Maryland Department of Natural Resources
Non-tidal Network Program
Nutrient and Sediment Load Trend Monitoring
Quality Assurance Project Plan
October 1, 2019 – September 30, 2020
Section 117(e)

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Preface

Maryland's Non-tidal Water Quality Monitoring Network began in July 2005 with funding from EPA Section 117(d). The network, presently, is composed of 24 sites where nutrient and sediment concentrations are sampled on a fixed monthly basis and eight times per year during high flow events. All sampling sites are located near a USGS stream gage, have a nearby bridge from which high flow samples may be obtained, are located at the outlet of major basins and represent watersheds with relatively high loads of nutrients and sediments.

Maryland's network was created as part of a coordinated effort, conducted by the Chesapeake Bay Program's Integrated Monitoring Networks Workgroup, to include all of the Chesapeake Bay watershed states in a network of stations with comparable collection and analysis protocols. Data from the Non-tidal Network will be used to estimate nutrient and sediment loads and trends in concentration for watershed management assessment purposes and for input to the CBP Watershed Model.

It is anticipated that the Non-tidal Network may be expanded spatially and upgraded as more funding becomes available.

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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's Monitoring Subcommittee – formerly AMQAW)
DNR - Maryland Department of Natural Resources
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
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
^oC - degrees Celsius

Distribution List

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Renee Karrh, MDNR
Shahla Ameli, MDH
Scott Phillips, USGS

PROJECT MANAGEMENT

A4 Project/Task Organization

This section lists the individuals and organizations responsible for the major aspects of the Maryland Non-tidal Network Program. The flow of project tasks is indicated in Figure 1.

Director and Principal Investigator: Bruce Michael, Resource Assessment Service, DNR. 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, implementation, and data analysis of the program.

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

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

Field Sampling Operations: Kristen Heyer, Environmental Program Manager, Annapolis Field Office. Monitoring and Non-tidal Assessment, DNR. 410-990-4600, kristen.heyer@maryland.gov

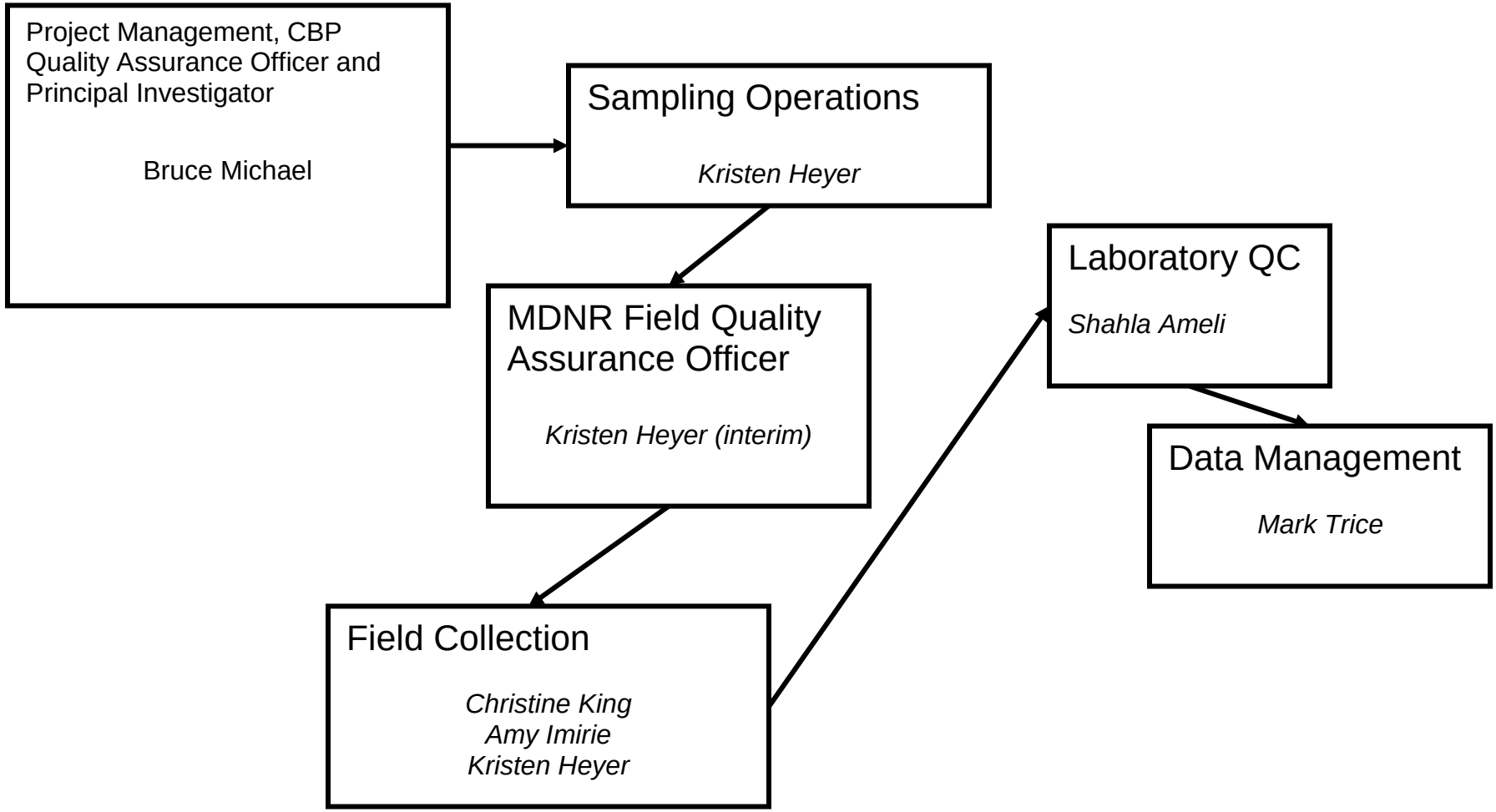
Responsibilities: This individual is responsible for administration of the field sampling activities including sample collection, sample storage, and sample delivery to laboratories.

Laboratory Analyses/Water Column Chemistry: Shahla Ameli, Laboratory Supervisor, MDH, Environmental Sciences Division. 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 project.

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

Figure 1. Organization Chart for the Maryland 117(e) Non-tidal Network Project



Responsibilities: This individual 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.

Sediment Analysis: Thomas Jeffords. USGS Indiana-Kentucky Water Science Center. 502-493-1916, tjeffords@usgs.gov .

Responsibilities: Determine suspended sediment concentration and sand/fine fractions on high flow event samples.

A5 Project Definition/Background

The Chesapeake Bay Program, under the Chesapeake 2000 agreement, is committed to reduce nutrient and sediment inputs into Chesapeake Bay. Nutrient and sediment allocations have been developed for tributary basins within the Bay watershed. This project is part of the cooperative effort of the Chesapeake Bay Program Integrated Monitoring Networks Workgroup to provide comparable data to assess progress in meeting nutrient and sediment reduction goals to meet water quality criteria in the Chesapeake Bay.

The main objectives of this monitoring program are to improve measurement of nutrients and sediment concentrations for the calculation of loads discharged to the Chesapeake Bay watershed, improve the accuracy of the watershed model, and help identify factors affecting nutrient and sediment loads. The project requires all participants to collect surface water samples by a method that generates horizontally and vertically integrated composite samples. This standard USGS collection method should provide data that better represents the concentration of nutrients and sediments. Since this project will specifically collect samples from eight storm events per year per station, our estimates of sediment loads contributed by Maryland's ten tributary strategy basins should be greatly improved.

Historically, Maryland's collection method at non-tidal sites was a single point grab sample. We will continue to collect a single point grab sample under our CORE/TREND network at historically collected locations. CORE/TREND sampling is conducted at co-located stations on different dates than the non-tidal network sites. We will collect an integrated sample every month under the Non-tidal Network using the USGS isokinetic sampling device if maximum velocity is 1.5 ft/sec or higher. A review of USGS discharge records indicates that for the majority of our stations, base and maximum flow is normally under 1.5 fps. Under low velocity conditions, a horizontally and vertically integrated composite sample will be generated. We anticipate that we will only collect isokinetic, equal-width increment samples during significant storm events.

Monthly measurements of in-situ parameters have also indicated that all sites except WIL0013 are well mixed. Since most of our sites are less than 110 feet wide, are 3 feet or less in depth and have a velocity under 1.5 fps, our historical data set may be a

reasonable representation of dry weather conditions. When using the historical data, discarding dates with an elevated discharge reading would provide data that are more representative of baseflow conditions.

