		
GWN0115 Gwynns Falls	C-20	S	0.0		
NPA0165 N BR Patapsco	C-21	S	0.0		
JON0184 Jones Falls	C-22	S	0.0		
GUN0125 Gunpowder Falls	C-23	S	0.0		

Please note anything out of the ordinary- late filtering, reasons for not sampling, extreme weather-

Appendix IX: CBL sheets for Cl + SO_4

Western MD Core **DNR-MANTA**
Day 1 **CBL**

DATE_____ **SCIENTIST SIGNOFF_____**

STATION	SAMPLE #	LAYER CODE	DEPTH (M)	TIME (MLTY)	SALINITY (ppt)
LYO0004 Little Youghiogheny	**C-11**	**S**	**0.0**		
NBP0534 N.Br. Potomac @ Bloomington	**C-13**	**S**	**0.0**		
GEO0009 Georges Creek	**C-15**	**S**	**0.0**		
NBP0461 N.Br. Potomac @ Keyser, WV	**C-16**	**S**	**0.0**		

Western MD Core **DNR-MANTA**
Day 2 **CBL**

DATE_____ **SCIENTIST SIGNOFF_____**

STATION	SAMPLE #	LAYER CODE	DEPTH (M)	TIME (MLTY)	SALINITY (ppt)
CCR0001 Cherry Creek	**C-9**	**S**	**0.0**		
	C-9 Dup	**S**	**0.0**		
YOU0925 Youghiogheny @ Friendsville	**C-8**	**S**	**0.0**		
NBP0103 N.Br. Potomac @ Spring Gap	**C-1**	**S**	**0.0**		
NBP0023 N. Br. Potomac @ Oldtown	**C-2**	**S**	**0.0**		
TOW0030 Town Creek	**C-3**	**S**	**0.0**		

Appendix III: Maryland Department of Health, Environmental Sciences Division: Standard Operating Procedures for Water Quality Parameters

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

SOP Title: Determination of Alkalinity by Titrimetry (Standard Method 2320 B)

SOP No.: CHEM-SOP-SM 2320 B

Revision: 4.2 Replaces: 4.1 Effective: 7/01/17

Laboratory: Inorganics Analytical Laboratory

POC: Lara Phillips
lara.johnson@maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

Standard Method 2320 B
Sop No.: CHEM-SOP-SM 2320 B

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	Taiyin Wei	6/2/08
1.0	12/09/09	Screen snapshots added to procedure, new Sample Run Log, tracking IDs for standards and reagents	Taiyin Wei	1/10
2.0	1/11	New SOP tracking number	Asoka Katumuluwa	1/11
3.0	4/12/12	Editorial and technical changes- Checklist update	S. Ameli J. Freeman-Scott	9/17/12
3.0	4/16/13	Reviewed The SOP	S. Ameli J. Freeman-Scott	6/16/13
4.0	10/31/14	Changed the format	A. Hamilton S. Ameli L. Phillips	12/01/14
4.1	6/1/15	Reviewed document, updated section 9.4	L. Phillips S. Ameli	7/1/15
4.1	5/2/16	Reviewed Document	L. Phillips S. Ameli	7/1/16
4.2	6/2/17	Reviewed Document and made organizational name changes	L. Phillips S. Ameli	7/1/17

TABLE OF CONTENTS

STANDARD OPERATING PROCEDURE

DETERMINATION OF ALKALINITY BY TITRIMETRY

Standard Method 2320 B

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to drinking, surface, and saline waters, and domestic and industrial wastewaters.

1.2 This method is suitable for all concentrations of alkalinity; however, appropriate aliquots should be used to avoid a titration volume greater than 50 mL. The sample must not be filtered, diluted, concentrated, or altered in any way.

1.3 Alkalinity is the acid-neutralizing or buffering capacity of a water body. The alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content; it is taken as an indication of the concentration of these constituents.

1.4 Measuring alkalinity is important in determining a stream's ability to neutralize acidic pollution from rainfall or wastewater. Alkalinity in excess of alkaline earth metal concentrations is significant in determining the suitability of water for irrigation.

2.0 SUMMARY OF METHOD

2.1 An unaltered sample is titrated to an electrometrically determined end-point of pH 4.5 using an automated system. The sample must not be filtered, diluted, concentrated, or altered in any way.

2.2 Alkalinity as $CaCO_3$ is determined from the volume required of a 0.02 N sulfuric acid (H_2SO_4) to titrate 50 mL of the sample. For samples with high alkalinities that require more than 50 mL of titrant smaller sample volumes are used.

2.3 For samples of alkalinities less than 20 mg/L, the amount of the acid required to reduce the pH exactly 0.30 pH units below pH 4.5 is measured and an extrapolation technique is used to determine the equivalence point.

3.0 INTERFERENCES

Soaps, oily matter, suspended solids, or precipitates may coat the glass electrode and cause a sluggish response. Clean the electrode occasionally. Do not filter, dilute, concentrate, or alter sample.

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during reagent preparation and instrument operation.

4.2 The use of a fume hood, protective eyewear and clothing and proper gloves are recommended when handling acids.

4.3 Each employee is issued a *Laboratory Safety Manual* and a *Quality Assurance plan* and is responsible for adhering to the recommendations contained therein.

4.4 Each chemical should be regarded as a potential health hazard. A reference file of material safety data sheet (MSDS) is available in the lab.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Mantech PC Titration system, consisting of

5.1.1.1 PC-Titrator with Auto-Sampler

5.1.1.2 System Controller with monitor

5.1.1.3 Printer

5.1.1.4 Electrode – Sure-Flow Combination pH electrode, glass body, with BNC connector, Man-Tech # PCE-80-PH1200 or equivalent.

5.1.2 Analytical balance – Mettler Toledo AG204 or equivalent

5.2 Supplies

5.2.1 Glass beakers – 100 mL

5.2.2 Graduated cylinder – class A, 50 mL

5.2.3 Volumetric flasks – class A, 50 mL, 100 mL, 500 mL, and 1000 mL

5.2.4 Pipetters – 100 – 1000 µL, 500 – 5000 µL, and 1 – 10 mL

5.2.5 Carboy – 5 L, with spigot, Nalgene

5.2.6 Transfer pipettes – Samco, cat. # 231

5.2.7 pH Electrode filling solution – follow manufacturer's recommendations

6.0 REAGENTS AND STANDARDS

6.1 Reagents

6.1.1 Deionized water

6.1.2 H_2SO_4, 0.02N – Fisher, cat. # SA 226-4

6.2 Standards

6.2.1 pH 4.0 buffer solution – Fisher, cat. # SB 101-500

6.2.2 pH 7.0 buffer solution – Fisher, cat. # SB 107-500

6.2.3 pH 10.0 buffer solution – Fisher, cat. # SB 115-500

6.2.4 Stock standard, 25,000 mg/L $CaCO_3$ (0.5N) – 10 mL/ 16 voluette ampoules, Hach, product # 14278-10

6.2.5 Intermediate standard, 5000 mg/L $CaCO_3$ – Pipet 5 mL of the stock standard (6.2.4) into a 25 mL volumetric flask. Bring to volume with deionized water. Prepare every two weeks.

6.2.6 Check standard, 50 mg/L $CaCO_3$ – Pipet 5 mL of intermediate standard (6.2.5) into a 500 mL volumetric flask. Bring to volume with deionized water. Prepare every two weeks.

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Samples are collected in 1 liter polyethylene cubitainers and iced or refrigerated to 4 ^{o}C. The holding time is 14 days

7.2 The sample must not be filtered, diluted, concentrated or altered in any way.

8.0 QUALITY CONTROL

8.1 The acceptable range for the slope of the calibration curve is -65 mV to -53 mV. Calibration has to be repeated if the slope falls outside this range.

8.2 A blank and a blank spike are analyzed at the beginning of the run. Blank concentration must be less than the reporting level of 1 ppm and the acceptable

value for the spike recovery is 90 – 110%. Blank, blank spike or sample spike not meeting the criteria is reanalyzed.

8.3 Every tenth sample is duplicated and spiked. The acceptable values for the relative percent difference (RPD) are ± 10 and for the spike recovery (SR) are 90 – 110%. If these do not fall within the accepted ranges, the corresponding analyses are repeated.

8.4 A check standard is run after every ten samples.

8.5 A QC sample is analyzed at the beginning and the end of each analytical run.

8.6 Data acceptance criteria are listed on the data review checklist. (Appendix A).

8.7 Laboratory participates in yearly ERA WatR Supply (WS) and WatR Pollution (WP) Proficiency Tests.

8.8 An initial demonstration of capability study is performed by each analyst performing the test.

9.0 PROCEDURE

9.1 Sample preparation

9.1.1 Prepare a list of samples to be analyzed on a Sample Run Log (Appendix B).

9.1.2 Pour approximately 60 mL of the pH 4, pH 7 and pH 10 buffers into each of the labeled 100 mL beakers.

9.1.3 Pour 50 mL portions of each well mixed sample, measured using a class "A" graduated cylinder, into labeled 100 mL beakers. Pour a duplicate of every tenth sample.

9.1.4 Spike blank and every tenth sample, or one sample per batch if analyzing less than 10 samples, by adding 1 mL of Intermediate standard solution (6.2.5) to 49 mL of deionized water and samples respectively.

9.2 Daily electrode preparation

9.2.1 Rinse the electrode with deionized water to remove crystal residue that may have formed on the surface during storage.

9.2.2 Check the electrolyte level in the reference cavity, which should be approximately ¼ inch below the fill-hole. If the electrolyte level is too

low, add filling solution (5.2.7) with a transfer pipet. Replace the cap, and then rinse clean the electrode again.

9.2.3 Remove fill-hole cover during calibration and measurement to ensure uniform flow of filling solution.

9.3 Weekly electrode maintenance

9.3.1 Disconnect the electrode from the unit. Empty the electrode with a transfer pipet. Rinse with deionized water and then, fill up with filling solution. Connect the electrode.

9.3.2 Soak electrode in pH 4 buffer for a minimum of one hour.

9.3.2.1 Follow the steps in 9.4.1 to 9.4.3

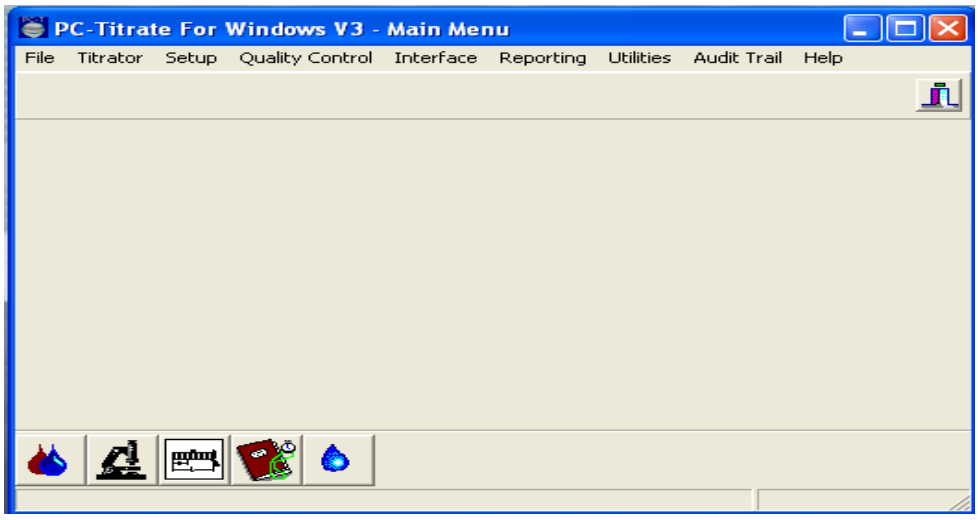
9.3.2.2 Place a beaker with pH 4 buffer in the # 1 position.

9.3.2.3 Select "Tubes" from "Zones", select "1" as the beaker number for "Tubes & the like". Click on "Go to this location XYZ" to send the probe to "1" position.

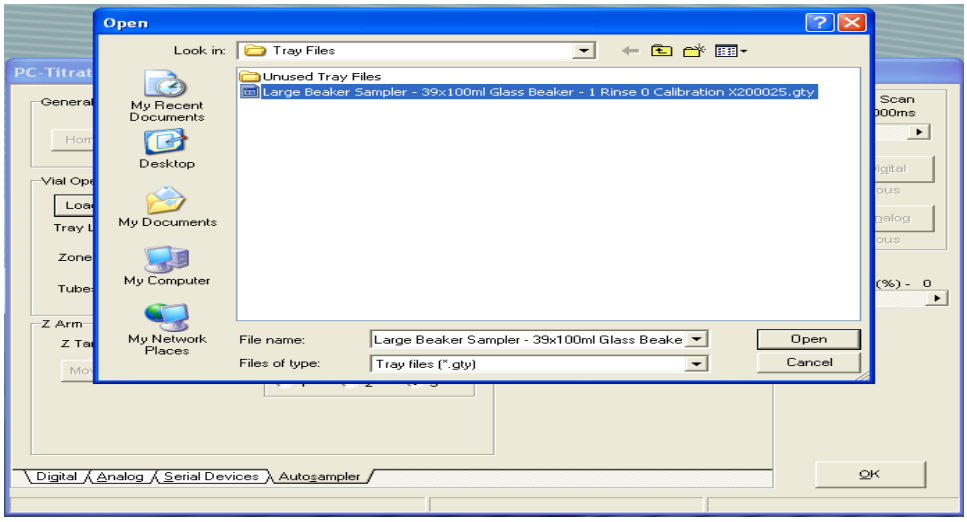
9.4 Instrument preparation

9.4.1 Check and fill the deionized bottle and acid bottle.

9.4.2 Turn on the computer and the autosampler. Double click on "PC-Titrate V3".

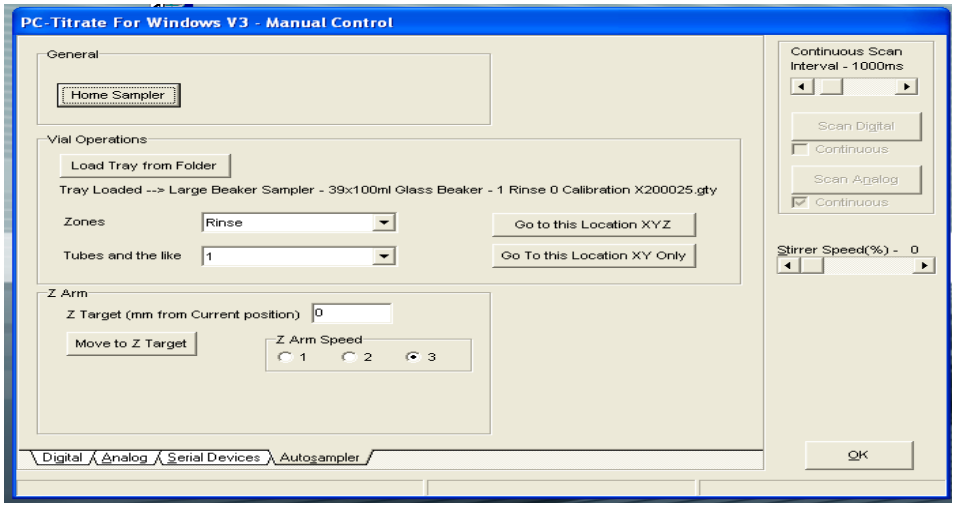


9.4.3 Click on "Titrator" and select "Manual control" from the pull down list. Select "Autosampler", "Load tray from folder", and "Large beaker sampler", and then click on "Open".

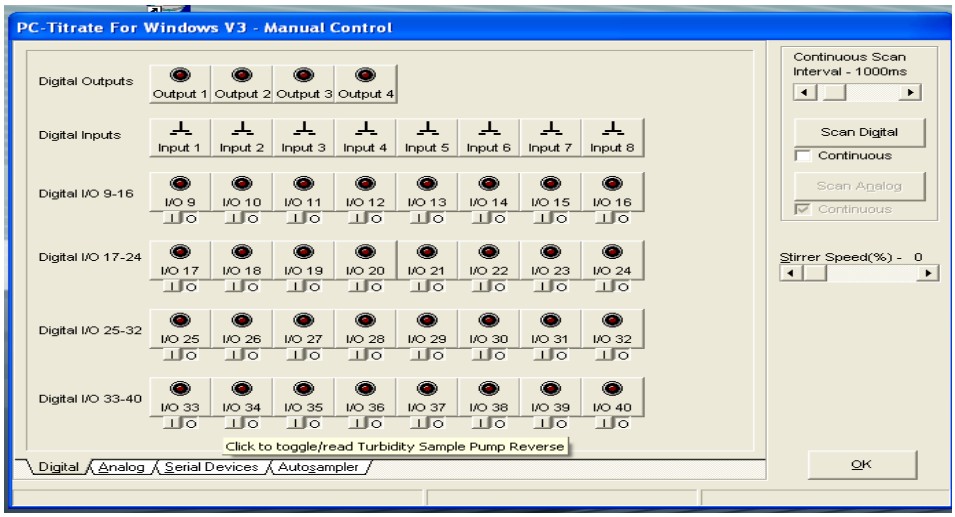


9.4.4 Click on "Home sampler" to send the probe to home position.

9.4.5 Select "Rinse" from "Zones" and "1" as the beaker number for "Tubes & the like". Click on "Go to this location XYZ" to send the probe to the rinse beaker.



9.4.6 Click on "Digit" tab and "Output 4" to rinse the probe and fill up the beaker. Click "Output 4" again to turn it off.



9.5 Buret preparation

9.5.1 Remove the titrant delivery line from the electrode block on the autosampler and place it into a waste beaker.

9.5.2 Check and fill the acid bottle.

9.5.3 Go to the “Serial devices”.

9.5.4 Click on button labeled “Dispense 10%” to dispense the 0.002 N H_2SO_4 through the titrant delivery line. Repeat 2 more times or until no bubbles are observed in the flow.

9.5.5 Fill up the syringe by clicking on “Syringe full down”.

9.5.6 Remove the dispenser tip from the waste beaker and return it to its position in the probe holder.

9.6 Daily electrode calibration and sample analysis

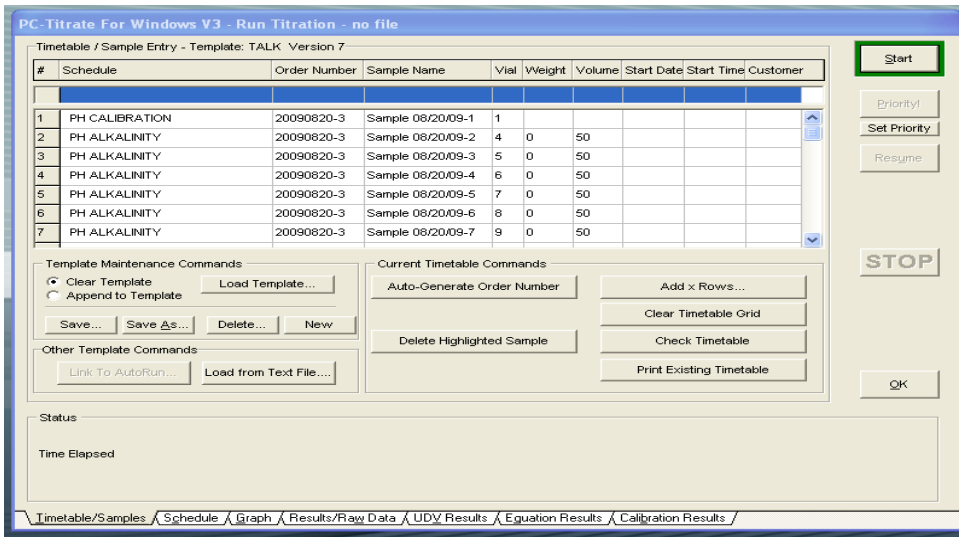
9.6.1 Place pH 4.0, 7.0 & 10.0 buffers into autosampler tray using position # 1, 2 & 3.

9.6.2 Click on the PC Titrate V3 tab.

9.6.3 Click on the book tab at the bottom labeled “pH cal 4-7-10” tab to call up the sample table.

9.6.4 Place the samples after the calibration: The template will have “4-7-10” under sample name at the first row reserved for a schedule of “pH calibration” with a 1 in the vial number box. Enter sample names

according to the sample run log (9.1.1) starting with the second row (vial # 4) a check standard, a blank, a blank spike, a QC, and samples to be analyzed. Enter a check standard, a blank, and a QC again at the end of the run. All other samples and checks are to be run with a "pH Alkalinity" schedule chosen.



9.6.5 Highlight each excess line, and then click on "Delete Highlighted Sample" to remove all unused sample information.

9.6.6 Highlight a line and click on "Add x lines" to add additional lines. Left click on the mouse to relocate the lines.

9.6.7 Click "Check Timetable" to verify information entered are valid. Roll down the table to make corrections if needed. Click "OK".

9.6.8 Load the samples according to the run list with the last sample followed by a beaker with the solution recommended by the probe's manufacturer.

9.6.9 Click on "Start".

9.6.10 To run a second tray using the same calibration: Double click on "pH Calibration" and replace it with "pH Alkalinity". Fill in sample names starting with the first row (vial #1). Make sure a set of the quality control samples: check standard, blank, and external QC is also being run at the beginning and at the end in the second tray.

9.6.11 Print the *Calibration Report* and a custom report of *Alkalinity Results* at the end of the run.

9.6.12 Recall each titration curve by clicking on "Titrator", "Titration Replay", "Load", and then, selecting date and sample name. Click on "Select" to observe the titration curve. Click "OK" to return to the main menu.

9.6.13 Results can also be printed out by clicking on "Equation results" tab, "Print", and then "OK".

9.6.14 Go to "Manual control" and select "Autosampler" tab. Select "tubes" from "Zones" and "1" as the number for "Tubes & the like". Click on "Go to this location XYZ" to send the probe to the # 1 beaker with the solution recommended by the probe's manufacturer.

9.6.15 Shut down the computer and turn off the autosampler.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 Alkalinities are calculated automatically by the PC-Titrate V.3 software based on 1 mL of 0.1N H_2SO_4 = 5.0 mg $CaCO_3$

10.1.1 Potentiometric titration to an end point of pH 4.5

$$\text{Alkalinity, mg } CaCO_3/L = \frac{\text{titrant dispensed, mL x 0.02N } (H_2SO_4) \text{ x 50,000}}{\text{sample volume, mL}}$$

10.1.2 Potentiometric titration of low alkalinity

$$\text{Total Alkalinity, mg } CaCO_3/L = \frac{(2B - C) \text{ x 0.02N } (H_2SO_4) \text{ x 50,000}}{\text{sample volume, mL}}$$

where:

B = mL titrant to first recorded pH
C = total mL titrant to reach pH 0.3 unit lower

10.2 Calculate the percentage spike recovery of the laboratory fortified blanks and samples as follows:

$$\%SR = \frac{\text{spiked sample conc.} - \text{sample conc., ppm}}{\text{amount of spike added to sample, ppm}} \text{ x } 100$$

10.3 Calculate the relative percentage difference of the duplicated samples as follows:

$$RPD = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \text{ x } 100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 Instrument maintenance, instrument calibration, and quality control data are kept in binders and test results are kept in the file cabinet.

11.2 Normal turnaround time for samples submitted to this lab will be 2 to 10 days from receipt. Results are reported in writing on a sample analysis request form.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 Excess reagents, samples and method process waste are poured into the sink with running water.

12.2 Actual reagent preparation volumes are to reflect anticipated usage and reagent stability.

13.0 REFERENCES

13.1 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater,* Method Number 2320B, 21st Edition, 2005

13.2 Man-Tech Associates inc., *PC-Titrate Windows Software Manual,* version 3.0, November 2004.

13.3 U.S. Environmental Protection Agency, *Monitoring and Assessing Water Quality, 5.10 Total Alkalinity,* November 2006

13.4 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.5 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX **A**

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist – Alkalinity

Standard Methods 2320 B

Lab Numbers[1]:_____

Date Collected: _____ Date Analyzed: _____ Analyst: _____

Procedure	Acceptance Criteria	Status*	Comments
Holding Time	14 days @ 4°C		
Calibration Results	Slope = –65.00 to –53.00 mV		
External QC[2]	Beginning and end of each run		
	Within acceptable range		
Reagent Blank	< Reporting level (1 mg/L)		
Blank Spike	1 per batch		
	Recovery = 90 – 110%		
Check Standard	After every 10th sample and at the end of the run		
	Concentration within 90 to 110% of the true value		
Duplicates/Replicates	Every 10th and the last sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
Matrix Spike	Every 10th and the last sample or 1/batch, if less than 10 samples		
	Recovery = 90 – 110%		
Decimal Places Reported	0		
Changes/Notes	Clearly stated		

* Check (√) if criteria are met.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

[2]External QC
Identification = _____
True Value = _____ ppm
Range = _____ ppm

APPENDIX **B**

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Sample Run Log – Alkalinity

Standard Method 2320 B

Date: _____ Analyst: _____

Tray 1 Cup #	Sample ID	Dilution
1	pH 4	
2	pH 7	
3	pH 10	
4	Ck Std	
5	Blank	
6	Blank -Spike	
7	QC	
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Tray 1 Cup #	Sample ID	Dilution
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		

Tray 2 Cup #	Sample ID	Dilution
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Sample Name	Tracking ID
pH 4 Buffer	
pH 7 Buffer	
pH 10 Buffer	
H_2SO_4, 0.02N	

Lab #	Average	RPD	% Spk Rec

Sample Name	Prep Log ID
Intermediate Std, 5,000 ppm	
Ck Std, 50 ppm	
QC:	

Standard Operating Procedures

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

SOP Title: Determination of Turbidity by Nephelometry (EPA Method 180.1)

SOP No.: CHEM-SOP-EPA 180.1

Revision: 3.2 Replaces: 3.1 Effective: July 1, 2017

Laboratory: Inorganics Analytical Laboratory

Author / POC: Jeffrey Fernandez
Jeffrey.Fernandez @maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

EPA Method 180.1
SOP No.: CHEM-SOP-EPA 180.1

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	Taiyin Wei	6/2/08
1.0	12/09	Tracking IDs for standards and reagents	Taiyin Wei	1/10
2.0	1/11	New SOP tracking number	Asoka Katumuluwa	1/10
2.0	6/13	Reviewed SOP	S. Ameli	6/13
3.0	10/20/14	Changed format	L.Phillips/J. Fernandez S. Ameli	12/1/14
3.0	6/1/15	Reviewed	L.Phillips S. Ameli	7/1/15
3.1	5/3/16	Reviewed and updated formatting and checklist	L.Phillips S. Ameli	7/1/16
3.2	5/2/17	Reviewed and updated formatting and checklist	L.Phillips/J. Fernandez S. Ameli	7/1/17

TABLE OF CONTENTS

STANDARD OPERATING PROCEDURES

DETERMINATION OF TURBIDITY BY NEPHELOMETRY

EPA Method 180.1

1.0 SCOPE AND APPLICATION

1.1 Turbidity is a principal physical characteristic of water and is an expression of the optical property that causes light to be scattered and absorbed by suspended matter or impurities that interfere with the clarity of the water.

1.2 Determination of turbidity is a common component of water quality assessments. This method is applicable to drinking, ground, waste and saline waters.

1.3 The applicable range of Hach 2100AN Turbidimeter is 0 to 4000 nephelometric turbidity units (NTU). Drinking water samples with turbidity values greater than 40 NTU are diluted and re-analyzed.

2.0 SUMMARY OF METHOD

2.1 This method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of light scattered, the higher the turbidity.

2.2 Readings in NTUs are made using a nephelometer. Detectors of the nephelometer are in place to measure the 90° scattered light, the forward scattered light, the back scattered light and the light transmitted through the sample. The laboratory measures the value in the "Ratio On" mode, in which the instrument's microprocessor uses a mathematical calculation to ratio signals from each detector. The benefits of applying "ratio" on measurements include better linearity, calibration stability, wide measurement range, and the ability to measure turbidity in the presence of color.

3.0 INTERFERENCES

3.1 Etched, scratched, or dirty sample vials or dust contamination within the sample cell compartment and optical compartment scatter light and give inaccurate readings.

3.2 Samples containing air bubbles, coarse debris, or floating sediments can cause erroneous readings.

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during inversion of sample and reading of sample result. Use absorbent towels if material is spilled and wash residual into drain.

4.2 Each employee is issued a ***Laboratory Safety Manual*** and is responsible for adhering to the recommendations contained therein.

4.3 Use absorbent towels if material is spilled and wash residual into drain.

4.4 A reference file of MSDS is available in room 7D1.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Hach Model 2100AN Laboratory Turbidimeter – consisting of a nephelometer with a tungsten-filament lamp for illuminating the sample and detectors to measure scattered light.

5.1.2 Computer – Dell, Microsoft Windows 98

5.1.3 Printer – Hewlett-Parker Deskjet 722C.

5.2 Supplies

5.2.1 Sample cells – 30 mL capacity, item # 20849-00, Hach Co.

5.2.2 Pipettes – Volumetric, class A, 5, 10, 20, and 25 mL.

5.2.3 Flasks – Volumetric, class A, 50 mL, 100 mL and 200 mL

5.2.4 Flasks – Erlenmyer, 50 mL and 100 mL

5.2.5 Gloves – Powder-free, nitrile, item #FF-700, Micro Flex.

5.2.6 Kimwipes – 14.7 x 16.6”, item #34721, Kimberly-Clark.

5.2.7 Carboy – 2 $\frac{1}{2}$ gal, with spigot, item # 23210020, Nalgene.

5.2.8 Container – Plastic, for liquid waste, 1 or 2 liter size.

6.0 REAGENTS AND STANDARDS

6.1 Reagents

6.1.1 Deionized water.

6.1.2 Hydrochloric acid, 6N – Fisher Scientific #LC15370-Z.

6.2 Standards

6.2.1 AMCO CLEAR Calibration Kit, for Hach 2100N/AN: 0, 20, 200, 1000, and 4000 NTU – Item # 85525, GFA Chemicals. Use freshly poured portions for calibrating the turbidimeter and discard the used standards prior to each new calibration. Rinse with DI water and new standard before pouring fresh standards.

6.2.2 AMCO CLEAR Sealed Standards: 0.5, 1.0, 2.0, 5.0, 20, 50, 100, and 200 NTU – Item # 86180, 86443, 86534, 86492, 86122, 85385, 86124, and 86123 respectively, GFA Chemicals. Read these standards at the beginning of each analytical run.

6.2.3 Quality Control Sample – QC-TUR-WS, Spex Certiprep Inc. Empty the entire contents into a small beaker and gently swirl to mix thoroughly. Do not rinse the ampule. Immediately transfer 10.0 mL of the concentrated solution into a 200 mL volumetric flask and bring to volume with deionized water. Mix well and use within 24 hours.

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Samples are collected in liter polyethylene cubitainers and refrigerated or iced to 4 ^{o}C until analysis to minimize microbiological decomposition of solids.

7.2 The holding time is 48 hours when preserved at 4 ^{o}C.

8.0 QUALITY CONTROL

8.1 Instrument Calibration

8.1.1 Primary standards (6.2.1) with concentrations ranging from 0 to 4000 NTU are used to calibrate the turbidimeter every two months.

8.1.2 Sealed secondary standards (6.2.2) with concentrations ranging from 0.5 to 200 NTU are analyzed before each day's run of samples. The instrument check is considered valid when each measured NTU value is within 90 – 110% of its true value. If the values do not fall within the acceptable range the instrument has to be recalibrated using the primary standards (6.2.1) or new standards should be ordered.

8.1.3 AMCO Clear standards are guaranteed to maintain the certified value for 1 year from ship date.

8.2 A mid-range check standard is analyzed after every ten samples and at the

end of each run. If a check standard fails, the samples between the last satisfactory check standard and the unsatisfactory standard are re-analyzed.

8.3 Every tenth sample is analyzed in duplicate. The accepted value for the relative percent difference (RPD) is ± 10 %. If the reading does not fall within the accepted ranges, the corresponding analysis is repeated.

8.4 Deionized water is run at the beginning, after every ten samples, and at the end of the run. The accepted value for the blank is less than 0.07 NTU. Routine maintenance includes periodically clean sample cells. Also see Section 9.5.3.

8.5 A quality control sample is analyzed quarterly. Results are kept in a binder next to the instrument.

8.6 A method detection limit (MDL) study is performed once a year by analyzing seven or more replicates of the 0.5 NTU standard spread out through three or more consecutive analytical runs. An MDL study is also performed by each new analyst and when any changes in the analytical procedure are made.

8.7 Data acceptance criteria are listed on the data review checklist (Appendix A).

9.0 PROCEDURE

9.1 Sample Cell Preparation

9.1.1 Clean the samples cells meticulously, both inside and out, and the caps.

9.1.2 Wash the sample cells with soap and rinse with deionized water.

9.1.3 After rinsing, immediately soak the sample cells in a 6N hydrochloric acid solution for a minimum of one hour.

9.1.4 After soaking, immediately rinse the sample cells with deionized water. Rinse a minimum of 15 times.

9.1.5 Immediately after rinsing the sample cells, cap the cells to prevent contamination from the air, and to prevent the inner cell walls from drying out.

9.1.6 Sample cells that are nicked or scratched must be replaced.

9.2 Index New Sample Cells

9.2.1 Fill clean sample cells with deionized water to the fill ring mark. Let samples stand for 30 seconds to allow bubbles to rise.

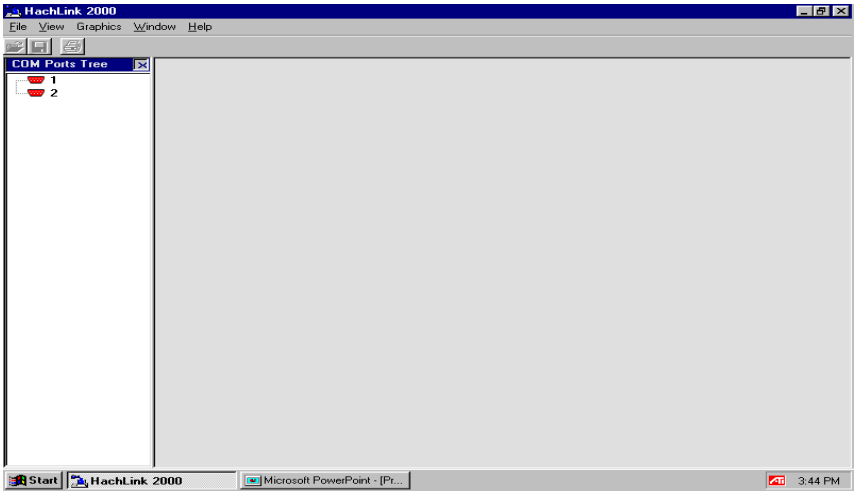
9.2.2 Measure the turbidity at several points of rotation, or as many points as needed, starting with placing the sample cell into the holder with the

diamond mark at 6 o'clock position. Mark the orientation where the turbidity reading is the lowest. Use this orientation to perform all sample measurements.

9.2.3 Use the same indexed sample cell, if possible, to measure all the samples.

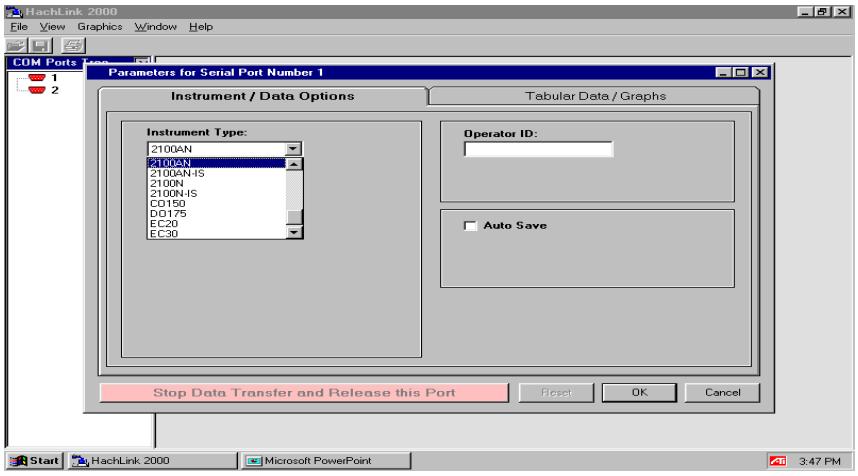
9.3 Instrument Start-up

9.3.1 Leave the turbidimeter on 24 hours a day if the instrument is used daily. Make sure "Ratio**"**, "Sample**"** and "Signal Average" keys are in "ON" mode displayed by a green light. Maintain "Range" key in "Auto" mode. Select "NTU" from "Units/Exit" key. Turn on the computer. Insert the disk marked as "Turbidity Data". Click on "Hachlink" on the desktop.

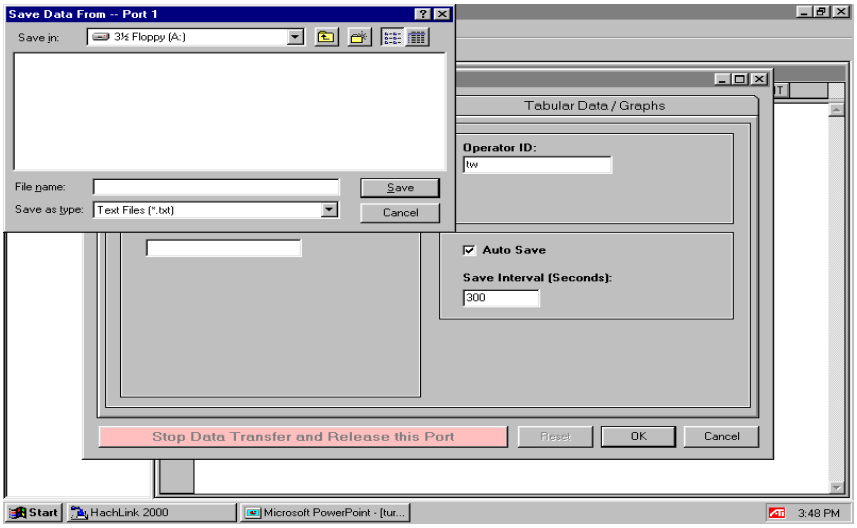


9.3.3 Select "COM Port 1" as the port type by clicking on "1".

9.3.4 Select "2100AN" from the pull down menu of instrument types.

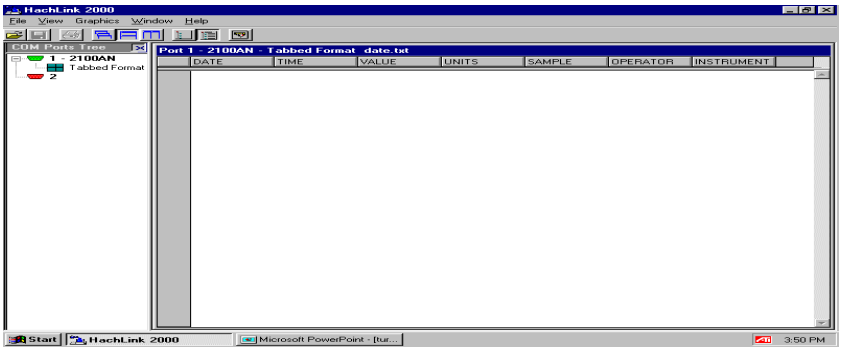


9.3.5 Enter operator I.D. and select "Auto Save".



9.4 Instrument Calibration

9.4.1 Select "Free Format" for calibration. Enter Date (Cal MM-DD-YY) as file name and click on "Save".



9.4.2 Press "Cal Zero". When 00 flashes in green display proceed. Do not shake or mix standards.

9.4.3 Place the "0" NTU standard into the cell holder, align the mark, then close the cell cover.

9.4.4 Press "Enter". The instrument display counts down from 60 to 0, and then makes a measurement.

9.4.5 The instrument automatically increments to the next standard, 01, as shown on screen in green display. Repeat steps 9.4.3 and 9.4.4 with the rest of the standards: 200, 1000 and 4000 NTU (When the instrument asks for 7500 NTU, press "Cal" to end it.)

9.4.6 Press "Cal Zero**"** again to store calibration information into memory. Press "Print". The instrument returns to the sample measurement mode.

9.4.7 Press "Cal" key to review Calibration Data. Use "Δ" key to scroll through the standards. Press the "Print" key prints all of the calibration data in effect. Press the "Units Exit" key to return to the operating mode.

9.4.8 Read sealed secondary standards

9.4.8.1 Follow step 9.3. Select "Tabled Format**"** for sample reading. Enter date as file name. Start with the deionized water as the blank. Thoroughly clean the outside of the sample cell and place it in the sample compartment. Close the sample holder cover.

9.4.8.2 Press "Enter", then press "Print" to save the reading.

9.4.8.3 Thoroughly clean each of the standard vials. Repeat steps 9.3.8.1 and 9.3.8.2 for all the standards: 0.5, 1.0, 2.0, 5.0, 20.0, 50.0, 100, and 200 NTU.

9.4.8.4 Press "Print". Keep the printouts in the binder marked "Instrument calibration data".

9.4.9 Check and fill the carboy with deionized water for rinsing the sample cell when performing sample measurements.

9.5 Sample Analysis

9.5.1 Prepare the list of samples for turbidity on the sample run log sheet (Appendix B) starting with blank, the daily check standards of 0.5, 1.0, 2.0, 5.0, 20, 50, 100, and 200 NTU, the deionized water, then enter each sample number. Measure one replicate, one check standard and one blank for every ten samples. Read check standards again at the end of the run.

9.5.2 Follow step 9.3. Select "Tabled Format**"** for sample reading. Enter date as file name.

9.5.3 Fill the clean and dry glass cell with deionized water. Wipe dry, then insert the cell. If the reading is greater than 0.07 NTU, the cell should be cleaned with detergent and the process repeated. Press "Enter" to clear all previous data, and then press "Print" to transmit data to computer and printer.

9.5.4 Place the 0.5 NTU sealed standard in the sample compartment. Close the cover. Press "Enter" and then press "Print".

9.5.5 Repeat for the rest of the standards.

9.5.6 Allow samples to reach room temperature to prevent fogging of the cell. Thoroughly mix the sample by gentle inversion. Do not shake. Quickly remove cap and pour approximately 20 ml of sample into the cell for rinse. Immediately fill cell with sample to volume line, wipe dry and insert into turbidimeter. Align the index mark (9.2) on the cell with the raised mark on the spill ring around the cell holder opening. Be sure the cell has been pushed down completely and is held in place by the spring clip. Close the cover.

9.5.7 Wait for 30 seconds. Check the turbidity reading of the sample from the digital display. Press "Enter", then press "Print" to save the first stable reading at approximately 15 seconds. If the turbidity reading fluctuates, take the cell out, invert to mix well and measure again. Observe the results in the display for accuracy.

9.5.8 Read the rest of the samples according to the run log sheet following step 9.5.6 and 9.5.7. Rinse the cell with deionized water, then rinsed with some of the sample before each sample measurement.

9.5.9 For drinking water sample with turbidities exceeding 40 NTU, dilute the sample with turbidity-free water until turbidity falls below 40 NTU.

9.5.10 After reading all samples, double click the blank area outside the table to go to "Microsoft Excel" table. Enter all sample identifications according to the run log sheet into the sample column. Print out the results.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 Calculate and report the average for the duplicated samples.

10.2 Multiply sample reading by the dilution factor to obtain the final result for diluted samples.

10.3 Calculate the relative percent difference for the duplicated samples as follows:

$$RPD = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \times 100$$

10.3 All results are reported to one decimal place. The reporting level (RL) is 0.5 NTU. All sample concentrations below this value are recorded as less than 0.5 NTU (< 0.5 NTU).

11.0 DATA AND RECORDS MANAGEMENT

11.1 Normal turnaround time for samples submitted to this lab will be 2 to 10 days from receipt. Results are reported in writing on a sample analysis request form.

11.2 Instrument maintenance, instrument calibration, and quality control data are kept in binders and test results are kept in the file cabinet.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 It is laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land buy minimizing and controlling all releases from fume and bench operation.

12.2 Samples and standards are poured down the drain while large amount of water is running.

13.0 REFERENCES

13.1 United States Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes,* Method 180.1 Revision 2.0, August, 1993.

13.2 Hach Company, *Model 2100AN Laboratory Turbidimeter Instruction Manual,* 1993.

13.3 Hach Company Technical Information Series – Booklet No. 11, *Turbidity Science,* 1998.

13.4 The American Public Health Association, *Standard Methods for the Examination of Water and Wastewater,* Method, 21thEdition, 2005.

13.5 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.6 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

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APPENDIX A

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist – Turbidity

EPA Method 180.1

Lab Numbers:_____

Date Collected: _____ Date Analyzed: _____ Analyst: _____

Procedure	Acceptance Criteria	Status*	Comments
Holding Time	48 hours @ 4 ºC		
Instrument Calibration[2] (0 – 4000 NTU)	Every two months		
Daily Calibration Checks[3] (0 – 200 NTU)	Within 90 to 110% of true values		
Blank	< 0.07 NTU		
Check Standards	After every 10th sample and at the end of the run		
	Concentrations within 90 to 110% of the true values		
Duplicates/Replicates	Every 10th and the last sample or 1/batch of drinking water samples and 1/batch of wastewater samples, if less than 10 samples of each kind		
	RPD ≤ 10 %		
External QC[4] Every two months	Within acceptable range		
	Last date analyzed:		
Decimal places reported	1		
Reporting Level	0.5 NTU; concentrations below this value reported as < 0.5 NTU		
Measured Values	Within range of 0 to 40.0 NTU for drinking water and 0 to 4000 NTU for others		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

***** Check (√) if criteria are met.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

[1]Include beginning and ending numbers; account for gaps by bracketing.

[2]Sample Name: AMCO CLEAR Calibration kit Tracking ID: _____

[3]Sample Name: AMCO CLEAR Standards Tracking ID: _____

[4]QC Sample: _____ Tracking ID: _____

True Value = _____ Acceptable Range = _____

APPENDIX B

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Sample Run Log – Turbidity

EPA Method 180.1

Date : _____ Analyst: _____

Sample #	Sample ID	Dilution	Conc. NTU
1	0.0 NTU		
2	0.5 NTU		
3	1.0 NTU		
4	2.0 NTU		
5	5.0 NTU		
6	20.0 NTU		
7	50.0 NTU		
8	100 NTU		
9	200 NTU		
10	DI Water		
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			

Sample #	Sample ID	Dilution	Conc. NTU
31			
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			
47			
48			
49			
50			
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			

QC Name	Prep Log ID

Lab #	Average	RPD

MDH - Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

SOP Title: Determination Particulate Carbon and Particulate Nitrogen (Exeter Analytical CE 440)

SOP No.: CHEM-SOP-CE 440

Revision: 2.3 Replaces: 2.2 Effective: 7/1/17

Laboratory: Inorganics Analytical Laboratory

Author / POC: Lara Phillips
Lara.johnson@maryland.gov
Jewel Freeman-Scott
Jewel.freeman-scott@maryland.gov

Laboratory Supervisor:	Signature	Date
QA Officer:	Signature	Date
Manager:	Signature	Date
Division Chief:	Signature	Date

EXETER METHOD CE 440
SOP No.: CHEM-SOP-CE 440

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	12/09	N/A	Taiyin Wei	1/10
1.0	1/11	New SOP tracking number	Asoka Katumuluwa	1/2010
1.1	8/11	Technical and editorial changes	Shahla Ameli	7/2011
2.0	10/14	Reviewed SOP-document control, editorial and technical changes	Shahla Ameli Lara Phillips	12/2014
2.1	6/10/15	Reviewed, Revised procedure, section 8.3	Lara Phillips Shahla Ameli	7/1/2015
2.2	5/9/16	Formatting changes, reviewed SOP	L Phillips J. Freeman-Scott S. Ameli	7/1/2016
2.3	7/1/17	Formatting changes, reviewed SOP, Revised sections 2.0, 4.2, 5.1.4,6.1, 9.2.6, 9.3.4.4.5, 9.4 and appendix B	L Phillips J. Freeman-Scott S. Ameli	7/1/2017

TABLE OF CONTENTS

STANDARD OPERATING PROCEDURES

DETERMINATION OF PARTICULATE CARBON & PARTICULATE NITROGEN

Exeter Analytical CE 440

1.0 SCOPE AND APPLICATION

1.1 This method is used to determine the carbon and nitrogen content in organic and inorganic compounds in surface and saline waters.

1.2 This instrument performs elemental analysis of material retained on filters used in water filtration applications.

2.0 SUMMARY OF METHOD

Particulate material on a pre-ignited glass fiber filter is combusted in an oxygen-helium atmosphere at 980°C. The products of combustion are passed over suitable reagents to undergo complete oxidation and removal of undesirable by-products. The remaining gas proceeds to a mixing chamber. This uniform gas mixture, then passes through a series of traps each bracketed with a pair of thermal conductivity detectors. The difference in the signals from each of these pairs of detectors is proportional to the amount of hydrogen (H in H_2O) and carbon (C in CO_2) present in each sample. The only remaining sample gas, nitrogen (N in N_2), is measured against the pure helium carrier gas.

3.0 INTERFERENCES

3.1 Sampling is the single largest determination of data quality. Duplicates or even triplicates sampling is recommended.

3.2 Filter blanks should be treated the same as filter samples in all respects.

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during instrument operation.

4.2 Combustion and reduction tubes are heated to 980°C and 650°C respectively. Wear heat resistant gloves and work on a heat resistant bench top when changing these tubes.

4.3 Wear insulated gloves and use tongs to remove hot crucibles from the furnace, and place them on a metal tray.

4.4 Each employee is issued a *Laboratory Safety Manual* and a *Quality Assurance Plan* and is responsible for adhering to the recommendations contained therein.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 CE-440 Elemental Analyzer

5.1.2 CEC-490 interface unit

5.1.3 PC computer

5.1.4 Drying oven, 45°C-55°C

5.1.5 Muffle furnace

5.1.6 Microbalance, Sartorius ME 5

5.2 Chemicals

5.2.1 Silver Tungstate-Magnesium Oxide on Chromosorb-A, 20 - 30 mesh

5.2.2 Silver Oxide-Silver Tungstate on Chromosorb-A, 20 – 30 mesh

5.2.3 Silver Vanadate on Chromosorb, 20 – 30 mesh

5.2.4 Ascarite, 20 mesh

5.2.5 Magnesium Perchlorate - slightly crush the irregular chunks to approx. 1/16” to 3/32” diameter

5.2.6 Copper wire

5.2.7 Compressed Oxygen gas

5.2.8 Compressed Helium gas

5.3 Supplies

5.3.1 Filters – Whatman GF/F glass fiber, 25 mm diameter, 0.7 µm particle retention

5.3.2 Nickel sleeves – 7 x 5 mm

5.3.3 Tin capsules – smooth, 6 x 2.9 mm

5.3.4 Desiccators and Desiccants

5.3.5 Microspectula – Hayman style, meets ASTM E 124, Fisher cat. no. 21-401-25A

5.3.6 Microforceps – smooth tips

5.3.7 Pinning forceps

5.3.8 Quartz wool

5.3.9 Vacuum grease

5.3.10 Gloves – heat resistant

5.3.11 Crucible dishes – 3" diameter

5.3.12 Crucible tongs

6.0 REAGENTS AND STANDARDS

6.1 Standard

Acetanilide ($C_6H_5NHCOCH_3$), Acros Organics or Exeter Analytical

6.2 External quality control samples

6.2.1 Domestic Sludge – Standard Reference Material 2781, National Institute of Standards & Technology

6.2.2 Marine Sediment Reference Materials (PACS-2) – National Research Council Canada

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

The filter pads (9.2.1 – 9.2.4) are kept frozen and dried pads (9.2.6) must be desiccated.

8.0 QUALITY CONTROL

8.1 The calibration series must be placed at the beginning of the wheel. (9.3.1)

8.2 Continue the sample run only after the calibration standards have been analyzed and confirmed that the calculated K_C and K_N are acceptable. KC = 18 – 25
KN = 7 – 10

8.3 Every tenth sample should be duplicated and followed by an Acetanilide standard.

8.4 The relative percent difference (RPD) for field and sample duplicates need to be calculated.

8.5 A standard series (standard, blank) should also be placed at the end of the wheel.

8.6 Data acceptance criteria are listed on the data review checklist (Appendix A).

8.7 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of the PC/PN pads provided by the client. MDL is calculated as follows:

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level
And a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made.

9.0 PROCEDURE

9.1 Preparation for Analysis

9.1.1 Filters – Place the filters in ceramic crucibles/dishes, combust at a temperature of 450 – 500°C for one hour, remove from oven and then place them in a desiccator to be cooled. Remove from the desiccator and store in a closed container. These filters are sent to the field for sample collection.

9.1.2 Nickel sleeves – Place the nickel sleeves in stainless cups and muffle at 900°C for one hour. Remove, cool down in a desiccator, and store in a capped glass jar. Pre-muffled sleeves can be purchased from Exeter Analytical, Cat # 6703-0499M.

9.2 Sampling, Filtration and Preparation (performed in the field)

9.2.1 Place a pre-combusted filter pad, with rough side up, in a vacuum filtration assembly.

9.2.2 Mix each sample well before pouring a known volume of sample (anywhere from 10 to 500 mL depending on the density of sample) and quickly pour sample into the filtration assembly.

9.2.3 Filter at a low pressure (15 inches Hg); vacuum to dryness and then break the seal of the vacuum.

9.2.4 Fold the filter in half (exposed surface inside), wrap in aluminum foil and label the sample with the date, ID, volume filtered, and scientist signature.

9.2.5 Freeze at –10°C until ready for analysis.

9.2.6 Prior to analysis, samples should be placed in a drying oven at 45°C-55°C for at least 12 hours. Once dried, leave samples in a desiccator until ready to use.

9.3 Sample Measurements

9.3.1 Prepare a sample run log (Appendix B) starting with a calibration series that is consisting of 1 nickel sleeve blank, 1 condition, 1 tin capsule blank, 1 condition, and then followed with 3 acetanilide standards.

9.3.2 Standard Preparation

9.3.2.1 Weigh out approximately 1500 µg of acetanilide into a tin capsule for each standard.

9.3.2.2 Quarterly; Weigh out 200 to 250 µg of domestic sludge into a pre-weighed tin capsule as the reference standard for particulate nitrogen (PN).

9.3.2.3 Quarterly; Weigh out about 1000 µg of PACS-2 into a pre-weighed tin capsule as a reference standard for particulate carbon (PC).

9.3.3 Sample Preparation

9.3.3.1 On a clean surface, place a 7 x 5 mm nickel sleeve into the filter loading die with a plastic loading funnel.

9.3.3.2 Fold the filter and squeeze it into the sleeve with a microforceps. Carefully pull out the microforceps. Use the 4 mm loading plunger to force the compressed filter into the nickel sleeve. Make sure no excess filter protrudes above the lip of the sleeve.

9.3.3.3 Transfer the standards and samples into the 64 sample wheel according to the run log (9.2.1).

9.3.4 Instrument Operation

9.3.4.1 On the main menu, click “Run” and select “Carbon, Hydrogen, Nitrogen” in the pull down list. Enter date (ddmmyy) as the run name, then click “Run” to open the sample information box.

9.3.4.2 Enter sample name and sample weight according to the run log. Enter 100 for the weight of the filter samples. Double check all entries.

9.3.4.3 Click "Run" to open the list of instructions.

9.3.4.4 Installation of the sample wheel

9.3.4.4.1 Open the manual purge valve on the injection box. Loosen the 4 cover screws and lift the lid. Remove the empty wheel if necessary.

9.3.4.4.2 Insert the loaded sample wheel with the locking pin in place (position 24). Tilt the wheel slightly, line up the scribe mark on the wheel with the ratchet in the housing. Make sure the mark on the tray is touching the triangular marker on the instrument. Lower the wheel, and make sure that it is properly seated. Place the locking pin in the center hole.

9.3.4.4.3 Close the cover, and tighten equally on all four screws.

9.3.4.4.4 Open and remove any spent capsules in the capsule receiver. Re-install the cover.

9.3.4.4.5 Check the helium pressure to be sure there is adequate gas to perform the run. Adjust the helium pressure to allow for a fill time near 30 (not < 20). The oxygen pressure is set around 25 psi with enough gas available to complete the run. The combustion temperature is set to 980°C, and reduction temperature at 650°C.

9.3.4.4.6 Close the valve. Click "OK" to start the run.

9.3.5 Data Analysis

9.3.5.1 Arrange the data print outs in order.

9.3.5.2 Open the Excel work book template from PCPN / Calculations / Year / Month.

9.3.5.3 Update the sample names and volumes in the spread sheet.

9.3.5.4 Enter the concentrations for PC and PN in their respective columns.

9.5.3.5 Confirm that the calculations are right and the formulae are ok and consistent. Save the file.

9.5.3.9 Double check all entries and print out the results.

9.4 Instrument maintenance

Replace and pack (Appendix C) reduction tube after 300 to 400 runs; combustion tube after 1000 runs; CO_2 or H_2O trap after 500 runs, and helium or oxygen scrubber after 2000 runs or sooner if necessary.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 Calculate the concentrations of PC or PN using the following formula:

$$\text{PC/PN, ppm} = \frac{\text{PC/PN, ug}}{\text{sample volume filtered, mL}}$$

10.2 Calculate the relative percent difference (RPD) for the duplicated samples as follows:

$$\text{RPD} = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \text{ x } 100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 Instrument maintenance, instrument calibration, and quality control data are kept in binders and test results are kept in the file cabinet.

11.2 Normal turnaround time for samples submitted to this lab for analysis will be 2 to 10 days from receipt. Results are reported in writing on a sample analysis request form.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

All spent capsules, combustion tube, reduction tube, and absorbent tubes are disposed of as regular trash.

13.0 REFERENCES

13.1 Exeter Analytical, Inc., *Model 440 CHN/O/S Elemental Analyzer Manual,* 1994.

13.2 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision15.0, August, 2016

13.3 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX A

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist – PC & PN

Exeter Method 440

Lab Numbers[1]:_____

Date Collected: _____ Date Analyzed: _____ Analyst: _____

Procedure	**Acceptance Criteria**	**Status***	**Comments**
Holding Time	28 days @ – 20°C		
2 Acetanilide Calibration Standards	KC = 18 – 25 KN = 7 – 10		
Blank	BC < 500 BN < 250		
Check Standard	After every 10th sample and at the end of the run		
	%C = 71.09 (Range = 70.005 – 71.569) %N = 10.36 (Range = 9.934 – 10.914)		
External PC QC[2] Analyze Quarterly	Within acceptable range		
	Last date analyzed:		
External PN QC[3] Analyze Quarterly	Within acceptable range		
	Last date analyzed:		
Field Filter Blank	PC < 25 µg; PN < 2 µg		
Field and Sample Duplicates	RPD Calculated		
Decimal Places Reported	3		
Sample Calculation	Done correctly		
Changes/Notes	Clearly stated		

* Check (√) if criteria are met. [1]Include beginning and ending numbers; account for gaps by bracketing.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

[2]PC QC Sample: PACS-2 Tracking ID: _____

True Value = _____ Acceptable Range =_____

[3]PN QC Sample: D. Sludge Tracking ID: _____

True Value = _____ Acceptable Range =_____

APPENDIX B

Division of Environmental Sciences

INORGANICS ANALYTICAL LABORATORY

Sample Run Log – Excel Template Particulate Carbon and Particulate Nitrogen

Exeter Method CE 440

Pos	Sample Name	wt (µg)	C-Result	H-Result	N-Result
1	Blank (B)	-			
2	cond (%)	1500			
3	Sleeve Blank (B)	-			
4	cond (%)	1500			
5	STD1 (K)	1500			
6	STD1 (K)	1500			

Position	Sample Name/No.		Sample wt (ug)	Sample vol (mL)	PC(ug)	PN(ug)	ppm PC	ppm PN	avg. PC	avg. PN
7	Acetanilide		1500	-			%C=		%N=	
8	Filter	a	-	-			ug PC=	#DIV/0!	ug PN=	#DIV/0!
9		b	-	-						
10			-				#DIV/0!	#DIV/0!		
11			-				#DIV/0!	#DIV/0!		
12			-				#DIV/0!	#DIV/0!		
13			-				#DIV/0!	#DIV/0!		
14			-				#DIV/0!	#DIV/0!		
15			-				#DIV/0!	#DIV/0!		
16			-				#DIV/0!	#DIV/0!		
17			-				#DIV/0!	#DIV/0!		
18			-				#DIV/0!	#DIV/0!		
19	SampleDup	a	-				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
20		b	-				#DIV/0!	#DIV/0!		
21	Acetanilide		1500	-			%C=		%N=	
22			-				#DIV/0!	#DIV/0!		
23			-				#DIV/0!	#DIV/0!		
24			-				#DIV/0!	#DIV/0!		
25			-				#DIV/0!	#DIV/0!		
26			-				#DIV/0!	#DIV/0!		
27			-				#DIV/0!	#DIV/0!		
28			-				#DIV/0!	#DIV/0!		
29			-				#DIV/0!	#DIV/0!		
30			-				#DIV/0!	#DIV/0!		
31	SampleDup	a	-				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
32		b	-				#DIV/0!	#DIV/0!		
33	Acetanilide		1500	-			%C=		%N=	
34	Blank		-	-	BC=		BH=		BN=	

APPENDIX C

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Tube Replacement – PC & PN

Exeter Method 440

CHN Mode Combustion Tube

Silver tungstate / Magnesium oxide on chromosorb

Silver Oxide / Silver tungstate on chromosorb

Silver vanadate on chromosorb

Silver gauze

Quartz wool

Platinum gauze

CHN Mode Reduction Tube

Copper wire

APPENDIX C (continued)

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Tube Replacement – PC & PN

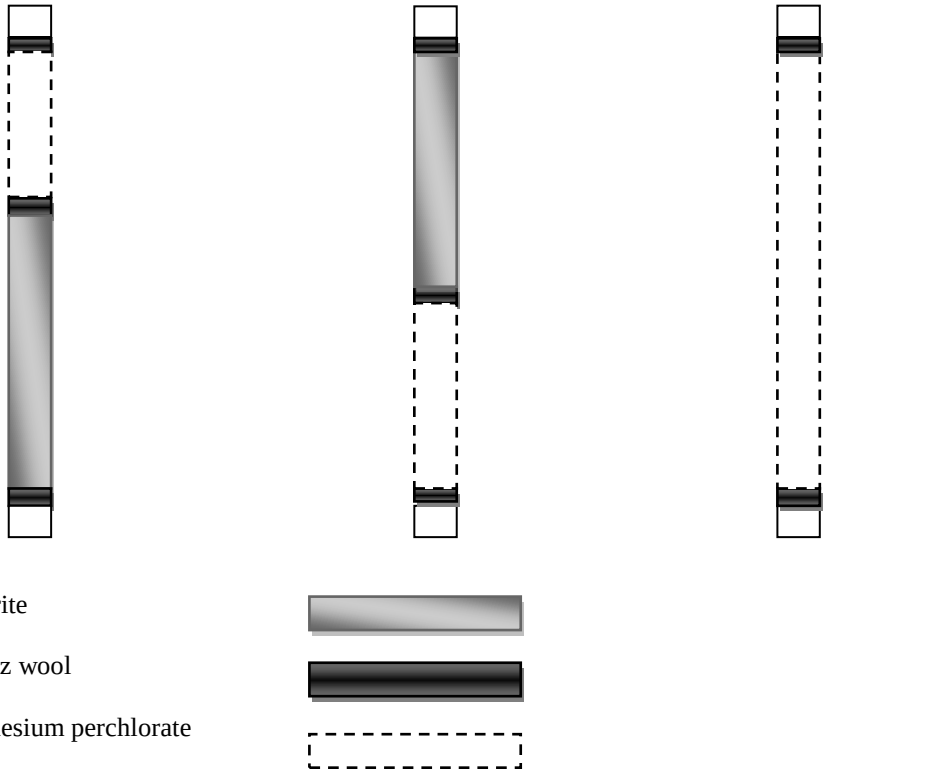
Exeter Method 440

CHN Mode Helium & Oxygen Scrubbers

CHN Mode CO_2 Trap

(This end up)

CHN Mode H_2O trap



Ascarite

Quartz wool

Magnesium perchlorate

SOP Title: Determination of Total Organic Carbon (Standard Method 5310 B)

SOP No.: CHEM-SOP-SM 5310B

Revision: 3.2 Replaces: 3.1 Effective: July 1, 2017

Laboratory: Inorganics Analytical Laboratory

Author / POC: Reza Hajarian
Reza.hajarian@maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

STANDARD METHOD 5310 B
SOP No.: CHEM-SOP-SM5310B

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	12/09	N/A	Taiyin Wei	1/10
1.0	8/11	New procedure section, new SOP tracking number	Reza Hajarian	8/11
2.0	9/12	Technical and editorial changes throughout the document	Reza Hajarian	9/12
3.0	11/14	Reviewed SOP-document control, editorial and technical changes	Reza Hajarian Lara Phillips Shahla Ameli	12/1/14
3.0	6/1/2015	Reviewed document and made changes to section 6.2	Reza Hajarian Lara Phillips Shahla Ameli	7/1/2015
3.1	5/5/2016	Reviewed document and made formatting changes	Lara Phillips Reza Hajarian Shahla Ameli	7/1/2016
3.2	5/5/2017	Reviewed document and made organizational name changes	Lara Phillips Reza Hajarian Shahla Ameli	7/1/2017

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STANDARD OPERATING PROCEDURES

DETERMINATION OF TOTAL ORGANIC CARBON

Standard Method 5310 B

6.0 SCOPE AND APPLICATION

6.1 This method is applicable to drinking, surface and saline waters, domestic and industrial wastes and provides a speedy and convenient way of determining the degree of organic contamination.

6.2 The fraction of total organic carbon (TOC) that passes through a 0.45 µm pore diameter filter is defined as dissolved organic carbon (DOC).

6.3 The method is applicable to measurement of organic carbon above 0.5 mg/L

2.0 SUMMARY OF METHOD

Organic carbon in a sample is converted to carbon dioxide (CO_2) by catalytic combustion at 680 ^oC. The carbon dioxide formed is purged from the sample, dried, and transferred with a carrier gas to a non-dispersive infrared gas analyzer (NDIR). The amount of carbon dioxide is directly proportional to the concentration of carbonaceous material in the sample.

3.0 INTERFERENCES

3.1 Carbonate and bicarbonate carbons represent interference under the terms of this test and must be removed or accounted for in the final calculation.

3.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a micro-liter type syringe. The opening of the syringe limits the maximum size of particles which may be included in the following measurements

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during reagent preparation and instrument operation. Use of gloves and eye protection is recommended when preparing solutions.

4.2 Each employee is issued a ***Laboratory Safety Manual*** and is responsible for adhering to the recommendations contained therein.

4.3 Each chemical should be regarded as a potential health hazard. A reference file of material safety data sheet (MSDS) is available in lab.

4.4 Gloves, lab coat, and protective eyewear must be used when removing the cover from the furnace and replacing the combustion tube.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Shimadzu TOC - V_{CPH} or TOC-L_{CPHCPN} Analyzer

5.1.2 Shimadzu ASI-V or ASI-L Autosampler

5.1.3 Computer

5.1.4 Printer

5.2 Supplies

5.2.1 Glass vials – 40 mL

5.2.2 Air – Compressed, ultra-zero, UN1002, GTS

5.2.3 Flasks – Volumetric, 200 mL, 1000 mL

5.2.4 Pipettes – Volumetric, 5 mL, 10 mL, 20 mL, 100 mL

5.2.5 Platinum Catalyst –Shimadzu Corp.

6.0 REAGENTS AND STANDARDS

6.1 Reagents

6.1.1 Deionized water free from analyte of interest is used to prepare all the reagents and the standards to reduce the carbon concentration of the blank.

6.1.2 2N Hydrochloric Acid – Dilute 166 mL of concentrated Hydrochloric Acid to 1 liter with deionized water.

6.2 Standards

TOC/TIC Standard - Custom made standard containing 10 mg/L of total organic carbon and 10 mg/L of total inorganic carbon, cat. # 092 Custom Standard, Environmental Resource Associates. To prepare this standard manually follow 6.2.1, 6.2.2 and 6.2.3steps.

6.2.1 Inorganic Carbon Stock Solution – Dissolve 4.41 g anhydrous Sodium Carbonate Na2CO3 in DI water then add 3.50 g Anhydrous Sodium Bicarbonate, NAHCO3. Dilute into a one liter volumetric flask and mix thoroughly. Prepare monthly and store in the refrigerator.

6.2.2 Organic Carbon Stock Solution – Dissolve 2.12 g KHP in to a 1000 mL flask. Bring up to volume with DI water and mix well. Prepare monthly and store in the refrigerator.

6.2.3 TOC/TIC Standard – Add 100 mL DI water to a 500 mL volumetric flask. Add 5 mL of 6.2.1 and 5 mL of 6.2.2 to the flask. Mix well and bring up to volume. Prepare monthly and store in the refrigerator.

6.2.4 Potassium Hydrogen Phthalate (KHP) stock standard solution, 1000 ppm – Weigh and Stir to dissolve 2.12 g of KHP in about 800 mL of deionized water in a 1 L volumetric flask. Fill to the mark with deionized water. Mix thoroughly. Transfer to a reagent bottle, label, and store at 4^{o}C. Prepare monthly.

6.2.5 KHP working standard, 10 ppm, 20 ppm – Dilute 10 mL and 20 mL of KHP 1000 ppm stock solution to 1 liter in volumetric flasks respectively and mix thoroughly. Transfer to reagent bottles, label, and store at 4 ^{o}C.

6.2.6 KHP working standards – Add 10.0 mL and 100 mL of the 10 ppm stock standard into two 200 mL volumetric flasks respectively. Dilute to mark with water and mix well. This makes working standards of 0.5 mg/L and 5.0, mg/L respectively.

6.2.7 Calibration Standards- By using a vial filled with blank (0 ppm) and a vial of 20 ppm of KHP in the beginning of the tray(see Pg. 6), the instrument automatically prepares the standards of 0.5 ppm, 1ppm, 5ppm, and 10ppm of KHP that will be used for generating the calibration curve.

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Refrigeration at 4^{o} C prior to analysis is required to minimize microbiological decomposition of solids. The holding time is 48 hours for unacidified samples or 28 days if sample is acidified at the time of collection.

8.0 QUALITY CONTROL

8.1 Reagent grade water is run as the blank control.

8.2 Replicates and spike are performed on every tenth sample or one replicate per run. Duplicated determinations should agree within 10% of their average.

8.3 Spike the sample with 5 ppm KHP by adding 100 μL of 1000 ppm stock solution into 20 mL of the sample. The acceptable spike recovery should be within 10% of the concentration added.

8.4 Quality control (QC) samples including check standard, spiked blank, and an external QC (An ERA QC with known expiration date, range and concentration is analyzed at the beginning and at the end of each run). Recoveries of check std, and blank spikes should be within 10% of its true value.

8.5 Instrument check solution, TIC/TOC, is analyzed at the beginning of each run. A reading of 10 ppm of TOC indicates the sample had been properly acidified and inorganic carbon had been successively removed.

8.6 All the standards and samples are analyzed at least three times from each tube. The concentrations reported for the samples are the mean of the triplicates, calculated by the computer program.

8.7 Every tenth sample is duplicated (analyzed from two different tubes) and spiked. The accepted value for the relative percent difference (RPD) or spike recovery is ± 10 %.

8.8 Data acceptance criteria are listed on the Data Review Checklist (Appendix A).

8.9 The laboratory annually participates in ERA water supply (WS) and water pollution (WP) proficiency studies

9.0 PROCEDURE

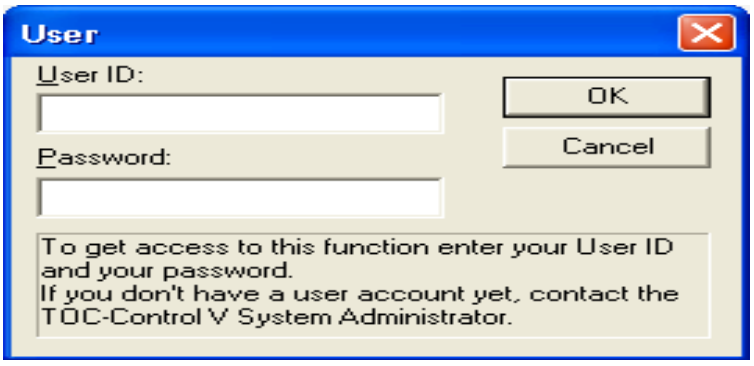
9.1 Analysis Flow

9.1.1 Checking the Dilution Water, Drain Vessel Water, Drain Vessel, and Humidifier water levels- Verify that water volumes are sufficient for analysis. If necessary, replenish water in all bottles.

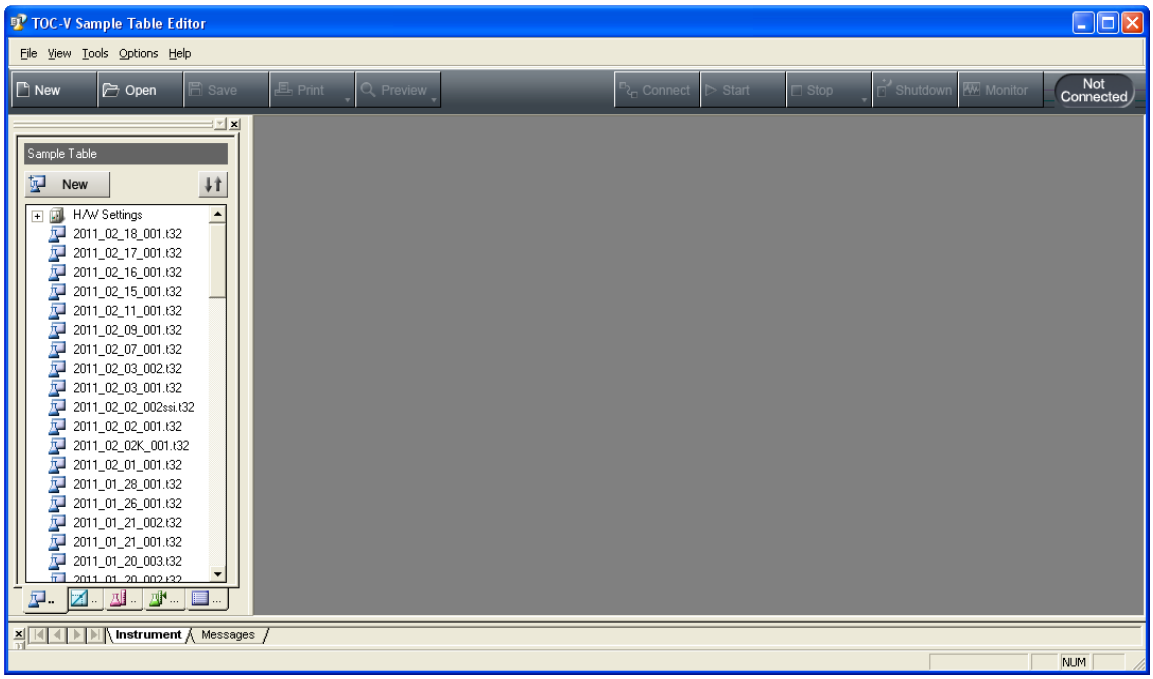
9.1.2 Switch on TOC-V_{cph} or $_{TOC}$-L_{CPHCPN} Analyzer, which undergoes the initialization sequence.

9.1.3 Turn on the computer.

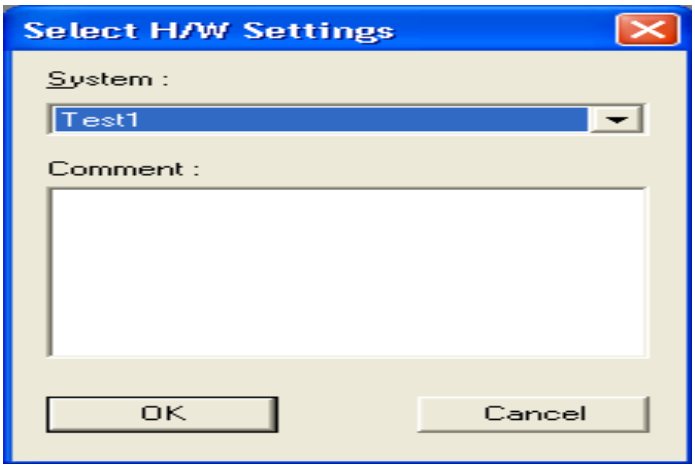
9.1.4 Double click on TOC Sample Table Editor icon on the Monitor. The "User" window is displayed.



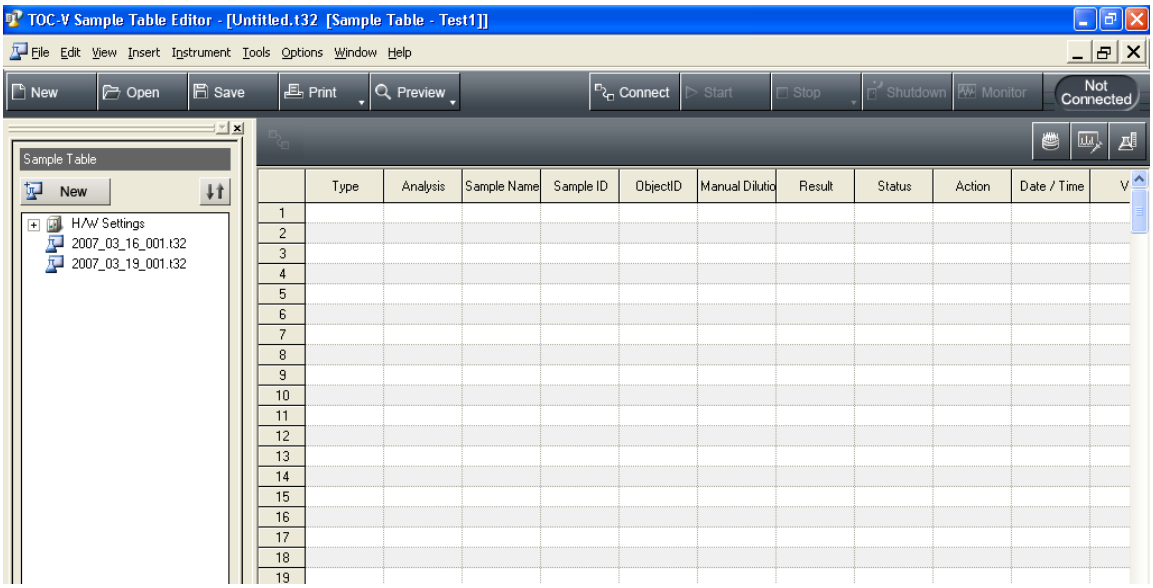
9.1.5 Click OK on the User. A TOC Sample Table Editor is opened.



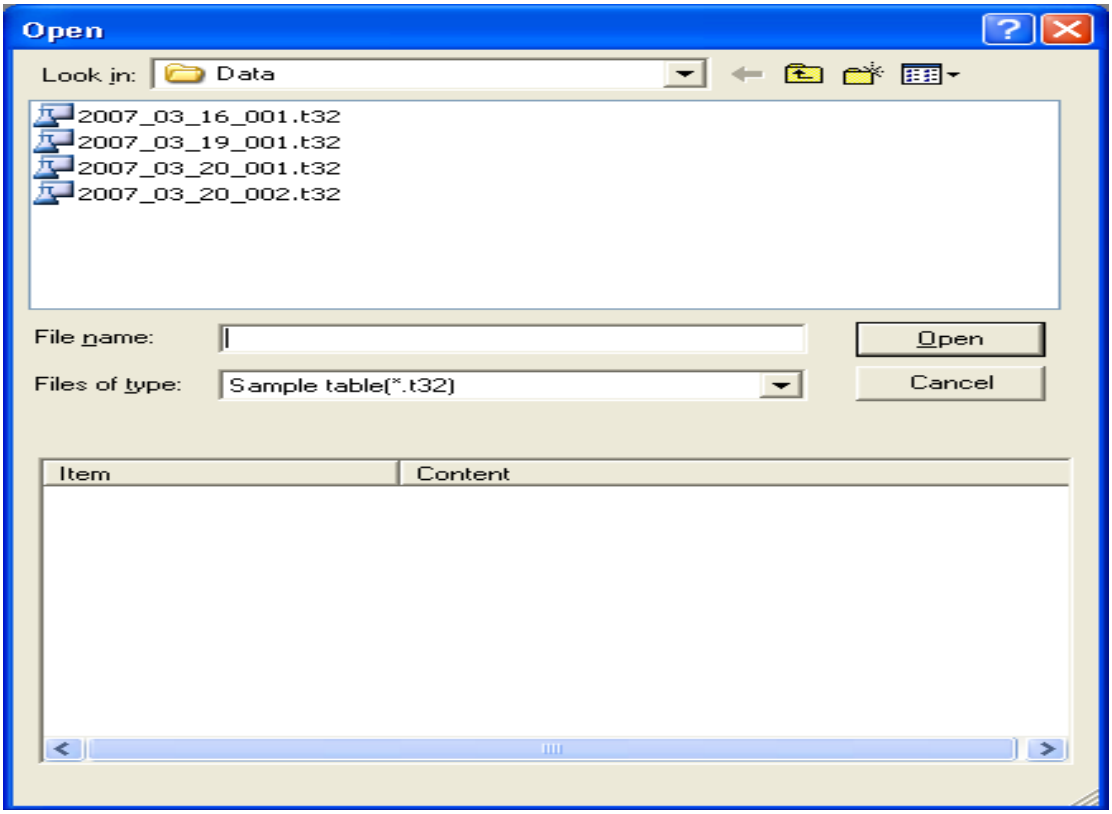
9.1.6 Click "New" on Sample Table Editor, The"Select H/W Settings" window is displayed.



9.1.7 Click OK, an Untitled.t32 [sample table-TOC] is opened in the Sample Table Editor.

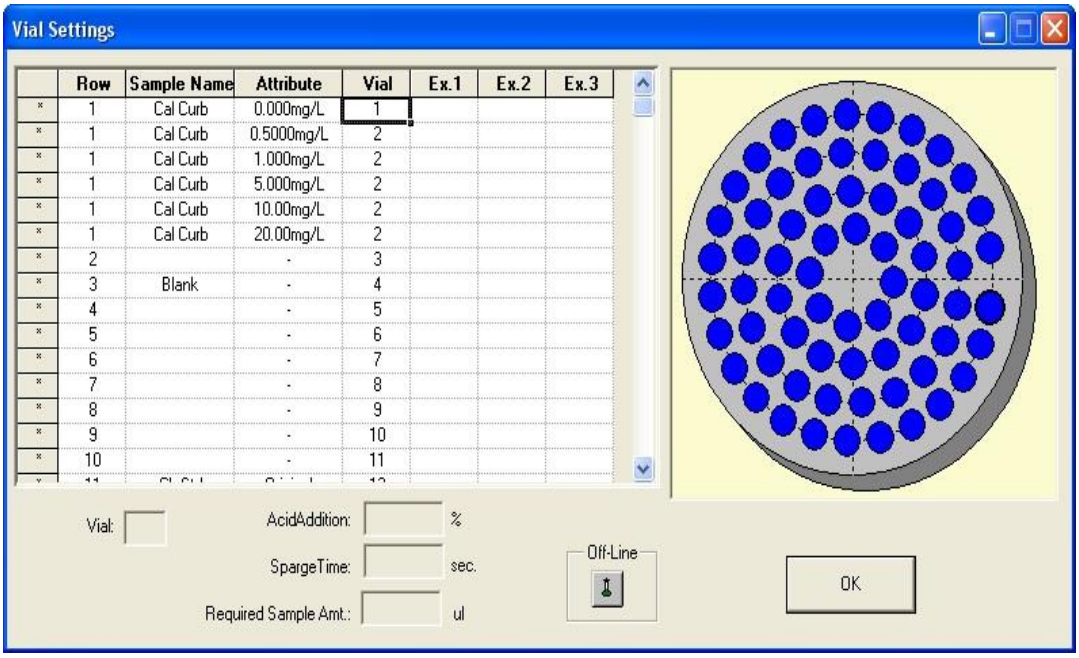


9.1.8 On TOC Sample Table Editor, select File, Import Schedule File in the menu Bar. The Open dialog is displayed.



9.1.9 Select the schedule file, and click Open. The schedule content is inserted into the sample table. It demonstrates calibration curve standards, quality control, check standard, blanks, etc.