Monthly sampling is performed at all sites. Sites classified as “primary” have, in addition to monthly sampling, approximately 8 samples taken per year during high flow events (defined in this project as at least a two-fold increase in flow). These additional “storm” samples provide site-specific information on the relationship between discharge and concentration necessary for load estimations. Site selection for both primary and supplemental (monthly sampling only) was based upon 1) the presence of an operating stream gage, 2) sites located at the outlet of a tributary strategy basin, 3) sites representing high load watersheds, 4) watershed area, and 5) the presence of a bridge for sampling during high flow events.

A6 Project Task/Description

Sample collection and analysis began in July 2005 and is expected to continue for at least five years provided there is continued funding from federal and state agencies. The network currently contains 24 primary (load) sites (Table 1). Routine (pre-determined date) monthly sampling is conducted at all sites. Eight samples collected during high flow conditions will be taken at all sites each year, preferably two storms per season. Storm events are targeted because large amounts of nutrients and sediments are moved during high flows. Routine sampling is conducted over a wide range in flow conditions to provide unbiased load calculations. Sites in the Maryland CBP Non-tidal network are shown on Figure 2.

Depth integrated samples will be taken at equal width intervals and composited at all sites any time the site is sampled for the non-tidal network. Parameters and analytical methods are listed in Table 2. An additional sample will be taken from the churn sample splitter for sediment concentration any time high flow conditions are sampled. A sand/fine analysis will be performed on the sediment sample on a quarterly basis.

The data collected under this project will be stored on a Maryland DNR server and submitted annually to the CBP for inclusion in the non-tidal network water-quality database of the Chesapeake Information Management System (CIMS). U.S. Geological Survey (USGS) staff will combine the concentration data collected by DNR with stream flow data to calculate nutrient and sediment loads using the USGS WRTDS model. USGS will calculate loads on an annual basis after enough years of data have been collected to accurately estimate the model parameters predicted by WRTDS. The loads can be used in CBP indicators and to demonstrate any improvements or deterioration in water quality to the public for the non-tidal network sites.

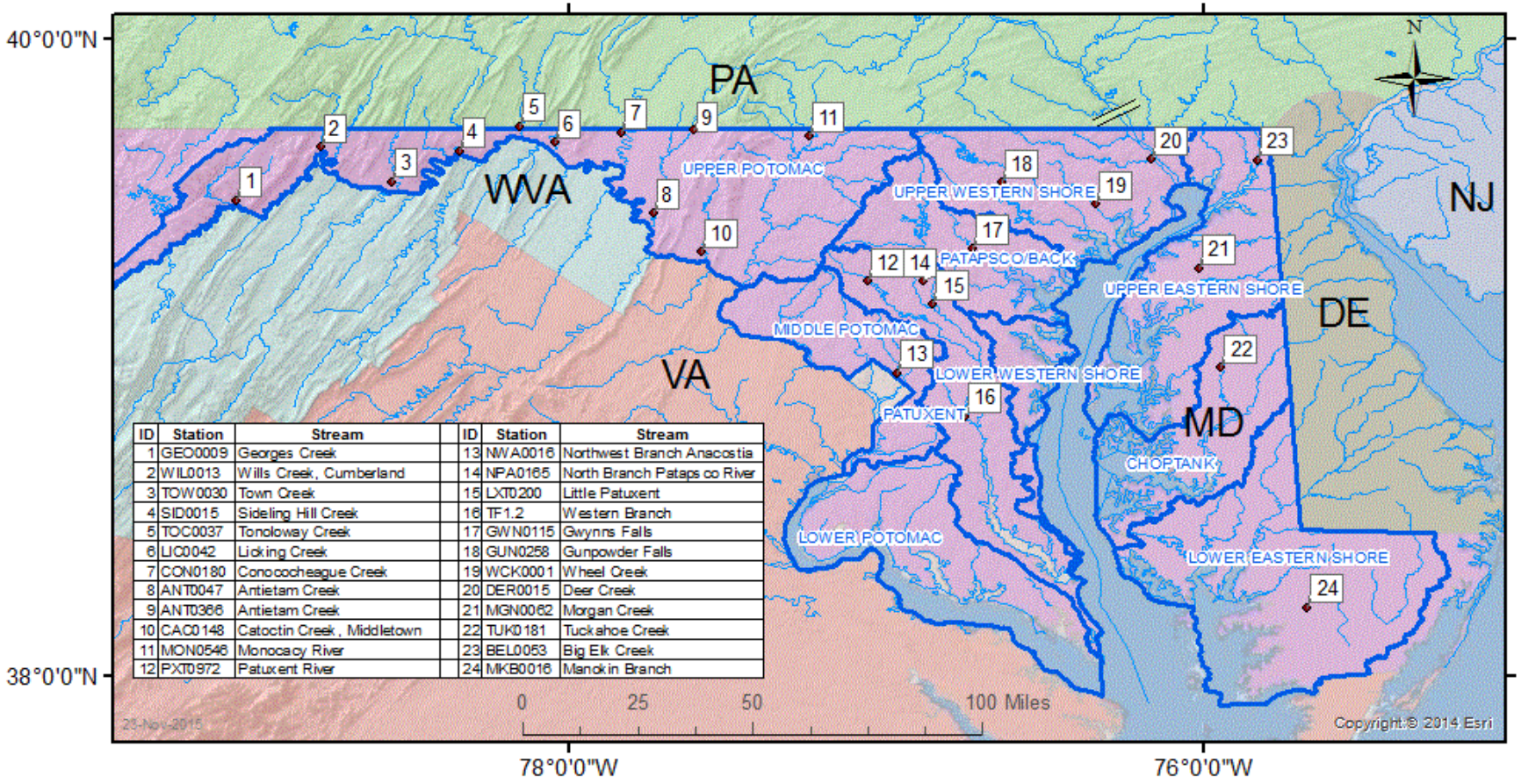
TABLE 1. Maryland CBP Non-tidal Network Program

Map #	MDNR Station ID	Stream Name	Lat (NAD83)	Long (NAD83)	Description of Sampling Location	USGS Gage #	Network Station Type
1	GEO0009	Georges Creek	39 29.6183083	079 02.6819417	Victory Street in Westernport, MD	01599000	Primary
2	WIL0013	Wills Creek	39 39.7110433	078 46.8174567	Locust Grove Road crossing Wills Creek in Cumberland, MD	01601500	Primary
3	TOW0030	Town Creek	39 33.1933333	078 33.3	Pack Horse Road near Oldtown, MD	01609000	Primary
4	SID0015	Sideling Hill Creek	39 38.9716667	078 20.6483333	Ziegler Raod near Bellegrove, MD	01610155	Primary
5	TOC0037	Tonoloway Creek	39 42.3816667	078 9.165	Timber Ridge Road near Hancock, MD	01613095	Primary
6	LIC0042	Licking Creek	39 40.5766667	078 2.51666667	Pecktonville Road, Pectonville, MD	01613525	Primary
8	ANT0047	Antietam Creek	39 27.240000	077 43.965	Burnside Bridge Road near Sharpsburg	01619500	Primary
9	ANT0366	Antietam Creek	39 42.975	077 36.3983333	Millers Church Road near Waynesboro, PA	01619000	Primary
10	CAC0148	Catoctin Creek	39 25.6212	077 33.3708	At bridge on MD 17, Middletown MD	01637500	Primary
11	MON0546	Monocacy River	39 41.7870000	077 14.368000	Bullfrog Road crossing the Monocacy	01639000	Primary
12	PXT0972	Patuxent River	39 14.3584867	077 03.3713467	At bridge on MD 97 near Unity	01591000	Primary
13	NWA0016	NW Branch Anacostia River	38 57.14	076 57.963333	Pedestrian Bridge, Queens Chapel Rd near Hyattsville,MD	01651000	Primary
14	NPA0165	North Branch Patapsco River	39 28.9671333	076 52.9250800	Bridge near gage, Cedarhurst, MD	01586000	Primary
15	LXT0200	Little Patuxent	39 10.065	076 51.075	Guilford Road near Guilford, MD	01593500	Primary
16	TF1.2	Western Branch	38 48.8580017	076 45.05207	At bridge on Water St. in Upper Marlboro	01594526	Primary
17	GWN0115	Gwynns Falls	39 20.5671783	076 43.5833000	At bridge on Essex Road in Villa Nova	01589300	Primary
18	GUN0258	Gunpowder Falls	39 33.0386351	076 38.1520258	Glencoe Road near Sparks,MD	01582500	Primary

TABLE 1. Maryland CBP Non-tidal Network Program (continued)

Map #	MDNR Station ID	Stream Name	Lat (NAD83)	Long (NAD83)	Description of Sampling Location	USGS Gauge #	Network Station Type
19	WCK0001	Wheel Creek	39 28.903333	076 20.431667	Near Abingdon, MD	0158175320	Primary
20	DER0015	Deer Creek	39 37.4085651	076 09.8863317	Stafford Road near Darlington MD	01580520	Primary
21	MGN0062	Morgan Creek	39 16.801667	076 0.873333	Perkins Hill Road near Kennedyville, MD	01493500	Primary
22	TUK0181	Tuckahoe Creek	38 58.0280000	075 56.5870000	Tuckahoe Creek at Crouse Mill Rd.	01491500	Primary
23	BEL0053	Big Elk Creek	39 37.2870000	075 49.7160000	Big Elk Creek at Rickett's Mill Road	01495000	Primary
24	MKB0016	Manokin branch	38 12.833333	075 40.283333	Near Princess Anne, MD	01486000	Primary

Figure 2. Maryland DNR non-tidal network nutrient and sediment load sites and tributary strategy basins.
Note: #7- CON0180 no longer being sampled



ID	Station	Stream	ID	Station	Stream
1	GEO0009	Georges Creek	13	NWA0016	Northwest Branch Anacostia
2	WIL0013	Wills Creek, Cumberland	14	NPA0165	North Branch Patapsco River
3	TOW0030	Town Creek	15	LXT0200	Little Patuxent
4	SID0015	Sideling Hill Creek	16	TF1.2	Western Branch
5	TOC0037	Tonoloway Creek	17	GWN0115	Gwynns Falls
6	LIC0042	Licking Creek	18	GUN0258	Gunpowder Falls
7	CON0180	Conococheague Creek	19	WCK0001	Wheel Creek
8	ANT0047	Antietam Creek	20	DER0015	Deer Creek
9	ANT0366	Antietam Creek	21	MGN0062	Morgan Creek
10	CAC0148	Catoctin Creek, Middletown	22	TUK0181	Tuckahoe Creek
11	MON0546	Monocacy River	23	BEL0053	Big Elk Creek
12	PXT0972	Patuxent River	24	MKB0016	Mankin Branch

TABLE 2. Parameters and Analytical Methods for the Chesapeake Bay Non-tidal Water-Quality Network

Parameters/Units	Holding Time and Condition	Method/ Reference	Method Detection Limit
Field (In situ)			
Temperature, ^{O}C	< 5 min.	Standard Methods 2550 B	0.1 ^{O}C
Dissolved Oxygen, mg/L	< 5 min.	Clark cell Standard Methods 4500-OG-2001 Hach LDO Method 10360	0.2 mg/L
pH	< 5 min.	Standard Methods 4500-H^{+}	0.1 units
Specific Conductance, umhos/cm	< 5 min.	Standard Methods 2510	1 umhos/cm
Laboratory			
Total Nitrogen, mg/L as N	Calculated	PN + TDN	N/A
Ammonium, mg/L as N	Frozen, 28 days/ 4 ^{O}C, 48 hrs.	EPA Method 350.1	0.0016 mg/L
Nitrite, mg/L as N	Frozen, 28 days/ 4 ^{O}C, 48 hrs.	EPA Method 353.2	0.0005 mg/L
Nitrate + Nitrite, mg/L as N	Frozen, 28 days/ 4 ^{O}C, 48 hrs.	EPA Method 353.2	0.0052 mg/L
Total Phosphorus, mg/L as P	Calculated	PP+TDP	N/A
Orthophosphate, mg/L as P	Frozen, 28 days/ 4 ^{O}C, 48 hrs.	EPA Method 365.1	0.0005 mg/L
Total Suspended Solids, mg/L	4 ^{o}C 7 days	Standard Methods 2540D	0.7 mg/L
Suspended Sediment (storms)	Dark Room 120 days	ASTM D3977-97(C)	0.5 mg/L
Sand/Fine Particles (storms)	Dark Room 120 days	ASTM 3977-97(C)	0.5 mg/L
Dissolved Organic Carbon, mg/L as C	Frozen, 28 days/ 4 ^{O}C, 48 hrs.	Standard Methods 5310B	0.0517 mg/L
Total Dissolved Phosphorus, mg/L as P	Frozen, 28 days/ 4 ^{O}C, 48 hrs.	EPA Method 365.1	0.0054 mg/L
Total Dissolved Nitrogen, mg/L as N	Frozen, 28 days/ 4 ^{O}C, 48 hrs.	EPA Method 353.2	0.0223 mg/L
Particulate Carbon, mg/L as C	Frozen 28 days	Exeter Analytical Model CE-440 Elemental Analyzer	0.032 mg/L
Particulate Nitrogen, mg/L as N	Frozen 28 days	Exeter Analytical Model CE-440 Elemental Analyzer	0.006 mg/L
Particulate Phosphorus, mg/L as P	Frozen 28 days	EPA Method 365.1	0.0005 mg/L

A7 Quality Objectives and Criteria

The Non-Tidal Network is designed to provide laboratory and field data that will help the state characterize water quality in headwater areas that affect the Chesapeake Bay. Assessment of the quality of the data collected through the program can be expressed in terms of representativeness, completeness, comparability, accuracy, and precision.

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 calls for monthly sampling on a pre-determined date, which is adequate for capturing long-term annual trends in concentration. Eight additional samples taken during high flow events are intended to determine the relationship between stream discharge and the parameters of interest so that annual loads may be calculated for primary sites.

Depth integrated samples using an isokinetic sampler or a weighted bottle sampler will be used at equal width intervals, the number of which is determined from Table 3. The type of sampler used is dependent upon flow velocity with the isokinetic sampler being used if the centroid flow velocity $\geq$ 1.5 ft/sec. Depth integrated samples are composited (i.e. combined for each width interval in a churn sample splitter). Sample bottles for nutrient and sediment analysis are filled from the churn splitter.

Table 3. Minimum Number of Vertical Samples at Primary Stations
(Routine and storm event samples)

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

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 Non-Tidal Network program must also be of comparable quality to the data generated by other states and laboratories participating in the program. Comparability among data sets is assured through the use of consistent field methods and protocols, participation in the Data Integrity Workgroup (formerly AMQAW), 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 the 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.

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 support Chesapeake Bay Program management decisions, Maryland's non-tidal network monitoring program strives to provide monitoring data of known and consistent quality to the CBPO 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 for field and lab measurements of <20 percent of the coefficient of variation; accuracy goals within 80 to 120 percent, and the completeness goals of 90 percent.

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. 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 the Department of Health's (MDH) participation in the Chesapeake Bay Coordinated Split Sample Program (CSSP), a split sampling program in which five 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. MDH follows the protocols in the *Chesapeake Bay Coordinated Split Sample Program Implementation Guidelines, Revision 4* (EPA, 2010) and its revisions. This document may be found on the Chesapeake Bay Program web site: (http://archive.chesapeakebay.net/pubs/quality_assurance/CSSP_Guidelines_12-17-10.pdf). Split samples are collected quarterly. Results are analyzed by appropriate statistical methods to determine if results differ significantly among labs. When a difference occurs, discussion begins regarding techniques and potential methods changes to resolve discrepancies and identify potential problems.

Additionally, MDH will participate two times per year in the United States Geological Survey (USGS) Standard Reference Sample Program and will permit USGS to release

the results to the Chesapeake Bay Program Quality Assurance Officer. Laboratory accuracy is 90-110% recovery.

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 analyzes and provides results on the corresponding request forms for the field duplicates. MDH performs precision calculations for laboratory duplicates, but not for the field 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 duplicates. 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.

A8 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 Standard Operating Procedures (Appendix A). Training for field personnel who collect samples for the Non-Tidal Network monitoring program was provided in a two-day USGS/CBP sponsored workshop on techniques required for obtaining a representative sample of nutrient and suspended sediment concentration in Harpers Ferry, WV in September 2004.

Training of personnel at MDH is conducted in the laboratory. Each new analyst is trained on the laboratory procedures he or she will be assigned to perform by an experienced analyst. Training is documented using the Division of Environmental Chemistry's training forms and signed by the Supervisor and the Division Chief.