9.1.10 To enter the vial numbers; enter a number in the sample ID column cell of sample table, click on the lower right part of the cell, and drag downward to enter a series of sample vial numbers all at once. Click on the first row of sample table, click Auto Sample icon, the Vial Setting dialog box is displayed. Select the cell where the last calibration standard is displayed and position the cursor precisely over the lower right corner of the selected cell until "+" is displayed. Click and drag. After all the samples viewed in vial setting, the vial numbers of the vials to be sampled in the ASI-V must be associated with the samples. Click OK in the lower left of "vial Settings."



9.2 Sample Loading

9.2.1 Place the blank vial in positions1 and the Calibration Standard 20ppm in position 2 of the turntable corresponding to the position specified in the ASI condition; i.e. vial 1-2. When automatic injection of acid is specified for NPOC measurement, the bottle containing 2N HCl must be placed next to analyzer.

9.2.2 Make a sample run list that starts with the 2 calibration check standards; a blank, the TIC/TOC check, and a QC, followed by sample names with

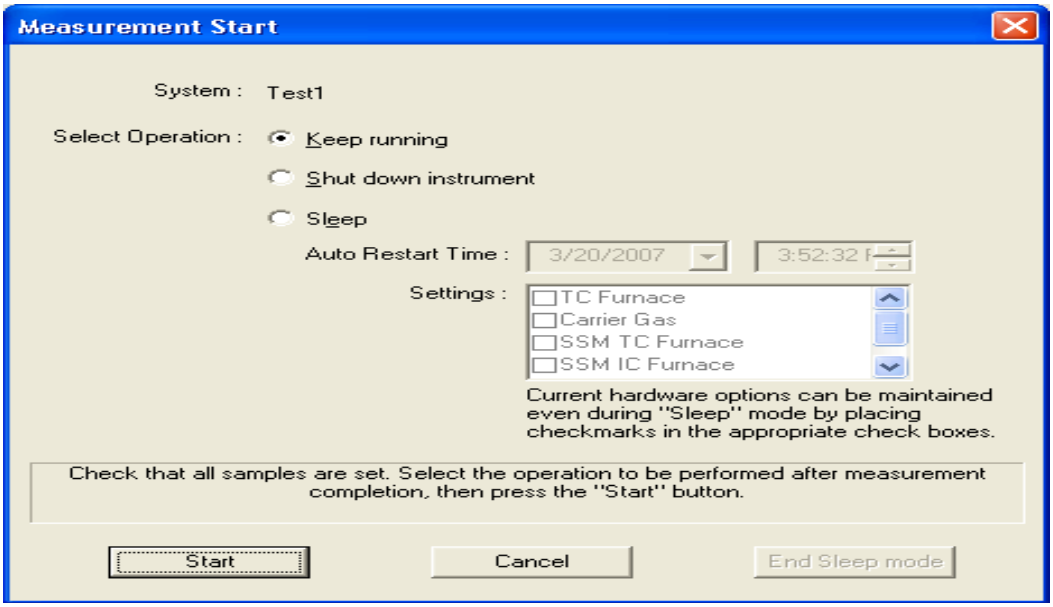
replicates and spikes for every ten samples. A blank, and a check standard are analyzed after every 10 samples. A blank, a check standard and a QC should also be included at the end of each run.

9.2.3 Load sample vials starting with position #1 on the turntable sequence as assigned in the sample worksheet.

9.2.4 The Sample Table must be connected to the connection instrument before analysis can be conducted. Click "Connect" either on the menu bar or click "Instrument-connect." The icon of the connected instrument and the "Ready" indicator in the Sample Table tab of the file viewer illuminate.

9.2.5 Check that the temperature is reaching 680 ^{o} C and humidifier temperature is 1 ^{o} C. Check the base line at range setting x1 and x50 for position, fluctuation, and noise

9.2.6 Starting the Instrument- Analysis can be started either by clicking "Start" of the menu bar or selecting "Instrument-Start Measurement". After clicking "Start", the "Measurement Start" window is displayed. Select the processing that will take place after analysis is complete. Finally, click the start icon at left corner of the Measurement Start window. Analysis starts. When measurement is complete, the results are displayed in the sample table, and analysis ends.



10.0 DATA ANALYSIS AND CALCULATIONS

10.1 Standard curve of 0.0 ppm to 10.0 ppm is established daily and is used directly without shifting to origin assuming the TOC content in water used in preparing standard solution is small enough, with respect to the standard solution concentration, to ignore.

10.2 Calculate % of spike recovery of the laboratory fortified samples as follows

$$\%\,SR = \frac{\text{spiked sample conc. ppm - sample conc., ppm}}{\text{amount of spike added to sample, ppm}} \text{ x } 100$$

10.3 Calculate the % of relative percent difference for the duplicated samples as follows:

$$RPD = \frac{\text{difference of the duplicates}}{\text{average of the duplicates}} \text{ x } 100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 Instrument maintenance, instrument calibration, and quality control data are kept in binders and test results are kept in the file cabinet.

11.2 Normal turnaround time for samples submitted to this lab will be 2 to 10 days from receipt. Results are reported in writing on a sample analysis request form or through Star Lims for drinking water samples.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 Samples and standards are poured down the drain while flushing with large amount of cold water.

12.2 Actual reagent preparation volumes are to be reflected anticipated usage and reagent stability.

13.0 REFERENCES

13.1 United States Environmental Protection Agency, *Methods for Chemical Analysis of Water and Waste,* Methods 415, August 1993

13.2 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater,* 21st Edition, 2005

13.3 Shimadzu Corporation, *Instrument Manual for Total Organic Carbon Analyzer Model TOC-5000*

13.4 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan*, SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.5 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX **A**

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist – TOC/DOC

Standard Method 5310 B

Lab Numbers[1]:_____

Date Collected: _____ Date Analyzed: _____ Analyst: _____

Procedure	**Acceptance Criteria**	**Status***	**Comments**
Holding Time	28 days @ – 20 ºC for filtered samples; 28 days @ 4 ºC for samples acidified to pH < 2 with HCl		
Calibration Curve	Corr. Coeff. $\geq$ 0.9950		
Sparge Check (TIC & TOC)	TOC = 9 – 11 ppm		
Reagent Blank	< Reporting level (0.50 mg/L)		
Matrix Spike	Every 10th and the last sample or 1/batch, if less than 10 samples		
	Recovery = 90 – 110%		
External QC	Beginning and end of each run		
	Within acceptable range		
Check Standard	After every 10th sample and at the end of the run		
	Concentration within 90 to 110% of the true value		
Duplicates/Replicates	Every 10th and the last sample or 1/batch, if less than 10 samples		
	RPD $\leq$ 10%		
Decimal Places Reported	2		
Measured Values	Within calibration range (0.50 to 20.00 ppm)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

* Check (√) if criteria are met. [1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

Sparge Check: TIC & TOC Tracking ID: _____

QC Sample: _____ Tracking ID: _____

True Value = _____ Acceptable Range = _____

APPENDIX **B**

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Sample Run Log –TOC/DOC

Standard Method 5310 B

Date: _____ Analyst: _____

Vial	Sample ID	Dil	Conc. (ppm)	Vial	Lab #	Dil	Conc. (ppm)	Vial	Sample ID	Dil	Conc. (ppm)
1	0-20 ppm		Cal. Std	27				53			
2	0-20 ppm		Cal. Std	28				54			
3	Blank			29				55			
4	Ex QC			30				56			
5	BLK QC			31				57			
6	TOC/TIC			32				58			
7	Blank			33				59			
8	BLK/SPK			34				60			
9	Blank			35				61			
10	MDL			36				62			
11	Blank			37				63			
12	CkStd 5ppm			38				64			
13	Blank			39				65			
14				40				66			
15				41				67			
16				42				68			
17				43				69			
18				44				70			
19				45				71			
20				46				72			
21				47				73			
22				48				74			
23				49				75			
24				50				76			
25				51				77			
26				52				78			

Sample Name	Prep Log ID
KHP Stock Std 1000 ppm	
KHP Std 20 ppm	
KHP Std 10 ppm	
KHP Std 5 ppm	
KHP Std 0.5 ppm	
QC:	

Lab #	Average	%RPD	% Spk Rec

Title: Determination of Total Suspended Solids (Standard Method 2540 D)

SOP No.: IAL-SOP-SM 2540 D

Revision: 3.2 Replaces: 3.1 Effective: 7/01/17

Laboratory: Inorganics Analytical Laboratory

POC: Amelie Hamilton / Reza Hajarian
amelie.hamilton@maryland.gov
reza.hajarian@maryland.gov

Laboratory Supervisor:	_____ Signature	_____ Date
QA Officer:	_____ Signature	_____ Date
Manager:	_____ Signature	_____ Date
Division Chief:	_____ Signature	_____ Date

STANDARD METHOD 2540 D
SOP No.: CHEM-SOP-SM 2540 D

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	12/09	N/A	Taiyin Wei	1/10
1.0	1/11	New SOP tracking number	Asoka Katumuluwa	
2.0	8/11	Updated procedure to include Proweigh filters	Moses Obura / S. Ameli	8/18/11
3.0	4/12/12	Technical and editorial changes to procedure	Reza Hajarian/ S. Ameli	4/12/12
3.0	6/24/13	Reviewed the document	S. Ameli	2013
3.1	10/9/14	Format changes	Lara Phillips S. Ameli	11/03/2014
3.1	5/4/2015	Reviewed SOP	L. Phillips A. Hamilton S. Ameli	7/1/2015
3.1	5/1/2016	Reviewed SOP	L. Phillips A. Hamilton S. Ameli	7/1/2016
3.2	6/1/2017	Reviewed SOP and made organizational name changes	L. Phillips A. Hamilton S. Ameli	7/1/2017

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STANDARD OPERATING PROCEDURES

DETERMINATION OF TOTAL SUSPENDED SOLIDS

Standard Method 2540 D

7.0

7.1 The total suspended solids (TSS) are present in sanitary wastewater and many types of industrial wastewater. There are also nonpoint sources of suspended solids, such as soil erosion from agricultural and construction sites.

7.2 TSS is the portion retained on a filer of 1.5 µm (or smaller) nominal pore size.

1.3 This method is suitable for the determination of solids in potable, surface and saline waters, as well as domestic and industrial wastewaters in the range up to 20,000 mg/L. This laboratory reports all values greater than 1 mg/L.

8.0 SUMMARY OF METHOD

A well mixed sample is filtered through a pre-washed and weighed microfiber filter with 1.5 µm pore size and the residue retained is then dried to a constant weight at 103-105 ^{o}C. The increase in weight of the filter represents the total suspended solids.

9.0 INTERFERENCES

3.0 Samples high in dissolved solids, such as saline water, brines and some wastes, may be subject to a positive interference. For such samples, the filter with sample should be washed thoroughly to ensure removal of dissolved solids from the filter.

3.1 Samples with large floating particles, submerged agglomerates of non-homogeneous materials or excessive residue on the filter may form a water-entrapping crust; limit the sample size to that yielding no more than 200 mg residues on the filter pad.

3.2 Samples high in oil and grease may be difficult to dry the residue to a constant weight in a reasonable amount of time.

10.0 HEALTH AND SAFETY

4.1 There is no apparent safety hazard associated with this analysis. However, it is advisable to wear disposable gloves and protective laboratory clothing when handling the samples and to wear autoclave gloves when taking metal trays in or out of the oven.

4.2 Each employee is issued a ***Laboratory Safety Manual*** and is responsible for adhering to the recommendations contained therein.

4.4 A reference file of material safety data sheet (MSDS) is available in lab.

11.0 EQUIPMENT AND SUPPLIES

5.2 Equipment

5.2.1 Balance – Analytical, XS 204, Mettler-Toledo

5.2.2 Computer and printer

5.2.3 Balance Data Transfer Software – *LabX direct balance*, V1.2, Mettler-Toledo

5.2.4 Adapter Cable – USB-RS232, Part # 11103691, Mettle-Toledo

5.2.5 Oven – Isotemp 500 series, 20 to 220^{o}C range, Fisher

5.2.6 Desiccator Cabinet – Stainless steel, cat # 08-645-11, Fisher

5.2.7 Desiccator – Glass with porcelain plate, cat # 08-615B, Fisher

5.2.8 Büchner flask, also known as a vacuum flask, 1000 ml, cat # 10-180F, Fisher

5.1.9 Filter Assembly for using commercially prepared pre-wash and pre-weigh filters

5.1.9.1 Filters – ProWeigh 47 mm glass fiber filters for Total suspended Solids, 1.5 µm pore size, cat # F93447MM-X, Environmental Express

5.1.9.2 Filter Holder – 47 mm polysulfone holder with funnel and base, cat # D0047P, Environmental Express

5.3 Supplies

5.3.1 Desiccants – Silica gel beads, cat # 08-594-17C, Fisher gel

5.3.2 Gloves – Autoclave, cat # 19-013-586, Fisher

5.3.3 Tongs – Stainless steel, cat # 15-186, Fisher

5.3.4 Thermometer – Oven, certified traceable, 20 to 130 ºC, cat # 15-171-5, Fisher

5.3.5 Trays – Stainless steel, cat # 13-361C, Fisher

12.0 REAGENTS AND STANDARDS

12.1 Deionized water

12.2 Quality control (QC) samples

12.3 QC SLD Solid Standards in Water, Inorganic Ventures.

12.4 Universal Solids Standard – Item # 2781, Environmental Express

13.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Non-representative matter such as large floating particles or submerged agglomerates of non-homogeneous materials should be excluded from the sample if it is determined that their inclusions are not desired in the final result.

7.2 Samples are collected in polyethylene cubitainer,

7.3 Refrigeration or icing at 4 ºC prior to analysis is required to minimize microbiological decomposition of solids. The holding time is 7 days at 4 ºC. However, it is recommended to begin the analysis as soon as possible.

14.0 QUALITY CONTROL

8.1 An initial Demonstration of Performance (IDP) to establish the instrument's capability is required prior to its use in analyzing client's samples. The linearity of calibration range (LCR) and the ability to quantify correctly Quality Control Samples are used to assess performance.

8.2 Deionized water is run as the blank control.

8.3 Replicates are performed on every tenth sample or one replicate per run.

8.4 A QC sample is run quarterly.

8.5 Data acceptance criteria are listed on data review checklist. (Appendix A)

8.6 Balance is professionally serviced and calibrated yearly and is checked with certified external weights and recorded daily.

8.7 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of a low concentration standard in one run. MDL is calculated as follows:

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made

15.0 PROCEDURE

9.1 Prepare a sample run log sheet (Appendix B) starting with a deionized water blank, replicates for every tenth samples or one per batch, and a QC when needed using the log. StarLIMS samples are recorded on the same run log.

9.2 Sample Analysis

9.2.1 Arrange the run log by Recording the identification number and weight of the ProWeigh filters that is going to be used for each sample. The dish/filter ID is listed in **Dish No**. column, record date of analysis under the **Date column** and enter the weight under **Initial Weight** column (appendix B).

9.2.2 Thoroughly mix sample by inversion. Use about 300ml for Potomac Boat samples and entire (supplied) volume for storm samples. Use a measuring cylinder to measure exact volume of sample filtered. Record the total volume filtered in the run log.

9.2.3 Using forceps, carefully place filter in the filter vacuum assembly. Squirt some distilled water in the filter to wet the filter paper and then turn on the vacuum pump.

9.2.4 Dispense measured volume in the corresponding filter as per your run log.

9.2.5 Rinse the graduated cylinder, filter, non-filterable residue and crucible wall with three successive 10-mL volumes of deionized water and add to the filter and continue suction for about 3 minutes after filtration is completed.

9.2.6 After all liquid has passed through the filter, first disconnect the suction flask from the pump tubing to release the pressure. Then turn off the vacuum. Remove filter and place it back in the original metal pan.

9.2.7 Dry at 103 ^{o} to 105 ^{o}C overnight, cool in a desiccator for 2 hours and determine the 1st final weight.

9.2.8 Turn on the computer. Click on the "**LabX direct balance**" icon.

9.2.9 Click to open TSS folder, select TSS template and enter the sample list. Click file and save the new file name by entering "**yy-mo-day**".

9.2.10 Check the balance with minimum three weights and record in the log book.

9.2.11 After determining the 1st final weight, repeat the cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg.

9.2.12 Return the filters into the oven for at least one hour, cool in desiccators for two hours, and determine the 2nd final weight.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 Sample result in the Microsoft Excel table (Appendix B) is formulated according to the following equation:

$$\text{TSS, ppm} = \frac{(\text{wt. of filter \& residue, g} - \text{wt. of filter, g}) \times 1000}{\text{Vol. of sample, mL}}$$

10.2 Calculate the % relative percent difference for the duplicated samples as follows:

$$\text{RPD} = \frac{\text{difference of the duplicates}}{\text{average of the duplicates}} \times 100$$

10.3 The detection limit for this method is 1 ppm.

11.0 DATA AND RECORDS MANAGEMENT

11.1 Instrument Maintenance, external QC and Ongoing Precision and Recovery, are kept in binders and test results are kept in the file cabinet.

11.2 Normal turnaround time for the analysis of samples submitted to this lab will be 2 to 10 days from receipt. Results are reported either in writing on a sample analysis request form or in a print out generated by StarLIMS.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

Samples are poured down the drain while flushing with large amount of cold water. Filters are disposed of as regular trash.

13.0 REFERENCES

13.2 United States Environmental Protection Agency, *Methods for Chemical Analysis of Water and Waste*, Method Number 160.2, August, 1993

13.3 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, Method Number 2540 D, 21st Edition, 2005

13.4 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan*, SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.5 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, Quality Manual, SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX A

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist – Total Suspended Solids (TSS)

Standard Method 2540 D

Lab Numbers[1]:_____

Date Collected: _____ Date Analyzed: _____ Analyst: _____

Procedure	**Acceptance Criteria**	**Status***	**Comments**
Holding Time	7 days @ 4°C		
Samples Analysis	Started within 5 working days		
Reagent Blank	< 1 mg/L		
Duplicates/Replicates	A minimum of 10% of the samples or 1/batch, if less than 10 samples		
	RPD ≤ 15%		
External QC[2] Analyze quarterly	Within acceptable range		
	Last date analyzed:		
Decimal Places Reported	DNR split samples: 3		
	All other DNR samples a. Results < 1 mg/L: 1 decimal place; report with "L" letter b. Results ≥ 1 mg/L: 0 decimal place		
	All samples on multiple sheets: 0		
Calculations	Done correctly		
Changes/Notes	Clearly stated		

***** Check (√) if criteria are met. **[1]**Include beginning and ending numbers, account for gaps by bracketing

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

[2]QC Sample: _____ Tracking ID: _____

True Value = _____ Acceptable Range = _____

APPENDIX B

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Sample Run Log – Total Suspended Solids (TSS)

Standard Method 2540 D

Analyst: _____

Lab No.	Vol. Filtered (L)	Dish No	Initial Wt Date	Initial Wt (gm)	Final Wt (1) Date	Final Wt (1) (gm)	Final Wt (2) Date	Final Wt. (2) (gm)	Net Wt (gm)

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

Title:	Determination of 5-Day Biochemical Oxygen Demand (Standard Method 5210 B)
SOP No.:	CHEM-SOP-SM 5210 B
Revision:	3.2 Replaces: 3.1 Effective: 7/01/2017
Laboratory:	Inorganics Analytical Laboratory
POC:	Yolanda Simms/ Lara Phillips Yolanda.simms@maryland.gov lara.johnson@maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

STANDARD ETHOD 5210 B
SOP N0. CHEM-SOP-SM 5210 B

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/4/08	N/A	Taiyin Wei	6/5/08
1.0	12/09	Tracking IDs for standards and reagents	Taiyin Wei	1/10
2.0	1/11	New SOP tracking number	Asoka Katumuluwa	1/11
3.0	2/12	New Procedure for new BOD analyzer	Cynthia Stevenson	12/12/12
3.1	10/31/14	Changed format	C. Stevenson S. Ameli	12/01/14
3.1	6/1/2015	Reviewed document	L. Phillips Y. Simms S. Ameli	7/1/2015
3.1	5/31/2016	Reviewed document	L. Phillips Y. Simms S. Ameli	7/1/2016
3.2	6/05/2017	Reviewed document and made organizational name changes, Updated 9.3.2-9.3.3, 9.6.1 and Run Log	L. Phillips Y. Simms S. Ameli	7/1/2017

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STANDARD OPERATING PROCEDURE

DETERMINATION OF 5 - Day Biochemical Oxygen Demand

Standard Method 5210 B

1.0 SCOPE AND APPLICATION

1.1 The biochemical oxygen demand (BOD) test is used for determining the relative oxygen requirement of wastewaters, effluents, polluted waters, and streams. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment system. The application of the test to organic waste discharges allows calculation of the effect of the discharges on the oxygen resources of the receiving water.

1.2 The BOD determination is an empirical test which measures the dissolved oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous irons. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The standard test conditions include dark incubation at 20 ^{o}C for five days. The actual environmental conditions of temperature, biological population, water movement, sunlight, and oxygen concentration cannot be actually reproduced in the laboratory. Results obtained must take into account the above factors when relating BOD results to stream oxygen demands.

2.0 SUMMARY OF METHOD

2.1 Appropriate dilutions of each sample and the quality control samples are incubated for 5 days (BOD_5) at 20 ^{o}C in the dark. The reduction in dissolved oxygen concentration during the incubation period yields a measure of the biochemical oxygen demand.

3.0 INTERFERENCES

3.1 Residual chorine can interfere in this determination and it is neutralized with Na_2SO_3, if present. Hach's USEPA-accepted DPD (N, N-diethyl-p-phenylenediamine) colorimetric method is used to detect any free chlorine in the sample.

3.2 The source water used for BOD sample dilution must be free of heavy metals, specifically copper, and toxic substances such as chlorine that can interfere with BOD measurements. Protect source water quality by using clean glassware, tubing, and bottles. Storage of prepared dilution water for more than 24 h after adding nutrients, minerals, and buffer is not recommended unless dilution water blanks consistently meet quality control limits.

3.3 Oxidation of reduced forms of nitrogen, mediated by micro-organisms, has been considered interference in the determination of BOD and can be prevented by an inhibitory chemical and reported results as carbonaceous biochemical oxygen demand (CBOD).

3.4 Exclude all light during the 5 day incubation period to prevent the possibility of photosynthetic production of dissolved oxygen (DO).

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during reagent preparation and instrument operation.

4.2 Each employee is issued a ***Laboratory Safety Manual*** and is responsible for adhering to the recommendations contained therein.

4.3 Use absorbent towels if material is spilled and wash residual into drain.

4.4 Each chemical should be regarded as a potential health hazard. A reference file of material safety data sheet (MSDS) is available in lab.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 YSI Model 5100 dissolved oxygen meter

5.1.1.1 Dissolved oxygen (DO) probe

5.1.1.2 Membrane replacement kits for DO probe

5.1.1.3 Mantech AutoMax 122 Autosampler with pumps

5.1.1.4 Computer and printer

5.1.2 Incubation room, thermostatically controlled at 20 ± 1^{o}C

5.1.3 pH meter – Accumet pH meter 15, Fisher Scientific

5.1.4 Magnetic stirrer

5.1.5 Buret – 50 mL

5.1.6 Drying oven – isotemp, gravity flow convection, 103 ^{o}C to 105 ^{o}C

5.1.7 Air compressor – 135 psi, Westward

5.2 Supplies

5.2.1 BOD bottles – 300 mL disposable bottles (cat. # D1001), botttle stoppers (cat. # D1025), and overcaps (cat. # D1050), Environmental Express

5.2.2 Carboy with spigot – 20 L capacity

5.2.3 Graduated Cylinders – 25, 50, 100, and 250 mL

5.2.4 Micropipetter – adjustable volume ranges from 1.0 to 5.0 mL

5.2.5 Pipet tips – 5000 µL

5.2.7 Plastic beakers – polypropylene, 1000 mL,

5.2.8 Membrane kit for BOD probe – cat. # 5906, YSI

5.2.9 Filter Unit, 0.45 µm – Nalgene disposable sterilization filter unit, cat. # 09-740-25B, Fisher

5.2.10 Tubes – polypropylene with snap caps, sterile, 14 mL, cat. # 14-959B, Fisher

5.2.11 Glass pipettes – volumetric, class A, 5 mL

5.2.12 Flasks – volumetric, class A, 500 mL and 1000 mL

5.2.13 Glass rods

5.2.14 Stirring bars

5.2.15 Weighing pans – aluminum, cat. #D57-144, Labsources, Inc.

6.0 REAGENTS

6.1 Dilution water

6.1.1 Aerate 19 liters (5 gallons) of deionized water in a 20 L carboy in the 20 ^{o}C room for 30 minutes. The dissolved oxygen concentration of water used for BOD test must be at least 7.5 mg/L. Following aeration, leave carboy to sit overnight in 20 ^{o}C room with the cap loosened to allow water to equilibrate.

6.1.2 Empty one premixed pillow of BOD Nutrient Buffer (Hach cat. # 14863-98) into aerated water (6.1.1) at 20 ^{o}C. Mix well. Prepare dilution water one hour before use.

6.2 Glucose-Glutamic acid (GGA) solution

6.2.1 Dry few grams each of glucose or dextrose and glutamic acid in aluminum weigh pans for 1 hour at 103 ^{o}C. Cool to room temperature in a dessicator.

6.2.2 Weigh out 0.15 g each of dextrose and glutamic acid and dissolve in 800 mL of deionized water in a 1 L volumetric flask. Dilute to mark and mix well. Prepare fresh immediately before use.

6.2.3 Instead of preparing fresh GGA solution each time, the solution prepared in 6.2.2 can be sterilized by filtering through a disposable sterilization filter unit, divided and stored in small volumes. If this procedure is followed, pour about 12 mL aliquots into each sterile 14 mL polystyrene tube, snap cap back on the tube, label, and store in the refrigerator. Prepare every two months.

6.2.4 Premade GGA is also available (Man-Tech Cat. No P17801). To prepare the standards, simply add the content of the 6 mL vial into each of the two BOD bottles marked for GGA.

6.3 Seeding material, prepare daily

6.3.1 One bottle of wastewater from the Cox Creek Wastewater Treatment Plant is delivered to the laboratory every Tuesday. Store the wastewater in the incubation room.

6.3.2 Pour the supernatant into an Erlenmeyer flask about an hour before beginning the run to allow solids to settle to the bottom of the flask. The amount of supernatant to be added to each BOD bottle is between 1.5 mL to 3.0 mL depending on the color, odor and density of the wastewater.

6.4 Sample pH

6.4.1 Calibration buffers – pH 4.0, pH 7.0, and pH 10.0 - Fisher cat. # SB105,

6.4.2 Sulfuric acid (H_2SO_4), 1M – Slowly and while stirring, add 2.8 mL of conc. H_2SO_4 to 80 mL of deionized water. Dilute to 100 mL. Mix well, label and store.

6.4.3 Sodium hydroxide (NaOH), 1N – Dissolve 4 g of NaOH in 80 mL of deionized water. Dilute to 100 mL.

6.5 Dechlorination

6.5.1 DPD free chlorine reagent power – cat. # 14070-99, Hach

6.5.2 Starch soluble for iodometry – cat. # 516-100, Fisher

6.5.3 Sodium sulfite solution (Na_2SO_3) – Dissolve 0.157 g of Na_2SO_3 in 100 mL of deionized water. This solution is not stable; prepare fresh daily.

6.5.4 Potassium iodide (KI) solution – Dissolve 10 g of KI in 100 mL deionized water. Mix well.

6.5.5 Acetic acid (CH_3COOH), 1:1 – Mix 20 mL deionized water with 20 mL glacial acetic acid.

6.5.6 Nitrification inhibitor – 2-chloro-6-(trichloro methyl) pyridine (TCMP), cat. # 2533, Hach

6.5.7 External Quality Control Sample – QC-DEM-WP, Spex Certiprep Inc.

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Keep samples at or below 4 ^oC from the time of collection and analyze within 48 hours after collection.

8.0 QUALITY CONTROL

8.1 Dilution water quality check: The dilution water blank, prepared in 6.1, serves as a check on quality of unseeded dilution water and cleanliness of incubation bottles. The DO uptake in 5 days must not be more than 0.20 mg/L. If this value exceeds 0.20 mg/L, then evaluate the cause and make appropriate corrections.

8.2 Glucose-glutamic acid check: The glucose-glutamic acid check solution is the primary basis for establishing precision and accuracy and is the principal measure of seed quality and analytical technique. For the 300 mg/L mixed primary standard, the average 5 days BOD must fall within the range of 198 ± 30.5 mg/L. If the average value falls outside this range, evaluate the reason and take appropriate actions. Consistently high values can indicate the use of too much seed suspension, contaminated dilution water, or the occurrence of nitrification. Consistently low values can indicate poor seed quality, use of insufficient quantity of seed suspension, or the presence of toxic materials. If low values persist, prepare a new mixture of glucose and glutamic acid and check the sources of dilution water and the seed.

8.3 Minimum residual DO and minimum DO depletion*:* Only the dilutions resulting in a DO depletion of at least 2.0 mg/L and a residual DO of at least 1.0 mg/L after 5 days of incubation are considered to produce valid data.

8.4 Seed Control: The DO uptake attributable to the seed should be between 0.6 -1.0 mg/L. The volume of seed added should be adjusted in order to meet the required range of 198 ± 30.5 mg/L for glucose-glutamic acid check.

8.5 An external quality control sample with a known BOD value is analyzed each quarter.

8.6 The YSI dissolved oxygen meter is calibrated in air (water saturated), i.e. the probe is parked in a BOD bottle containing 1" of water.

8.7 Data acceptance criteria are listed in the data review checklist (Appendix A).

8.8 Laboratory participates in ERA WatR Pollution (WP) Proficiency Testing annually.

9.0 PROCEDURE

9.1 Sample preparation:

9.1.1 Prepare the sample run list for checking color, odor, pH and chlorine and for dilutions. (Appendix B)

9.2 Check samples for residual chlorine.

9.2.1 Using the Hach Swiftest dispenser, insert DPD free chlorine reagent powder into each test tube, add about 10 mL of sample and observe for any color change occurring within a few seconds. A pink color indicates presence of chlorine and therefore the samples(s) must be dechlorinated.

9.2.2 Determine the required volume of Na2SO3 needed to dechlorinate on a 50 mL portion of the pH adjusted sample. Add 0.5 mL of 1:1 acetic acid (6.5.5), 0.5 mL of KI solution (6.5.4) and a few drops of starch solution to sample. Using a 50 mL buret, titrate with Na2SO3 (6.5.3) solution to the starch-iodine (blue) end point. Record the volume used. Calculate and add the required volume of Na2SO3 solution to the pH adjusted portion of the sample (9.3.3).

9.3 Check sample pH

9.3.1 Label 1 L polypropylene beakers with the sample numbers. Pour about 500 mL of samples into 1 L beakers. Pour 100 mL of sample if it has strong sewage odor.

9.3.2 Calibrate the pH meter as stated in the meter directions. Standardize the pH meter using pH 4, 7 and 10 buffers. Record the slope and temperature in the logbook. Read each buffer after the calibration and record the results in the pH meter log.

9.3.3 Read the pH of each sample making sure they are stirred during the measurement. Adjust the pH of each sample to a final reading between 6.5 to 7.5. with 1N NaOH or 1M H_2SO_4. Record the final pH. Leave the pH meter on standby when finished.

9.4 Sample dilution:

9.4.1 Bring samples to BOD room temperature (20 °C) before making dilutions.

9.4.2 Check samples for color and odor.

9.4.3 Dilutions are prepared directly in BOD bottles. Transfer 200 and 100 mL aliquots of each prepared stream sample, 50, 25, 10 and 5 mL aliquots of each prepared sewage sample, and 10, 5, 1 and 0.5 mL aliquots of each prepared strong industrial wastes, as appropriate, into labeled BOD bottles using class A graduated cylinders and volumetric pipets. Rinse the cylinder between samples. Dilutions may need to be adjusted to reflect the qualities of the sample. Place the bottles in the correct order in the rack.

9.5 Nitrification inhibition:

9.5.1 If nitrification inhibition is desired add 3 mg of TCMP (6.5.6) to each 300 mL bottle before capping.

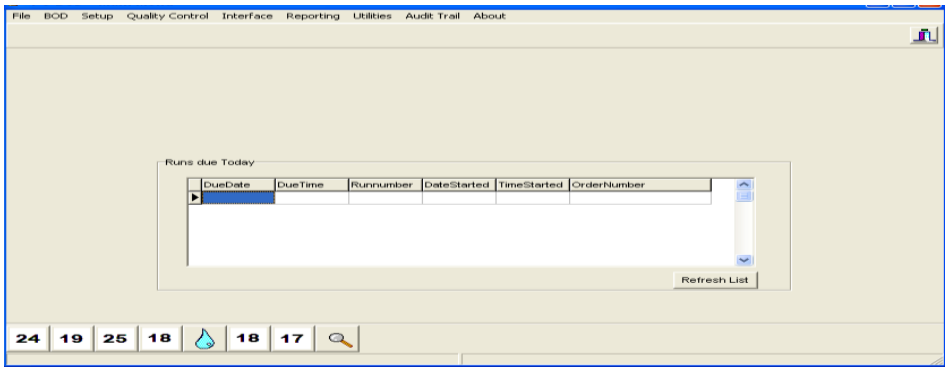
9.5.2 Note the use of nitrification inhibition in the reporting results.

9.6 Prepare autosampler using the "PC-BOD" software:

9.6.1 Push the bottle containing 1" of water up to the probe to create a seal. Warm up YSI 5100 for at least 30 minutes. Calibrate the probe. Ensure that it is set in **REMOTE** mode.

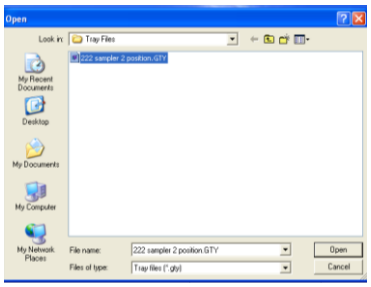
9.6.2 On the computer desktop locate the software icon.

Double click on the icon and the software will open to the main screen. If the icon is not present, open the software by clicking on the desktop 'Start' menu, followed by 'All Programs' and select 'PC-BOD'.



9.6.3 Under **BOD** select **MANUAL.**

9.6.3.1 Click on the '**Load Tray from Folder**' button. The window shown at right will appear.

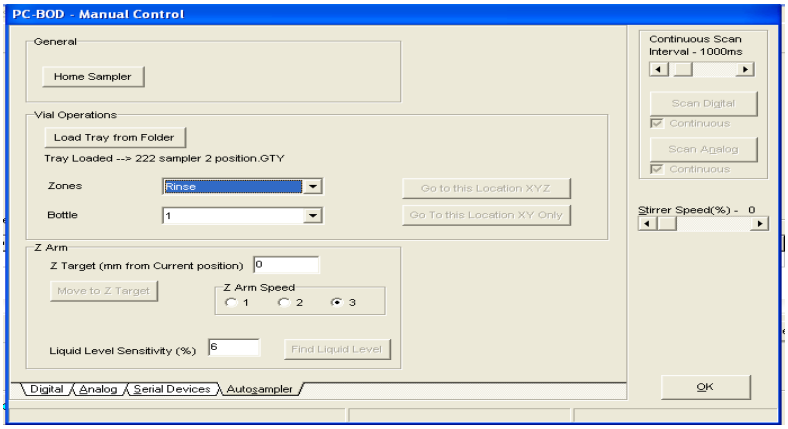


9.6.3.2 Click on the tray file named *271 sampler…* and then click on the '**Open**' button.

9.6.3.3 The'**Home Sampler**' button will become active and the 'zones' and 'bottle' windows will be filled in.

9.6.3.4 Click on the '**Home Sampler**' button. The sampler will move to the home position and the buttons to the right of'**zones**' & **'bottles'** will become active. If the sampler is already in the home position, it will appear that nothing is happening but within a few seconds the two buttons will become active. Remove the rack and place a waste beaker in bottle position 3.

9.6.3.5 To move the autosampler to a specific location first select the following zone: -Bottle: allows DO probe to go into a bottle



9.6.3.6 Select the bottle location to move to by using the drop down menu. For example selecting 'Bottle' and '3' will allow the DO probe to go to the 3rd bottle position.

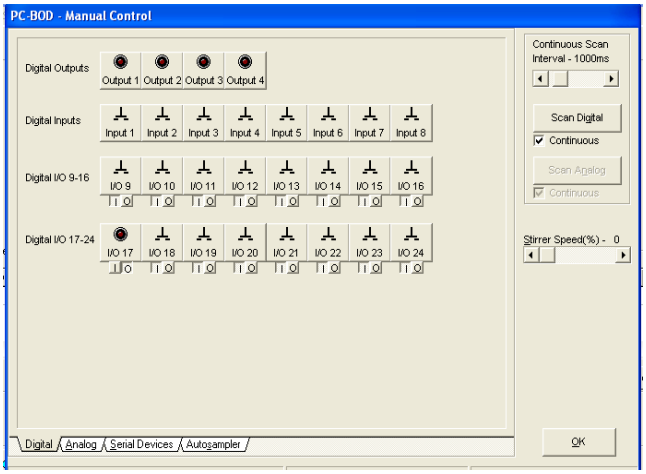
9.6.3.7 To move the autosampler to the specified location click on **'Go to this location XY only'** to move above the bottle position.

9.6.3.8 To move the autosampler only in the Z direction (up and down), enter the number of millimeters to move in the box next to '**Z Target (mm from current position)**'.Use a '–' sign before the number of millimeters to indicate moving in a downward direction. Click '**Move to Z Target'** to move the sampler. For example, entering -43 will move the Z arm down 43mm.

9.6.3.9 Move the autosampler to bottle location **3** and lower it into a waste collection beaker by moving the autosampler down the Z axis by three **-43mm** increments.

9.6.4.0 Prime the pumps with the seed, nutrient water and rinse water.
9.6.4.1 Open '**Digital**' Tab: This tab allows the pumps and stirrer to be turned on or off. Click on the button listed below to turn the device on or off.

Output 1 – dilution pump
Output 2 – seed pump
Output 3 – inhibitor pump
Output 4 – DO probe stirrer
Output 17 – rinse pump



9.6.4.2 Turn on the seed pump,(**Output2**), ensuring that all the rinse water left in the line has been emptied into the waste beaker and the seed is being drawn completely through the line. Turn it off when the seed is dripping into the waste beaker.

9.6.4.3 Turn on the dilution pump, (**Output 1**), ensuring that all the rinse water left in the line has been emptied into the waste beaker and the nutrient water is being drawn through the line. Turn it off.

9.6.4.4 Return the Autosampler to the home position.

1. Open the **Autosampler** tab.
2. Click on '**home sampler'**
3. Click '**OK**'.

9.7 Choose Calibration Schedules and Method.

9.7.1 From the run screen (**BOD/Run BOD**), click on the '**Calibration Schedule' and** choose '**YSI1500 Barometer Cal**'from the drop down menu.

9.7.2 Select the method to use by clicking on the **'Schedule'** button. Choose pump schedule '**3PUMPSJBF**'

9.8 Setting up a run manually.

9.8.1 From the run screen (**BOD/Run BOD**), click on the '**Edit'** followed by the '**Add X Rows**' button. Enter the number of rows that need to be added to the one already

on the grid to give one row per bottle in the run. Click '**OK**' and the rows will be added.

9.8.2 Build the batch starting with one stabilizing water blank, two duplicated water blanks, three seeds at 10, 15 and 20 mL, two duplicated mixtures of 5 mL of G/G with 2-3 mL of seeds followed by 2 to 5 different dilutions of each sample plus 2-3 mL of seeds. See appendix C for an example run and enter as shown.

9.8.3 Fill in the columns on the template. To remove extra lines, click the '**Delete Highlighted Sample**' button. Do not leave blank lines in the template.

9.8.4 Click '**Done Edit**' and the batch will be set up.

9.8.5 Click the '**Auto-Generate Order Number'** button. Enter operator's initials in the box in the upper left corner of the screen.

9.8.6 Load marked bottles into the autosampler racks.

9.8.7 Place the rack containing the first samples onto the autosampler.

9.8.8 Press the '**Start**' button to begin calibration and sample analysis. When prompted enter the rack number currently on the autosampler and press '**OK**'.

9.8.9 Following the screen prompts with regard to calibrating the autosampler and recording the results in the book.

9.8.10 Continue following the screen prompts to allow the auto dilutor to seed, dilute and take an initial D.O. reading of all the samples in the rack. If there are multiple racks the program will prompt for insertion of them at the correct time.

9.9 When a sample is supersaturated.

9.9.1 Stop, delete initial DO readings higher than 9.2.

9.9.2 Shake the diluted, seeded sample in the designated container to remove excess DO

9.9.3 Restart the run and the autosampler will begin with the first sample without a reading.

9.10 Incubation: After all the samples in a rack have been diluted, seeded and had an initial DO reading taken, remove the rack from the autosampler. Place a stopper and cap on each bottle before incubating the sealed bottles for 5 days in the 20^{o}C incubation room with the lights turned off.