A9 Documentation and Records

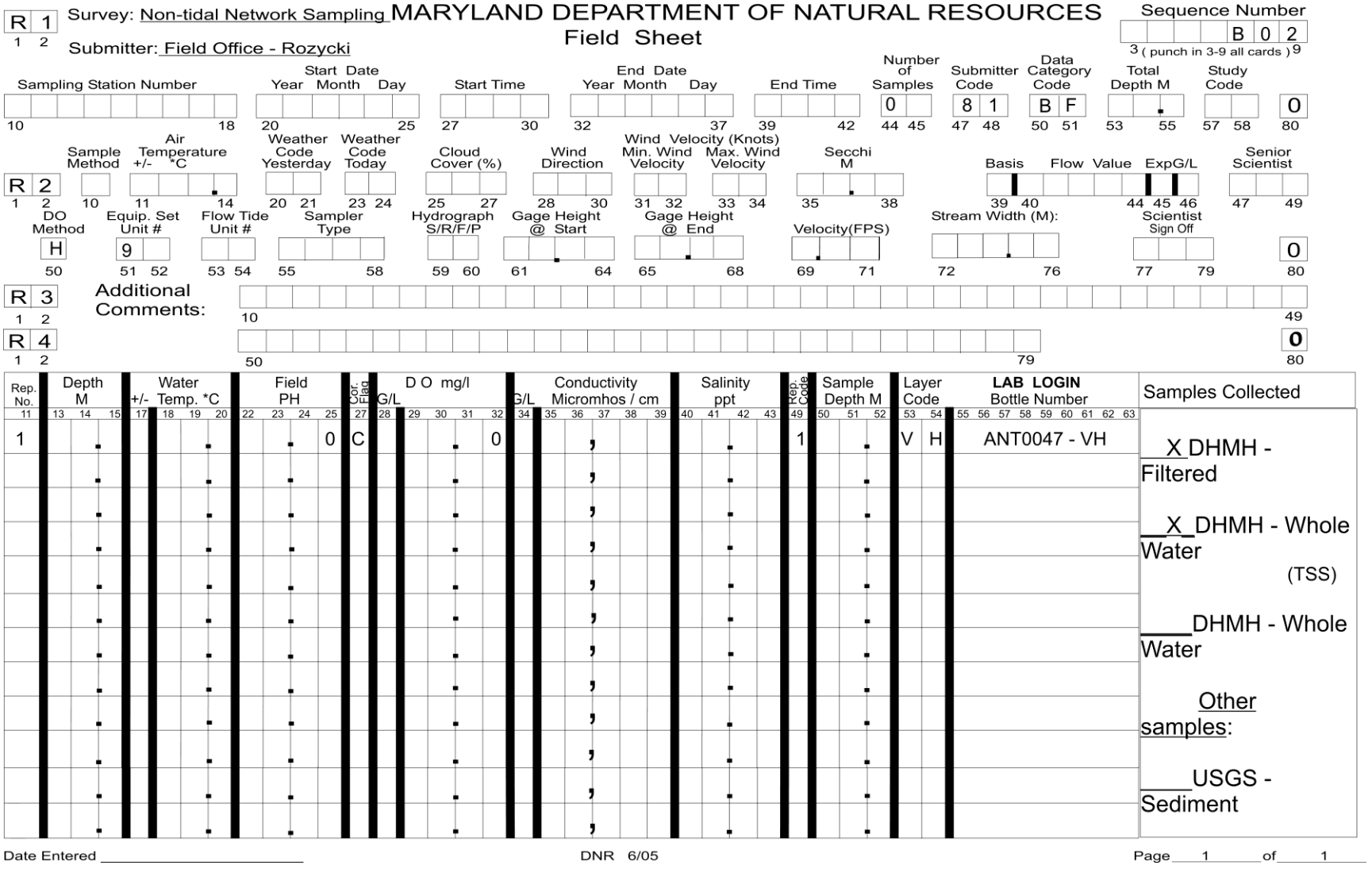
Documenting sampling events is an important component of the Non-Tidal Network monitoring program. Field crews document all data obtained in the field on field sheets (Figure 3). Since the data generated by this program are not used for legal purposes, a formal chain of custody sheet is not required. Field sheets and any information concerned with specific problems and/or events during a sampling run, as well as modifications to the sampling program will be maintained by field office staff.

The water quality monitoring field sheet is completed on arrival at each station. This form is used to record sample collection depth, weather, flow, field measurements (pH, water temperature, dissolved oxygen, and conductivity) and data pertinent to the

collection of samples such as type of sample, and date and time of collection. A field notebook 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 (c.f. distribution list). The QAPP will be updated annually. Any modifications to the QAPP will be reviewed and approved by the personnel conducting the sampling, the principal investigators and DNR's Quality Assurance Officer. Once approved, the final version will be made available to all interested parties by placing downloadable copies in the Chesapeake Bay Program's and DNR's websites. Project reporting to management will be accomplished by semi-annual progress reports of activities. Electronic summaries of provisional instantaneous water quality data will be provided on an annual basis.

Figure 3. Non-tidal Network Field Sheet



DATA GENERATION AND ACQUISITION

B1 Sampling Process Design

Sampling locations are described in Table 1 and shown on Figure 2. In order for a site to be considered for the non-tidal network program, it needed to: 1) have an operating stream gage, 2) represent tributary strategy basins, 3) represent watersheds with high nutrient and sediment loads, 4) be among Maryland's larger watersheds, and 5) have a safe bridge from which storm samples could be taken. Stations from DNR's historical network were reviewed to see if they met these criteria and stations in under-sampled regions of the state were also considered. Data are available for the ten initial primary sites (ANT0047, BEL0053, GWN0115, DER0015, NPA0165, TF1.2, MON0546, TUK0181, WIL0013, and GEO0009) starting July 2005. Three supplemental sites were added in October 2005 (GUN0258, PXT0972, and CAC0148). The three supplemental sites all have the same attributes as primary sites and were upgraded to primary sites with storm event sampling in January 2007. Five new primary sites (MKB0016, MGN0062, WCK0001, NWA0016, LXT0200) were added in October 2011. Three additional sites (CVA0046, WIL0065, and 01636500) were added to the non-tidal network in the spring of 2012 following receipt of Chesapeake and Atlantic Coastal Bays Trust Fund money. Sampling at the three Trust Fund sites ended in July 2015 due to a lack of funding. In October 2014, six sites (CON0180, TOC0037, TOW0030, SID0015, LIC0042, ANT0036) were transferred from PA Department of Environmental Protection (PA DEP) to DNR. The six PA DEP sites have been sampled since July 2005 with the exception of CON0180 which was dropped in October 2018. A comparison study was conducted in 2015 to compare laboratory results between MDH and PA DEP labs. Given the nature of this project, the only matrix sampled at these stations is water. The parameters of interest are presented in Table 2.

Data were collected at the ten initial DNR primary sites starting in January 2005; however, data from January 2005 through June 2005 are not included in the data base due to concerns that a step trend could be introduced following a laboratory methods change that occurred in July 2005.

Routine monitoring (pre-determined date) is conducted on a monthly basis to obtain data over a range of flows. Eight storm event samples are collected per year at each station because the highest nutrient and sediment loads are generated during high flows. Ideally, two storm event samples are collected in each season. This sampling protocol was designed to provide adequate data for use in the U.S. Geological Survey WRTDS model, which is used to calculate loads and trends.

B2 Sampling Methods

Sampling protocols for the Maryland non-tidal network can be found in the Maryland *Non-Tidal Network Program Standard Operating Procedures* (Department of Natural Resources, 2019) (Appendix A). Routine and storm event samples are collected using a modified version of the U.S. Geological Survey equal width interval method that reduces the number of vertical samples from ten to an odd number based on the width of the stream being sampled (Table 3).

Routine samples: Equal width interval, depth integrated samples are taken monthly at primary sites. Vertical sampling is done with a DH-81 if the stream is wadable or a DH-95 (a DH-59 was used in 2005) where samples are accessible only from a bridge. A weighted bottle, WBH-96, is used when the velocity is less than 1.5 ft/second. Samples are composited in a 4- or 8-liter churn splitter from which subsamples are drawn. A single whole water sample bottle is drawn and sent to MDH for TSS analysis. A second whole water sample bottle is drawn and field processed for dissolved nutrients and particulate analysis. A sample is also collected for total suspended sediment analysis and sent to the USGS Sediment Laboratory in Kentucky if routine sample collection occurs during a high flow event.

Storm samples: Sampling during high flow events is accomplished from a bridge by use of a DH-95 at primary sites. All vertically integrated samples are collected in the churn splitter and, in addition to the TSS, dissolved nutrients and particulate filters for analysis at MDH, a whole water sample is drawn to send to the USGS Sediment Laboratory in Kentucky for suspended sediment analysis.

Field Measurements: Dissolved oxygen, pH, specific conductance and temperature are measured monthly and during high flow events by using a Hydrolab or YSI. Measurements are taken at the center of each equal width interval and the median value is recorded for the sample. Average stream flow during the sampling period, as reported by USGS, is also recorded. See the attached Non-tidal Network Monitoring Program Standard Operating Procedures for more detailed information.

B3 Sample Handling and Custody

With the exception of sediment samples, laboratory samples are placed on ice and transported in coolers from monitoring stations to the field office in Annapolis by Maryland Department of Natural Resources (DNR) Monitoring Program field personnel. Samples are either frozen for later delivery or taken directly to the MDH laboratory by field personnel or left with a courier for delivery to the MDH laboratory. Data sheets accompany these samples to MDH (Figure 4). Sample bottles for sediment analysis are shipped to the USGS Water Science Center Sediment Laboratory in Louisville, KY on an intermittent basis. These data are not used for legal purposes, therefore chain of custody forms are not used.

Note that DNR does not currently acidify samples for ammonium (NH4), nitrate+nitrite (NO23), or dissolved organic carbon (DOC) as described in the Code of Federal Regulations (40 CFR Part 136). DNR has completed an assessment of the effects of

preservation method on NH4 concentration by analyzing samples that have been preserved with sulfuric acid (acidification), freezing, or keeping the samples on ice and delivering them to the laboratory for analysis within 24 hours. The results indicated that acid-preserved field samples are biased low compared to frozen samples and samples kept on ice for analysis within 24 hours. Acid-preserved samples were also biased low compared to known high and known low NH4 concentrations of samples prepared in the laboratory. The results of this study have been documented in a data analysis issues tracking system (DAITS) report (DAITS050). DAITS050 is available on the Chesapeake Bay Program web site (http://www.chesapeakebay.net/). DAITS050 was approved by the Analytical Methods and Quality Assurance Workgroup (now Data Integrity Workgroup), therefore DNR will continue to preserve NH4 samples by keeping them on ice if they can be delivered to the lab for analysis within 24-hours, or by freezing for longer holding times.

DNR has also reviewed data that compared the effect of preservation method on NO23 and DOC. The results of that study are documented in DAITS051, which was approved by the Analytical Methods and Quality Assurance Workgroup (now DIWG) in September 2013. Although a statistically significant difference was detected between iced (the current method) and acid-preserved samples there was far more variability in NO23 concentrations among seasons and stations than among preservation methods. Statistically significant differences were also detected among preservation methods for DOC; however a test of multiple comparisons grouped acid and iced together. Mean differences between acid and iced DOC samples were less than the method detection limit and differences between acid and frozen only slightly exceeded the method detection limit. Given the equivalency between chilling for 24 hours or freezing for longer periods and acid preservation, DNR will continue to use the historic methods.

B4 Analytical Methods

All Hydrolab and YSI instruments are calibrated both prior to and after their use for measuring temperature, pH, dissolved oxygen, and conductivity. All calibration checks are recorded in field logbooks. Laboratory personnel follow EPA guidelines on quality control and quality assurance. Minimum detection limits for field measurements are described in Table 2.

R 5 study code: 0 9 Replicate:

Maryland Department of Natural Resources

Sequence Number

Survey: NTN: Antietam Creek

Collector: _____

Laboratory Analysis Sheet (Non-tidal Network Program)

AFO-410-990-4526/C. Rozycki

Data Code 1B

Sample Station Number: A N T 0 0 4 7

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

Start Depth M: 0 0 | End Depth M | Start Time

Bottle Numbers:

Type of Sample: Whole (1 quart bottle): A N T 0 0 4 7 V H | Filtered (8 ounce bottle): A N T 0 0 4 7 V H | PC/PN/PP Foils: S E P S H E E T 0

R 6 | Sample Method: 6 | Data Category Code: B F | Sample Layer: V H | 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									
	BOD 5 day mg/l	B O D 5									
	Turbidity NTU (W)	T U R B									
	Total Alkalinity (W) mg/l	T A L K									
X	Total Susp. Solids (W) mg/l	T S S									
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									

Date Reported (Year Month Day) | Final Lab sign off | QA/QC sign off | Transcriber sign off | Send Results To:

R 8 | 0

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

Date Entered: _____

DNR 5/2005

Figure 4. Non-tidal Network Laboratory Analysis Sheet

B5 Quality Control

The data collected as part of the Non-tidal Network are used in making management decisions regarding Chesapeake Bay water quality as described in the Introduction. 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. General discussions of quality assurance and quality control aspects associated with accuracy, precision, audits, and bias are provided in the subsections below.

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 five laboratories involved in Chesapeake Bay monitoring analyze the coordinated split samples.

Precision: Precision of the chemical analytical methods is determined and documented from duplicate analyses. 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 samples consist of a set of two samples that are collected and processed so they can be considered identical samples. Field duplicate samples are collected to estimate the reproducibility of water quality samples and to provide additional water quality data. Twenty-four duplicate samples will be processed per water year. Each sample site has a minimum of one duplicate sample per water year. Sixty percent (60%) of the duplicates will be collected as routine samples. The remaining forty percent (40%) will be collected as storm event samples. This breakdown of duplicate sampling mimics the percentages that each site is sampled per water year. The duplicate will be drawn from the same churn splitter as the original sample and processed identically.

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.

Bias: Non-Tidal Network field blanks consist of de-ionized water which is placed in a sample container in the field and treated as a sample in all respects, including exposure to site sampling conditions, processing, preservation, storage, and laboratory analysis. The Non-tidal Workgroup assumes that if a field blank concentration is $\geq$ 10 percent of the associated water quality concentration, the water quality concentration is considered

biased and water quality samples collected on that day will be assigned an analytical problem code of “BM.” Field blanks will be collected in a randomized fashion at a frequency of one per station per year**,** for a total of 24.

Reporting: Quality assurance information for field duplicate samples in the mainstem and tributaries is stored on the routine computerized water quality data sets as replicate observations that can be used to assess precision. Laboratory quality assurance/control information on duplicates and spikes is stored on a computerized data set at the laboratory. To estimate laboratory precision, low concentration field duplicate and blank results should be reported un-rounded to at least three figures. For example, the value 0.005 has only one significant figure and at least three are needed.

B6 Instrument/Equipment Testing, Inspection, and Maintenance

Field crews carry two calibrated Hydrolab or YSI meters 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 under special remarks.

B7 Instrument/Equipment Calibration and Frequency

The procedures outlined here refer to the Hydrolab Series 5 instruments. The detailed calibration procedures will be performed as described in the Hydrolab Operation Instructions Manual.

I. Calibration

A. Set up a calibration logbook for each unit, with make, model, and serial number and purchase date. Assign a letter for DNR 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 any parameter has drifted.

C. Specific conductance calibration shall be made using standards generated by the field office from dry KCl and deionized water. The standard used for sampling the non-tidal waterways of Maryland is 292 microsiemens/cm (microsiemens=microS=ΦS); or 0.002, molar KCl (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. Dissolved Oxygen calibration uses the air-saturated water method. After correcting for barometric pressure and temperature, the oxygen content of the air-saturated water can be checked against standard DO tables. The membrane (YSI) or cap (LDO) is also visually checked every time the meter is pre- or post-calibrated. If any damage is observed, the instrument is pulled for servicing and a replacement instrument is used for sampling (this is not common).

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

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

DNR does not follow the DO probe calibration procedures that are described in Chapter V (Non-tidal water quality monitoring) of the Recommended Guidelines for Sampling and Analysis in the Chesapeake Bay Monitoring Program: (http://www.chesapeakebay.net/committee_analyticalmethodsworkgroup_projects.aspx?menuitem=16701). The DO field measurement protocol calls for a calibration at the beginning and end of each day to ensure there has been no drift in the sensor. DNR conducted a year long analysis of optical DO sensor post-calibration drift data and found that generally there were differences of ≤0.1 mg/L DO between pre- and post-calibration readings even after a week. In 2011, DNR received approval from the Chesapeake Bay Program Non-tidal Workgroup to continue with weekly calibrations.

B8 Inspection/Acceptance of Supplies and Consumables

The deionized water at the Field Office 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 in line 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.

Deionized water blanks are submitted to the laboratory with every sampling run. The field staff rinses a 16 ounce bottle three times with deionized water directly from the storage container in the field vehicle. The blank is filled to the bottle shoulder to leave

space for expansion if the samples are frozen. The blank is then submitted to the laboratory along with field samples for analysis.