9.11 Read final DO:

9.11.1 Turn on the YSI 1500 and allow to warm up for 30 minutes. Press the '**Mode**' button then choose '**Remote**' from among the options.

9.11.2 Loading an Existing Run in the computer.

9.11.2.1 Open the '**PC BOD'** program.

9.11.2.2 On the main screen click on '**BOD**' and then select '**Run BOD**'

9.11.2.3 Choose the '**Load Existing Runs**' tab. On this screen there are 4 buttons which indicate runs in various stages of completion. Choose '**Finals Due Today'** and highlight the row containing the appropriate run.

9.11.2.4 Click on '**Load Selected**' Enter the operators initials in upper right window.

9.11.2.5 Place the rack with the samples to be run onto the autosampler.

9.11.2.6 To begin the run click on the '**Start**' button and enter the number of the rack currently on the autosampler when prompted.

9.11.2.7 Follow the program prompts to calibrate the probe and record the readings in the log book.

9.11.2.8 Continue following the program prompts to take the final D.O. readings.

9.12 Monthly maintenance of BOD probe

9.12.1 Prepare the oxygen probe electrolyte by filling the bottle included with the kit to neck with deionized water. Shake well until crystals are dissolved.

9.12.2 Remove the old membrane cap assembly from the probe. Wipe clean the metal tip of the probe.

9.12.3 Take a new membrane cap assembly and fill in with the fresh electrolyte solution and then screw the cap assembly onto the probe.

9.12.4 Always park the probe in a BOD bottle containing one inch of D.I. water when not in use.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 All the calculations are preformed automatically by BOD Analyst software using the following equations:

10.1.1 Amount of dissolved oxygen consumed during the incubation period:

O_2 Depletion (mg/L) = Initial DO – Final DO

10.1.2 Seed factor used for correcting the BOD test for oxygen depletion resulting from the presence of seed:

$$\text{Seed Factor (mg/L)} = \frac{O_2 \text{ Depl in seed control}}{\text{Vol seed in seed control}} \text{ x Vol seed in sample}$$

10.1.3 BOD of the samples:

$$\text{BOD (mg/L)} = \frac{O_2 \text{ Depl in sample} - \text{Seed Factor}}{\text{Sample Volume, ml}} \text{ x Bottle Volume, mL}$$

10.2 If more than one sample dilution meets the acceptance criteria, report the average calculated by the software program.

10.3 If the O_2 depletion is less than 2 mg/L with 200 mL portion (maximum sample volume) of the sample, report the result from this dilution.

10.4 If all the sample dilutions produce a final DO of less than 1.0 mg/L, report the result from the highest dilution with a > sign.

11.0 DATA AND RECORDS MANAGEMENT

11.1 All Quality Control data are kept in a binder labeled as "Quarterly QC for BOD".

11.2 Normal turnaround time for BOD samples submitted to this lab is 7 to 10 days from receipt with a sample holding time of 2 days. Results are reported in writing on a sample analysis request form.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 It is laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land buy minimizing and controlling all releases from fume and bench operation.

12.2 Samples and standards are poured down the drain while large amount of water is running.

13.0 REFERENCES

13.1 United States Environmental Protection Agency, *Methods for Chemical Analysis of Water and Waste*, Method Number 405.1, August, 1993.

13.2 American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 21st Edition, Method 5210 B, 2005.

13.3 YSI BODANALYST Operations Manual, 1999.

13.4 YSI 5905/5010 BOD Probe Instruction Manual, 1999.

13.5 PC-BOD Operator's Manual – Man Tech 2009

13.6 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.7 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX A

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist – BOD_5

Standard Method 5210 B

Lab Numbers[1]: _____

Date Collected: _____ Date Analyzed: _____ Analyst: _____

Procedure	Acceptance Criteria	Status*	Comments
Holding Time	48 hours @ 4 °C		
Chlorine	Neutralized if present		
pH	Between 6.5 to 7.5; adjusted if out of range		
Initial DO	< 9.20 mg/L at 20 °C		
Incubation Period	5 days		
DO uptake of dilution water	< 0.20 mg/L		
DO uptake of seeded dilution water (seed factor)	0.60 to 1.00 mg/L		
BOD_5 for Glucose/Glutamic Acid (G/GA) solution	198 $\pm$ 30.5 mg/L		
Sample dilutions	Meet the requirements: Final DO $\geq$ 1.00 mg/L and DO depletion $\geq$ 2.00 mg/L		
	Decide on the value to be reported if requirements are not met.		
External QC[2] Analyzed quarterly	Last date analyzed		
	Within acceptable range		
Decimal Places Reported	1		
Reported Values	$\geq$ 2 mg/L; concentrations below this value reported with < sign for Chesapeake Bay samples; as < 2 mg/L for all other samples.		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

[2]QC Sample: _____ Tracking ID: _____
True Value = _____ Acceptable Range = _____

APPENDIX B

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Sample Run Log –BOD_5
Standard Method 5210 B

Date: _____ Analyst: _____

Lab #	Sample Type	Dilution	Color	Odor	pH	pH Adj. to	Chlorine	Chl. Neutr

Sample Name	Tracking ID
pH 4 Buffer	
pH 7 Buffer	
pH 10 Buffer	
Seeds	

Sample Name	Prep Log ID
H_2SO_4, 1M	
NaOH, 1N	
G/GA	
Dilution water	

APPENDIX C

Division of Environmental Chemistry
INORGANICS ANALYTICAL LABORATORY

EXAMPLE OF BATCH

Bottle#	Sample name	Sample Volume (mL)	Seed Volume(mL)
1	Calib		
2	Blank		
3	Blank		
4	Blank		
5	Seed		10
6	Seed		15
7	Seed		20
8	BOD GGA	5	3
9	BOD GGA	5	3
10	WW 1111	100	3
11	WW 1111	200	3
12	WW E12001111001	50	3
13	WW E12001111001	100	3
14	WW E12001111001	200	3
15			

APPENDIX D

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Troubleshooting

PROBLEM	CAUSE	SOLUTION
Autosampler jam.	Tangled lines.	Straighten the lines. Exit the Run. Home the Sampler. Reload the Run.
D.O. readings inconsistent/ unexpected.	Probe membrane no longer intact.	Change membrane.
Initial Blank readings too high.	Dilution water supersaturated.	Degas carboy with Helium gas for 30 seconds.
Initial Sample readings too high.	Sample is supersaturated.	Stop the Run. Pour diluted sample into a shaker and shake for 30 seconds. Return to BOD bottle and replace in rack. Delete the D.O. reading in EDIT mode. Restart the run.

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

SOP Title: Determination of Particulate Phosphorus by Flow Injection Colorimetric Analysis (EPA Method 365.1)

SOP No.: CHEM-SOP-EPA 365.1

Revision: 3.2 Replaces: 3.1 Effective: 7/1/2017

Laboratory: Inorganics Analytical Laboratory

Author / POC: Rickey Carpenter/Cynthia Stevenson
rickey.carpenter@maryland.gov
cynthia.stevenson@maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

EPA METHOD 365.1
SOP No.: CHEM-SOP-EPA 365.1

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	Shahla Ameli	6/2/08
1.0	12/09	Screen snapshots added to procedure, new Sample Run Log, tracking IDs for standards and reagents	Shahla Ameli	1/10
2.0	8/7/11	New SOP tracking number, editorial and technical changes	Shahla Ameli	8/18/11
2.1	8/13	Reviewed SOP	Shahla Ameli	8/18/11
3.0	11/18/2014	Document control and editorial changes.	C. Stevenson S. Ameli R. Carpenter	12/01/2014
3.0	6/1/2015	Reviewed SOP	C. Stevenson S. Ameli R. Carpenter	7/1/2015
3.1	5/5/16	Changes to include commercial standard (6.2.1)	C. Stevenson S. Ameli R. Carpenter	7/1/16
4.1	6/1/2016	Reviewed and made organizational name changes	C. Stevenson S. Ameli R. Carpenter	7/1/2017

TABLE OF CONTENTS

STANDARD OPERATING PROCEDURES

Particulate Phosphorus

EPA Method 365.1

16.0 SCOPE AND APPLICATION

16.1 This method is applicable to the determination of particulate phosphorus in surface and saline waters.

16.2 The applicable range is 0.05 to 1.0 mg P/L.

17.0 SUMMARY OF METHOD

17.1 Samples for particulate phosphorus are collected by filtering known volumes of water samples through the filters in the field. The filters are folded, placed in aluminum foil pouches, and kept frozen until the analysis time.

2.2 Filters are combusted at 550ºC for 1.5 hours and treated with 1 N hydrochloric acid for 24 hrs.

17.2 The supernatant is analyzed for orthophosphate using Lachat Method *12-115-01-1-F*, where ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

18.0 INTERFERENCES

18.1 High iron concentrations (Fe^{3+} greater than 50 mg/L) can cause precipitation of, and subsequent loss, of phosphorus.

18.2 Silica forms a pale blue complex, which also absorbs at 880nm. This interference is generally insignificant as a silicate concentration of approximately 1 mg/L SiO_2/L would be required to produce a 0.3 µg P/L positive error in orthophosphate.

18.3 Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus.

18.4 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other processing apparatus that bias analyte response.

19.0 HEALTH AND SAFETY

19.1 Good laboratory practices should be followed during reagent preparation and instrument operation.

19.2 The use of a fume hood, protective eyewear and clothing and proper gloves are required when handling acids.

20.0 EQUIPMENT AND SUPPLIES

20.1 Equipment

20.1.1 Flow injection analysis equipment (Lachat 8500 series, QuikChem), consisted of the following modules, designated to deliver and react sample and reagents in the required order and rations:

20.1.1.1 Sampler

5.1.1.2 Multi-channel proportioning pump

5.1.1.3 Reaction unit or manifold

5.1.1.4 Colorimetric detector with a 10 mm, 800 µL glass flow cell and an 880 nm interference filter

5.1.1.5 Computer with Omnion 3.0 Data System and Printer

5.1.2 Isotemp Muffle Furnace (Fisher Scientific cat. no. 10-505-10)

20.1.2 Analytical Balance

20.1.3 Automatic Shaker (Thermo Scientific MaxQ 2508)

20.2 Supplies

20.2.1 Test tubes, 13 x 100 mm (Fisher Scientific cat. no. 14-961-27)

20.2.2 Volumetric flasks, Class A

20.2.3 Volumetric pipettes, Class A

20.2.4 Centrifuge tubes, 50 mL, with caps (Fisher Scientific cat. no. 14-432-22)

5.2.6 Test tubes, 16 x 125 mm (Fisher Scientific cat. no. 14-961-30)

5.2.7 Sera filters (TechniServe cat. no. 510-4055-P01)

5.2.8 Aluminum weighing pans (Fisher Scientific cat. no. 08-732)

5.2.9 Glass Microfiber Filters, Whatman GF/F; 47 mm, 0.7 μm pore size (Fisher Scientific cat. no. 1825-047)

21.0 REAGENTS AND STANDARDS

21.1 Reagents

6.1.1 Reagent Water – Use deionized (18 megohm) water when preparing all reagents and standards. Degas deionized water and all reagents, except standards, to remove dissolved gases.

6.1.2 Stock Ammonium Molybdate Solution- In a 1 L volumetric flask, dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)6Mo7O_{24}.4H_2O$] in approximately 800 ml DI water. Dilute to the mark and let stir for 4 hours. Store in a plastic container and refrigerate. May be stored up to two months when kept refrigerated.

6.2.1 Stock Antimony Potassium Tartrate Solution- In a 1 L volumetric flask, dissolve **3.22 g antimony potassium tartrate Trihydrate $K(SbO)C_4H_4O_6.3H_2O$)** or dissolve **3.0 g antimony potassium tartrate hemihydrate $K(SbO)C_4H_4O_6.1/2H_2O$),** in approximately 800 ml DI water. Dilute to the mark and let stir for few minutes. Store in a dark bottle and refrigerate. This stock may be used up to two months when kept refrigerated.

6.1.4 Molybdate Color Reagent – Add 106.5 mL stock ammonium molybdate and 36.0 mL stock antimony potassium tartrate to about 250 mL of deionized water in a 500 mL volumetric flask. Dilute to the mark with deionized water and invert to mix, store in dark container and prepare weekly. Degas with helium.

6.1.5 Ascorbic Acid Reducing Solution – Dissolve 60.0 g ascorbic acid in about 800 mL deionized water in a 1 L volumetric flask. Mix on a magnetic stirrer and dilute to the mark with deionized water. Prepare fresh weekly.

6.1.6 1.0 M Hydrochloric Acid (Carrier/Diluent for Standards) – Add 83.0 mL of concentrated hydrochloric acid (37%, ACS Reagent Grade, d=1.200) to about 800 mL of deionized water in a 1L volumetric flask. Dilute to mark with deionized water after cooling to room temperature. Mix well, prepare monthly.

6.1.7 Sodium Hydroxide - EDTA Rinse Solution – Dissolve 65.0 g sodium hydroxide and 6g tetrasodium ethylenediamine tetraaceticacid (Na_4EDTA) in about 800 deionized water in a 1 L volumetric flask. Mix on a magnetic stirrer until it is completely dissolved. Dilute to the mark with deionized water and mix.

6.2 Standards

6.2.1 Phosphorous standard (1000) mg P/L, purchased from an approved source. If this stock is not available prepare 100 ppm P/L as detailed in (6.2.2).

6.2.2 Stock Standard 100 mg P/L in 1.0 M Hydrochloric Acid – Add 10 mL of Phosphorus 1000 ppm stock standard (6.2.1) to about 60 mL of 1.0 M Hydrochloric Acid in a 100 mL volumetric flask, dilute to mark, and mix well. If the 1000 ppm P stock is not available, prepare the 100 ppm stock by dissolving 0.4394 g potassium phosphate monobasic (KH_2PO_4) that has been dried for two hours at 110°C, in about 800 mL of 1 M hydrochloric acid (6.1.6) in a 1 L volumetric flask. Dilute to the mark with 1.0M hydrochloric acid and mix. Prepare monthly.

6.2.3 Intermediate Standard Solution (10 mg P/L) – Add 10 mL of stock standard (6.2.2) to 60 mL of 1 M hydrochloric acid (6.1.6) in a 100 mL volumetric flask and dilute to 100 mL mark and mix. Prepare weekly

6.2.4 Working standards – Prepare the standards according to the following chart; dilute each with1.0 M hydrochloric acid (6.1.6) and mix. Prepare every 48 hours.

6.2.5 Spiking solution – Use stock standard, 100 mg P/L (6.2.2) to spike 10 ml of blank (1M HCl) with 50 µL of this solution (Blank Spike)

Concentration mg N/L	**Intermediate Std 10ppm, mL**	**Final Volume, mL**
1.0	10	100
0.5	5	100
0.4	4	100
0.3	3	100
0.2	2	100
0.1	1	100
0.05	500µL	100
0.00	0	100

22.0 COLLECTION, PRESERVATION, AND STORAGE

22.1 Samples are collected by the field personnel on Whatman GF/F filters (47 mm; 0.7 µm pore size) by filtering known volumes of water samples through the filters. The filters are folded, placed in aluminum foil pouches, and kept frozen until analysis.

23.0 QUALITY CONTROL

8.1 A demonstration of capability study is performed by each analyst performing the test by analyzing four replicates of an external quality control sample during one or two analytical runs.

8.2 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of the PP pads provided by the client, over three consecutive analytical runs. MDL is calculated as follows:

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made.

8.3 In the analytical run, every tenth sample is duplicated followed a blank. The accepted value for the relative percent difference (RPD) is ± 10%.

8.4 Blank filters are processed and analyzed when provided by the field personnel.

8.5 One mid-range standard (0.40 mg P/L) is analyzed for every 10 samples.

8.5 An external quality control sample is analyzed at the beginning and at the end of each analytical run.

- 8.5.1 For each analytical run prepare the external quality control sample.
- 8.5.2 Ignite MESS-4 marine sediment in a 550ºC for 90 minutes. When cool, store in desiccator for up to a year.
- 8.5.3 Weigh out **0.025g** of the ignited MESS-4 in the desiccator and add 10mL of 1M HCl in a capped centrifuge tube.
- 8.5.4 Shake all samples well manually to have all filters soaked in the solution. Place the samples on the automatic shaker overnight along with the QC sample. After shaking overnight allow sediment to settle to the bottom of the tube.
- 8.5.5 Prepare a X10 dilution of the QC by pipetting off 5mL of the top layer from the centrifuge tube mixture, making sure to avoid the sediment, and adding it to a 50mL volumetric flask.
- 8.5.6 Bring up to volume with 1M HCl. Invert to mix well.

8.6 Blank used for PP is 1M HCl is analyzed at the beginning of each run and after every 10^{th} samples.

24.0 PROCEDURE

9.1 Sample Preparation

9.1.1 Place filters (samples and blanks, if provided) in labeled aluminum weighing pans and combust in a muffle oven at 550^{o}C for 1½ hours. Label the pans by impressing numbers on the bottom of the pan. Any ink would burn off

.

9.1.2 Cool to ambient temperature, then transfer the combusted filters to labeled 50 mL screw cap centrifuge tubes. Use forceps to insert the pad into the bottom of the conical tube to ensure digestion.

9.1.3 Add 10 mL 1M hydrochloric acid to each tube

9.1.4 Cap tubes and shake well, making sure that all filters are soaked in the solution. Shake all tubes before placing them on the automatic shaker. on an automatic shaker for a 24 hour period.

9.1.5 Pour samples into 16 x 125 mm tubes and filter using Sera filters.

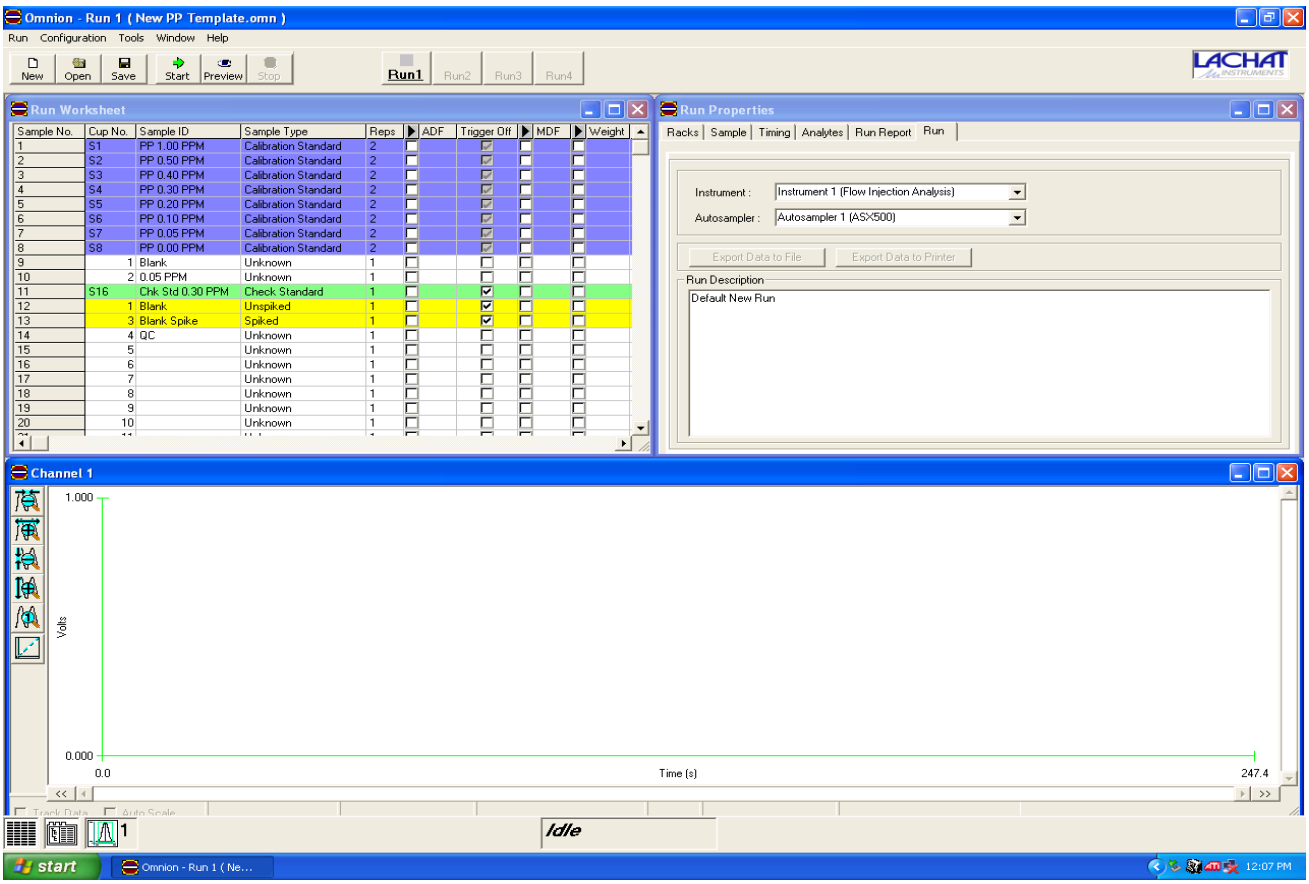
9.1.6 Transfer the filtrate to auto sampler tubes following the order of the run worksheet.

9.2 Instrument set-up and sample analysis

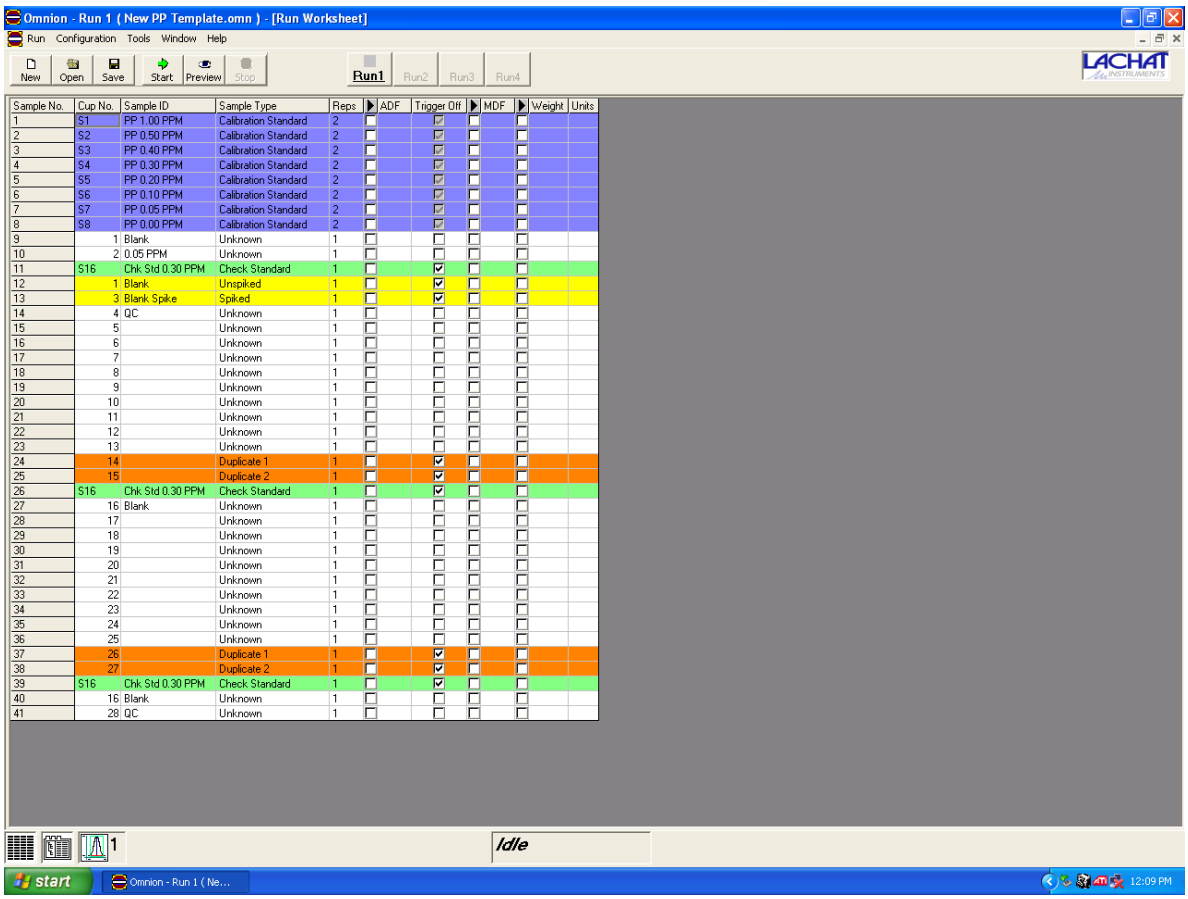
9.2.1 Set up manifold as described in the method.

9.2.2 Turn on the Lachat instrument, computer, monitor, and printer.

9.2.3 Double click on Omnion and then double click on **"LL PP"** to open the template, which consists of three windows.



9.2.4 Maximize the **"Run Worksheet"** window at the top left hand corner of the screen by clicking on the middle square on that screen to open the worksheet template. Enter sample identifications in the sample identification column, making sure that all duplicates are entered in the appropriate locations of the worksheet. Identifications of the quality control samples analyzed at the beginning of the run and the locations of the duplicates and the spiked have been saved on the template. Press the **"Enter"** key after each entry in order to save all entries.

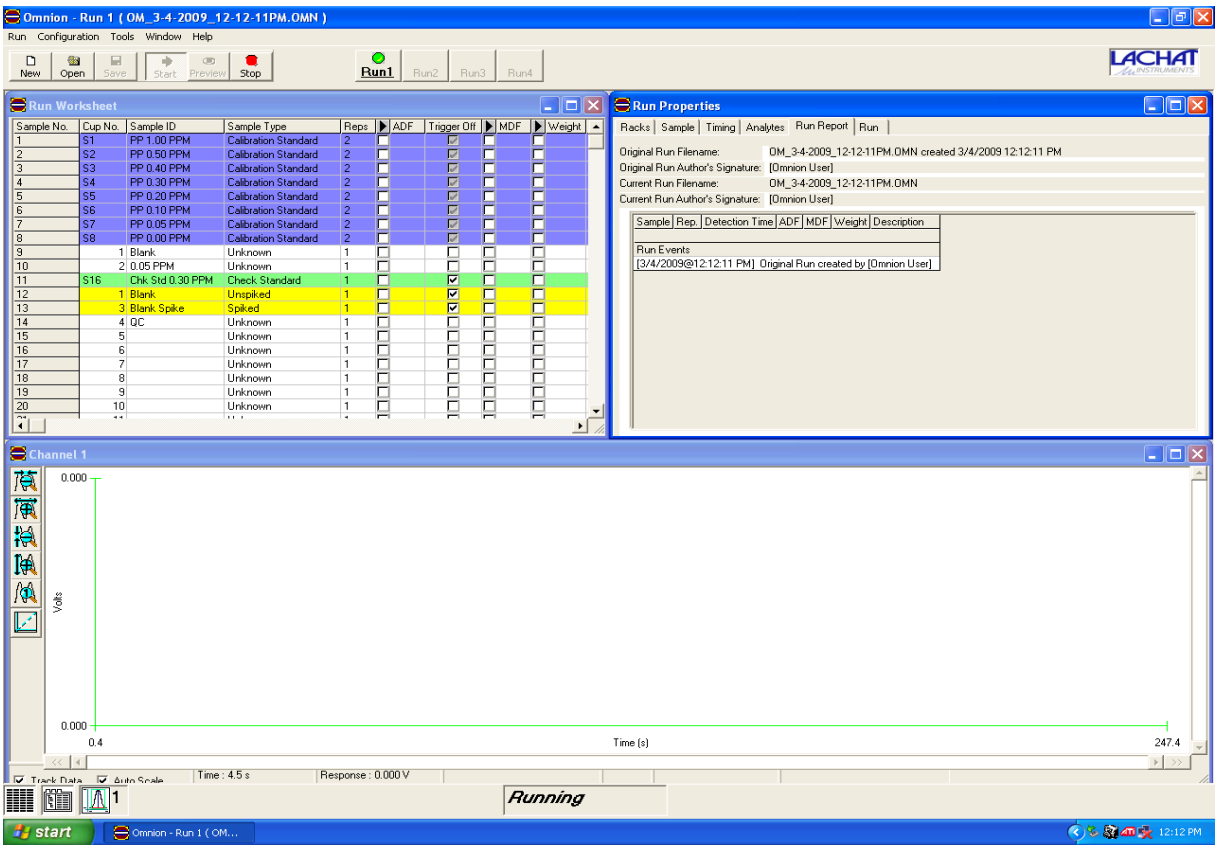


9.2.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

9.2.6 Click on **"Window"** tab and then, click on **"Tile"** to return to the screen with three windows.

9.2.7 Place standards in standard vials, then in the standard rack in the order of decreasing concentration from positions 1 to 8. Place the check standard vial in position 16 (S16) of the standard rack. Pour samples and quality control samples into 13 x 100 mm test tubes and place them in the sample racks according to the worksheet prepared in 9.2.4.

9.2.8 Pump deionized water through all reagent lines for 5 – 10 minutes and check for leaks and smooth flow. Switch to reagents and continue pumping for about 10 minutes. Click on **"Preview"** tab to monitor the baseline.



9.2.9 Once a stable baseline is achieved, click on **"Stop"** tab to stop monitoring the baseline. Click on **"Start"** tab to begin the analysis.

9.2.10 If the calibration passes, instrument will continue to analyze the samples. If failed, take appropriate corrective actions and recalibrate before proceeding to analyze samples.

9.2.11 Samples with concentration exceeding the calibrated range will be manually diluted by 1M HCl and reanalyzed.

9.2.12 After the run is complete, remove the reagent lines and place them in DI water and rinse for about 15 minutes. If necessary, rinse the system with the NaOH – EDTA rinse solution (6.1.7) for not more than 5 minutes followed by DI water of 10 – 15 minutes. Pump the tubes dry, release the pump tubing, and turn off the pump.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 All the calculations are performed by the Omnion 3.0 software system. The amount of color is plotted against the known concentrations and the line that best fits among the data points is the calibration curve. The concentration of unknown

samples are determined automatically by plugging the amount of color (response) in the calibration curve equation. All standards are analyzed in duplicate and data points are used for the calibration curve. Samples with phosphorous concentrations greater than 1.00 ppm are diluted manually by 1.0 M HCl and reanalyzed.

10.2 Calculate the actual concentration of particulate phosphorus in samples (mg/L) by multiplying the concentration of sample mg /L X10mL / volume of sample (mL) used in the filtrate. Use the template below for calculations.

Particulate Phosphorus Calculation

Date:

(concentration*10)/volume of sample= result

Sample #	conc.	volume	value		Sample #	conc.	volume	value	
			#DIV/0!	###				#DIV/0!	###

10.3 Calculate the relative percentage difference for the duplicated samples as follows:

$$\%\,\mathrm{RPD} = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \times 100$$

10.4 The reporting level for this method is the concentration of the lowest standard, which is 0.05 ppm.

10.5 Report results for all routine samples to three decimal places and for performance evaluation (PE) samples to three significant figures.

11.0 DATA AND RECORDS MANAGEMENT

11.1 The analysis data for the calibration blank, external QC and instrument performance check solution (IPC), i.e. the mid-range check standard, is kept on file with the sample analysis data.

11.2 Normal turnaround time for samples submitted to this lab is 2 to 10 days from the receipt. Results are reported in writing on the sample analysis request form.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 It is laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume and bench operation.

12.2 Samples and standards are poured down the drain and flushed with running water.

13.0 REFERENCES

13.1 United States Environmental Protection Agency, *Methods for Chemical Analysis of Water and Waste, EPA/600/R-93/100, Method 365.4, May 1993.*

13.2 Lachat Instruments, *Determination of Total Phosphate in Ashed Soil Samples by Flow Injection Analysis, Method 12-115-01-1-F, September 2003.*

13.3 Chesapeake Biological Laboratories, *Particulate Phosphorus Method, February 2004.*

13.4 EPA Method 365.1, *Methods for the Determination of Inorganic Substances in Environmental Samples*, August 1993.

13.5 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater,* 21st Edition, Method 4500- P E, 2005.

13.6 Lachat Instruments, *Operating Manual for the Quikchem Automated Ion Analyzer*.

13.7 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. Revision15.0, August 2016.

13.6 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1,2015.

APPENDIX **A**

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist- Particulate Phosphorus

EPA Method 365.1

Lab Numbers[1]:_____ Analyst: _____

Date Collected: _____ Date Digested: _____ Date Analyzed: _____

Procedure	Acceptance Criteria	Status*	Comments
Holding Time	28 days @ –15°C		
Calibration Curve	Corr. Coeff. ≥ 0.9950		
Reagent Blank	< Reporting level (0.05 ppm)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
Check Standard	After every 10th sample and at the end of the run		
	Recovery = 90–110%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.05– 1.00 ppm)		
Diluted Samples	Correct final calculations		
External QC[2]	Beginning and end of each run		
	Within acceptance range		
Changes/Notes	Clearly stated		

* Check (✓) if criteria are met. [1]Include beginning and ending numbers, account for gaps by bracketing

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

Reagents	ID
1M HCl	_____
Ascorbic Acid	_____
Color Reagent	_____

External QC

Identification = _____
True Value = _____ ppm
Range = _____ ppm

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

Title: Determination of Total Dissolved Nitrogen
Flow Injection Colorimetric Analysis
(EPA Method 353.2)

SOP No.: (CHEM-SOP-EPA 353.2 TDN)

Revision: 4.1 Replaces: 4.0 Effective: 7/1/2017

Laboratory: Inorganics Analytical Laboratory

POC: Jewel Freeman-Scott/Cynthia Stevenson
Jewel.freeman-scott@maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

EPA METHOD 353.2
SOP No.: CHEM-SOP-EPA 353.2

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	S. Ameli	6/2/08
1.0	12/09	Screen snapshots added to procedure, new Sample Run Log, tracking IDs for standards and reagents	S. Ameli	1/10
2.0	8/11	New SOP tracking number, technical and editorial changes	S. Ameli	8/18/11
2.1	8/13	Reviewed SOP	S. Ameli	8/18/11
3.0	11/2014	New tracking numbers, technical and editorial changes	S. Ameli C. Stevenson	12/2014
3.0	6/1/2015	Reviewed SOP	C. Stevenson	7/1/2015
4.0	5/5/2016	Technical and editorial changes. Added commercial stock standard. (6.2.1)	S. Ameli C. Stevenson J. Freeman-Scott	7/1/2016
4.1	6/1/2016	Reviewed and made organizational name changes	S. Ameli C. Stevenson J. Freeman-Scott	7/1/2017

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STANDARD OPERATING PROCEDURES

DETERMINATION OF TOTAL DISSOLVED NITROGEN IN ALKALINE PERSULFATE DIGESTS BY AUTOMATED COLORIMETRY

EPA Method 353.2

1.0 SCOPE AND APPLICATION

24.1 This method is applicable to seawater, brackish water, and non-saline water.

24.2 The applicable range is 0.1 to 5.0 mg N/L.

2.0 SUMMARY OF METHOD

The nitrate is quantitatively reduced to nitrite by passing the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide under acidic conditions followed by coupling with N-(1-naphthyl) ethylene diamine dihydrochloride (Marshall's reagent) to form a reddish-purple azo dye which is measured colorimetrically at 520 nm. Per manufacture's recommendation, water samples are digested for one hour with alkaline persulfate to oxidize all the nitrogen compounds present in the sample to nitrate (NO_3^-). A single multi-analyte standard is prepared for nitrate, nitrite and phosphorus as they are analyzed concurrently on the same instrument.

3.0 INTERFERENCES

3.1 Total carbon concentrations in excess of 20 mg C/L or Chemical Oxygen Demand (COD) concentrations in excess of 50 mg/L will result in complete oxidation of nitrogen compounds.

3.2 The presence of sulfide and chloride produced by seawater oxidation would reduce the column life as compared to non-saline samples.

3.3 Turbidity and colored samples that absorb at 520 nm will interfere in the colorimetric determination. Turbidity of the samples can be removed by filtration prior to analysis.

4.0 HEALTH AND SAFETY

4.1 Good laboratory practices should be followed during reagent preparation and instrument operation.
The use of a fume hood, protective eyewear, lab coat and proper gloves are required when preparing reagents.

4.2 Sodium hydroxide, hydrochloric acid, and phosphoric acid used in this determination have the potential to be highly toxic or hazardous. Consult Material Safety Data Sheets (MSDS) for detailed explanations.

4.3 Each employee is issued a *Laboratory Safety Manual* and is responsible for adhering to the recommendations contained therein.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Lachat QuickChem 8500 Automated Ion Analyzer, which includes the following:

5.1.1.1 Automatic sampler

5.1.1.2 Multi-channel proportioning pump

5.1.1.3 Injection module with a 12.5 cm x 0.5 mm ID sample loop

5.1.1.4 Manifold

5.1.1.5 Colorimetric detector

5.1.1.5.1 Flow cell, 10 mm path length

5.1.1.5.2 Interference filter, 520 nm

5.1.1.6 Computer with Omnion 3.0 Data system and printer

5.1.2 Analytical balance capable of accurately weighing to the nearest 0.0001 g

5.1.3 Top loading balance for weighing chemicals for reagents

5.2 Supplies

5.2.1 Class A volumetric flasks, 50 – 1,000 mL

5.2.2 Class A volumetric pipettes, 1– 10 mL

5.2.3 Automatic pipetters, 100 µL- 10 mL

5.2.4 Digestion tubes – 40 mL vials (Fisher 14-959-37C or equivalent) with autoclavable caps lined with Teflon liners (Kimble Caps Cat # 03-340-77E)

5.2.5 Beakers, disposable, polypropylene, 50 mL(Fisher 01-291-10)

5.2.6 Test tubes, glass, 13 x 100 mm and 16 X 125 mm

5.2.7 Reagent storage bottles, plastic or glass

5.2.8 Cadmium Reduction Column (Lachat Part No. 50237A)

5.2.9 Ultra High Purity Helium gas for degassing

6.0 REAGENTS AND STANDARDS

6.1 Reagents

Use deionized water (DI) for preparing all solutions. Prevent the bubble formation by degassing deionized water and all reagents with helium for one minute and half.

6.1.1 15 N Sodium Hydroxide – Gradually add 150 g NaOH in a beaker of about 200 mL DI water. Mix well and ensure dissolution. Let the solution reach to room temperature, and store in a plastic container**.**

6.1.2 Ammonium Chloride Buffer, pH 8.5 – While working In a fume hood, dissolve 85.0 g ammonium chloride (NH_4Cl) and 1.0g disodium ethylenediamine tetraacetic acid dihydrate ($Na_2EDTA.2H_2O$) in about 800 mL DI water, in a 1L volumetric flask. Mix well and dilute to the mark. Adjust the pH to 8.5 with 15 N sodium hydroxide solution and then filter the reagent and refrigerate. This solution is stable for one month.

6.1.3 Sulfanilamide Color Reagent – Add about 600 mL of DI water into a 1 L volumetric flask. Then add 100 mL 85% phosphoric acid (H_3PO_4), 40.0 g sulfanilamide, and 1.0 g N- (1-naphthyl) ethylenediamine dihydrochloride (NED). Stir for about 30 minutes until dissolved. Dilute to the mark, filter and store in a dark bottle in a refrigerator. This solution is stable for one month.

6.1.4 Alkaline Persulfate Oxidizing Reagent - In a 1L volumetric flask, dissolve 20.1 g potassium persulfate ($K_2S_2O_8$), and 3g sodium hydroxide (NaOH) in about 600 mL DI water. Dilute to mark and mix. Prepare fresh daily before use.

6.1.5 Borate Buffer, 1.0 M, pH 7.5 - dissolve 61.8 g boric acid and 8.0 g sodium hydroxide in about 800 mL DI water in a 1 L volumetric flask. Mix for about four hours until it is completely dissolved. Dilute to the mark with DI water and mix. This solution is stable for two months.

6.2 Standards

6.2.1 Nitrate standard (1000 mg N/L), purchased from an approved source. If this stock is not available prepare by dissolving 0.722 g of potassium nitrate (dried in the oven for two hours at 110 ºC) in about 60 mL of DI water in a 100 mL volumetric flask. Dilute to mark and mix. Prepare monthly.

6.2.2 Phosphorous standard (1000) mg P/L, purchased from an approved source. If this stock is not available prepare in lab as described in TDP SOP.

6.2.3 Stock phosphorous (100 mg P/L) Standard Solution Add 10 mL of Phosphorus 1000 ppm stock standard (6.2.2) to about 60 mL of DI water in a 100 mL volumetric flask, dilute to mark, and mix well. Store in a dark bottle and prepare monthly.

6.2.4 Combined Intermediate Standard Solution (1 mg P/L and 10 mg N/L) – Add 10 mL of (6.2.3) 100 mg P/L (stock standard solution for total dissolved phosphorus determination) and 10 mL of 1000 mg N/L stock nitrate standard solution (6.2.1) to about 800 mL DI water in a 1L volumetric flask. Dilute to mark and mix. Prepare weekly.

6.2.5 Combined Working Standard Solutions (5.0, 2.0, 1.0, 0.5, 0.2, 0.1and 0.0 ppm) - Use the following table to prepare standards. Prepare per run and standards are good for 48 hours.

Concentration, mg N/L	**Combined Working Standard, mL**	**Final Volume, mL**
5.0	50	100
2.0	20	100
1.0	20	200
0.5	5	100
0.2	2	100
0.1	1	100
0.0	0	100

Note: ***The analyst may save a set of standards from a previous run for troubleshooting purposes only***

6.2.6 Stock Nitrite Standard Solution for Cadmium check (1000 mg N/L) - Purchased from an approved source. If not available, weigh and dissolve 0.6072 g potassium nitrite in about 80 mL of DI water in a 100 mL volumetric flask. Dilute to mark and mix. Prepare monthly.