MDH uses a water system provided and serviced by SIEMENS. 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. All supplies and consumables for laboratory analyses are described in the applicable standard operating procedure section for the parameter in question (see Appendix D).

B9 Non-direct Measurements

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

B10 Data Management

Data collection for the chemical and physical properties component of the Non-Tidal Network program begins when measurements from field recording instruments are entered onto field data sheets. A senior field scientist ensures that all measurements are taken 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 DNR 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, laboratory, and suspended sediment results to expect. See Appendix B for a sample Cross Reference Sheet and associated documentation.

Nutrient laboratory data sheets are also initiated in the field. These nutrient laboratory sheets list each parameter requested for analysis and include basic information about the sample, such as station, date, time, depth, and volume filtered. The sheets serve as sample transfer sheets, traveling with the samples to the Maryland Department of Health laboratory (MDH) for nutrient analysis or to the USGS Kentucky Water Science Center for sediment analysis. Both the sheets and the samples are logged in at their respective laboratories.

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 nutrient lab 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 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. 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.

Sediment data analyzed by the USGS Kentucky lab are transmitted to DNR directly as Excel spreadsheet files. Because of the time lag between processing field and MDH laboratory data and sediment samples, the sediment results are reviewed by a DNR biologist using a separate computer program at a later time. The sediment data management program reads data from the USGS file and merges it with the field and MDH laboratory data. The sediment program flags records that do not successfully merge with field and MDH laboratory data and it is up to the data reviewer to resolve these discrepancies and ensure that sediment data are valid. Once all data are deemed valid, they are uploaded to DNR’s Access water quality data base.

The final data set combining the field, lab, and sediment 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 data base 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 are also transferred to the Chesapeake Bay Program Data Center.

Files submitted by DNR are uploaded and further screened by the Bay Program's non-tidal Data Upload and Evaluation Tool (DUET). DUET 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 data base and prevents files that have fatal errors from being entered into CIMS. Non-fatal errors, such as those with values falling out of a historical range, are reviewed and accepted or rejected. Data are checked for consistency and will be assigned a problem code if they fail. Examples of consistency checks include testing whether NH4 + NO23 is greater than TDN and whether PO4 exceeds TDP. Water quality data will be reported if the difference is less than the practical quantitation limit (PQL) and assigned a "QQ" to the appropriate analysis problem code. Water quality data will be reviewed if the difference exceeds the PQL and an "NQ" analysis problem code will be assigned. A "G" code will be assigned when the reported value is between the MDL and the PQL (or reporting limit) and an "IQ" will be assigned if it cannot be determined if the part exceeds the whole value and whether or not the difference is within analytical precision,

Once data are entered into the CIMS data base, they are available to the public via the Bay Program data-hub (www.chesapeakebay.net). The data management process is diagramed in Figure 5.

Data management flow chart
Data Entry through production of Final
Master Data Set

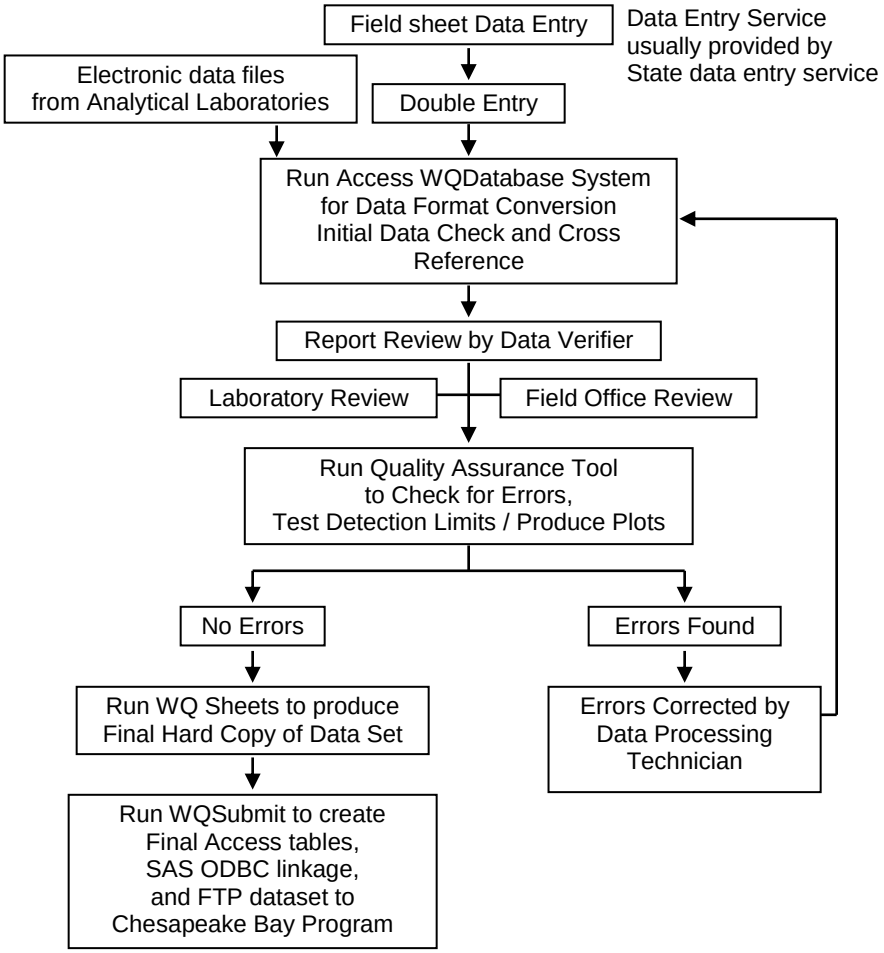


Figure 5. Data management flow chart

ASSESSMENT AND OVERSIGHT

Program and performance audits verify that procedures specified in the Project Plan are being followed. These audits ensure the integrity of the reported data.

C1 Assessments and Response Action

Field: 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 the Data Integrity Workgroup (formerly AMQAW) and possible solutions are offered. The Field QA Officer regularly reviews Equipment Log Books to ensure that all field staff are following QC procedures. Standard maintenance recommended by Hydrolab is performed at six-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.

Laboratory: Instrument preventive maintenance, repairs, and analytical corrective actions are documented on laboratory note books or the Division's Analytical Corrective Action forms. Corrective actions are initiated by the laboratory 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 (Appendix C) 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 3 or Chesapeake Bay Program Office staff.

Data Management: The Data Input Editor is the first line of defense for data correction. A DNR Administrative Aide reviews all incoming data and compares the data to the cross-reference file. The Administrative Aide verifies the submitted data and applies corrections to the physical data-sheet if errors are identified. During the data-import process, the Data Processing Programmer makes all corrections to the data and key fields as they are imported into the Water Quality Database System. The Data Processing Programmer 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 Water Quality 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

Reports to management are contained in the outline of deliverables in the project Scope of Work. Any changes to the QAPP or to the SOPs referenced herein will be

documented and approval of the DNR Principal Investigator and Quality Assurance Officer will be obtained prior to implementation.

Compiling the final datasets involves matching of field data, water quality data and sediment data. The sediment samples are processed by USGS Kentucky Lab, and in the past receipt of the sediment data has been the last step completed before DNR can compile the final datasets, QAQC the data and then submit. Data are generally submitted by USGS to DNR as a group of three or four months at a time, with generally a delay of five or six months (so for example, October 2014- January 2015 data was submitted on March 14, 2015). Once the data are received from USGS, there are still data management and QAQC steps that are required, generally within another month. As a result, DNR feels it will take about six months (following the end of the first month in the group) to be able to submit the data in a final format to CBP. DNR proposes the following deliverable dates and has included them in the grant proposal as a placeholder:

Data submission for period July-September 2018	1/30/2020
Data submission for period October - December 2018	4/30/2020
Data submission for period January -March 2019	7/31/2020
Data submission for period April -June 2019	10/31/2020

DNR will work with USGS to meet the deliverable dates as much as feasible.

DATA REVIEW AND USABILITY

D1 Data Review, Verification, and Validation

These procedures are described in Section B10, Data Management and C1, Assessments and Response Action, above. The MDH Environmental Sciences Division uses data review checklists for data validation (Figure 6).

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 programmer who makes the changes (Figure 5).

D2 Verification Validation Methods

These procedures are described in Section B10, above.

D3 Reconciliation with User Requirements

Data collected under this program will initially be analyzed by staff of the USGS to calculate loads and estimate trends in nutrients and total suspended sediment using the Weighted Regressions on Time, Discharge, and Season model developed by Hirsch et al., (2010). USGS ensures data quality by prescreening the input data through an examination of scatter plots. Preliminary output from the model is also screened for outliers by examining plots of residuals and box plots.