6.2.6 Nitrite working standard for Cadmium check (2.5ppmN/L)
Pipette 0.5 ml of (6.2.6) into 200 mL volumetric flask. Mix and dilute to mark. Prepare weekly

6.2.7 Nitrate working Standard for Cadmium check (2.5 mg N/L) – Pipette 0.5 mL of (6.2.1) to about 100 mL DI water in a 200 ml volumetric flask. Dilute to mark and mix. Prepare monthly.

6.2.8 Spiking Solution – Pipette 50 µl of a combined solution of 5 mL of 1000 mg/L N (6.2.1) and 5 mL of 100 mg/L P (6.2.3) into 10 mL of sample (sample spike)or 10 mL of DI water (blank spike). Prepare monthly.

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Samples are filtered in the field, collected in plastic bottles or cubitainers, and are preserved by cooling to 4^{o} C.

7.2 Samples are analyzed within 48 hrs after collection. If they cannot be analyzed within this time period, they may be frozen at -20^{o} C as soon as possible at the laboratory. The holding time for frozen samples is 28 days.

8.0 QUALITY CONTROL

8.1 An initial Demonstration of Performance (IDP) to establish the instrument's capability is required prior to its use in analyzing client's samples. The linearity of calibration range (LCR) and the ability to quantify correctly Quality Control Samples are used to assess performance.

8.2 A mid-range check standard and a calibration blank is analyzed immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. The acceptable concentrations for the check standard must be within ± 10% of the actual concentration of the check standard. If a check standard fails, the samples between the last satisfactory check standard and the unsatisfactory standard are re-analyzed. Blank concentration must be less than the reporting level of 0.1 ppm. Blanks that do not meet this criterion are reanalyzed.

8.3 Every tenth sample is duplicated (analyzed from two different tubes) and spiked. The accepted values for the relative percent difference (RPD) must fall within ± 10 % and for spike recovery between 90 - 110%. If these do not fall within the accepted ranges, the corresponding analyses are repeated.

8.4 A QC sample with a known concentration and a known range is analyzed at the beginning and at the end of each analytical run. Follow the vendor's procedure for preparation of solution. QC samples that do not fall within the accepted range are repeated.

8.5 Samples with a concentration exceeding the calibrated range are diluted manually and reanalyzed.

8.6 Data acceptance criteria are listed on the data review checklist (page 15)

8.7 The laboratory annually participates in USGS, Chesapeake Bay Laboratory (CBL), Water Supply (WS) and Water Pollution (WP) proficiency studies.

8.8 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of the 0.1 ppm standard spread over three analytical runs. MDL is calculated as follows: Note: The analyst might save a set of stds from previous run for troubleshooting purposes.

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made.

8.9 A demonstration of capability study is performed by each analyst performing the test by analyzing four replicates of an external quality control sample during one or two analytical runs.

8.10 The efficiency of the cadmium column before and after sample run is calculated by running 2.5 ppm NO_2-N (6.2.6) and 2.5 ppm NO_3-N (6.2.7) standards and using the formula (NO_3-N/NO_2-N) x 100. The accepted range for the cadmium column efficiency is 90-110%. If the efficiency is out of this range, new standards are prepared and efficiency is re-evaluated. If the efficiency is still out of range then the column is replaced.

9.0 PROCEDURE

9.1 Sample preparation

9.1.1 Make a list of samples to be analyzed and pour aliquots of samples into labeled 16 mm x 125 mm test tubes.

9.1.2 Pipette 10 mL of each standard or sample into digestion tubes.

9.1.3 Pipette 10 mL of a mid-range (0.1 mg P/L and 1.0 mg N/L) standard, a blank, a blank spike, and an external quality control sample into digestion tubes. With each tray prepare a duplicate and a spike of every 10^{th} sample.

9.1.4 Pipette 10 mL of the nitrate and nitrite standards for cadmium column check (6.2.6 and 6.2.7) into digestion tubes.

9.1.5 Add 5 mL of digestion solution to each tube, screw the caps on tightly and mix each. Digest the standards, samples, and all the quality control samples in the autoclave for **60 minutes** at 121 ^{o}C (250 ^{o}F) @ 17 psi after it reaches the set temperature and pressure.

For Autoclave Operation please see the manual

9.1.6 After one hour, turn off the autoclave and let the digests cool for 5 minutes. Remove the tubes from the autoclave and let the digests come to room temperature. Add 1 mL of borate buffer, vortex, and pour into 13 x 100 mm culture tubes analyze them as soon as possible.

9.1.7 If samples cannot be analyzed same day, do not add the borate buffer. Refrigerate the digests at 4^{o}C. Refrigerated digests will be brought up to room temperature, and subsequently 1 mL borate buffer (6.1.5) is added to each tube and mixed thoroughly by a vortex.

9.1.8 Analyze the digests using the procedure described in 9.2.

9.2 Instrument Calibration and Sample Analysis

9.2.1 Set up manifold according to the manifold diagram.

9.2.2 Pump deionized water through all reagent lines and check for leaks and a smooth flow. Switch to reagents and allow the system to stabilize and a stable baseline is achieved.

9.2.3 Enter sample information required by the data system.

9.2.4 Place standards, blanks, samples, quality controls, etc. in the auto sampler according to the run table.

9.2.5 Initiate the analytical run.

9.2.6 At the end of the run, review the calibration curve statistics and the results for the quality control samples. Acceptable values for the correlation coefficient are $\geq$ 0.9950. Other quality control criteria are described in 8.0.

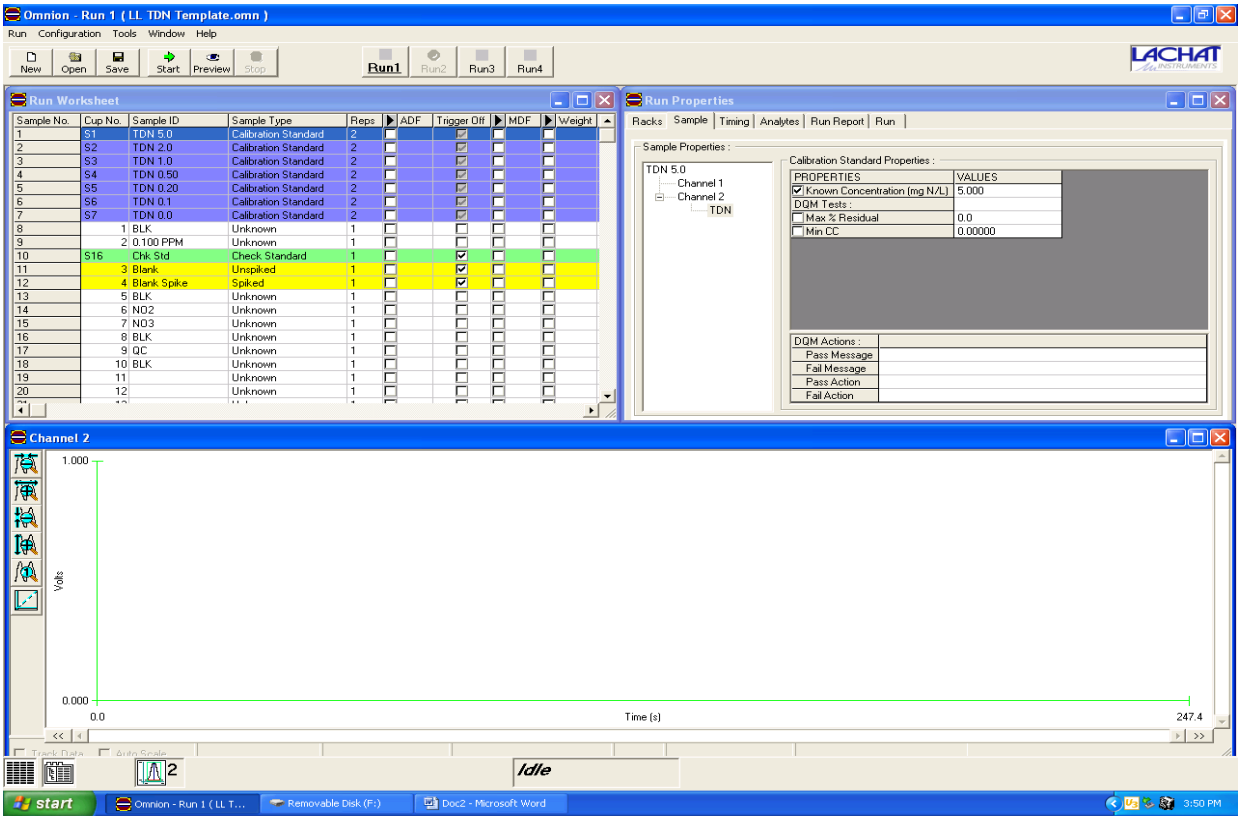
9.2.7 Get the data reviewed by a designated scientist, and then, report the results on the Analysis Request Forms.

9.3 Instrument set-up and sample analysis

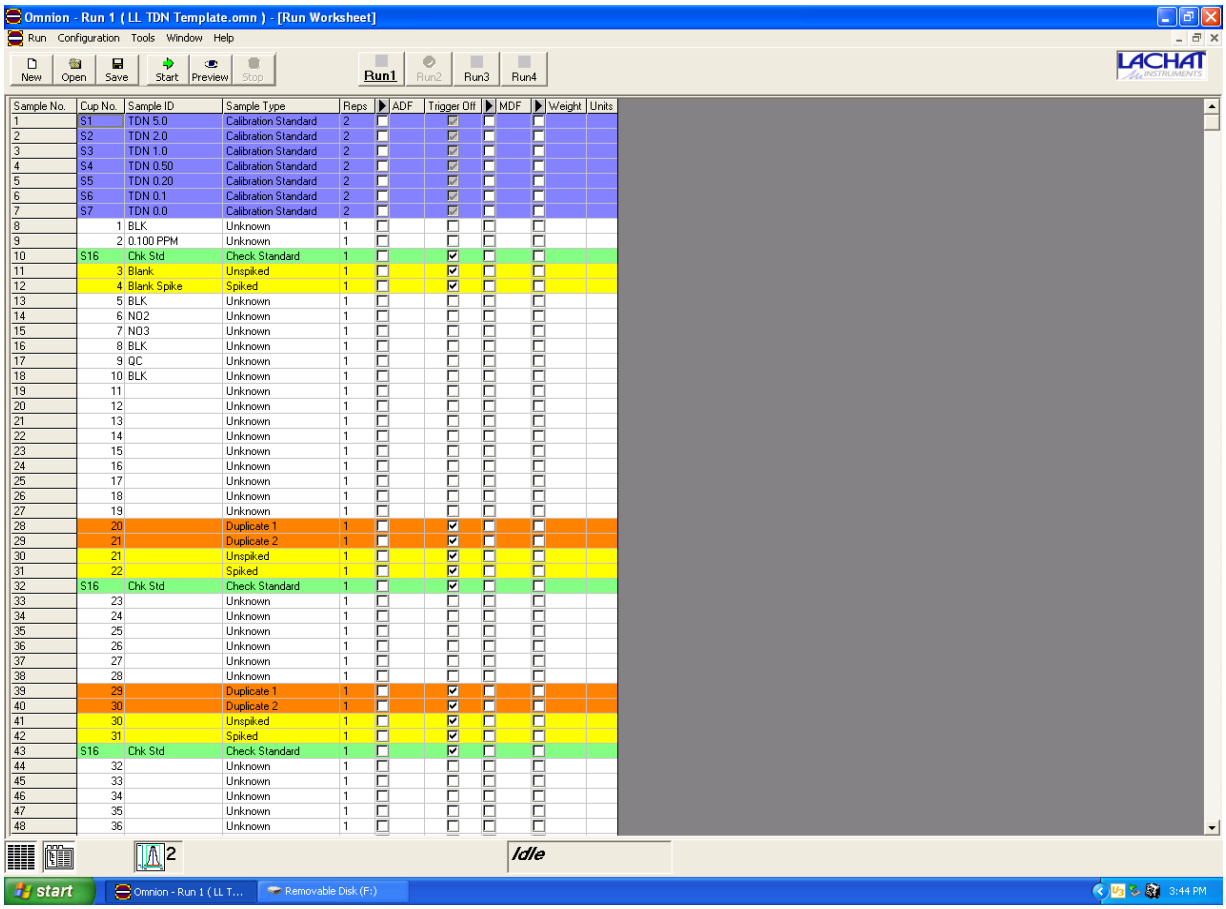
9.3.1 Set up manifold as in the diagram.

9.3.2 Turn on the Lachat instrument, computer, monitor, and printer.

9.3.3 Double click on Omnion and then on **"LL TDN"** to open the template, which consists of three windows.



9.3.4 Maximize the **"Run Worksheet"** window at the top left hand corner of the screen by clicking on the middle square on that screen to open the worksheet template. Enter sample identifications in the sample identification column, making sure that all duplicates and spikes are entered in the appropriate locations of the worksheet. Identifications of the quality control samples analyzed at the beginning of the run and the locations of the duplicates and the spiked have been saved on the template. Make sure that **"Enter"** key is pressed after each entry in order to save all entries.

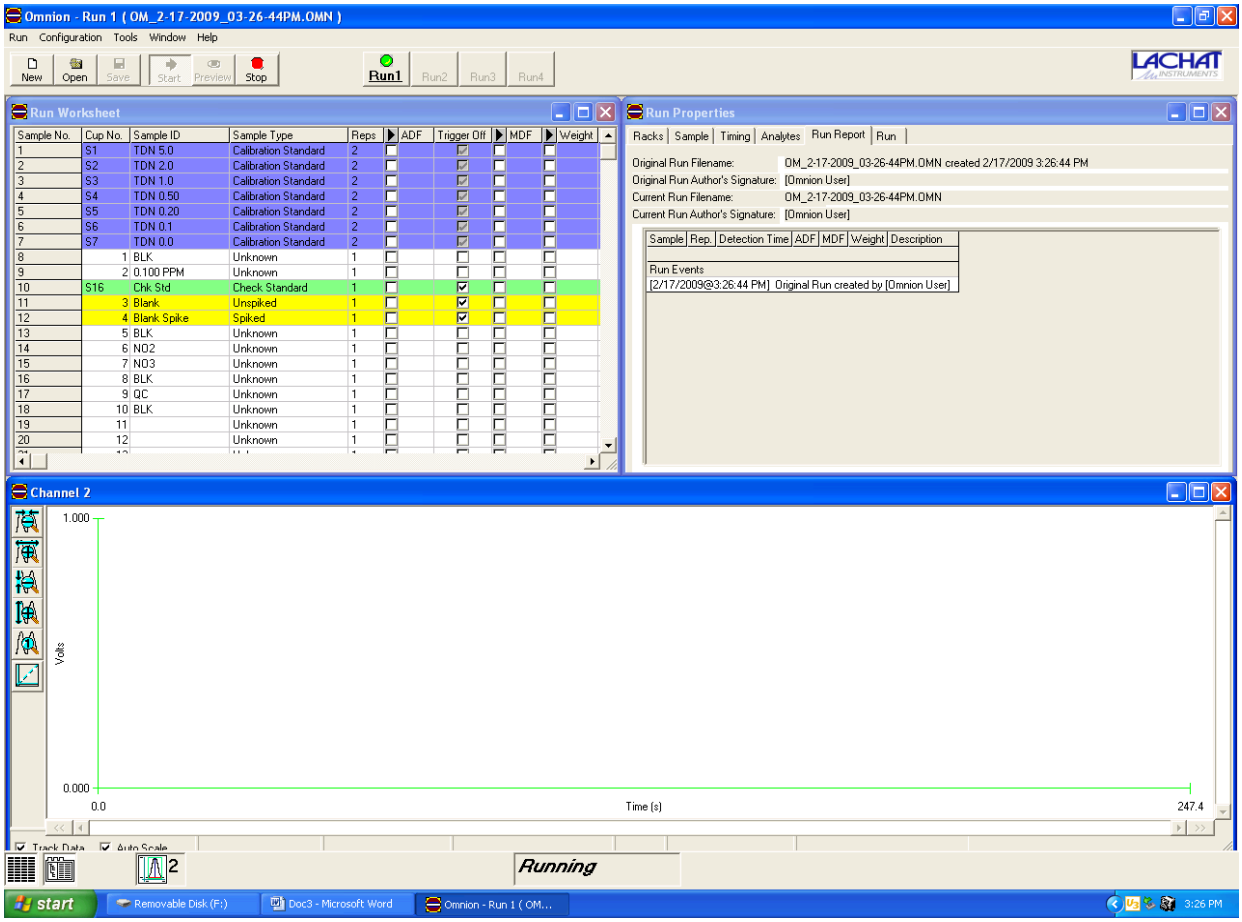


9.3.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

9.3.6 Click on **"Window"** tab and then, click on **"Tile"** to return to the screen with three windows.

9.3.7 Place standards in standard vials in the standard rack in order of decreasing concentration in positions 1 to 7 (STD 7 is DI water -0.00 mg/L). Place the check standard vial in position 16 (S16) of the standard rack. Pour samples and quality control samples into 13 x 100 mm test tubes and place them in the sample racks according to the worksheet.

9.3.8 Pump deionized water through all reagent lines for 10 – 15 minutes and check for leaks and smooth flow. Switch to reagents and continue pumping for about 10 minutes. Click on **"Preview"** tab to monitor the baseline.



9.3.9 Once a stable baseline is achieved, click on **"Stop"** tab to stop monitoring the baseline. Click on **"Start"** tab to begin the analysis.

9.3.10 If the calibration passes, curve fit of calibration solutions/response based on regression, the instrument will continue to analyze the samples. If it fails, take appropriate corrective actions and recalibrate before proceeding to analyze samples.

9.3.11 Manual dilution will be performed to reanalyze samples with concentrations exceeding the calibrated range.

9.3.12 After the run is complete, remove the reagent lines and place them in DI water and rinse for about 15 minutes. If necessary, rinse the system with the NaOH – EDTA rinse solution for not more than 5 minutes, followed by DI water rinse for 10 – 15 minutes. Pump the tubes dry, release the pump tubing, and turn off the pump.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 All the calculations are performed by the Omnion 3.0 software system. The amount of color is plotted against the known concentrations and the line that best fits among the data points is the calibration curve. The concentration of unknown samples is determined automatically by plugging the amount of color (response) in the calibration curve equation. All standards are analyzed in duplicate and all data points are used for the calibration curve. Samples with total dissolved nitrogen concentrations greater than 5.00 ppm are diluted manually by 1.0 M HCl and reanalyzed.

10.2 The reduction efficiency of the cadmium column is calculated as followings:

$$\% \text{ RE} = \text{NO3/NO2 X } 100$$

10.3 Calculate the percentage spike recovery of the laboratory fortified samples as follows:

$$\%\,SR = \frac{(\text{spiked sample conc.} - \text{sample conc.}),\ \text{ppm}}{\text{amount of spike added to sample, ppm}} \times 100$$

10.4 Calculate the relative percentage difference for the duplicated samples as follows:

$$\%\,RPD = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \times 100$$

10.5 The reporting level for this method is the concentration of the lowest standard, which is 0.1 ppm.

10.6 Report results for all routine samples to three decimal places and for performance evaluation (PE) samples to three significant figures.

11.0 DATA AND RECORDS MANAGEMENT

11.1 The analysis data for the calibration blank, external QC and instrument performance check solution (IPC), i.e. the mid-range check standard, is kept on file with the sample analysis data.

11.2 Normal turnaround time for samples submitted to this lab is 2 to 10 days from the receipt. Results are reported in writing on the sample analysis request form.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 It is laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume and bench operation.

12.2 Samples and standards are poured down the drain and flushed with running water.

13.0 REFERENCES

13.1 EPA Method 353.2, *Methods for the Determination of Inorganic Substances in Environmental Samples*, August 1993.

13.2 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, Method 4500- P E, 2005.

13.3 Lachat Instruments, *Methods Manual for the Quikchem Automated Ion Analyzer*, Method 10-107-04-4-A.

13.4 Lachat Instruments, *Operating Manual for the Quikchem Automated Ion Analyzer*.

13.5 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.6 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX A

Division of Environmental Sciences

INORGANICS ANALYTICAL LABORATORY

Data Review Checklist Total Dissolved Nitrogen (TDN)/Alkaline Persulfate Digestion

EPA Method 353.2

Lab Numbers[1]:_____ Analyst: _____

Date Collected: _____ Date Digested: _____ Date Analyzed: _____

Procedure	Acceptance Criteria	Status*	Comments
Holding Time	48 hours @ 4°C; 28 days @ –20°C		
Calibration Curve	Corr. Coefficient. $\geq$ 0.9950		
Reagent Blank	< Reporting level (0.100 ppm)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptance range		
Check Standard	After every 10th sample and at the end of the run		
	Recovery = 90–110%		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
Cadmium Column Check	NO_3/NO_2 X 100=90-110%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.100–5.00 ppm)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____

Analyst's Signature & Date Reviewer's Signature & Date

_____

Supervisor's Signature and Date

Reagents	ID	Reagents	ID	External QC	
Ammonia Buffer	_____	Oxidizing Reagent	_____	Identification =	_____
Color Reagent	_____	Borate Buffer	_____	True Value =	_____ ppm
				Range =	_____ ppm

APPENDIX B

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist LL Total Dissolved Phosphorus TDP/ LL Total Dissolved Nitrogen TDN

EPA Method 353.2 and EPA 365.1

Lab Numbers: [1] _____ Analyst: _____

_____ Date Digested: _____

Dates Collected: _____ Date Analyzed _____

Procedure	Acceptance Criteria	Status (√)	Comments
Holding Time	48 hours @ 4ºC; 28 days @ –20ºC		
Calibration Curve	Corr. Coeff. $\geq$ 0.9950		
Reagent Blank	< Reporting Level (0.010 ppm for TDP; 0.100 ppm for TDN)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10 samples		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptable range		
Check Standard	After every 10th sample and at the end of the run		
	Recovery = 90–110%		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
Cadmium Column Check	NO_3/NO_2 X 100=90-110%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.010–0.500 ppm for TDP; 0.100–5.000 ppm for TDN)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____

Analyst's Signature and Date Reviewer's Signature and Date

_____

Supervisor's Signature and Date

Reagents	ID	Reagents	ID
Ammonia Buffer		Sulfanilamide	
Ascorbic Acid		Color Reagent	
Borate Buffer		Molybdate	
1M HCl		Color Reagent	

External QC

Identification = _____

True Value = TDN /TDP ppm

Range = TDN ppm

Range = TDP ppm

Oxidizing Reagent _____ _____

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

SOP Title:	Determination of Total Dissolved Phosphorus Flow Injection Colorimetric Analysis (EPA Method 365.1)
SOP No.:	CHEM-SOP-EPA 365.1 TDP
Revision:	4.1 Replaces: 4.0 Effective: 7/1/2017
Laboratory:	Inorganics Analytical Laboratory
POC:	Jewel Freeman-Scott Jewel.freeman-scott@maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

EPA METHOD 365.1
SOP No.: CHEM-SOP-365.1TDP

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	Shahla Ameli	6/2/08
1.0	12/09	Screen snapshots added to procedure, new Sample Run Log, tracking IDs for standards and reagents	Shahla Ameli	1/10
2.0	1/11	New SOP tracking number, technical and editorial changes	J Freeman-Scott Shahla Ameli	8/18/2011
2.0	1/13	Reviewed the SOP	Shahla Ameli	1/13/2013
3.0	10/09/14	New SOP tracking number, technical and editorial changes, formatting changes	C. Stevenson S. Ameli	12/2014
3.0	6/1/2015	Reviewed SOP	C. Stevenson	7/1/2015
4.0	6/13/16	Technical and editorial changes. Added commercial stock standard. (6.2.1)	S. Ameli C. Stevenson J. Freeman-Scott	7/1/2016
4.1	6/1/2016	Reviewed and made organizational name changes	S. Ameli C. Stevenson J. Freeman-Scott	7/1/2017

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STANDARD OPERATING PROCEDURES

TOTAL DISSOLVED PHOSPHORUS IN ALKALINE PERSULFATE DIGESTS

EPA Method 365.1

25.0 SCOPE AND APPLICATION

25.1 This method is applicable to seawater, brackish water, and non-saline water.

25.2 The applicable range is 0.01 to 0.5 mg P/L.

26.0 SUMMARY OF METHOD

Water samples are digested for one hour with alkaline persulfate to convert all of the phosphorus present in the sample to orthophosphate (PO_4^{3-}). Approximately 1.3 mL of this digest is injected onto the manifold, where orthophosphate reacts with ammonium molybdate and antimony potassium tartrate under acidic condition and then reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. Per manufacture's recommendation, water samples are digested for one hour with alkaline persulfate to oxidize all the phosphorus compounds present in the sample to orthophosphate (PO_4^{3-}). The absorbance is directly proportional to the concentration of phosphorus in the sample.

27.0 INTERFERENCES

27.1 Silica forms a pale blue complex which also absorbs at 880 nm. A silica concentration of 4000 ppm would produce a 1 ppm positive error in orthophosphate.

27.2 Glassware should be washed with 1:1 HCl and rinsed with deionized water in order to prevent possible contamination problems in low level phosphorus determinations.

28.0 HEALTH AND SAFETY

28.1 Good laboratory practices should be followed during reagent preparation and instrument operation.

28.2 The use of a fume hood, protective eyewear, lab coat and proper gloves must be used when preparing reagents.

28.3 Sodium hydroxide, hydrochloric acid, and sulfuric acid used in this determination have the potential to be highly toxic or hazardous. Consult MSDS for detailed explanations.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Lachat QuickChem 8500 Automated Ion Analyzer, which includes the following:

5.1.1.1 Automatic sampler

5.1.1.2 Multi-channel proportioning pump

5.1.1.3 Injection module with a 12.5 cm x 0.5 mm ID sample loop

5.1.1.4 Manifold

5.1.1.5 Colorimetric detector

5.1.1.5.1 Flow cell, 10 mm path length

5.1.1.5.2 Interference filter, 880 nm

5.1.1.6 Computer with Omnion 3.0 Data system and printer

5.1.2 Analytical balance capable of accurately weighing to the nearest 0.0001 g

5.1.3 Top loading balance for weighing chemicals for reagents

5.2 Supplies

5.2.1 Class A volumetric flasks, 50 – 1,000 mL.

5.2.2 Class A volumetric pipettes, 1– 10 mL.

5.2.3 Automatic pipetters, 100 µL- 10 mL

5.2.4 Digestion tubes – 40 mL vials (Fisher 14-959-37C or equivalent) with autoclavable caps lined with Teflon liners (Kimble Caps Cat # 03-340-77E)

5.2.5 Beakers, disposable, polypropylene, 50 mL(Fisher 01-291-10)

5.2.7 Test tubes, glass, 13 x 100 mm and 16 X 125 mm

5.2.8 Reagent storage bottles, plastic or glass

5.2.8 Ultra High Purity Helium gas for degassing

6.0 REAGENTS AND STANDARDS

6.1 Reagents

Use deionized water (DI) for preparation of all solutions. Prevent the bubble formation by degassing deionized water and all reagents except the standards with helium for two minutes.

6.1.1 Alkaline Persulfate Oxidizing Reagent - In a 1L volumetric flask, dissolve 20.1 g potassium persulfate ($K_2S_2O_8$), and 3g sodium hydroxide (NaOH) in about 600 mL DI water. Dilute to mark and mix. Prepare fresh daily before use.

6.1.2 Hydrochloric Acid, 1.0 M – Add 83.3 mL concentrated hydrochloric acid (37%, ACS Reagent Grade, d = 1.200) to about 800 mL of DI water in a 1L volumetric flask in a fume hood. Dilute to mark, mix well and prepare monthly.

6.1.3 Stock Ammonium Molybdate Solution – Dissolve 40.0 g ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}$, 4H2O) in about 800 mL DI water in a 1 L volumetric flask. Dilute to the mark and stir until completely dissolved; this may take about 4 hours. Store in plastic and refrigerate. This stock may be used up to two months when kept refrigerated.

6.1.4 Stock Antimony Potassium Tartrate Solution – Dissolve 3.22 g antimony potassium tartrate trihydrate ($K(SbO)C_2H_4O_6.3H_2O$) in about 600 mL DI water in a 1L volumetric flask. Dilute to mark and mix. Store in a dark bottle and refrigerate. This stock may be used up to two months when kept refrigerated.

6.1.5 Molybdate Color Reagent – In a hood, carefully add 70.0 mL concentrated sulfuric acid to about 500 mL water in a 1 L volumetric flask and mix well. Then, add 72.0 mL stock antimony potassium tartrate (6.1.4) and 213 mL stock ammonium molybdate (6.1.3). Dilute to the mark with DI water. Prepare weekly and degas with helium.

6.1.6 Ascorbic Acid Reducing Solution – Dissolve 75.0 g ascorbic acid in about 800 DI water in a 1 L volumetric flask. Mix on a magnetic stirrer and dilute to the mark with DI water. Prepare fresh weekly.

6.1.7 Borate Buffer, 1.0 M, pH 7.5 - Dissolve 61.8 g boric acid and 8.0 g sodium hydroxide in about 800 mL DI water in a 1 L volumetric flask. Mix on a magnetic stirrer until it is completely dissolved. Dilute to the mark with DI water. This stock may be used up to two months

6.1.8 Carrier Solution - Combine 300 mL of oxidizing reagent (6.1.1),

60.0 mL 1 M hydrochloric acid (6.1.2), and 60.0 mL borate buffer (6.1.7) in a 1 L volumetric flask, dilute to volume, and stir well. Degas the solution with helium. It is recommended that the carrier is degassed within 4 hours of use and prepared same day of analysis.

6.1.9 Sodium Hydroxide – EDTA Rinse - In a 1L flask, dissolve 65.0 g sodium hydroxide (NaOH) and 6 g tetrasodium ethylenediamine tetraacetic acid (Na_4EDTA) in about 800 deionized water. Mix on a magnetic stirrer until it is completely dissolved. Dilute to the mark with deionized water. Prepare as needed.

6.2 Standards

6.2.1 Phosphorous standard (1000) mg P/L, purchased from an approved source with expiration date. If this stock is not available prepare 100 ppm P/L as detailed in 6.2.2 below.

6.2.2 Stock Standard Solution (100 mg P/L) - Add 10 mL of Phosphorus 1000 ppm stock standard (6.2.1) to about 60 mL of DI water in a 100 mL volumetric flask, dilute to mark, and mix well. If the 1000 ppm P stock is not available, prepare the 100 ppm stock by dissolving 0.4394 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4) which has been dried for two hours at 110^{o}C in about 800 mL deionized water. Dilute to the mark and invert to mix. Prepare monthly.

6.2.3 Combined Intermediate Standard Solution (1 mg P/L and 10 mg N/L) - Add 10 mL of stock standard (6.2.2) and 10 mL of 1000 mg N/L (stock standard solution for total dissolved nitrogen determination) to about 800 mL DI water in a 1L volumetric flask. Dilute to mark and mix. Prepare weekly.

6.2.4 Spiking Solution – Mix 5 mL of 1000 mg/L N and 5 mL of 100 mg/L P (6.2.2) in a small vial with cap. Mix well and pipette 50 µl of this solution into 10 mL of sample (sample spike) or 10 mL of DI water (blank spike). Prepare monthly.

6.2.5 Combined Working Standard Solutions - Use the following table to prepare standards. Dilute each to100 mL and mix well. DI water is used as the last standard (0.00 ppm). Prepare per run and standards are good for 48 hours.

Concentration mg P/L	Combined Working Standard, mL	Final Volume, mL
0.5	50	100
0.2	20	100
0.1	20	200
0.05	5	100
0.02	2	100
0.01	1	100
0.00	0	100

Note: ***The analyst may save a set of standards from a previous run for troubleshooting purposes only.***

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Samples are filtered in the field, collected in plastic bottles or cubitainers and preserved by cooling to 4^{o} C.

7.2 Samples are analyzed within 48 hrs after collection. If they cannot be analyzed within this time period, they may be frozen at -20^{o} C as soon as possible at the laboratory. The holding time for frozen samples is 28 days.

8.0 QUALITY CONTROL

8.1 An initial Demonstration of Performance (IDP) to establish the instrument's capability is required prior to its use in analyzing client's samples. The linearity of calibration range (LCR) and the ability to quantify correctly Quality Control Samples are used to assess performance.

8.2 A mid-range check standard and a calibration blank are analyzed Immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. The acceptable concentrations for the check standard must be within ± 10% of the true value. If a check standard fails, the samples between the last satisfactory check standard and the unsatisfactory standard are re-analyzed. Blank concentration must be less than the reporting level of 0.01 ppm. Blanks that do not meet this criterion are reanalyzed.

8.3 Every tenth sample is duplicated (analyzed from two different tubes) and spiked. The accepted values for the relative percent difference (RPD) must fall within ± 10 % and for spike recovery between 90 - 110%. If these do not fall within the accepted ranges, the corresponding analyses are repeated.

8.4 A QC sample with a known concentration and a range is analyzed at the beginning and at the end of each run. QC samples that do not fall within the accepted range are repeated.

8.5 Samples with a concentration exceeding the calibrated range are diluted manually and reanalyzed.

8.6 Data acceptance criteria are listed on the data review checklist (Appendix A).

8.7 The laboratory annually participates in USGS, CBL, ERA Water Supply (WS) and Water Pollution (WP) proficiency studies.

8.8 A demonstration of capability study is performed by each analyst performing the test by analyzing four replicates of an external quality control sample during one or two analytical runs.

8.9 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of the 0.01 ppm standard spread over three analytical runs. MDL is calculated as follows:

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made

9.0 PROCEDURE

9.1 Sample preparation

9.1.1 Make a list of samples to be analyzed and pour aliquots of samples into labeled 16 mm x 125 mm test tubes.

9.1.2 Pipette 10 mL of each standard or sample into digestion tubes.

9.1.3 Pipette 10 mL of a mid-range standard (0.3 mg P/L and 3.0 mg N/L), a blank, a blank spike, and an external quality control sample into digestion tubes with each tray of 24 samples. Prepare a duplicate and a spike of every 10^{th} sample.

9.1.4 Pipette 10 mL of the nitrate and nitrite standards for cadmium column check (6.2.5 and 6.2.6) into digestion tubes. DONE FOR TDN ONLY

9.1.5 Add 5 mL of Alkaline Persulfate Oxidizing Reagent (6.1.1) to each tube, screw the caps on tightly and mix each. Digest the standards, samples, and all the quality control samples in the autoclave for 60 minutes after the autoclave reaches the set temperature and pressure of 121 ^{o}C (250 ^{o}F) @ 17 psi. *Please see the manual for Autoclave Operation.*

9.1.6 The autoclave will turn off automatically. Allow the digests to cool for 5 minutes. Remove the tubes from the autoclave and let the digests come to room temperature. Add 1 mL of borate buffer, vortex, and pour into 13 x 100 mm culture tubes analyze them as soon as possible.

9.1.7 If samples cannot be analyzed same day, **do not add the borate buffer**, refrigerate the digests at 4^{o}C. Refrigerated digests will be brought up to room temperature, and then 1 mL borate buffer (6.1.7) is added to each tube and mixed.

9.1.8 Analyze the digests using the procedure described in 9.2.

9.2 Instrument Calibration and Sample Analysis

9.2.8 Set up manifold according to the manifold diagram.

9.2.9 Pump deionized water through all reagent lines and check for leaks and a smooth flow. Switch to reagents and allow the system to stabilize and a stable baseline is achieved.

9.2.10 Enter sample information required by the data system.

9.2.11 Place standards, blanks, samples, quality controls, etc. in the auto sampler according to the run table.

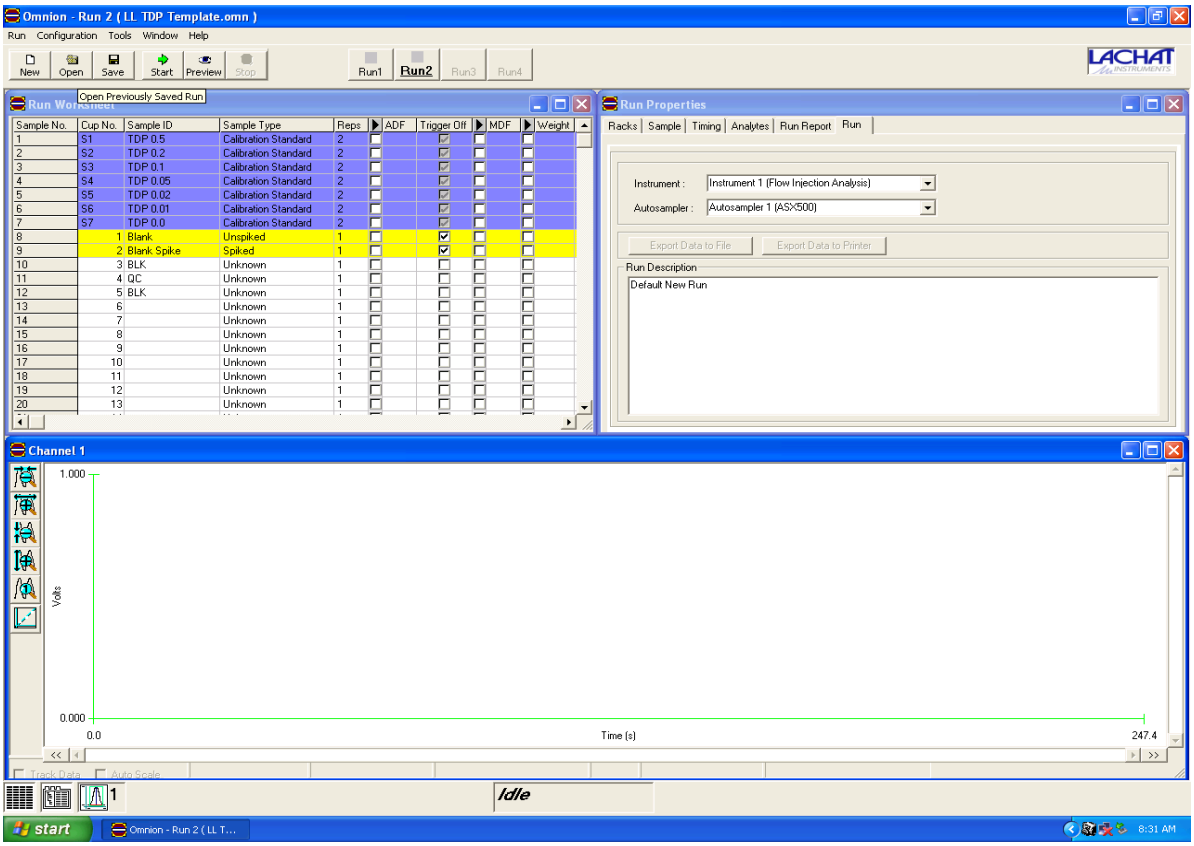
9.2.12 Click on **"Start"** tab to begin the analysis.

9.3 Instrument set-up and sample analysis

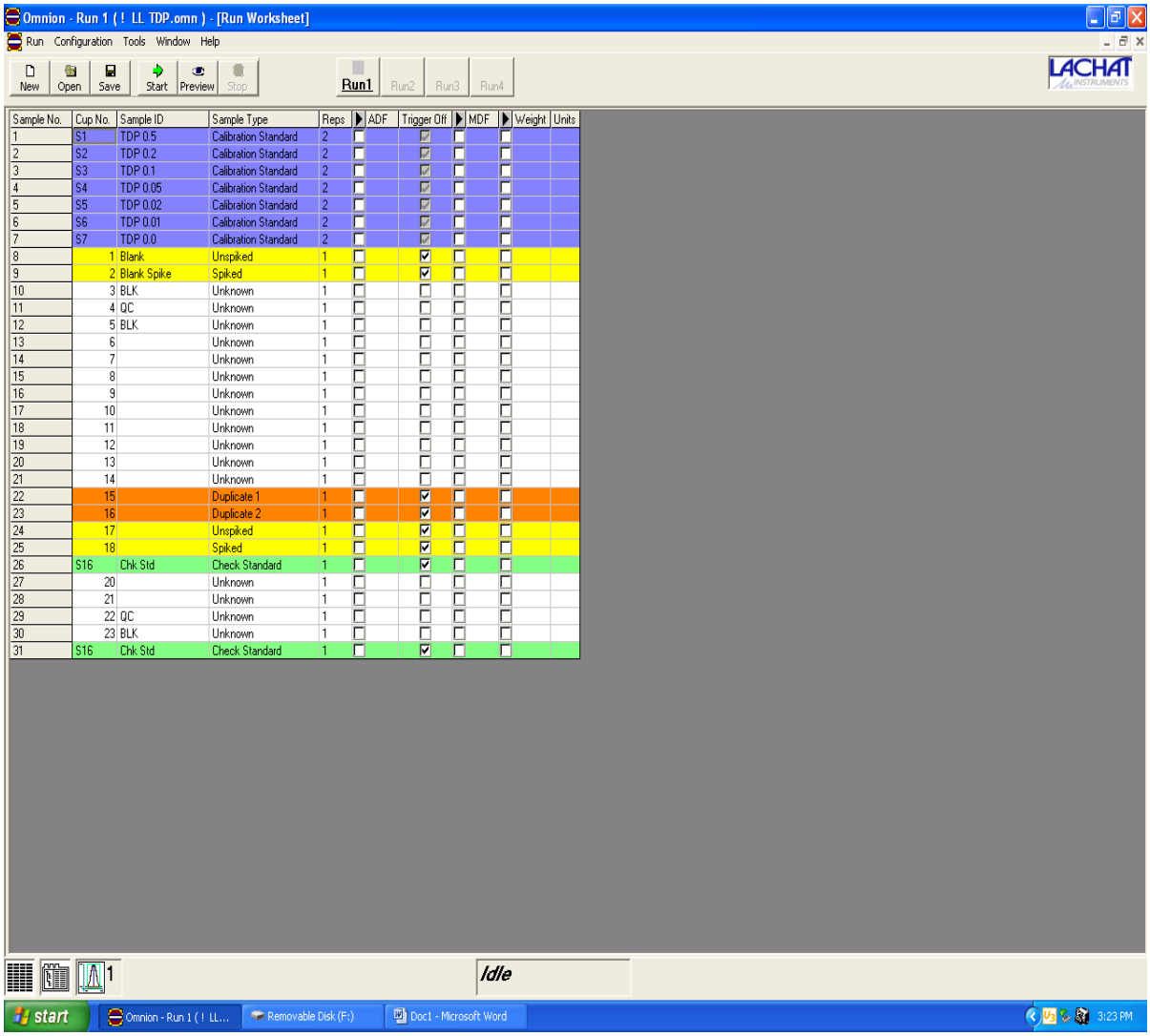
9.3.1 Set up manifold as in the diagram.