USGS developed the monitoring protocols and load and trend estimation techniques that will be employed to analyze data collected under this grant. These monitoring and data analysis techniques have been applied by USGS to data they collect under the River Input Monitoring Program, which started in the mid-1980s. As a result, the Non-Tidal Network data should directly meet the objectives for which it is collected.

State of Maryland
DHMH - Laboratories Administration
DIVISION OF ENVIRONMENTAL CHEMISTRY
Nutrients Section

Figure 6. Data Review Checklist

LL Orthophosphate/EPA Method 365.1
LL Ammonia/ EPA Method 350.1

Lab Numbers[1]:_____

Date Collected: _____ Date Analyzed: _____ Analyst: _____

Procedure	Acceptance Criteria	Status *	Comments
Holding Time	48 hours @ 4°C; 28 days @ –20°C		
Samples Analyzed	Within 5 working days		
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 samples		
	Recovery = 90–110%		
External QC[2]	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 for OP; 0.008–0.500 ppm for NH_3)		
Diluted Samples	Correct final calculations		
Changes/Notes	Clearly stated		

Table 4. Non-tidal network stations and expected parameters for 2019-2020.

AGENCY	SOURCE	STATION	USGS_GAGE_STATION	DESCRIPTION	STATION_TYPE
MDDNR	MDDNR	ANT0047	01619500	Antietam Creek near Sharpsburg, MD	Primary
MDDNR	MDDNR	BEL0053	01495000	Big Elk Creek at Elk Mills, MD	Primary
MDDNR	MDDNR	CAC0148	01637500	Catoctin Creek near Middletown, MD	Primary
MDDNR	MDDNR	DER0015	01580520	Deer Creek near Darlington, MD	Primary
MDDNR	MDDNR	GEO0009	01599000	Georges Creek at Franklin, MD	Primary
MDDNR	MDDNR	GUN0258	01582500	Gunpowder Falls at Glencoe, MD	Primary
MDDNR	MDDNR	GWN0115	01589300	Gwynns Falls at Villa Nova, MD	Primary
MDDNR	MDDNR	LXT0200	01593500	Little Patuxent River at Guilford, MD	Primary
MDDNR	MDDNR	MGN0062	01493500	Morgan Creek near Kennedyville, MD	Primary
MDDNR	MDDNR	MKB0016	01486000	Manokin Branch near Princess Anne, MD	Primary
MDDNR	MDDNR	MON0546	01639000	Monocacy River at Bridgeport, MD	Primary
MDDNR	MDDNR	NPA0165	01586000	North Branch Patapsco River at Cedarhurst, MD	Primary
MDDNR	MDDNR	NWA0016	01651000	NW Branch Anacostia River near Hyattsville, MD	Primary
MDDNR	MDDNR	PXT0972	01591000	Patuxent River near Unity, MD	Primary
MDDNR	MDDNR	TF1.2	01594526	Western Branch at Upper Marlboro, MD	Primary
MDDNR	MDDNR	TUK0181	01491500	Tuckahoe Creek near Ruthsburg, MD	Primary
MDDNR	MDDNR	WCK0001	0158175320	Wheel Creek near Abingdon, MD	Primary
MDDNR	MDDNR	WIL0013	01601500	Wills Creek near Cumberland, MD	Primary
MDDNR	MDDNR	TOC0037	01613095	Tonoloway Creek, Hancock, MD	Primary
MDDNR	MDDNR	TOW0030	01609000	Town Creek, Oldtown, MD	Primary
MDDNR	MDDNR	SID0015	01610155	Sideling Hill Creek, Belle Grove, MD	Primary
MDDNR	MDDNR	LIC0042	01613525	Licking Creek, Pectonville, MD	Primary
MDDNR	MDDNR	ANT0366	01619000	Antietam Creek, near Waynesboro, PA	Primary
MDDNR	USGSMD	01493112	01493112	Chesterville Branch near Crumpton	Primary
MDDNR	USGSMD	01491000	01491000	Choptank River near Greensboro, MD	Primary_RIM
MDDNR	USGSMD	01578310	01578310	Susquehanna River at Conowingo, MD	Primary_RIM
MDDNR	USGSMD	01594440	01594440	Patuxent River near Bowie, MD	Primary_RIM
MDDNR	USGSMD	01646580	01646580	Potomac River at Chain Bridge, Washington, DC	Primary_RIM

Table 4. Non-tidal network stations and expected parameters for 2019-2020 (continued).

WTEMP (P00010)	SPCOND (P00095)	DO (P00300)	PH (P00400)	TURB_NTU, TURB_FTU, TURB_FNU, or TURB_NTRU	SALINITY (P00480)
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---
X	X	X	X	---	---

Table 4. Non-tidal network stations and expected parameters for 2019-2020 (continued)

TN (P00600)	PN (P00601)	TDN (P00602)	TKNF (P00623)	TKNW (P00625)	TON (P00605)	PON (P?????)	DON (P00607)	DIN (P?????)	NH4F (P00608)	NH4W (P0061
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
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CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---
CALC	X	X	**---**	CALC	CALC	---	CALC	CALC	X	---

Table 4. Non-tidal network stations and expected parameters for 2019-2020 (continued)

NO2W (P00615)	NO3F (P00618)	NO23F (P00631)	NO23W (P00630)	TP (P00665)	PP (P00667)	TDP (P00666)	DOP (P00673)	HPO4F (P00677)	PIP (P49568)	PO4F (P0067
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	CALC	X	X	CALC	---	---	X
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---	CALC	X	---	CALC	X	X	CALC	---	---	X
---	CALC	X	---	X	CALC	X	CALC	X	---	X
---	CALC	X	---	X	CALC	X	CALC	X	---	X
---	CALC	X	---	X	CALC	X	CALC	X	---	X
---	CALC	X	---	X	CALC	X	CALC	X	---	X
---	CALC	X	---	X	CALC	X	CALC	X	---	X

Table 4. Non-tidal network stations and expected parameters for 2019-2020 (continued)

TSS (P00530)	FSS (P00540)	VSS (P00535)	SSC_TOTAL (P80154)	SSC_SAND (No parameter code)	SSC_FINE (No parameter code)	SSC_%FINE (P70331)
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	---	---	STORM	---	---	STORM
X	CALC	X	STORM	---	---	STORM
X	CALC	X	STORM	---	---	STORM
X	CALC	X	STORM	---	---	STORM
X	CALC	X	STORM	---	---	STORM
X	CALC	X	STORM	---	---	STORM

Table 4. Non-tidal network stations and expected parameters for 2019-2020 (continued)

PC (P00694)	TOC (P00680)	DOC (P00681)	POC (P00689)	PIC (P00688)	TALK (P00410)	ALKF (P39086)	SIF (P00955)
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
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X	---	X	---	---	---	---	---
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X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	---	X	---	---	---	---	---
X	CALC	X	CALC	X	---	X	X
X	CALC	X	CALC	X	---	X	X
X	CALC	X	CALC	X	---	X	X
X	CALC	X	CALC	X	---	X	X
X	CALC	X	CALC	X	---	X	X

Table 4. Non-tidal network stations and expected parameters for 2019-2020 (continued)

HARDNESS (P00900)	CHLA (P32211)	BOD5W (P00310)	CLW (P00940)	CLF
---	---	---	---	---
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---	---	---	---	---
---	X	---	---	---
---	X	---	---	---
---	X	---	---	---
---	X	---	---	---
---	X	---	---	---