9.3.2 Turn on the Lachat instrument, computer, monitor, and printer.

9.3.3 Double click on the short-cut for **"LL TDP"** to open the template, which consists of three windows.



9.3.4 Maximize the **"Run Worksheet"** window at the top left hand corner of the screen by clicking on the middle square on that screen to open the worksheet template. Enter sample identifications in the sample ID column, making sure that all duplicates and spikes are entered in the appropriate locations of the worksheet. Identifications of the quality control samples analyzed at the beginning of the run and the locations of the duplicates and the spiked have been saved on the template. Press **"Enter"** key after each entry in order to save all entries.



Sample No.	Cup No.	Sample ID	Sample Type	Reps	ADF	Trigger Off	MDF	Weight	Units
1	S1	TDP 0.5	Calibration Standard	2					
2	S2	TDP 0.2	Calibration Standard	2					
3	S3	TDP 0.1	Calibration Standard	2					
4	S4	TDP 0.05	Calibration Standard	2					
5	S5	TDP 0.02	Calibration Standard	2					
6	S6	TDP 0.01	Calibration Standard	2					
7	S7	TDP 0.0	Calibration Standard	2					
8	1	Blank	Unspiked	1					
9	2	Blank Spike	Spiked	1					
10	3	BLK	Unknown	1					
11	4	QC	Unknown	1					
12	5	BLK	Unknown	1					
13	6		Unknown	1					
14	7		Unknown	1					
15	8		Unknown	1					
16	9		Unknown	1					
17	10		Unknown	1					
18	11		Unknown	1					
19	12		Unknown	1					
20	13		Unknown	1					
21	14		Unknown	1					
22	15		Duplicate 1	1					
23	16		Duplicate 2	1					
24	17		Unspiked	1					
25	18		Spiked	1					
26	S16	Chk Std	Check Standard	1					
27	20		Unknown	1					
28	21		Unknown	1					
29	22	QC	Unknown	1					
30	23	BLK	Unknown	1					
31	S16	Chk Std	Check Standard	1					

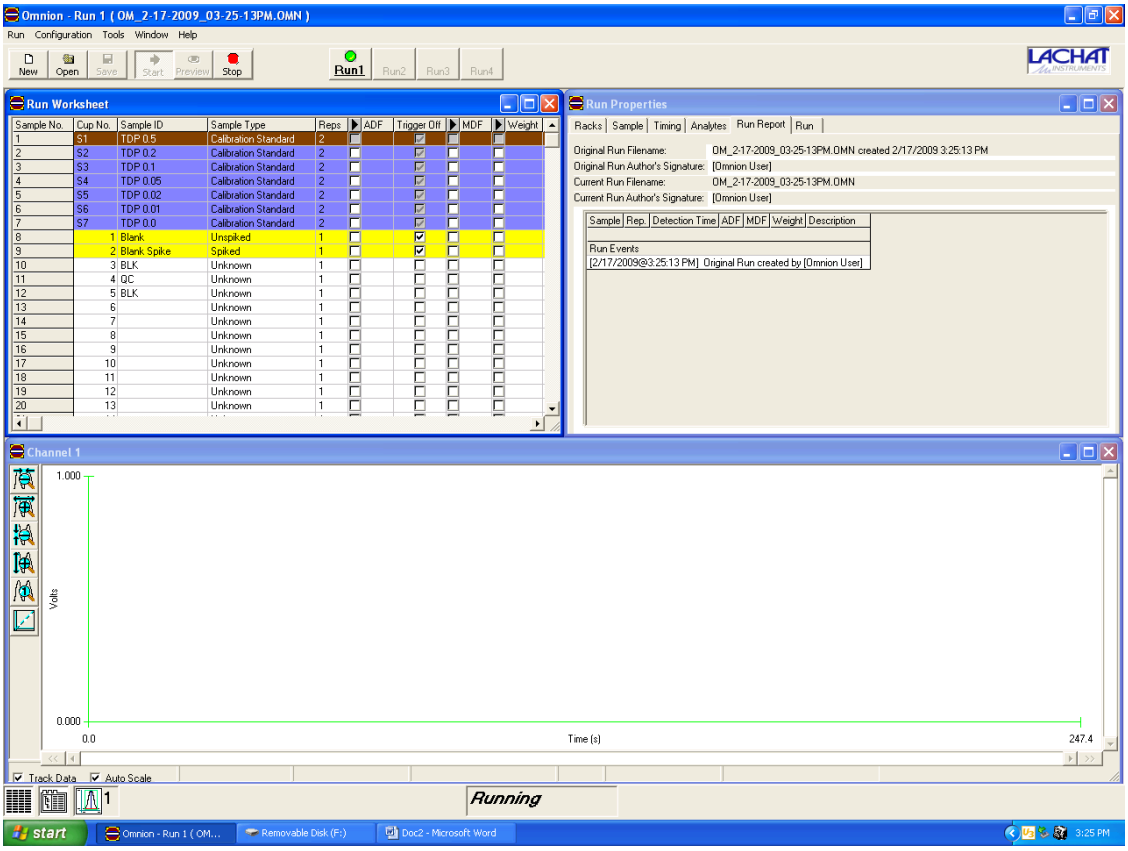
9.3.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

9.3.6 Click on **"Window"** tab and then, click on **"Tile"** to return to the screen with three windows.

9.3.7 Place standards in standard vials in the standard rack in order of decreasing concentration in positions 1 to 7 (std 7 is 0.00 mg/L). Place the check standard vial in position 16 (S16) of the standard rack. Pour samples and quality control samples into 13 x 100 mm test tubes and place them in the sample racks according to the worksheet prepared in 9.2.4.

9.3.8 Pump deionized water through all reagent lines for 15 – 20 minutes and check for leaks and smooth flow. Switch to reagents and continue

pumping for about 10 minutes. Click on **"Preview"** tab to monitor the baseline.



9.3.9 Once a stable baseline is achieved, click on **"Stop"** tab to stop monitoring the baseline. Click on **"Start"** tab to begin the analysis.

9.3.10 If the calibration passes, instrument will continue to analyze the samples. If it fails, take appropriate corrective actions and recalibrate before proceeding to analyze samples.

9.3.11 Manual dilution using the digested blank will be performed to reanalyze samples with concentrations exceeding the calibrated range.

9.2.12 After the run is complete, remove the reagent lines and place them in DI water and rinse for about 15 minutes. If necessary,
rinse the system with the NaOH – EDTA rinse solution (6.1.5) for about 5 minutes followed by DI water for 10 – 15 minutes. Pump the tubes dry, release the pump tubing, and turn off the pump.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 All the calculations are performed by the Omnion 3.0 software system. It uses a calibration graph of peak area versus concentration orthophosphate concentrations in the samples. All standards are analyzed in duplicate and all data points are used for the calibration curve. Samples with orthophosphate concentrations greater than 0.5 ppm are manually diluted and reanalyzed.

10.2 Calculate the percentage spike recovery of the laboratory fortified samples as follows:

$$\%\,SR = \frac{(\text{spiked sample conc.} - \text{sample conc.}),\ ppm}{\text{amount of spike added to sample},\ ppm} \times 100$$

10.3 Calculate the relative percentage difference for the duplicated samples as follows:

$$\%\,RPD = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \times 100$$

10.4 The reporting level for this method is the concentration of the lowest standard, which is 0.01 ppm.

10.5 Report results for all routine samples to three decimal places and for performance evaluation (PE) samples to three significant figures.

11.0 DATA AND RECORDS MANAGEMENT

11.1 The analysis data for the calibration blank, external QC and instrument performance check solution (IPC), i.e. the mid-range check standard, is kept on file with the sample analysis data.

11.2 Normal turnaround time for samples submitted to this lab is 2 to 10 days from the receipt. Results are reported in writing on the sample analysis request form.

11.3 The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 It is laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume and bench operation.

12.2 Samples and standards are poured down the drain and flushed with running water.

13.0 REFERENCES

13.1 EPA Method 365.1, *Methods for the Determination of Inorganic Substances in Environmental Samples*, August 1993.

13.2 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, Method 4500- P E, 2005.

13.3 Lachat Instruments QuickChem Method 30-115-01-4-A, *Determination of Total Phosphate by Flow Injection Analysis.*

13.4 Lachat Instruments, *Operating Manual for the Quikchem Automated Ion Analyzer*.

13.5 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. Revision15.0, August 2016.

13.6 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX A

Division of Environmental Sciences

INORGANICS ANALYTICAL LABORATORY

Data Review Checklist LL Total Dissolved Phosphorus (TDP)/Alkaline Persulfate Digestion

EPA Method 365.1

Lab Numbers: [1] _____ Analyst: _____

_____ Date Digested: _____

Dates Collected: _____ Date Analyzed _____

Procedure	Acceptance Criteria	Status (✓)	Comments
Holding Time	48 hours @ 4ºC 28 days @ –20ºC		
Calibration Curve	Corr. Coeff. $\geq$ 0.9950		
Reagent Blank	< Reporting level (0.010 ppm)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptance range		
Check Standard	After every 10th sample and at the end of the run		
	Recovery = 90–110%		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.010–0.500 ppm)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____

Analyst's Signature & Date Reviewer's Signature & Date

_____

Supervisor's Signature & Date

Reagents ID Reagents ID External QC

Color Reagent _____ Oxidizing Reagent _____ Identification = _____

Ascorbic Acid		Borate Buffer		True Value =	ppm
1M HCl				Range =	ppm

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

Title: Determination of Nitrate/Nitrite and Nitrite (Low Level)
Flow Injection Colorimetric Analysis
(EPA Method 353.2)

SOP No.: CHEM-SOP - EPA METHOD 353.2

Revision: 3.2 **Replaces:** 3.1 **Effective:** 7/1/2017

Laboratory: Inorganics Analytical Laboratory

POC: Rickey Carpenter/Cynthia Stevenson
rickey.carpenter@maryland.gov
Cynthia.stevenson@maryland.gov

Laboratory Supervisor:	Signature	Date
QA Officer:	Signature	Date
Manager:	Signature	Date
Division Chief:	Signature	Date

EPA METHOD 353.2
SOP No.: CHEM-SOP-EPA 353.2

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	Shahla Ameli	6/2/08
1.0	12/09	Screen snapshots added to procedure, new Sample Run Log, tracking IDs for standards and reagents	Shahla Ameli	1/10
2.0	8/11	New SOP tracking number, editorial and technical changes	Shahla Ameli	8/18/2011
2.1	12/10/12	Check list correction, addition to section 9.0	Shahla Ameli	12/10/2012
2.2	7/13	Reviewed SOP	C. Stevenson Shahla Ameli	12/10/2013
3.0	10/14	New SOP tracking number, editorial changes	C. Stevenson R. Carpenter Shahla Ameli	12/10/14
3.0	6/1/15	Reviewed SOP	C. Stevenson R. Carpenter Shahla Ameli	7/1/2015
3.1	5/5//16	Reviewed SOP, formatting changes	C. Stevenson R. Carpenter Shahla Ameli	7/1/2016
3.2	6/1/2017	Reviewed and made organizational name changes	C. Stevenson S. Ameli R. Carpenter	7/1/2017

TABLE OF CONTENTS

STANDARD OPERATING PROCEDURES

Nitrate/Nitrite and Nitrite (Low Level) Flow Injection Colorimetric Analysis

EPA Method 353.2

29.0 SCOPE AND APPLICATION

29.1 This method determines nitrite, or nitrate/nitrate in drinking, ground, surface, domestic waters and industrial waste.

29.2 The range of this method is from 0.020 mg/L to 4.00 mg/L for nitrate–nitrite and 0.002 mg/L to 0.400 mg/L for nitrite.

29.3 Nitrate is found only in small amounts in domestic wastewater, but in the effluent of nitrifying biological treatments nitrate may be found in concentrations up to 30 mg/L.

30.0 SUMMARY OF METHOD

Nitrate is reduced quantitatively to nitrite by passage through a copperized cadmium column. The reduced nitrate plus original nitrite is further determined by coupling with N-(naphthyl) ethylenediamine dihydrochloride. The dye produced has magenta color and is read at 520nm, and is directly proportional to the concentration of analyte. Determination of nitrite takes place through the same procedure as Nitrate/Nitrite without a cadmium column.

31.0 INTERFERENCES

31.1 Interference from iron, copper or other metals is eliminated by addition of EDTA to the ammonium chloride buffer.

31.2 Suspended matter in the column will restrict sample flow. Remove suspended solids by filtration.

31.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that can bias analyte response.

32.0 HEALTH AND SAFETY

32.1 Accepted laboratory safety practices should be followed during reagent preparation and instrument operation. Use of lab coats, fume hoods, gloves and eye protection are required.

32.2 The following chemicals have the potential to be highly toxic or hazardous.

32.2.1 Cadmium

32.2.2 Phosphoric acid

32.2.3 Hydrochloric acid

32.2.4 Sodium Hydroxide

32.3 A reference file of Material Safety Data Sheet (MSDS) is available to all personnel involved in the chemical analysis.

5.0 EQUIPMENT AND SUPPLIES

5.1 Equipment

5.1.1 Lachat QuickChem 8500 Automated Ion Analyzer, which includes the following:

5.1.1.1 Automatic sampler

5.1.1.2 Multi-channel proportioning pump

5.1.1.3 Injection module with a 12.5 cm x 0.5 mm ID sample loop

5.1.1.4 Manifold

5.1.1.5 Colorimetric detector

5.1.1.5.1 Flow cell, 10 mm path length

5.1.1.5.2 Interference filter, 520 nm

5.1.1.6 Computer with Omnion 3.0 Data system and printer

5.1.2 Analytical balance capable of weighing to the nearest 0.0001 g

5.1.3 Top loading balance for weighing chemicals for reagents

5.2 Supplies

5.2.1 Class A volumetric flasks, 50 – 1,000 mL

5.2.2 Class A volumetric pipettes, 1– 10 mL

5.2.3 Automatic pipetters, 100 µL- 10 mL

5.2.4 Beakers, disposable, polypropylene, 50 mL(Fisher 01-291-10)

5.2.5 Test tubes, glass, 13 x 100 mm and 16 X 125 mm

5.2.6 Reagent storage bottles, plastic or glass

5.2.7 Cadmium Reduction Column (Lachat Part No. 50237A)

5.2.8 Ultra High Purity Helium gas for degassing

6.0 REAGENTS AND STANDARDS

6.1 Reagents

6.1.1 Ammonium Chloride buffer, pH 8.5, 2 L - Dissolve 170 g of NH_4Cl and 2.0 g of disodium ETDA ($Na_2EDTA'2H_2O$) in about 1600 mL DI water in a 2 L beaker. Mix using a stir bar. Adjust the pH to 8.5 with 15 N sodium hydroxide solution and bring up to volume. Use filter paper to remove all the small particles from the reagent and refrigerate. Prepare monthly.

6.2.1 Sulfanilamide color Reagent, 1 L - Add carefully, while mixing, 100 mL 85% phosphoric acid (H_3PO_4) to 700 mL DI water in a 1 L volumetric flask. Add 40 mg sulfanilamide and 1g N-1-naphthyl ethylenediamine dihydrochloride (NED) and stir to dissolve. Bring up to 1 L with DI water. Filter, store in amber bottle and refrigerate. This solution is stable for one month.

6.2.1 15 N Sodium Hydroxide – Add 150 g NaOH very slowly to 180 mL DI water in a 250 mL volumetric flask. CAUTION: The solution will get very hot! Mix until dissolved. Cool and store in a *plastic* bottle.

6.2 Standards

6.2.1 Nitrate Stock Standard (1000 mg /L of nitrate nitrogen) - Purchased from approved commercial supplier with expiration date. If this standard is not available, then weigh 0.7218g of dried potassium nitrate KNO_3 (1000 mg/ L of nitrate nitrogen) in 100 mL volumetric flask. Prepare monthly.

6.2.1 Nitrite Stock Standard (1000 mg/L of nitrite nitrogen) - Purchased from approved commercial supplier with expiration date. If this standard is not available, then weigh 0.6072g of dried potassium nitrite KNO_2 (1000 mg/L of nitrite nitrogen) in 100 mL volumetric flask. Prepare weekly.

6.2.1 Combined Intermediate Standard, 90 mg/L nitrate nitrogen and 10 mg/L nitrite nitrogen - Pipete 9 mL of 6.2.1 and 1 mL of 6.2.2 into about 70 mL DI water in a 100 mL volumetric flask. Bring up to volume with DI water, mix, and store at 4°C. Use this standard as spiking solution.

6.2.1 Nitrate Cadmium check, 0.5 ppm – Dilute 100 µL of reagent 6.2.1 to 200 mL with DI water in a 200 mL volumetric flask. Prepare weekly.

6.2.1 Nitrite Cadmium check, 0.5 ppm – Dilute 100 µL of reagent 6.2.2 to 200 mL with DI water in a 200 mL volumetric flask. Prepare weekly.

6.2.1 Working Standards- The working standards are prepared by diluting the combined intermediate standard (6.2.3) in 100 mL volumetric flasks using the following table. Working standards are good for 48 hours.

Std Concentration NO3+NO2 ppm	Std Concentration NO2 ppm	Combined Intermediate Std
4.000	0.400	4 mL
2.000	0.200	2 mL
1.000	0.100	1 mL
0.500	0.050	500 µL
0.200	0.020	200 µL
0.080	0.008	80 uL
0.020	0.002	10 mL of 0.200/0.020 ppm
0.000	0.000	DI H_2O

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Samples are collected in plastic bottles or cubitainers, and are preserved by cooling to 4^{o} C. These samples are never acidified and their pH is usually between 5 to 9.

7.2 Samples are analyzed within 48 hours after collection. If they cannot be analyzed within this time period, they should be frozen at –20^{o}C on the first day they arrive to lab. The holding time for frozen samples is 28 days.

8.0 QUALITY CONTROL

8.1 An initial Demonstration of Performance (IDP) to establish the instrument's capability is required prior to its use in analyzing client's samples. The linearity of calibration range (LCR) and the ability to quantify correctly Quality Control Samples are used to assess performance.

8.2 All the standards are run in duplicates: that is, two analyses from each tube. A linear calibration is performed daily before the sample run.

8.3 A mid-range check standard and a calibration blank is analyzed following

daily calibration, after every ten samples (or more frequently, if required) and at the end of the sample run. If a check standard fails, the samples between the last satisfactory check standard and the unsatisfactory standard are re-analyzed.

8.4 Every tenth sample is duplicated (analyzed from two different tubes) and spiked. The accepted value for the relative percentage relative difference (RPD) and spike recovery is ± 10 %. Prepare sample spikes by adding 50 µL of 6.2.3 to 10 mL of samples. If these do not fall within the accepted ranges, the corresponding analyses are repeated.

8.5 An external quality control is analyzed at the beginning and at the end of each analytical run.

8.6 A deionized water blank is run in the beginning and after every tenth sample. Results for blanks should be <0.002 for NO2 and <0.02 for NO3+NO2 mg N/L.

8.7 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of the 0.1 ppm standard spread over three analytical runs. MDL is calculated as follows:

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made.

8.8 A demonstration of capability study is performed by each analyst performing the test by analyzing four replicates of an external quality control sample during one or two analytical runs.

9.0 PROCEDURE

9.1 Sample preparation

9.1.1 Prepare a list of samples to be analyzed and pour samples into labeled tubes (16 mm x 125 mm test tubes).

9.1.2 Spike the blank and every tenth sample by adding 50 µL of combined standard (6.2.3) to 10 mL of sample or DI water.

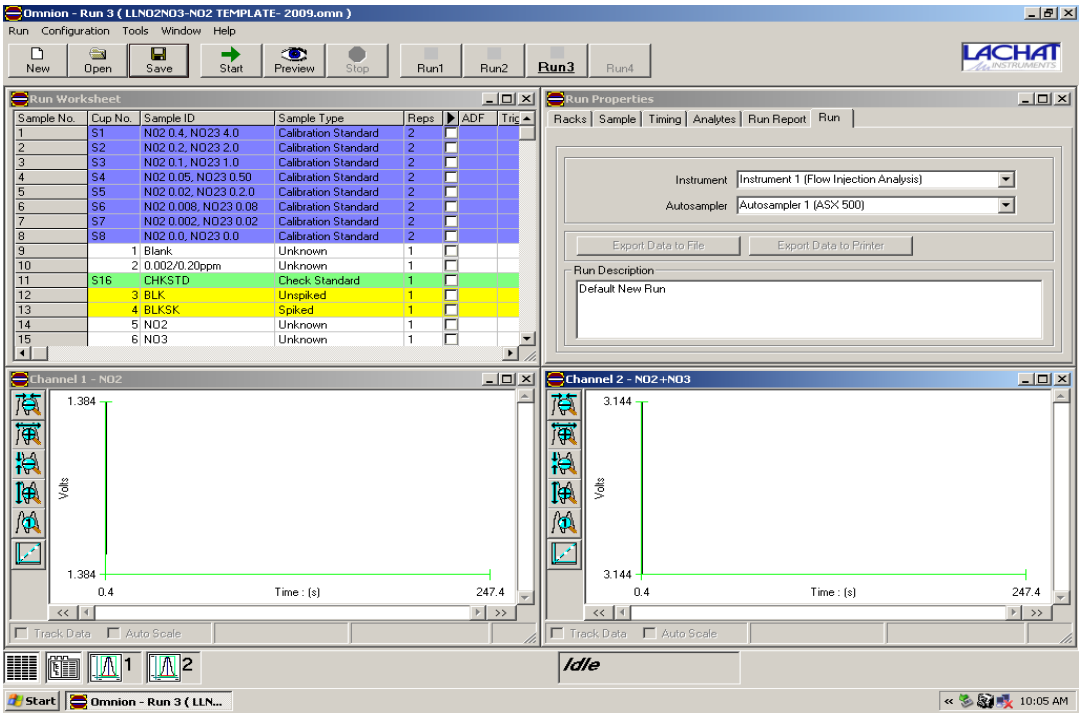
9.1.3 To prevent bubble formation, degas all reagents with helium, except those specified not to. Use helium at 140 Pa (20lb/in2)

9.2 Instrument calibration and sample analysis

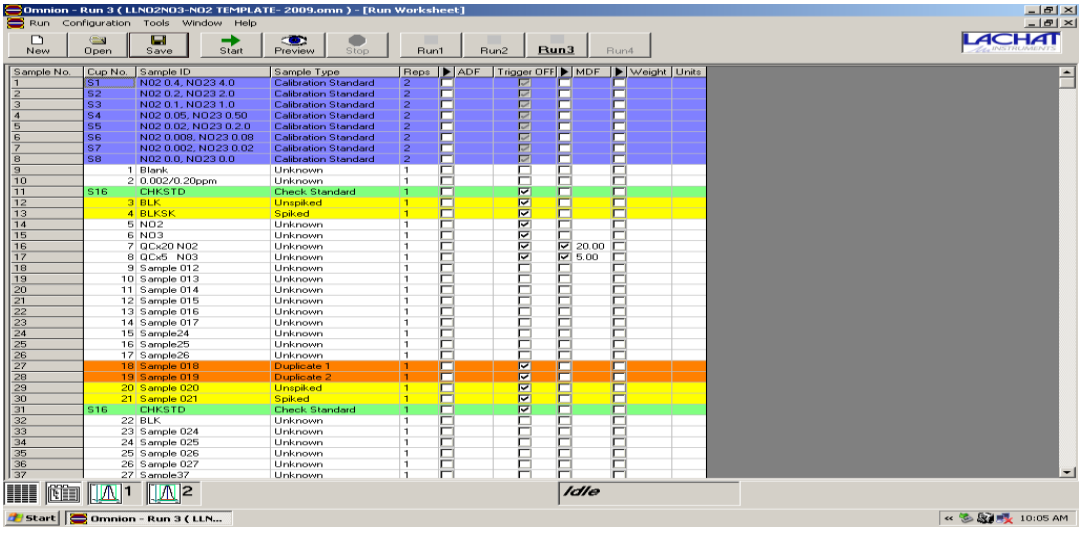
9.2.1 Set up manifold as in the method's manifold diagram.

9.2.2 Turn on the Lachat instrument, computer, monitor, and the printer.

9.2.3 Double click on Omnion and open the **"LL NO_3+NO_2/ NO_2"** folder to find the template, which consists of four windows.



9.2.4 Maximize the **"Run Worksheet"** window at the top left hand corner of the screen by clicking on the middle square on that screen.



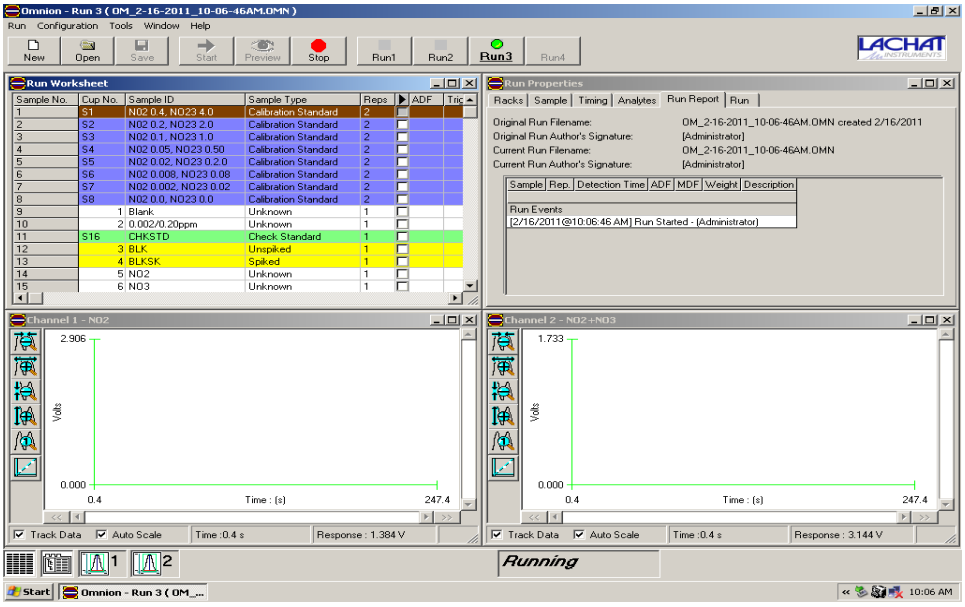
9.2.5 Enter all the sample, sample spike, QC and standard information in the run worksheet and print out the list.

9.2.6 Click on **"Window"** tab and then, click on **"Tile"** to return to the screen with three windows (9.2.3).

9.2.7 Place standards in standard vials in the standard rack in order of decreasing concentration in positions 1 to 8. Place the check standard vial in position 16 (S16) of the standard rack. Pour samples and quality control samples into 13 x 100 mm test tubes and place them in the sample racks according to **"Sample Run Log**".

9.2.8 Pump deionized water through all reagent lines for 15 – 20 minutes and check for leaks and smooth flow. Switch to reagents, turn on the Cadmium switching valve on and allow the buffer to rinse it for 5-10 minutes (Note: Lachat columns come as ready to use and need to be flushed for about 10 minutes with buffer after installing on the system.) Click on **"Preview"** tab to monitor the baseline.

9.2.9 Once a stable baseline is achieved, click on **"Stop"** tab to stop monitoring the baseline. Click on **"Start"** tab to begin the analysis.



9.2.10 If the calibration passes, instrument will continue to analyze the samples. If it fails, take appropriate corrective actions and recalibrate before proceeding to analyze samples.

9.2.11 Auto dilution will trigger on to reanalyzed samples with concentration exceeding the calibrated range.

9.2.12 When the run is complete, remove the reagent lines and place them in DI water and rinse for about 15 minutes. For extra rinse a reagent of Disodium EDTA can be used followed by DI rinse. Then all the reagent lines should be air dried and released from the pump.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 All the calculations are performed by the Omnion 3.0 software system. It uses a calibration graph of peak area versus concentration to calculate the "nitrate + nitrite nitrogen" concentrations in the samples. All standards are analyzed in duplicate and all data points are used for the calibration curve. Samples with "nitrate + nitrite nitrogen" concentrations greater than 4 ppm are automatically diluted and reanalyzed.

10.2 The reduction efficiency of the cadmium column is calculated as following:

$$\% \text{ Recovery} = NO3/\ NO2\ X100$$

10.3 Calculate % of spike recovery of the laboratory fortified samples as follows:

$$\%\,SR = \frac{\text{spiked sample conc.} - \text{sample conc., ppm}}{\text{amount of spike added to sample, ppm}} \text{x}\,100$$

10.4 Calculate the relative percent difference for the duplicated samples as follows:

$$RPD = \frac{\text{difference between the two duplicates}}{\text{average of the duplicates}}\ \text{x}\,100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 All the results are reported on the sample request forms to three decimal places and for performance evaluation (PE) samples to three significant figures. Normal turnaround time for samples submitted to this lab is 2 to 10 days from receipt.

11.2 Completed data packages are scanned and stored electronically before being placed in the appropriate binders in the lab.

11.3 Results are reported in writing on a sample analysis request form. The Division shall retain all pertinent laboratory records for each sample for a period of five years. At the conclusion of this period, clients shall be given the option to take custody of their sample records. The Division shall not be responsible for retrieving, copying, or transferring laboratory records exceeding the five years custody period.

12.0 WASTE MANAGEMENT

12.1 It is the laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume and bench operation.

12.2 Samples and standards are poured down the drain while large amount of water is running.

12.3 Compliance with state's sewage discharge permits and regulations is required. For more information consult the "Waste Management manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4600.

13.0 REFERENCES

13.1 EPA Method 353.2, *Methods for the Determination of Inorganic Substances in Environmental Samples*, Revision 2.0, August 1993.

13.2 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater, 21st Edition, p. 4-125, Method 4500- NO_3^-, 2005*

13.3 Lachat Instruments, Methods Manual for the *Quikchem Automated Ion Analyzer*, Method 10-107-04-1-A

13.4 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision 15.0, August 2016

13.5 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015

APPENDIX **A**

Division of Environmental Sciences

INORGANICS ANALYTICAL LABORATORY

Data Review Checklist – Nitrite, Nitrate + Nitrite Nitrogen (Low Level)

EPA Method 353.2; Revision 2.0

Lab Numbers: [1] _____ Analyst: _____

Dates Collected: _____ Date Analyzed: _____

Procedure	Acceptance Criteria	Status (√)	Comments
Holding Time	48 hours @ 4°C 28 days @ –20°C		
Calibration Curve	Corr. Coefficient. ≥ 0.9950		
Reagent Blank	< 0.02 ppm for NO23 and < 0.002 ppm for NO2		
Blank Spike	1 per batch		
	Recovery = 90 – 110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10 samples		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptable range		
Check Standard	After every 10th sample and at the end of the run		
	Concentrations = 90–110% of the true value		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
NO_3/NO_2 Cadmium Column Check	90–110%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.020–4.000 ppm for NO_2+NO_3; 0.002–0.400 ppm for NO_2)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

***** Check (√) if criteria are met. **[1]**Include beginning and ending numbers, account for gaps by bracketing

_____ _____

Analyst's Signature & Date Reviewer's Signature & Date

_____

Supervisor's Signature & Date

Reagents ID External QC

Ammonia Buffer _____ Identification = _____

Color Reagent		True Value =	NO2	NO2+3	ppm
		Range =	NO2	NO2+3	ppm

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

Title: Determination of Ammonia – Low Level
Flow Injection Colorimetric Analysis
EPA Method 350.1

? No.: CHEM-SOP-EPA 350.1

Revision: 4.0 Replaces: 3.2 Effective: 3/26/2018

Laboratory: Inorganics Analytical Laboratory

POC: Clair Vares
clair.vares@maryland.gov

Laboratory Supervisor: _____ Signature _____ Date

QA Officer: _____ Signature _____ Date

Manager: _____ Signature _____ Date

Division Chief: _____ Signature _____ Date

EPA METHOD 350.1
SOP No.: CHEM-SOP-EPA 350.1

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	Shahla Ameli	6/2/08
1.0	12/09	Screen snapshots added to procedure, new Sample Run Log, tracking IDs for standards and reagents	Shahla Ameli	1/10
2.0	8/11	New SOP tracking number and technical and editorial changes	Clair Vares Shahla Ameli	8/18/11
2.1	12/12/12	Section 6.0 and technical and editorial changes	Clair Vares Shahla Ameli	12/12/12
2.1	8/2013	Reviewed SOP	C. Stevenson Shahla Ameli	12/12/12
3.0	10/09/14	New SOP tracking number and technical and editorial changes	C. Stevenson C. Vares Shahla Ameli	12/01/114
3.0	6/1/15	Reviewed SOP	C. Stevenson	7/1/2015
3.1	5/5/16	Reviewed and formatted SOP	C. Stevenson	7/1/2016
3.2	6/1/17	Reviewed and organizational name changes	S. Ameli/C. Vares/ C. Stevenson	7/1/2017
4.0	3/15/18	Adopted Salycilate Method	S. Ameli / C. Vares/ C. Stevenson	3/26/2018

TABLE OF CONTENTS

STANDARD OPERATING PROCEDURE

DETERMINATION OF AMMONIA (LOW LEVEL) FLOW INJECTION COLORIMETRIC ANALYSIS

EPA Method 350.1

33.0 SCOPE AND APPLICATION

33.1 This method determines Ammonia in industrial samples, drinking, ground and surface waters.

33.2 The applicable range of this method is 0.008 to 0.500 mg N/L.

34.0 SUMMARY OF METHOD

2.1 The salicylate method is a variation of the Berthelot-Phenate method but does not require the use and disposal of toxic phenol. When ammonia is heated with salicylate and hypochlorite in an alkaline phosphate buffer an emerald green color is produced which is proportional to the ammonia concentration. The color is intensified by the addition of sodium nitroprusside.

The salicylate method involves a three-step reaction sequence. The first reaction step involves the conversion of ammonia to monochloroamine by the addition of chlorine. The monochloroamine then reacts with salicylate to form 5-aminosalicylate. Finally, the 5-aminosalicylate is oxidized in the presence of sodium nitroferricyanide (a catalyst) to form a blue-green colored dye that absorbs light at 650nm. In the assay described below, the colorimeter uses an ammonia standard curve to determine the amount of ammonia in samples.

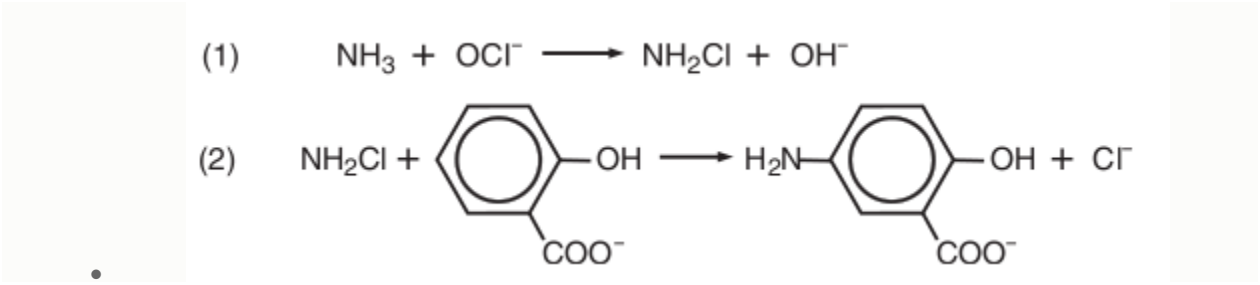


Fig 1: Ammonia compounds are initially combined with hypochlorite to form monochloramine (1),which then reacts with salicylate to form 5-aminosalicylate (2).

35.0 INTERFERENCES

3.1 In alkaline solution, calcium and magnesium will interfere by forming a precipitate, which scatters light. EDTA is added to the buffer to prevent this interference.

3.2 Non-volatile amines such as cysteine, ethanolamine and ethylenediamine

cause a decrease in ammonia sensitivity.

3.3 Lauryl sulfate and some detergents can cause low ammonia recoveries.

3.4 Color, turbidity and certain organic species may interfere. Turbidity is removed by filtration and sample color can be corrected for by running the samples through the manifold without color formation.

3.5 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that can bias analyte response, especially in low level detection of Ammonia. To eliminate this problem, wash glassware with 1:1 HCl and rinse with DI water.

36.0 HEALTH AND SAFETY

4.1 Accepted laboratory safety practices should be followed during reagent preparation and instrument operation. Use of gloves, eye protection, and lab coat are required when preparing reagents.

4.2 The following chemicals have the potential to be highly toxic or hazardous.

3.3.1. Sodium Hydroxide

3.3.2. Sodium Nitroprusside

4.3 A reference file of Material Safety Data Sheet (MSDS) is available to all personnel involved in the chemical analysis.

37.0 EQUIPMENT AND SUPPLIES

37.1 Equipment

37.1.1 Lachat Quick Chem FIA 8500 series.

5.1.1.2 XYZ Auto sampler ASX-520 series with sample, standard and dilution racks

5.1.1.3 Manifold or reaction unit

5.1.1.4 Multichannel Reagent Pump RP-100 series

5.1.1.5 Colorimetric Detector

5.1.1.5.1 Flowcell, 10 mm, 80uL, glass flow cell

5.1.1.5.2 660 nm interference filter

5.1.1.6 Computer, monitor, printer and The Flow Solution software.

5.2 Supplies

5.2.1 13x100 mm test tubes, Fisher # 14-961-27

5.2.2 16x125 mm test tubes, Fisher # 14-961-30

38.0 REAGENTS AND STANDARDS

6.1 Reagents

6.1.1 Buffer - In a 1 L volumetric flask dissolve 30.0 g sodium hydroxide (NaOH), 25.0 g ethlylenediaminetetraacetic acid, disodium salt dihydrate, and 67 g sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) in about 900 mL DI water. Dilute to the mark with DI water and invert three times. Prepare fresh bi-weekly.

6.1.2 Salicylate Nitroprusside Color Reagent - In a 1 L volumetric flask, dissolve 144 g sodium salicylate [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$] and 3.5 g sodium nitroprusside [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about 800 mL DI water. Dilute to the mark with DI water and invert to mix. Store in a light proof bottle. Prepare monthly.

6.1.3 Hypochlorite Reagent - In a 1 L volumetric flask, dilute 60 mL 5.25% sodium hypochlorite (NaOCl), to the mark with DI water. Invert to mix. Prepare weekly.

6.1.4 Sodium Hydroxide – EDTA Rinse - In a 1 L volumetric flask, dissolve 65 g sodium hydroxide (NaOH) and 6 g tetrasodium ethylenediamine tetraacetic acid (Na4EDTA) in 800 mL of water. Dilute to the mark after all is dissolved. This is used for cleaning both OP and NH3 manifold.

6.1.5 Diluent/ Carrier for non Preserved Samples - Use Millipore ultra pure water as carrier. Degas for one minute.

6.1.6 Diluent/ Carrier for Preserved Samples - In a **1 L** volumetric flask, dilute **2 mL Concentrated sulfuric Acid (H_2SO_4).** Dilute to the mark with **DI water**. Invert to mix. Scale up according to need.

6.2 Standards

6.2.1 Ammonia Stock Standard (1000) mg N/L - This standard is pre-made and purchased from an approved commercial supplier with expiration date. If this stock standard is not available, prepare it by dissolving 0.3819 g ammonium chloride (NH_4Cl) that has been dried in the oven for two hours at 105 ^{o} C, in about 80 ml of DI water. Bring up to the 100 mL mark with DI water and store at 4^{o} C. Prepare this reagent monthly.

6.2.3 Intermediate Standard (100 mg N/L) - Pipette 10 ml of standard 6.2.1 into a 100 ml volumetric flask. Bring up to mark with DI water. Store at 4^{o} C. Make weekly.

6.2.4 Spiking Solution (100 mg N/L) - This is the same as the intermediate standard, which is used to spike the samples. Pipette 30 uL of the spiking solution (standard 6.2.2) into 10 mL of DI water or 10 mL of sample, in order to make the blank spike and sample spike. The concentration of spiking solution is 0.30 mg/L

6.2.5 Working Standards - The working standards are prepared according to the following table and they are good for 48 hours:

Ammonia ppm	Combined Intermediate Std	Final Volume
0.000	DI water	100 ml
0.008	2.67 ml of Std 0.30ppm	100 ml
0.020	20 ml std 0.100ppm	100 ml
0.100	100 µl	100 ml
0.200	200 µl	100 ml
0.300	600 µl	200 ml
0.400	400 µl	100 ml
0.500	500 µl	100 ml

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.3 Samples are collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water.

7.4 Never use acid preservation for samples to be analyzed for Low Level Ammonia.

7.5 Samples to be analyzed for ammonia only are cooled to 4^{o} C and analyzed within 48 hours. For short-term preservation, freeze at –20^{o} C for no more than 28 days.

8.0 QUALITY CONTROL

8.1 An Initial Demonstration of Performance (IDP) to establish the instrument's capability is required prior to its use in analyzing client's samples. The Linearity of Calibration Range (LCR) and the ability to quantify the Quality Control Samples correctly are used to assess performance.

8.2 A demonstration of capability study is performed by each analyst performing the test by analyzing four replicates of an external quality control sample during one or two analytical runs.

8.3 All the standards are run in duplicates: that is, two analyses from each tube. A linear calibration is done daily before the sample run. See the attached checklist for the acceptance criteria.

8.4 A mid-range check standard and a calibration blank is analyzed Immediately following daily calibration, after every ten samples (or more frequently, if required) and at the end of run. If a check standard fails, the samples between the last satisfactory check standard and the unsatisfactory standard are re-analyzed.

8.5 Every tenth sample is duplicated (analyzed from two different tubes) and spiked. The accepted value for the relative percent difference (RPD) or spike recovery is ± 10 %. If these do not fall within the accepted ranges, the corresponding analyses are repeated.

8.6 A known QC is analyzed for ammonia in the beginning and at the end of each run.

8.7 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of the 0.008 ppm standard spread over three analytical runs. MDL is calculated as follows:

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made.

9.0 PROCEDURE

9.1 Sample preparation

9.1.1 Prepare a list of samples to be analyzed and pour samples into labeled tubes (16mm x 125 mm test tubes).

9.1.2 Spike every tenth sample by adding 30 uL of 100 ppm N/L (Intermediate standard) into 10 mL DI water (blank spike) or 10 mL of sample (sample spike).

9.1.3 Filter the turbid samples by inserting the Sera Filter inside the 16 X 125 mm test tubes containing the sample. Press the filter down and pour the filtered sample collected on the top inside a 13 x 100 mm test tube for analysis.

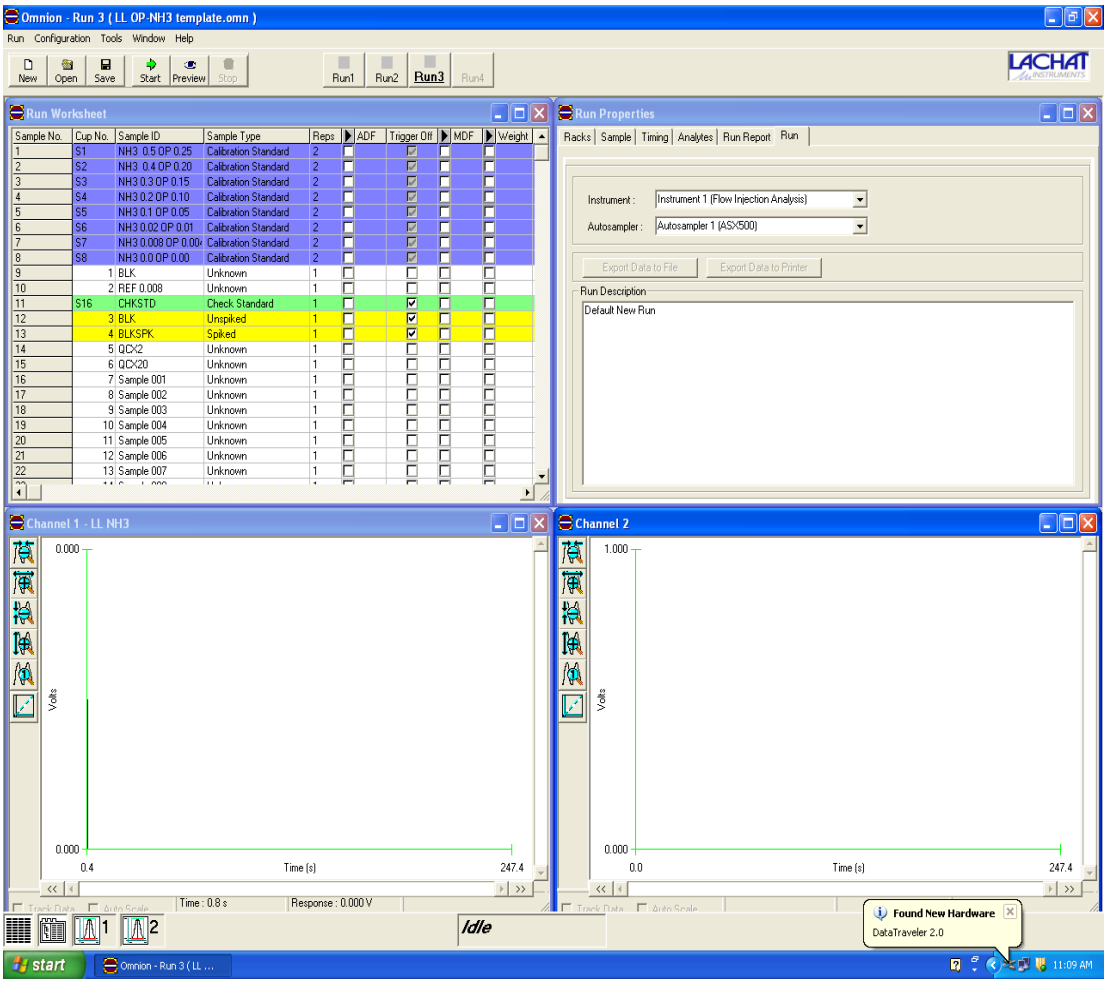
9.1.4 To prevent bubble formation, degas all reagents with helium for one minute. Use Helium at 140 kPa (20lb/in2) through a helium degassing tube or a pipette.

9.2 Instrument set-up and sample analysis

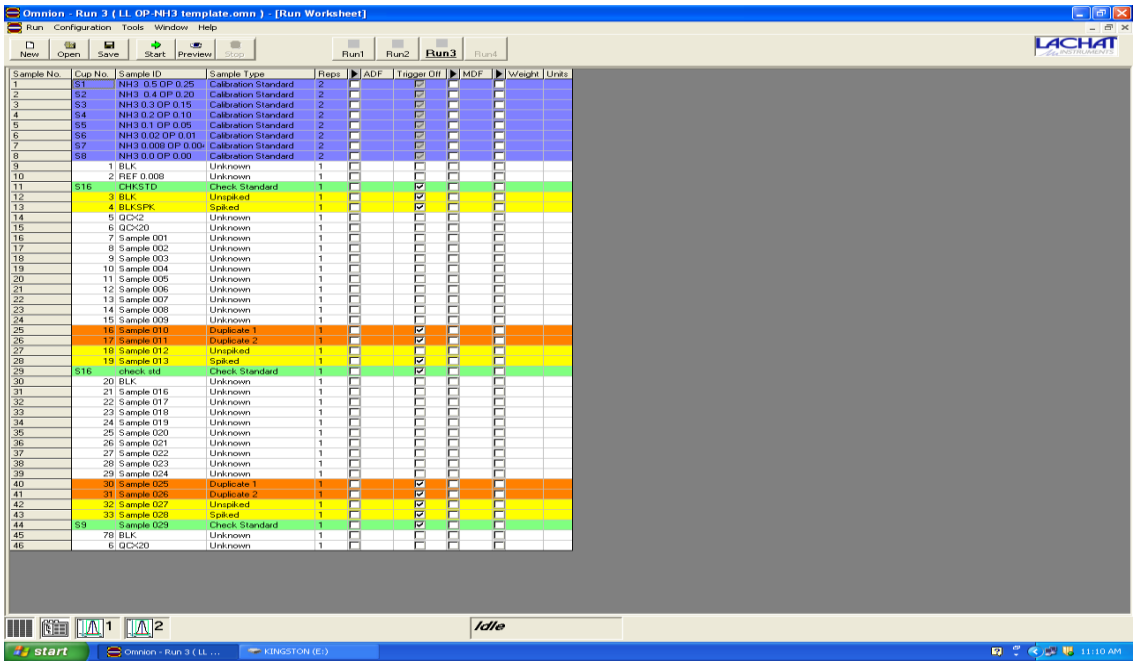
9.2.1 Set up manifold as in the diagram.

9.2.2 Turn on the Lachat instrument, computer, monitor, and printer.

9.2.3 Double click on **"LL OP/NH3"** to open the template, which consists of four windows. Samples are analyzed consecutively for orthophosphate and ammonia on the same system.



9.2.4 Maximize the **"Run Worksheet"** window at the top left hand corner of the screen, by clicking on the middle square on that screen to open the worksheet template. Enter sample identifications in the sample ID column, making sure that all duplicates and spikes are entered in the appropriate locations of the worksheet. Identifications of the quality control samples analyzed at the beginning of the run and the locations of the duplicates and the spiked have been saved on the template. Press **"Enter"** key after each entry in order to save all entries.

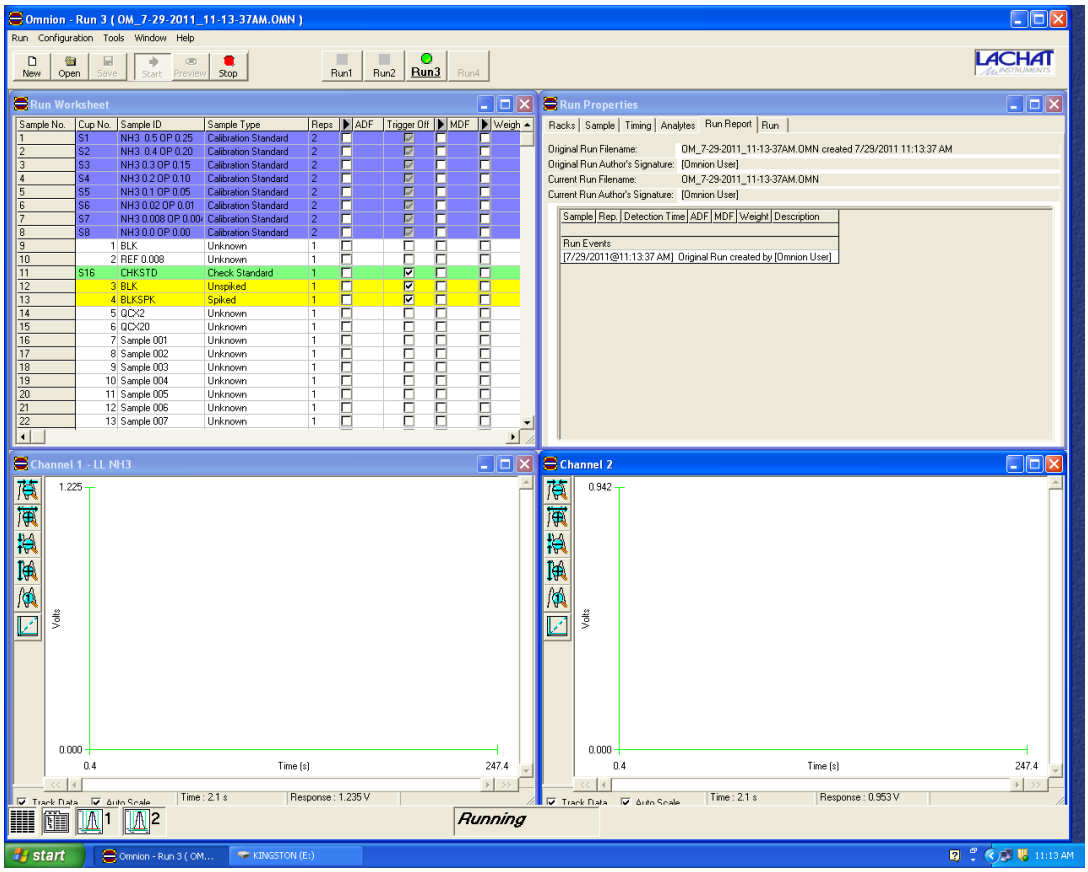


9.3.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

9.3.6 Click on **"Window"** tab and then, click on **"Tile"** to return to the screen with four windows.

9.3.7 Place standards in standard vials in the standard rack in order of decreasing concentration in positions 1 to 8 (STD 8 is DI water-0.00 mg/L). Place the check standard vial in position 16 (S16) of the standard rack. Pour samples and quality control samples into 13 x 100 mm test tubes and place them in the sample racks according to the worksheet prepared in 9.2.4.

9.3.9 Pump deionized water through all reagent lines for 15 – 2 minutes and check for leaks and smooth flow. Switch to reagents in the order of 1. Buffer, 2. Phenol, 3. Bleach, 4. Nitroprusside. If analyzing for orthophosphate, then include; 5. Ascorbic Acid and 6. Color Reagent and continue pumping for about 10 minutes. Click on **"Preview"** tab to monitor the baseline.



9.3.9 Once a stable baseline is achieved, click on **"Stop"** tab to stop monitoring the baseline. Click on **"Start"** tab to begin the analysis.

9.3.10 If the calibration passes, the instrument will continue to analyze the samples. If it fails, take appropriate corrective actions and recalibrate before proceeding to analyze samples.

9.3.11 Manual dilution will be performed to reanalyze samples with concentrations exceeding the calibrated range.

9.2.12 After the run is complete, remove the reagent lines in the reverse order that they were inserted and place them in DI water and rinse for about 15 minutes. For extra rinse of NH3 channel, a reagent of 1M HCl can be used for 5 minutes followed by DI rinse for 10 – 15 minutes. Pump the tubes dry, release the pump tubing, and turn off the pump.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 All the calculations are performed by the Omnion 3.0 software system. It uses a calibration graph of peak area versus concentration to calculate the ammonia nitrogen concentrations in the samples. All standards are analyzed in duplicate and all data points are used for the calibration curve. Samples with nitrogen concentrations greater than 0.500 ppm are manually diluted and reanalyzed.

10.2 Calculate the percentage spike recovery of the laboratory fortified samples as follows:

$$\%\,SR = \frac{(spiked\,sample\,conc. - sample\,conc.), ppm}{amount\,of\,spike\,added\,to\,sample, ppm} x\,100$$

10.3 Calculate the relative percentage difference for the duplicated samples as follows:

$$\%\,RPD = \frac{difference\,between\,the\,duplicates}{average\,of\,the\,duplicates}\,x\,100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 Completed data packages are scanned and stored electronically before being placed in the appropriate binders in the lab.

11.2 Report only those results that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard are manually diluted and reanalyzed.

11.3 Sample results for are reported in mg N/L. Report results to three decimal places. Report results below the lowest calibration standard as <0.008. For the Chesapeake Bay Program only, report all calculated results with the "L" sign for concentrations less than that of the lowest standard.

12.0 WASTE MANAGEMENT

12.1 It is laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume and bench operation. Compliance with all sewage discharge permits and regulations is also required.

12.2 Samples and standards are poured down the drain while a large amount of water is running. Reagent waste lines are also washed down through the drain with water running.

12.3 For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

13.0 REFERENCES

13.1 EPA Method 350.1, *Methods for the Determination of Inorganic Substances in Environmental Samples*, August 1993

13.2 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, Method 4500- NH3 H, 2005

13.3 *Lachat Instruments QuickChem Method 10-107-06-2-O,* Determination of Ammonia by Flow Injection Analysis

13.4 Lachat Instruments, *Operating Manual for the Quikchem Automated Ion Analyzer*

13.5 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.6 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 4.1, January 2018.

APPENDIX A

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist-LL Ammonia

EPA Method 350.1

Lab Numbers: [1] _____ **Analyst:** _____

Dates Collected: _____ **Date Analyzed:** _____

Procedure	Acceptance Criteria	Status(√)	Comments
Holding Time	48 hours @ 4ºC 28 days @ –20ºC		
Calibration Curve	Corr. Coeff. ≥ 0.9950		
Reagent Blank	< Reporting level (0.008 ppm)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10 samples		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptable range		
Check Standard	After every 10th sample and at the end of the run		
	Concentration = 90–110% of the true value		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.008–0.500 ppm)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature and Date

Reagents	ID	Reagents	ID
Buffer			
Salicylate Nitroprusside			
Sodium Hypochlorite			

External QC
Identification = _____
True Value = _____ ppm
Range = _____ ppm

APPENDIX B

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist LL Orthophosphate/ LL Ammonia

EPA Method 365.1/ EPA Method 350.1

Lab Numbers: [1] _____

Analyst: _____

Dates Collected: _____ **Date Analyzed:** _____

Procedure	Acceptance Criteria	Status(√)	Comments
Holding Time	48 hours @ 4°C; 28 days @ –20°C		
Calibration Curve	Corr. Coeff. $\geq$ 0.9950		
Reagent Blank	< Reporting Level (0.004 ppm for OP; 0.008 ppm for NH_3)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptable range		
Check Standard	After every 10th sample and at the end of the		
	Concentration = 90–110% of the true value		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10		
	RPD ≤ 10%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.004–0.250 ppm for OP; 0.008–0.500 ppm for NH_3)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature and Date

NH_3 Reagents	ID	OP Reagents	ID
Buffer		Color Reagent	
Salicylate Nitroprusside		Ascorbic Acid	
Sodium Hypochlorite			

External QC

Identification = _____
True Value = _____ NH_3 / OP _____ ppm
NH_3 Range = _____ ppm
OP Range = _____ ppm

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

SOP Title: Determination of Orthophosphate- Low Level Flow Injection Colorimetric Analysis (EPA Method 365.1)

SOP No.: CHEM-SOP-EPA 365.1

Revision: 3.2 Replaces: 3.1 Effective: 7/1/2017

Laboratory: Inorganics Analytical Laboratory

Author / POC: Clair Vares
clair.vares@maryland.gov

Laboratory Supervisor:	_____ Signature	_____ Date
QA Officer:	_____ Signature	_____ Date
Manager:	_____ Signature	_____ Date
Division Chief:	_____ Signature	_____ Date

EPA METHOD 365.1
SOP No.: CHEM-SOP-EPA 365.1

REVISION RECORD

Revision	Date	Changes	Made By	Effective Date
0.0	6/3/08	N/A	Shahla Ameli	6/2/08
1.0	12/09	Screen snapshots added to procedure, new Sample Run Log, tracking IDs for standards and reagents	Shahla Ameli	1/10
2.0	8/11	New SOP tracking number, technical and editorial changes	Shahla Ameli	8/18/11
2.1	12/12/12	Technical and editorial changes	Clair Vares/ Shahla Ameli	12/12/12
2.1	7/13	Reviewed SOP	C. Stevenson S. Ameli	12/12/12
3.0	11/19/14	Formatting and document control changes	C. Stevenson R. Carpenter S. Ameli	12/01/14
3.0	6/1/2015	Reviewed SOP	C. Stevenson	7/1/2015
3.1	5/5/2016	Reviewed and changes to 9.3.8	C. Vares S. Ameli C. Stevenson	7/1/2016
3.2	6/1/2017	Reviewed and made organizational name changes	C. Vares S. Ameli C. Stevenson	7/1/2017

TABLE OF CONTENTS

Standard Operating Procedure

Low Level Orthophosphate (Flow Injection Colorimetric Analysis)

EPA Method 365.1

39.0 SCOPE AND APPLICATION

39.1 This method determines orthophosphate (PO_4^{3-}) in drinking, ground, surface, domestic waters and industrial waste.

39.2 The applicable range of this method is 0.004 to 0.250 mg P/L.

40.0 SUMMARY OF METHOD

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex which is further reduced by ascorbic acid to form a blue complex, which absorbs light at 880 nm. The absorbance is directly proportional to the concentration of orthophosphate present in the sample.

41.0 INTERFERENCES

41.1 Silica forms a pale blue complex, which also absorbs at 880 nm. This interference is insignificant on the silica concentration of 1 mg SiO_2/L. If the silicate concentration is higher than 1 ppm, the sample result will not be reliable within the calibration range of the method.

41.2 Concentrations of ferric iron (Fe^{3+}) greater than 50 mg/L will cause a negative error due to precipitation of and subsequent loss of orthophosphate.

41.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that can bias analyte response especially in low level detection of OP. To eliminate this problem wash glassware with 1:1 HCl and rinse with DI water.

42.0 HEALTH AND SAFETY

42.1 Accepted laboratory safety practices should be followed during reagent preparation and instrument operation. The use of a fume hood, protective eyewear, lab coat and proper gloves is required when preparing reagents.

42.2 The following chemical has the potential to be highly toxic or hazardous.

42.2.1 Sulfuric Acid

42.3 A reference file of Material Safety Data Sheets (MSDS) is available to all personnel involved in the chemical analysis.

43.0 EQUIPMENT AND SUPPLIES

43.1 Equipment

43.1.1 Lachat Quick Chem FIA 8500 series.

5.1.1.1 XYZ Auto sampler ASX-520 series with sample, standard and dilution racks

5.2.1.2 Manifold or reaction unit

5.2.1.3 Multichannel Reagent Pump RP-100 series

5.2.1.4 Colorimetric Detector

5.2.1.4.1 Flowcell, 10 mm, 80uL, glass flow cell

5.2.1.4.2 880 nm interference filter

5.2.1.5 Computer, monitor, printer and The Flow Solution software.

5.3 Supplies

5.2.1 13x100 mm test tubes, Fisher # 14-961-27

5.2.2 16x125 mm test tubes, Fisher # 14-961-30

6.0 REAGENTS AND STANDARDS

6.1 Reagents

6.1.1 Stock Ammonium Molybdate Solution- In a 1 L volumetric flask, dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)6Mo7O_{24}.4H_2O$] in approximately 800 ml DI water. Dilute to the mark and let stir for 4 hours. Store in a plastic container and refrigerate. May be stored up to two months when kept refrigerated.

6.2.1 Stock Antimony Potassium Tartrate Solution- In a 1 L volumetric flask, dissolve **3.22 g antimony potassium tartrate Trihydrate $K(SbO)C_4H_4O_6.3H_2O$)** or dissolve **3.0 g antimony potassium tartrate hemihydrate $K(SbO)C_4H_4O_6.1/2H_2O$),** in approximately 800 ml DI water. Dilute to the mark and let stir for few minutes. Store in a dark bottle and refrigerate. This stock may be used up to two months when kept refrigerated.

6.2.1 Molybdate color Reagent. 1 L- Add carefully, while mixing, 35 ml sulfuric acid to about 500 ml DI water. When the temperature is cool add 72.0 mL Stock Antimony potassium Tartrate and 213 mL Stock

Ammonium Molybdate Solution. Dilute to the mark and mix well by inverting. Store in dark bottle. Degas with helium for 1 minute. Prepare fresh weekly. A prepared reagent can also be purchased from HACH Company, catalog number 52002.

6.2.1 Ascorbic Acid Reducing Solution, 0.33 M – In a 1 L volumetric flask, dissolve 60.0 g granular ascorbic acid in about 700 ml DI water. Bring to volume and invert to mix. Add 1.0 g dodecyl sulfate (CH_3 $(CH_2)_{11}OSO$ 3Na). Use degassed water to prepare this reagent. Prepare fresh weekly. Discard if the solution becomes yellow.

6.2.1 Sodium Hydroxide – EDTA Rinse - Dissolve 65 g sodium hydroxide (NaOH) and 6 g tetrasodium Ethylenediamine tetraacetic acid (Na4EDTA) in 1.0L DI water. Used for cleaning OP manifold lines.

6.2.1 Carrier – Use DI water for carrier degassed for one minute.

6.2 Standards

6.2.1 Orthophosphate Stock Standard (1000 mg P/L) - This standard is pre-made and purchased from RICCA CHEMICALS (cat. no. 5839.1-16). If this stock standard is not available, prepare by dissolving 4.396 g of primary standard grade anhydrous potassium phosphate monobasic (KH2PO4) that has been dried in the oven for one hour at 105 ^{o} C in about 500 ml of DI water. Bring up to 1000 mL mark with DI water and store at 4^{o} C. Prepare this reagent monthly.

6.2.1 Intermediate Standard (50 mg P/L) - Pipette 5 ml of standard 6.2.1 into a 100 ml volumetric flask. Bring up to mark with DI water. Store at 4^{o} C. Make weekly.

6.2.1 Spiking Solution (50 mg P/L) - This is the same as the intermediate standard, which is used to spike the samples. Pipette 30 uL of the spiking solution (standard 6.2.2) into 10 mL of DI water or 10 mL of sample, in order to make the blank spike and sample spike. The concentration value for spiking solution is 0.15 mg/L.

6.2.1 Working Standards - The working standards are prepared according to the following table every 48 hours:

Orthophosphate ppm	Combined Intermediate Std	Final Volume
0.000	DI water	100 ml
0.004	2.67 ml of std 0.150 ppm	100 ml
0.010	20 mL of std 0.050 ppm	100 ml
0.050	100 uL	100 ml
0.100	200 uL	100 ml
0.150	600 uL	200 ml
0.200	400 uL	100 ml
0.250	500 uL	100 ml

7.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.6 Samples are collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water.

7.7 Never use acid preservation for samples to be analyzed for LL/HL OP.

7.8 Samples to be analyzed for Orthophosphate only are cooled to 4^{o}C and analyzed within 48 hours. For short-term preservation freeze at –20^{o}C for not more than 28 days.

8.0 QUALITY CONTROL

8.1 An initial Demonstration of Performance (IDP) to establish the instrument's capability is required prior to its use in analyzing client's samples. The linearity of calibration range (LCR) and the ability to quantify the Quality Control Samples correctly are used to assess the performance.

8.2 A demonstration of capability study is performed by each analyst performing the test by analyzing four replicates of an external quality control sample during one or two analytical runs.

8.3 Immediately following daily calibration, a mid-range check standard and a calibration blank is analyzed, also after every ten samples (or more frequently, if required) and at the end of run. If a check standard fails, the samples between the last satisfactory check standard and the unsatisfactory standard are re-analyzed.

8.4 Every tenth sample is duplicated (analyzed from two different tubes) and spiked. The accepted value for the relative Percent Difference (RPD) or spike recovery is ±

10 %. If these do not fall within the accepted ranges, the corresponding analyses are repeated.

8.5 A known QC sample for Orthophosphate is run in the beginning and at the end of each run.

8.6 A method detection limit (MDL) study is performed once a year by analyzing seven replicates of the 0.004 ppm standard spread over three analytical runs. MDL is calculated as follows:

MDL = (t) x (S) where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates] and S = standard deviation of the replicate analyses.
Each new analyst also performs an MDL study or when any changes in the analytical procedure or instrument are made.

8.7 All the standards are run in duplicates: that is, two analyses from each tube. A linear calibration is performed daily before the sample run.

9.0 PROCEDURE

9.1 Sample preparation

9.1.1 Prepare a list of samples to be analyzed.

9.1.2 Spike every tenth sample by adding 30 uL of 50 ppm P/L (Intermediate Standard) into 10 mL DI water or 10 mL of sample.

9.1.3 Filter the turbid samples by inserting the Sera Filter inside a 16 X 125 mm test tubes containing the sample. Press the filter down and pour the filtered sample collected in the top into a 13 x 100 mm test tube for analysis.

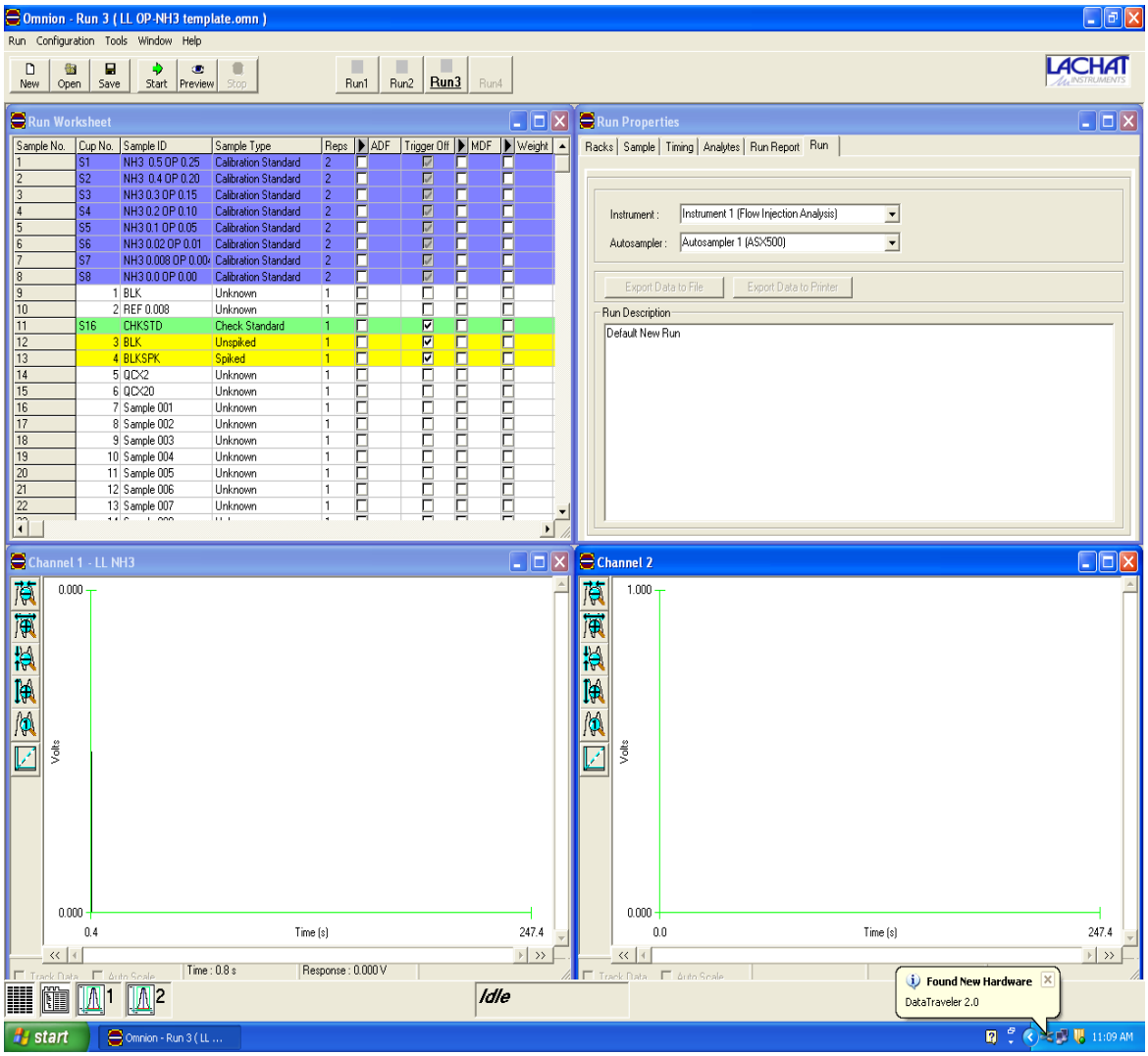
9.1.2 To prevent bubble formation, degas all reagents, except those specified by the method with helium. Use He gas at 140 kPa ($20 lb/in^2$) through a helium degassing tube or a pipette for 1.5 minutes.

9.2 Instrument set-up and sample analysis

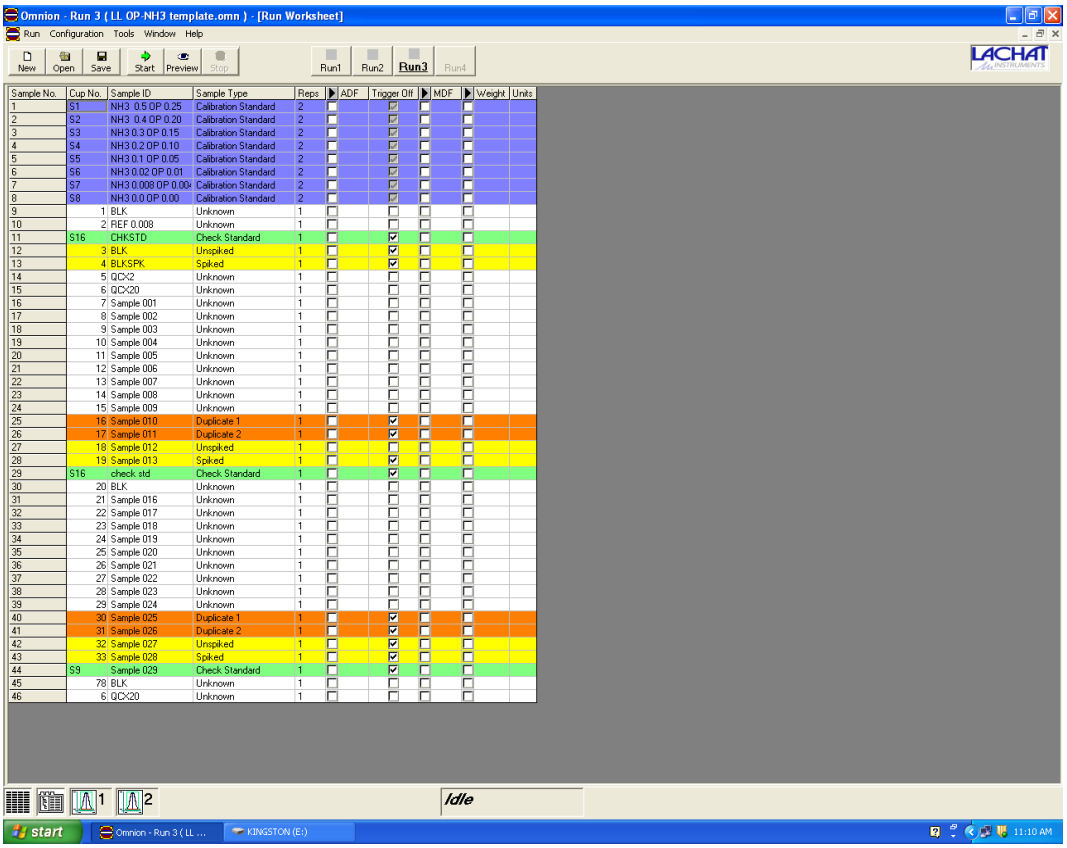
9.2.1 Set up manifold as in the diagram.

9.2.2 Turn on the Lachat instrument, computer, monitor, and printer.

9.2.3 Double click on **"LL OP/NH3"** to open the template, which consists of four windows. Samples are analyzed consecutively for orthophosphate and ammonia on the same system.



9.2.4 Maximize the **"Run Worksheet"** window at the top left hand corner of the screen by clicking on the middle square on that screen to open the worksheet template. Enter sample identifications in the sample ID column, making sure that all duplicates and spikes are entered in the appropriate locations of the worksheet. Identifications of the quality control samples analyzed at the beginning of the run and the locations of the duplicates and the spiked have been saved on the template. Press **"Enter"** key after each entry in order to save all entries.

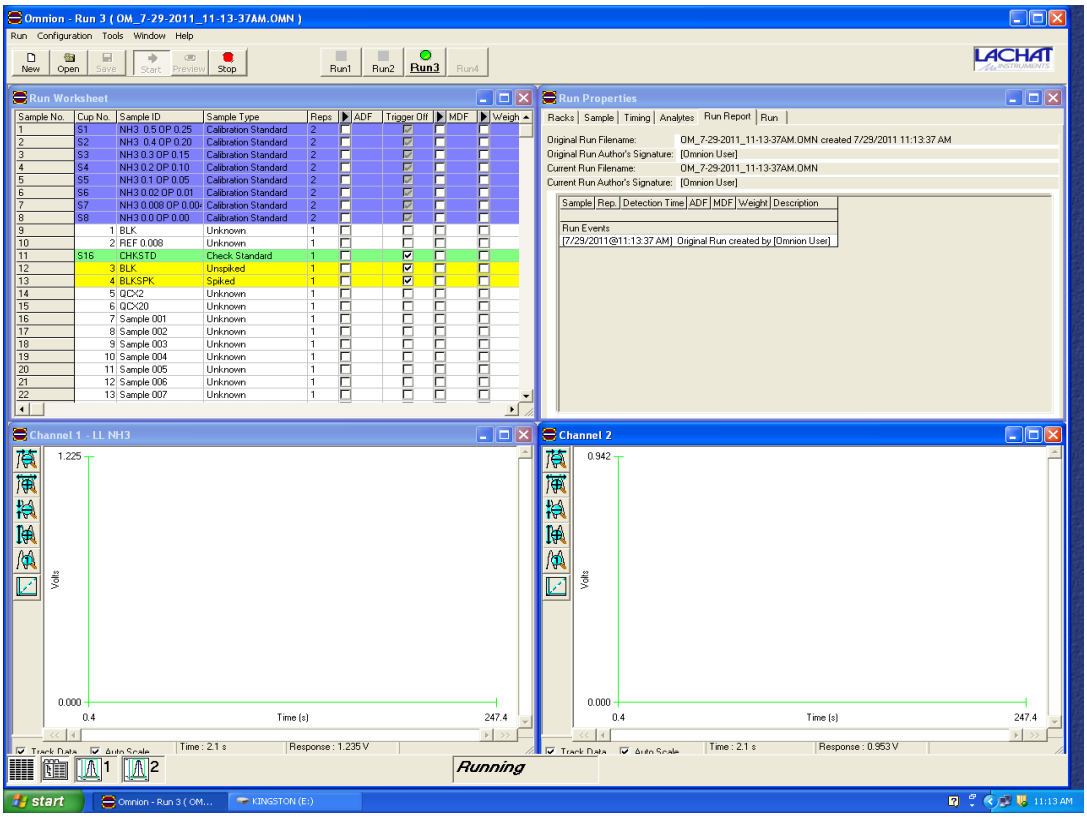


9.2.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

9.2.6 Click on **"Window"** tab and then, click on **"Tile"** to return to the screen with three windows.

9.2.7 Place standards in standard vials in the standard rack in order of decreasing concentration in positions S1 to S8 (position S8 is DI water-0.00 mg/L). Place the check standard vial in position 16 (S16) of the standard rack. Pour samples and quality control samples into 13 x 100 mm test tubes and place them in the sample racks according to the worksheet prepared in 9.2.4.

9.2.8 Pump deionized water through all reagent lines for 15 – 20 minutes and check for leaks and smooth flow. Switch to reagents in the following order: 1. Ascorbic Acid, 2. Color Reagent. Continue pumping for about 10 minutes. Click on **"Preview"** tab to monitor the baseline.



9.2.9 Once a stable baseline is achieved, click on **"Stop"** tab to stop monitoring the baseline. Click on **"Start"** tab to begin the analysis.

9.2.10 If the calibration passes, instrument will continue to analyze the samples. If it fails, take appropriate corrective actions and recalibrate before proceeding to analyze samples.

9.2.11 Manual dilution will be performed to reanalyze samples with concentration exceeding the calibrated range.

9.2.12 After the run is complete, remove first the Color Reagent line and then the Ascorbic Reagent line placing them in DI water and rinsing for about 15 minutes. If necessary, rinse the OP reagent lines with the NaOH – EDTA rinse solution (6.1.5) for about 5 minutes followed by DI water for 10 – 15 minutes. Pump the tubes dry, release the pump tubing, and turn off the pump.

10.0 DATA ANALYSIS AND CALCULATIONS

10.1 All the calculations are performed by the Omnion 3.0 software system. It uses a calibration graph of peak area versus concentration to calculate the phosphorus concentrations in the samples. All standards are analyzed in duplicate and all data points are used for the calibration curve. Samples with phosphorus concentrations greater than 0.250 ppm are manually diluted and reanalyzed.

10.2 Calculate the percentage spike recovery of the laboratory fortified samples as follows:

$$\%\,SR = \frac{(\text{spiked sample conc.} - \text{sample conc.}),\ \text{ppm}}{\text{amount of spike added to sample, ppm}} \times 100$$

10.3 Calculate the relative percentage difference for the duplicated samples as follows:

$$\%\ RPD = \frac{\text{difference between the duplicates}}{\text{average of the duplicates}} \times 100$$

11.0 DATA AND RECORDS MANAGEMENT

11.1 Completed data packages are scanned and stored electronically before being placed in the appropriate binders in the lab.

11.2 Report only those results that fall between the lowest and the highest Calibration standards. Samples exceeding the highest standard are manually diluted and reanalyzed.

11.3 Sample results for OP are reported in mg P/L. Report results to three decimal places. Report results below the lowest calibration standard as <0.004. For the Chesapeake Bay Program, only report all calculated results with the "L" sign for concentrations less than that of the lowest Standard.

12.0 WASTE MANAGEMENT

12.1 It is laboratory's responsibility to comply with all state, federal and local regulations governing waste management, particularly the hazardous waste identification rules and disposal restrictions, and to protect the air, water and land buy minimizing and controlling all releases from fume and bench operation.

12.2 Samples and standards are poured down the drain while large amount of water is running. For more information consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's

Department of Government Regulations and Science Policy, 1155 Street N. W., Washington D. C. 20036, (202) 872-4477.

13.0 REFERENCES

13.1 EPA Method 365.1, *Methods for the Determination of Inorganic Substances in Environmental Samples*, August 1993.

13.2 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, Method 4500- P E, 2005.

13.3 *Lachat Instruments QuickChem Method 10 – 115 – 01 – 1 – M,* Determination of Orthophosphate by Flow Injection Analysis.

13.4 Lachat Instruments, *Operating Manual for the Quikchem Automated Ion Analyzer*.

13.5 Division of Environmental Chemistry, Maryland Department of Health and Mental Hygiene, *Quality Assurance Plan,* SOP No. CHEM-SOP-QAP Revision15.0, August 2016

13.6 Division of Environmental Chemistry and Division of Microbiology, Maryland Department of Health and Mental Hygiene, *Quality Manual,* SOP No. QA-SOP-QM Revision 1.1, July1, 2015.

APPENDIX **A**

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist LL Orthophosphate

EPA Method 365.1

LabNumbers[1]:_____

Date Collected: _____ Date Analyzed: _____Analyst: _____

Procedure	Acceptance Criteria	Status (✓)	Comments
Holding Time	48 hours @ 4ºC/ 28 days @ –20ºC		
Calibration Curve	Corr. Coefficient. ≥ 0.9950		
Reagent Blank	< Reporting level (0.004 ppm)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10 samples		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptable range		
Check Standard	After every 10th sample and at the end of the run		
	Concentration = 90–110% of the true value		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10 samples		
	RPD ≤ 10%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.004–0.250 ppm)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____
Analyst's Signature & Date Reviewer's Signature & Date

_____
Supervisor's Signature & Date

Reagents	ID
Color Reagent	
Ascorbic Acid	

External QC
Identification = _____
True Value = _____ ppm
Range = _____ ppm

APPENDIX B

Division of Environmental Sciences
INORGANICS ANALYTICAL LABORATORY

Data Review Checklist LL Orthophosphate/ LL Ammonia

EPA Method 365.1/ EPA Method 350.1

Lab Numbers: [1] _____

Analyst: _____

Dates Collected: _____ Date Analyzed: _____

Procedure	**Acceptance Criteria**	**Status(✓)**	**Comments**
Holding Time	48 hours @ 4°C; 28 days @ –20°C		
Calibration Curve	Corr. Coeff. $\geq$ 0.9950		
Reagent Blank	< Reporting Level (0.004 ppm for OP; 0.008 ppm for NH_3)		
Blank Spike	1 per batch		
	Recovery = 90–110%		
Matrix Spike	Every 10th sample or 1/batch, if less than 10		
	Recovery = 90–110%		
External QC	Beginning and end of each run		
	Within acceptable range		
Check Standard	After every 10th sample and at the end of the		
	Concentration = 90–110% of the true value		
Duplicates/Replicates	Every 10th sample or 1/batch, if less than 10		
	RPD ≤ 10%		
Decimal Places Reported	3		
Measured Values	Within calibration range (0.004–0.250 ppm for OP; 0.008–0.500 ppm for NH_3)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

[1]Include beginning and ending numbers, account for gaps by bracketing.

_____ _____

Analyst's Signature & Date Reviewer's Signature & Date

_____

Supervisor's Signature and Date

NH_3 Reagents	ID	OP Reagents	ID	External QC	
Sodium Phenolate		Color Reagent		Identification =	
Sodium Nitroprusside		Ascorbic Acid		True Value =	NH_3 / OP ppm
Sodium Hypochlorite				NH_3 Range =	ppm
EDTA Buffer				OP Range =	ppm

Appendix IV: Maryland Department of Health:
Divisional Analytical Corrective Action Form

MDH - Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

DIVISIONAL ANALYTICAL CORRECTIVE ACTION FORM
Quality Assurance Program

✓ NONCONFORMANCE

Customer:		**Samples(s) :**	
Test:	**Method**	**Instrument:**	**Date of Occurrence**
---- Failed Tuning ---- Failed Calibration ---- Instrument Instability ---- Instrument Malfunction ---- Other	---- Power Failure ---- Broken or Lost Aliquot ---- Insufficient Volume ---- Poor Aliquot Preservation	__ Exceeded Holding Time ---- Matrix Interference --- Out-of-Control QC Param.	
Detailed:			
Signature of Originator:		**Date:**	

✓ CORRECTIVE ACTION TAKEN

---- Instrument Returned ---- Instrument Recalibrated ---- Instrument Serviced	--- Sample(s) Re-poured --- Sample(s) Reanalyzed --- Lab Management Notified --- Other
	Date of Completion:
Signature of Person Responsible:	**Date:**

❒ VERIFICATION OF NONCONFORMANCE AND CORRECTIVE ACTION

Signature of Supervisor	**Date**

❒ **NOTIFICATION**

Customer Contact Required? Yes ---- No	**SMA / Date of Contact**
Detailed Description	
Signature of Notifier	**Date**

❒ **IS FURTHER INVESTIGATION / MONITORING NEEDED?** ☐ **YES** ☐ **NO**

If YES, Please Forward To	Date

❒ **DESCRIBE RESULTS OF FURTHER INVESTIGATION**

❒ **WAS PROBLEM FINALLY CORRECTED?** ☐ **YES** ☐ **NO**

If It Was Not Corrected, Explain

Corrective Action Reviewed By Supervisor	Date

Corrective Action Reviewed By Division Chief	Date

Corrective Action Reviewed By QA Officer	Date

❒ **ACKNOWLEDGEMENT**

Signature of QA Officer	**Date**

Copies: QA Officer
Laboratory QA File

Appendix V: Chesapeake Biological Laboratory, Nutrient Analytical Services: Standard Operating Procedures

University of Maryland Center for Environmental Science
Chesapeake Biological Laboratory
Nutrient Analytical Services
146 Williams St., Solomons, MD 20688 http://nasl.cbl.umces.edu/

Standard Operating Procedure for Spectrophotometric Determination of Chlorophyll α in waters and sediments of Fresh/Estuarine/Coastal Areas.

(References: SM10200H, EPA 446.0)

Document #: NASLDoc-034

Revision 2018-1
Replaces Revision 2017-4
Effective May 1, 2018

I attest that I have reviewed this standard operating procedure and agree to comply with all procedures outlined within this document.

Employee (Print)	Employee (Signature)	Date
Employee (Print)	Employee (Signature)	Date
Employee (Print)	Employee (Signature)	Date
Employee (Print)	Employee (Signature)	Date

Revised by: _____ Date: _____

Reviewed by: _____ Date: _____

Laboratory Supervisor: _____ Date: _____

Revisions 2018

9.2.2 Replaced “Student’s *t* value for the 99% confidence level with n-1 degrees of freedom (*t* = 3.14 for 7 replicates)” with “the Student’s t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom.”

1. SCOPE and APPLICATION

1.1 This is an acetone extraction method to determine chlorophyll α in fresh, estuarine waters, and coastal waters.

1.2 A Method Detection Limit (MDL) of 0.62 µg/L active chlα and 0.74 µg/L phaeophytin was determined using the Student's *t* value (3.14, n=7) times the standard deviation of a minimum of 7 replicates. If more than seven replicates are used to determine the MDL, refer to the Student's *t* test table for the appropriate n-1 value.

1.3 The quantitation limit for chlα is dependent upon the volume of sample filtered. The reporting limit is equal to the MDL.

1.4 This procedure should be used by analysts experienced in the theory and application of chlorophyll analysis. A three month training period with an analyst experienced in the analysis using the spectrophotometer is required.

1.5 This method can be used for all programs that require spectrophotometric analysis of chlorophyll α.

1.6 This procedure is based on Standard Methods 10200H, 19th Edition and EPA Method 446.0.

2. SUMMARY

2.1 Chlorophyll α is extracted from phytoplankton cells using a 90% solution of acetone. The samples are refrigerated in the dark from 2 to 24 hours (over night is preferable). After the appropriate time, the samples are centrifuged to separate the sample material from the extract. Because the waters of the Maryland portion of the Chesapeake Bay are relatively turbid, the sample extract is filtered through a 0.45 um PTFE or nylon syringe filter and transferred into a clean tube, and centrifuged again for 20 minutes. The extract is analyzed on a spectrophotometer. To determine phaeophytin and active chlα, the extract is then acidified using 1N HCl, and reread. The concentrations are then calculated using Lorenzen's modified monochromatic equation. Uncorrected chlorophyll may be determined using the Jeffrey and Humphrey trichromatic equation.

3. DEFINITIONS

3.1 Absorbance – A measure of the amount of light at a specific wavelength absorbed by a liquid.

3.2 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.3 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and

systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.4 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.5 Analytical Range – The analytical range is dependent on the volume of water filtered and the volume of acetone used in the extraction.

3.6 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.7 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.8 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration – The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.10 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.11 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.12 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

3.12.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

3.12.2 Initial Calibration Verification (ICV) – An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.12.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 10-15 field sample analysis.

3.13 Certified Reference Material (CRM) – A reference material one or more of whose 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. (ISO 17025)

3.14 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.15 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.16 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.17 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.18 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)

3.19 External Standard (ES) – A pure analyte (anacystis nidulans algae, or equivalent) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.20 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.21 Field Reagent Blank (FRB) – An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.22 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.23 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.24 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1

and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.25 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., 90% acetone) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.

3.26 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intralaboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.27 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank (ACS), also known as MDL.

3.28 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired. Also known as Quantitation Limit.

3.29 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.30 Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.31 May – Denotes permitted action, but not required action. (NELAC)

3.32 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.

3.33 Monochromatic equation – Also known as Lorenzen's modified monochromatic equation, it requires the absorbance values of 664 and 665 nm before and after an acidification step of 90 seconds to calculate the amount of chlorophyll α and phaeophytin in the sample. The chlorophyll a is reported as corrected for phaeophytin. Chlorophyll *b* and *c* cannot be calculated using this equation.

3.34 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.35 Path Length – The path length is the width of the cuvette cell (length between optical non-frosted sides). For this method, 5 and 1 cm path length cuvettes are used.

3.36 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality

indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.37 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.38 Quality Control Sample (QCS) – A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials and is also known as the CRM.

3.39 Run – One sample analysis from start to finish, including printout.

3.40 Run Cycle – Typically a day of operation – the entire analytical sequence of runs from the first run to the last run.

3.41 Sample Volume – Volume of water filtered.

3.42 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.43 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.44 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.45 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique. Also known as CRM.

3.46 Trichromatic equation – Also known as Jeffrey and Humphrey's Trichromatic Equations, they require absorbance values at 664, 647, and 630 nm to calculate the amount of uncorrected chlorophyll α in a sample. Chlorophyll *b* and *c* pigments can also be determined. No acidification is required and phaeophytin cannot be calculated from this equation.

4. INTERFERENCES

4.1 Light and heat cause the chlorophyll molecule to break down. Therefore, the samples should be kept cold in the dark and care should be taken when grinding the samples so as not to overheat the sample. When ready to analyze, the extract must be at room temperature and the analysis performed under reduced lighting.

4.2 Any compound that absorbs light between 630 and 665 nm may interfere with chlorophyll measurement. The absorbance measurement at 750 nm is subtracted from the sample's other measured absorbances (665, 664, 647, and 630 nm) to account for the turbidity of the clarified sample. If the absorbance at 750 nm is above 0.007 absorbance units (AU), the sample may be filtered one more time.

4.3 The spectral overlap of chlorophyll α, *b*, and *c* and phaeophytin can cause over or under-estimation of chlorophyll and/or phaeophytin. The amount of chlorophyll *b* and *c* in a sample is dependent on the taxonomic composition of the phytoplankton it contains. In the trichromatic equation, chlorophyll α may be overestimated in the presence of phaeophytin. In the monochromatic equation, chlorophyll α may be slightly overestimated in the presence of chlorophyll *b* and phaeophytin may be overestimated in the presence of carotenoids.

5. SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Associate Director of Administration and Facilities of the incident. Contact the CBL Associate Director of Administration and Facilities if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS). Table 1:

Chemical	Health Hazard	Fire Hazard	Instability Hazard	Specific Hazard	
Hydrochloric Acid	3	0	2	ACID, COR	
Acetone	1	3	0		

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)

HAZARD RATING
Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly hazardous, 0 – normal material

Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200° F, 1 – above 200° F, 0 – will not burn

Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 – violent chemical change, 1 – unstable is heated, 0 - stable

Specific Hazard - White: Acid = ACID, Alkali = ALK, Corrosive = COR, Oxidizer = OXY

6. EQUIPMENT AND SUPPLIES

6.1 A scanning spectrophotometer capable of measuring wavelengths within the visible range. This laboratory uses Shimadzu UV2401PC and UV2450PC spectrophotometers.
6.2 Freezer, capable of maintaining -20° ± 5° C.
6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives.
6.4 A centrifuge.
6.5 A Teflon pestle for grinding, either by hand or power, and/or a sonicator.
6.6 5-cm path length and 1-cm path length cuvettes of either special optical glass or quartz.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.
7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.
7.3 Acetone ($H_2C=O=CH_2$), 90% v/v

Acetone, reagent grade	900 ml
Reagent water	100 ml

Using a graduated cylinder, add 100 ml reagent water to 900 ml acetone.

7.4 Hydrochloric Acid, 1N –

Hydrochloric acid (HCl), concentrated,	8.6 ml
Reagent water, q.s.	100 ml

In a 100 ml volumetric flask, add 8.6 ml of concentrated hydrochloric acid to ~60 ml of reagent water. Dilute to 100 ml with reagent water.

7.5 Blanks – A reagent blank of 90% acetone is used.

7.6 Standards – Standards used are one of the following:

7.6.1 Chlorophyll α from Anacystis nidulans algae, PN C6144-1MG, ordered from Sigma/Aldrich. If chlorophyll from algae is not available, chlorophyll α from spinach may be substituted.

7.6.2 Turner Designs Spectrophotometric chlorophyll α standard, PN10-950. Standard Package includes one 20mL ampoule with a known concentration of Chlorophyll α Primary Standard in 90% acetone solution

Shelf Life: 1-year from manufacturing date, un-opened and stored in freezer at -20°C.

7.7 Quality Control Sample (QCS) – For this procedure, the QCS can be any certified sample which is obtained from an external source. If a certified sample is not available, then use the standard material.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for chlα should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 µm), or equivalent.

8.2 Water collected for chlα should be filtered as soon as possible. If immediate filtration is not possible, the water samples should be kept on ice in the dark and filtered within 24 hours.

8.3 The filtered sample is kept frozen at -20° C or lower. Filter pads should be folded in half and may be stored in folded aluminum foil pouches.

8.4 Frozen chlα filters should be extracted within 4 weeks. Once the sample is extracted, the clarified extract should be stored at -20° C or lower and should be analyzed within the original holding time.

9. QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

9.2.1 The initial demonstration of capability (DOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2 Method Detection Limits (MDLs) – MDLs should be established for chlα using a low level ambient water sample. To determine the MDL values, analyze a minimum of seven replicate filtered aliquots of water. Perform all calculations defined in the procedure (Section 11) and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = St_{(n-1,1-\alpha=0.99)}$$

Where,
t(n-1,1-α=0.99) = the Student's t-value appropriate for a single-tailed 99th percentile
t statistic and a standard deviation estimate with n-1 degrees of freedom.
n = number of replicates
S = Standard Deviation of the replicate analyses.

9.2.3 MDLs should be determined yearly. If more than 7 replicates are analyzed, use the appropriate n-1 value obtained from the table for the Student's *t* test.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory reagent blank is analyzed at the beginning of each sample run, after every tenth sample, and at the end of the run. The LRB consists of 90% acetone treated the same as the samples. LRB data are used to assess contamination from the laboratory environment.

9.4 Data Assessment and Acceptance Criteria for Quality Control Measures

9.4.1 The instrument optical performance is checked quarterly using a didymium reference standard which presents a wide range of crisply resolvable peaks which are easily used to correlate the wavelength indicator on the spectrophotometer to the known peak. Each peak reading should fall within the manufacturer's tolerance of the wavelength readout. If the criteria are not met, the instrument must be seen by a service technician.

9.5 Corrective Actions for Out of Control Data

9.5.1 The sample is first analyzed using the 5 cm path length cuvette. If the 665 nm reading is above 1.000 absorbance units, the sample should be reread using the 1 cm cuvette.

9.5.2 If the absorbance of the LRB shows an upward trend, AUTOZERO and re-BASELINE, then reread that LRB.

10. CALIBRATION AND STANDARDIZATION

10.1 Calibration – Quarterly optical performance checks are performed using a certified reference material such as Didymium or Holmium Oxide cell or filter used to check wavelength accuracy.

11. PROCEDURE

11.1 Sample Preparation – water column

11.1.1 Filter a known volume of water through a Whatman GF/F filter pad (nominal pore size 0.7 μm). Good color is needed on the pad. Do not rinse the pad.

11.1.2 Fold pad in half, sample inside, wrap in aluminum foil, label and freeze for analysis within 4 weeks.

11.1.3 Before analysis, briefly thaw pads, and then place in a 15 ml centrifuge tube. Add 10 ml of 90% acetone. Work under subdued lighting.

11.1.4 Write all information in the lab bench sheet.

11.1.5 Using a Teflon pestle, grind the filter against the side of the tube until the filter is well ground. If hand grinding, 10-15 seconds is all that is necessary. Power grinding requires vigilance, because excess heat will degrade the chlorophyll. Allow the sample to extract for 2 - 24 hours in the dark under refrigeration. Overnight is recommended.

11.1.6 Remove tubes from refrigerator.

11.1.7 Shake tubes, and then centrifuge at 500-675g for 30 minutes. Using a syringe, withdraw the sample and filter into the cuvette. If the samples are not analyzed that day the extract must be transferred to another tube. Pull the extract from the first tube and filter while transferring to a second numbered centrifuge tube. The extract is filtered through 0.45 um PTFE or nylon syringe filters. The transferred samples may be stored in the freezer for up to 30 days after extraction. When ready to analyze the samples, centrifuge again for 20 minutes at 500-675g.

To calculate rpm use this formula:

$$RCF = 1.12r(rpm/1000)^2,$$

Where: RCF = relative centrifugal force

r = radius of the rotor in millimeters (usually found on the manufacturer's website)
rpm = speed of rotation

11.2 Pollution Prevention and Waste Management

11.2.1 This method generates hazardous waste.

11.2.2 Acetone waste is stored in 4 liter jugs in the cabinet under the hood and transferred to the hazardous waste area of the Storage Facility on campus.

11.2.3 Do not pour acetone down the sink.

11.2.4 Decant the waste acetone into the waste jugs, and then allow the remaining ground filter pad or sediment to dry in the hood.

11.2.5 The dried waste may then be put in the trash.

11.3 Using the Shimadzu UVProbe software:

11.3.1 Turn on the spectrophotometer (either the UV2401 or the UV2450) and the computer. Open the UVProbe software. Select photometric mode and connect to the instrument to turn on the lamps. Allow the instrument to run the lamp check and click OK. Allow the lamps to warm up for a minimum of 45 minutes before beginning sample analysis. Press GO TO WL and change the wavelength to 750 nm. Open the Method.

11.3.2 Using the 5 cm path length cuvettes, fill both the reference and sample cuvettes with 90% acetone. Wipe the windows of the cuvettes carefully with lens paper to dry. Click on AUTOZERO, then run a BASELINE. When the baseline is complete, label the first line of the sample table as blk1. Click on READ UNK (unknown) or press F9 to begin scanning. All wavelengths should be very close to zero. If not, AUTOZERO again, and rerun the BASELINE. Run blk2 if needed.

11.3.3 The reference cuvette is filled with 90% acetone and is left in place. Periodically check the liquid level, adding more 90% acetone as needed.

11.3.4 Begin analyzing samples. Enter the sample name in the sample table twice, once with a "b" designation for before acid, and again with an "a" designation for after acid.

11.3.5 Dispense sample into the sample cuvette. Wipe the windows of the cuvette carefully with lens paper and place in the cell holder.

11.3.6 Check the absorbance at 750 nm. If it is at 0.007 or below, press F9 to start the scan. If it is above 0.007, the sample may be filtered one more time through a 0.45 um ptfe syringe filter. If the 750 nm absorbance is still not below 0.007, proceed with the scan. It may

be necessary to recheck the zero if several samples in a row start above 0.007 at the 750 nm reading.

11.3.7 After the first scan is read, add enough 1N HCl to the sample to achieve a concentration of 0.003 N HCl within the sample. One drop of acid is used in the 1-cm path length cuvettes and 3 drops in the 5-cm cuvettes. Gently stir the sample for 30 seconds and wait another 30 seconds before starting the scan. A total of 90 seconds is needed to complete the reaction before reading. A 30 second wait is built into the method.

11.3.8 Rinse the sample cuvette with acetone after each sample. Then rinse with a small amount of sample before filling.

11.3.9 Repeat steps 11.3.4 through 11.3.6 for all samples, adding a blank after every 10 samples.

11.3.10 Run a blank at the end

11.3.11 Save the file. Right click on Properties.

11.3.12 Hide columns TYPE, EX, and CONC. Print file.

11.3.13 Save the file again as a text file to be imported into a spreadsheet for calculation.

12. CALCULATIONS

Chlorophyll corrected for phaeophytin (µg/L):

$$\text{Chlorophyll } \alpha \text{ corrected (ug/L)} = \frac{26.7(664_B - 665_A) \times V_1}{V_2 \times L}$$

Phaeophytin (µg/L):

$$\text{Phaeophytin } \alpha \text{ (ug/L)} = \frac{26.7\,[1.7(665_A) - 664_B] \times V_1}{V_2 \times L}$$

Uncorrected chlorophyll (µg/L):

$$\text{Chlorophyll } \alpha \text{ uncorrected (ug/L)} = \frac{[11.85(664_B) - 1.54(647_B) - 0.8(630_B)]xV1}{V_2 \times L}$$

Chlorophyll/Phaeophytin ratio:

Absorption peak ratio: $664_B/665_A$

Where: 664_B = Subtract 750 nm value (turbidity correction) from absorbance at 664 nm before acidification.

665_A = turbidity corrected absorbance at 665 nm after acidification.

647_B = turbidity corrected absorbance at 647 nm before acidification.

630_B = turbidity corrected absorbance at 630 nm before acidification.

V_1 = volume of extract (mL)

V_2 = volume of sample filtered (L)

L = path length (cm)

13 REFERENCES

13.1 APHA, Standard Methods for the Examination of Water and Wastewater, Method
#10200H, 19th Edition.

13.2 EPA Method 446.0.

CHLOROPHYLL A SPEC BENCH DATA SHEET			BOX #	CRUISE:	
SPEC ID UV2450	LOADED BY:			LOAD DATE:	
SPEC ID UV2401	ANALYZED BY:		ANALYSIS DATE:		
		FIRST	TRANSFER		
SAMPLE ID	DATE	TUBE #	TUBE #	VOL. FILTERED	COMMENTS
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UNIVERSITY OF MARYLAND CENTER FOR ENVIRONMENTAL SCIENCE CHESAPEAKE BIOLOGICAL LABORATORY NUTRIENT ANALYTICAL SERVICES LABORATORY
146 Williams St., Solomons MD 20688 www.umces.edu/nutrient-analytical-services-laboratory

Standard Operating Procedure for Determination of Anions By Ion Chromatography in Fresh/Estuarine/Coastal Waters (References Standard Methods 4110B)

Document #: NASLDoc-026

Revision 2019-1
Effective May 1, 2019

I attest that I have reviewed this standard operating procedure and agree to comply with all procedures outlined within this document.

_____	_____	-
Employee (Print)	Employee (Signature)	Date
_____	_____	-
Employee (Print)	Employee (Signature)	Date
_____	_____	-
Employee (Print)	Employee (Signature)	Date
_____	_____	-
Employee (Print)	Employee (Signature)	Date

Revised by: _____ Date: _____

Reviewed by: _____ Date: _____

Laboratory Supervisor: _____ Date: _____

Determination of Anions by Ion Chromatography in Fresh/Estuarine/Coastal Waters

1. SCOPE and APPLICATION

1.1 Ion Chromatography is a process that separates ion based on their affinity to the ion exchanger. The separated ion passes through a suppressor where they are converted to their highly conductive acid forms. The ions are identified based on retention times and measured by peak area.

1.2 A Method Detection Limit (MDL) of 0.08 mg Cl^-/L, 0.09 mg SO_4/L, 0.02 mg Br/L was determined using the MDL method as specified in the EPA Federal Register 821-R-16-006, titled Definition and Procedure for the Determination of the Method Detection Limit, Revision 2.

1.3 The Quantitation Limit/Reporting Limit for Cl^- was set at 0.80 mg Cl^-/L, SO_4 was set at 0.90 mg SO_4/L, Br was set at 0.08 mg Br/L

1.4 The method is suitable for Cl^- concentrations 0.08 to 200 mg Cl^-/L, 0.09 to 200 mg SO_4/L, and 0.02 to 2.0 mg Br/L.

1.5 This procedure should be used by analysts experienced in the theory and application of Ion Chromatography analysis. Three months experience with an analyst, experienced in the analysis of anions in aqueous samples, is required.

1.6 This method can be used for all programs that require analysis of anions.

1.7 This procedure references Standard Methods 4110B.

2. SUMMARY

2.1 Filtered water samples are injected into a stream of eluent and passed through a series of ion exchangers. The separated anions are then passed through a suppressor device and are measured by conductivity.

3. DEFINITIONS

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4 Analytical Range – 5.0-200 mg Cl^-/L, 5.0-200 mg SO_4/L, 0.06-2 mg Br/L.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. An

analytical batch is composed of prepared environmental samples (extracts, digestates, concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Blank – A volume of reagent water fortified with the same matrix as the calibration standards, without analyte added.

3.10 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.11 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.12 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

3.12.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

3.12.2 Initial Calibration Verification (ICV) – An individual standard, which may be the same compound used as the calibrating standard, but not from the same vendor unless confirmed as different lots, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.12.3 Continuing Calibration Verification (CCV) – An individual standard, this may be the same as the calibrating standard, and is analyzed after every 10 field sample analyses.

3.13 Certified Reference Material (CRM) – A reference material one or more of whose 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. (ISO 17025)

3.14 Conditioning Blank- Reagent water (ASTM Type I) analyzed before the calibration curve to decrease the instrument blank and stabilize the column conditions.

3.15 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.16 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.17 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.18 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

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3.22 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.23 Injection- the sample aliquot that is drawn into the syringe and injected into the stream of eluent.

3.24 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.25 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., Reagent water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.

3.26 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard 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. (NELAC)

3.27 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.28 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD such that it is ≥ the lower standard. This is also referred to as the Quantitation Limit.

3.29 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.30 Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.31 May – Denotes permitted action, but not required action. (NELAC)

3.32 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero (Standard Methods).

3.33 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.34 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.35 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.36 Quality Control Sample (QCS) – A sample of analyte of known and certified concentration. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials. Also referred to as CRM.

3.37 Run Cycle – Typically a day of operation – the entire analytical sequence from sampling the first standard to the last sample of the day.

3.38 Sample Volume- Amount of sample injected into the stream of eluent.

3.39 Sample Tray –Metal tray that holds auto analyzer vials containing samples or standards. The user identifies each vial in the operating software.

3.40 Sample Tray Holder – An automated carousel that contains up to six sample segments. This carousel spins in clockwise manner to move the sample trays into position for analysis.

3.41 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.42 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.43 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.44 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique. Also referred to as CRM.

4. INTERFERENCES

4.1 Any substance in a sample that has a retention time that coincided with the retention time of any anion.

4.2 A high concentration of anion can cause interference with resolution and possibly cause carryover to other ions. Dilute sample and reanalyze.

4.3 Contaminants in reagent water.

5. SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes must be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Associate Director of Administration and Facilities Maintenance of the incident. Contact the CBL Associate Director of Administration and Facilities Maintenance if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Table 1

Chemical	Health Hazard	Fire Hazard	Instability Hazard	Specific Hazard	
Potassium Sulfate	0	0	0		
Sodium Chloride	1	0	0		
Sodium Bromide	2	0	0		
Methane sulfonic Acid	3	1	0	COR,IRR	
Potassium Hydroxide	3	0	0	COR,IRR	

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)

HAZARD RATING

Health Hazard - Blue: 4 – deadly, 3 – extreme danger, 2 – hazardous, 1 – slightly hazardous, 0 – normal material

Fire Hazard - Red: Flash Points: 4 – below 73° F, 3 – below 100° F, 2 – below 200° F, 1 – above 200° F, 0 – will not burn

Instability Hazard - Yellow: 4 – may detonate, 3 – Shock and heat may detonate, 2 – violent chemical change, 1 – unstable is heated, 0 - stable

Specific Hazard - White: Acid = ACID, Alkali = ALK, Corrosive = COR, Oxidizer = OXY

6. EQUIPMENT AND SUPPLIES

6.1 Dionex ICS-5000+ Reagent-Free Ion Chromatography System. Chromeleon 7.2 operating software on a computer running Microsoft Windows 7 operating system.

6.2 Refrigerator, capable of maintaining 4 +/- 2^{o}C.

6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc.) should be sufficiently clean for the task objectives. This laboratory cleans all lab ware related to this method with a 10% HCl (v/v) acid rinse.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.

7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.

7.3 Methane Sulfonic Acid

Doinex EGC III MSA Methanesufonic Acid Eluent Generator Cartridge purchased from Thermo Fisher Scientific. Product#074535, CAS#131058-3.

7.4 Potassium Hydroxide

Dionex EGC III KOH Potassium Hydroxide Eluent Generator Cartridge purchased from ThermoFisher Scientific. Product#074532, CAS#73-75-2.

7.5 Bromide Stock Standard–

Sodium Bromide	0.6438 g
Reagent water	up to 500 mL

In a 500 mL volumetric flask, dissolve 0.6438 g of Sodium Bromide in ~400 mL of reagent water. Dilute to 500 mL with reagent water (1mg Br/mL). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 6 months.

7.6 Chloride Stock Standard –

Sodium Chloride	0.8243 g
Reagent water	up to 500 mL

In a 500 mL volumetric flask, dissolve 0.8243 g of Sodium Chloride in ~400 mL of reagent water. Dilute to 500 mL with reagent water (1mg Cl^- /mL). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 6 months.

7.7 Sulfate Stock Standard –

Potassium Sulfate	0.9070 g
Reagent water	up to 500 mL

In a 500 mL volumetric flask, dissolve 0.9070 g of Potassium Sulfate in ~400 mL of reagent water. Dilute to 500 mL with reagent water (1mg SO_4/mL). Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 6 months.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for anions should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.

8.2 Water collected for anions should be stored at 4°C. The sample container should be clean and sample rinsed.

8.3 Anion samples may be stored up to 28 days at 4°C.

9. QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Performance

9.2.1 The initial demonstration of capability (iDOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2 Linear Dynamic Range – LDR (Linear Calibration Range) should be established using appropriate six or seven point calibration curve.

9.2.3 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and every batch, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3*s* of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and

corrected before either proceeding with the initial determination of MDL_s or continuing with analyses.

9.2.4 Method Detection Limits (MDL_s) – Initial MDL_s should be established for Anions using a spiked water sample, typically two to ten times the estimated MDL and no more than ten times higher than the estimated MDL. Process a minimum of seven spiked samples and seven blank samples through all steps of the method. The samples used for the MDL must be prepared and analyzed in at least three batches on three separate calendar dates. If multiple instruments are used for this analysis, MDL_s must include data/calculations from all instruments.

9.2.4.1 Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.

9.2.4.2 Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

9.2.4.3 Compute the MDL_s (the MDL based on spiked samples) as follows:

$$MDL_s = t_{(n-1,\ 1-\alpha=0.99)} S_S$$

Where:

MDL_s = the method detection limit based on spiked samples

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = the Student's t-value appropriate for a single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom.

S_S = sample standard deviation of the replicate spiked sample analyses.

9.2.4.4 Compute the MDL_b (the MDL based on method blanks) as follows:

If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) commonly observed when a peak is not present in chromatographic analysis.

If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99^{th} percentile of the method blank results. For "n" method blanks where $n \geq 100$, sort the method blanks in rank order. The (n * 0.99) ranked method

blank result (round to the nearest whole number) is the MDL_b. For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are ... 1.5, 1.7, 1.9, 5.0, and 10, then 164 x 0.99 = 162.36 which rounds to the 162nd method blank result.

Therefore, MDL_b is 1.9 for n =164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result). Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.

If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$MDL_b = \bar{X} + t(n-1, 1-\alpha=0.99)S_b$$

Where:
MDL_b = the MDL based on method blanks
$\bar{X}$ = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = the Student's t-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with n-1 degrees of freedom.
S_b = sample standard deviation of the replicate method blank sample analyses.

9.2.4.5 The verified MDL is the greater of the MDL_s or MDL_b. If the verified MDL is within 0.5 to 2.0 times the existing MDL and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the Initial MDL determination with six degrees of freedom.)

9.2.4.6 The MDL should be determined annually, whenever there is a significant change in instrumental response or a significant change in instrument configuration. Data for annual MDL calculation and verification is analyzed at least quarterly, throughout the year.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of Nanopure water treated the same as the samples. Analyte found in LRB indicates possible reagent or laboratory environment contamination. LRB data are used to assess and correct contamination from the laboratory environment

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning of the run and every batch, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3*s* of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of the MDL or continuing with the analyses. The results of these QCS/SRM samples shall be used to determine batch acceptance.

9.3.3 The QCS are obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4 Control Charts – The Accuracy Control Chart for QCS/SRM samples is constructed from the average and standard deviation of the 20 most recent QCS/SRM measurements. The accuracy chart includes upper and lower warning levels (WL=±2*s*) and upper and lower control levels (CL=±3*s*). These values are derived from stated values of the QCS/SRM. The standard deviation (*s*) is specified relative to statistical confidence levels of 95% for WLs and 99% for CLs. Enter QCS/SRM results on the chart each time the sample is analyzed

9.3.5 Calibration Verification, Initial and Continuing (ICV/CCV) – Immediately following calibration (ICV) and following every 10 samples (CCV), one calibration verification of a CRM falling within the middle of the curve is analyzed to assess instrument performance. The CCVs are to be within the expected value ± 3*s*. Failure to meet the criteria requires correcting the problem, including reanalysis of any affected samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery - Percent Recovery

9.4.1 Analyte recovery is assessed through percent recoveries of laboratory spikes of samples.

9.4.2 Percent Recovery = (Actual value/Expected value) X 100.

9.5 Assessing Analyte Precision – Relative Percent Difference

9.5.1 Analyte replication is assessed through duplicate analyses of samples – Relative Percent Difference.

9.5.2 RPD = (‖Laboratory Duplicate Result 1 – Laboratory Duplicate Result 2)‖/[(Laboratory Duplicate Result 1 + Laboratory Duplicate Result 2)/2] X 100

9.6 Corrective Actions for Out of Control Data

9.6.1 Control limit – If one measurement exceeds Accuracy Control Chart CL, repeat the analysis immediately. If the repeat measurement is within the CL, continue analyses; if it exceeds the CL, discontinue analyses and correct the problem.

9.6.2 Warning limit – If two out of three successive points exceed Accuracy Control Chart WL, analyze another sample. If the next point is within WL, continue analyses; if the next point exceeds the WL, evaluate potential bias and correct the problem.

9.6.3 Trending – If seven successive Accuracy Control Chart measurements are on the same side of the central line, discontinue analyses and correct the problem.

9.6.4 When external QCS samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.6.5 When external CCV samples are out of control, correct the problem. Reanalyze the samples analyzed between the last in-control measurement and the out-of-control one.

9.7 General Operation - To assure optimal operation and analytical results, the Reagent Blank and CCV are tracked daily in the raw data file, copied to Reagent Blank and CCV Control Charts.

Table 2

QC Indicator	Acceptance/ Action Limits	Action	Frequency (Batch)
Correlation Coefficient	≥ 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.
Quality Control Sample (QCS)/ Certified Reference Material (CRM)	± 10%	If QCS value is outside □ 10% of the target value reject the run, correct the problem and rerun samples.	Beginning of run and every 20 samples.
Initial Calibration Verification (ICV)	± 10%	Recalibrate if outside acceptance limits.	Beginning of run following standard curve.

Continuing Calibration Verification (CCV)	± 10%	If outside 10%, correct the problem. Rerun all samples following the last in-control CCV.	After every 20 samples.
Method Blank/Laboratory Reagent Blank (LRB)	≤ Method Quantitation Limit	If the LRB exceeds the quantitation limit, 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 and after every 20 samples following the CRM.
Laboratory Fortified Sample Matrix Spike	± 10%	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.	1/10 (Spike or duplicate)
Laboratory Duplicate	±10%	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.	1/10 (Spike or duplicate)

10. CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Six or seven point calibrations are used with the calibrations that cover the analytical range. The following outlines the preparation of the working standards.

10.2 Chloride Working Standards-

In a 100 mL volumetric flask add the corresponding volume of stock standard from Table 3 in ~40 mL reagent water. Dilute to 100 mL with reagent water. Write name of preparer, preparation date, standard

manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 3 months. Store at 4º C.

Table 3

Chloride Stock Standard Volume (mL)	Chloride Concentration (mg/mL)
0.5	5.0
1.0	10.0
2.0	20.0
3.0	30.0
4.0	40.0
10.0	100.0
20.0	200.0

10.3 Sulfate Working Standards-

In a 100 mL volumetric flask add the corresponding volume of stock standard from Table 4 in ~40 mL reagent water. Dilute to 100 mL with reagent water. Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 3 months. Store at 4º C.

Table 4

Sulfate Stock Standard Volume (mL)	Sulfate Concentration (mg/mL)
0.5	5.0
1.0	10.0
2.0	20.0
3.0	30.0
4.0	40.0
10.0	100.0
20.0	200.0

10.4 Bromide Working Standards –

In a 100 mL volumetric flask add the corresponding volume of stock standard from Table 5 in ~40 mL reagent water. Dilute to 100 mL with reagent water. Write name of preparer, preparation date, standard manufacturer, manufacturer lot number in the Analytical Standard log book. Make fresh every 3 months. Store at 4º C.

Table 5

Bromide Stock Standard Volume (mL)	Bromide Concentration (mg/mL)
0.00625	0.0625
0.0125	0.125
0.025	0.25
0.05	0.50
0.10	1.00
0.20	2.00

10.2 The instrument prepares a standard curve for each set of calibrators. A graph plotting measured µS*min against standard concentration is presented. One standard value for each and every standard is incorporated in the curve. The coefficient of determination (Pearson's r value) for the calibration curve as well as the calculated concentration of each calibrator is reviewed. The calculated value of each calibrator must be within ten percent of the expected value. The coefficient of determination (Pearson's r value) for the calibration curve must be greater than 0.995.

11. PROCEDURE – DAILY OPERATIONS QUALITY CONTROL

11.1 Instrument constantly runs in idle mode. Initiate Chromeleon 7.2 software.
11.2 In the software on the Home tab turn off the pump flow, eluent generator and suppressor by clicking the toggle switch next to each module.
11.3 Discard any water remaining in the eluent reagent bottle from the previous analytical run. Fill the eluent reagent bottle with fresh reagent water.
11.4 Discard any water remaining in the syringe bottle from the previous run. Fill the syringe bottle with fresh reagent water.
11.5 Prime the pump by opening the purge valve ¼ turn.
11.6 Under the instrument tab in the software, in UMD ICS5000 Anions tab under Pump 1 tab prime pump for at least 500 seconds at a flow rate of 5.00 mL/min, click prime button to initiate pump prime.
11.7 Once prime is completed close purge valve.
11.8 Under the home tab turn pump on and set flow rate at 0.250 mL/min.
11.9 Under the home tab turn on eluent generator and set concentration to 10mM KOH.
11.10 Under the home tab turn on suppressor and set current at 13mA.
11.11 Temperature controls should remain on.
11.12 Check the pressure under the pump_1 tab. Pressure must be at ~2000psi otherwise the pump will shut off.
11.13 Under the sampler tab prime syringe at least 20 cycles to ensure no bubbles are in the syringe and water line.
11.14 Allow instrument to equilibrate for one hour.
11.15 After one hour, under the CD-Left tab, total signal should be at ~0.300 µS.
11.16 Gather working standards from refrigerator. Assess standards and remake anything that has exceeded the time over which it is considered stable.
11.17 Begin daily bench sheet documentation.
11.18 Check salinity values for each sample. Based on the salinity values the sample may require dilution.

Table 6

Salinity Value (ppt)	Dilution Factor
0.3-0.4	X2
0.5-1.75	X5

1.76-3.9	X10
>3.9	X20

11.19 Load working standards and samples into clean sample rinsed 10mL auto sampler vials.

11.20 Under the sampler tab tray controls, remove each tray from instrument and load each tray with standards and samples. Instrument is loaded as follows: standards, CRM, blank then samples. Throughout the analytical batch, samples are chosen as laboratory duplicates and laboratory spikes to assess analyte precision and analyte recovery, respectively. Insert corresponding trays back into instrument.

11.21 Under the Data tab in the Anions folder for 2019 create a sequence from the menu bar. A window will pop up click on Anions, enter the number of samples and name the folder the analysis date.

11.22 Copy and paste the Instrument Method, Processing Method, and Report Template from a previous sequence into the sequence just created.

11.23 Under the new sequence in the name column enter the standards and the names of the samples loaded. Instrument is loaded as follows: Two conditioning blanks, standards, CRM, blank then samples. Standard Reference Material (SRM) samples are analyzed every 20 samples, and Laboratory Reagent Blanks (LRB) are analyzed every 20 samples following CRM.

11.24 In the instrument method column select the 10-45mMGrad_1 method. Copy down for all.

11.25 In the processing method column select Anions_1. Copy down for all.

11.26 The last injection should be water blank and the instrument method should be LowFlow20Mm_Iso.

11.27 In the type column for the standards change the drop down selection to read calibration standard. All other samples should remain as unknown.

11.28 In the level column for the standards change the drop down selection to one for the lowest standard through seven for the highest standard.

11.29 In the position column enter the tray position that each standard and sample corresponds with.

11.30 Volume column should be set at 10µL.

11.31 Save run.

11.32 Click start.

11.33 As calibration curves are produced by the instrument, review them for acceptability. The instrument software prepares a standard curve for each Anion. A graph plotting measured µS*min against standard concentration is presented. If acceptance criteria are not met the entire curve shall be reanalyzed. One standard value for each and every calibrator is incorporated in the curve.

11.34 If results fall outside acceptance limits, the sample should be reanalyzed. If sample result exceeds the highest standard of the highest calibration range, the samples will need to be diluted and reanalyzed.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Upon completion of all analysis, under the Report Designer tab print Summary Report. Under the Electronic Report tab export Peak integration Report, Calibration Report, and Summary Report. Results are exported to a DATA folder on the desktop. The file is named by the analysis date. The report file for analytical batch of January 1, 2017 would be named 010117. The file is saved as a PDF file.

12.2 The instrument software has calculated final sample concentration from the designated standard curve. Dilution will require further calculation before the data can be finalized. The analyst examines each row of data. Results are eliminated that are outside the limits of the calibration range.

13. REFERENCES

13.1 Parsons, T.R.,Y. Maita and C.M. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis, Pergamon Press, Elmsford, N.Y.

13.2 Kerouel, R. and A. Aminot. 1987. Procédure optimisée horscontaminations pour l'analyze des éléments nutritifs dissous dans l'eau de mer. Mar. Environ. Res 22:19-32.