PROGRAM	PROJECT	AGENCY	SOURCE	LAB	PARAMETER (expected)	CIMS METHOD CODE	LAB METHOD	FILTER – For Defined Particulate (Filter), Dissolved (Filtrate) or Seive Analysis, Provide Filter or Seive type (plate or pleated), diameter (mm) and effective pore size (microns).	MDL	UNIT	MDL START DATE	MDL END DATE	Reporting Level	Reporting Level START DATE	Reporting Level END DATE
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	DOC	L01	Determination of Total Organic Carbon Standard Method 5310B	Glass fiber plate, 47 mm diameter, 0.7 micron pore size, field filtered	0.0517	mg/L	1/1/18	12/31/18	0.5	1/1/18	12/31/18
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	NH4F	L01	Determination of Ammonia, Low Level, Flow Injection Colorimetric Analysis EPA Method 350.1	Glass fiber, plate, 47 mm diameter, 0.7 micron pore size; field filtered	0.0016	mg N/L	1/1/18	12/31/18	0.008	1/1/18	12/31/18
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	NO23F	L01	Determination of Nitrate/ Nitrite, Low Level, Flow Injection Colorimetric Analysis EPA Method 353.2	Glass fiber, plate, 47 mm diameter, 0.7 micron pore size; field filtered	0.0052	mg N/L	1/1/18	12/31/18	0.02	1/1/18	12/31/18
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	NO2F	L01	Determination of Nitrite, Low Level, Flow Injection Colorimetric Analysis EPA Method 353.2	Glass fiber, plate, 47 mm diameter, 0.7 micron pore size, field filtered	0.0005	mg N/L	1/1/18	12/31/18	0.002	1/1/18	12/31/18
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	PC	L01	Determination of Particulate Carbon and Nitrogen Exeter Analytical CE-440 Elemental Analyzer	Glass fiber plate, 25 mm diameter, 0.7 micron pore size, field filtered	0.032	mg/L	1/1/18	12/31/18	NA	NA	NA
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	PN	L01	Determination of Particulate Carbon and Nitrogen Exeter Analytical CE-440 Elemental Analyzer.	Glass fiber plate, 25 mm diameter, 0.7 micron pore size, field filtered	0.006	mg/L	1/1/18	12/31/18	NA	NA	NA
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	PO4F	L02	Determination of Orthophosphate, Low Level, Flow Injection Colorimetric Analysis EPA Method 365.1	Glass fiber, plate, 47 mm diameter, 0.7 micron pore size, field filtered	0.0005	mg P/L	1/1/18	12/31/18	0.004	1/1/18	12/31/18
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	PP	L01	Determination of Particulate Phosphorus by Flow Injection Colorimetric Analysis EPA Method 365.1	Glass fiber, plate, 47 mm diameter, 0.7 micron pore size, field filtered	0.0005	mg/L	1/1/18	12/31/18	NA	NA	NA
NTWQM	NTN	**MDDNR**	**MDDNR**	**USGS KYSL**	SSC_%FINE	L01	ASTM (2002). American Society for Testing and Materials D3977-97(C), Percent of Suspended Sediment Particles Passing Through a 62 µm Sieve.	The fraction of sample passing through a 62 micron mesh size metal sieve is analyzed by the vacuum filtration method, using Whatman #934-AH glass-fiber crucible filters (1.5 micron pore size).		PCT					

PROGRAM	PROJECT	AGENCY	SOURCE	LAB	PARAMETER (expected)	CIMS METHOD CODE	LAB METHOD	FILTER – For Defined Particulate (Filter), Dissolved (Filtrate) or Seive Analysis, Provide Filter or Seive type (plate or pleated), diameter (mm) and effective pore size (microns).	MDL	UNIT	MDL START DATE	MDL END DATE	Reporting Level	Reporting Level START DATE	Reporting Level END DATE
NTWQM	NTN	**MDDNR**	**MDDNR**	**USGS KYSL**	SSC_TOTAL	L02	ASTM (2002). American Society for Testing and Materials D3977-97(C) Suspended sediment is calculated from separately analyzed Sand and Fine Fractions, [SSC_TOTAL] = [SSC_SAND] + [SSC _FINE]	Sample is poured onto a 62 micron sieve into a dish. The coarse (sand) fraction retained on the sieve is transferred, dried at 103 °C for 2-3 hours, then weighed. The fine fraction passing through the sieve is analyzed by the filtration method, using Whatman #934-AH glass-fiber crucible filters (1.5 micron pore size).	0.5	mg/L					
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	TDN	L01	Determination of Total Dissolved Nitrogen, Flow Injection Colorimetric Analysis EPA Method 353.2	Glass fiber plate, 47 mm diameter, 0.7 micron pore size, field filtered	0.0223	mg N/L	1/1/18	12/31/18	0.1	1/1/18	12/31/18
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	TDP	L01	Determination of Total Dissolved Phosphorus, Flow Injection Colorimetric Analysis EPA Method 365.1.	Glass fiber, plate, 47 mm diameter, 0.7 micron pore size, field filtered	0.0054	mg P/L	1/1/18	12/31/18	0.01	1/1/18	12/31/18
NTWQM	NTN	**MDDNR**	**MDDNR**	**MDH**	TSS	L01	Determination of Total Suspended Solids Standard Method 2540 D	Glass fiber plate, 47 mm diameter, 1.5 micron effective pore size	0.7	mg/L	1/1/18	12/31/18	NA	NA	NA

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Quality Assurance Project Plan for the Maryland Department of Natural Resources Chesapeake Bay Water Quality Monitoring Program Chemical and Physical Properties Component for the period July 1, 2019 - June 30, 2020.

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U.S. Environmental Protection Agency (EPA). 1996. ***Recommended Guidelines for Sampling and Analysis in the Chesapeake Bay Monitoring Program***. Chesapeake Bay Program, August 1996. CBP/TRS 148/96; EPA 903-R-96-006.

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Appendix A

Non-tidal Network Program
Field Standard Operating Procedures

Maryland Department of Natural Resources

Non-tidal Network Program
Standard Operating Procedures

Prepared by: _____ Date: _____
Natural Resource Biologist

Approved by: _____ Date: _____
Water Quality Monitoring Program Chief

May 2019

Table of Contents

MD-DNR
Non-tidal Network Program Protocols

Maryland's Non-tidal Water Quality Monitoring Network currently includes 24 load sites where nutrient and sediment concentrations are sampled monthly in addition to 8 times throughout the year during high flow events. A detailed description of sampling methods can be found in Chapter V (Non-tidal water quality monitoring) of the Methods and Quality Assurance for Chesapeake Bay Water Quality Monitoring Programs.
https://www.chesapeakebay.net/documents/CBPMethodsManualMay2017.pdf

Load Sites: GEO0009, ANT0047, MON0546, BEL0053, TUK0181, WIL0013, NPA0165, GWN0115, DER0015, TF1.2, CAC0148, PXT0972, GUN0258, MKB0016, MGN0062, WCK0001, NWA0016, LXT0200, ANT0366, LIC0042, TOC0037, TOW0030, SID0015.

Procedure: We will use a modified version of the USGS equal width interval assignment for our load sites. The PA USGS modified protocol reduces the number of verticals collected from 10 to a lower number based on the width of the stream being sampled.

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

The number of verticals that will be used for MD-DNR sampling is dependent on the width of the stream at the time of sampling. The PA USGS modification assumes that the number of verticals required can be reduced because the stream is well mixed across the horizontal direction. To check this assumption each time a station is sampled four in-situ parameters (oxygen, pH, specific conductance and temperature) will be measured at each selected vertical sampling point. If the stream appears well mixed, the composite sample will be collected from vertical samples drawn at these selected sampling points. This will be done by running the full nutrient suite on a sample generated at each vertical sampling point and on the normally generated vertical/horizontal composite. If variability is excessive, additional vertical sampling points (max 9) will be required for that station. MD-DNR frequently does more vertical sampling points than is required by the chart above.

Once stream variability has been assessed, collection of a water sample that is both horizontally and vertically integrated will begin. If the maximum stream velocity observed is greater than or equal to 1.5 ft/sec and under 7.0 ft/sec an isokinetic equal width increment (EWI) composite sample will be generated using an approved USGS sampler (DH95 or DH81). If maximum stream velocity is under 1.5 ft/sec a non-isokinetic equal width increment composite sample will be generated using an approved USGS sampler (WBH-96 or modified DH81). At this time, MD DNR does not have the capability to collect an isokinetic sample at sites with velocities over 7 ft/sec.

The EWI composite sample will be generated by collecting individual depth integrated samples at each specified vertical sampling point. These samples will be composited in either a 4L or 8L

churn splitter. Two to three sample bottles will then be drawn from the churn splitter for field processing and /or delivery to the appropriate laboratory. One sample bottle will be processed in the field to generate both filter pads for the particulate nutrient parameters and bottles of filtered sample water for the dissolved nutrient parameters. A second bottle will be filled for TSS analysis. All of the water in the TSS bottle will be used for the analysis. The TSS samples will be analyzed by the Maryland Department of Health (MDH). During storm events a third sample will be drawn and sent to the USGS Sediment Lab in Kentucky for suspended sediment analysis.

Please see the Instrument Sampling Procedures for a detailed explanation of how the in-situ parameters are sampled.

Please see Non-tidal Network Program Sampling Procedures for details on the collection of the EWI composite sample.

Please see the Non-tidal Network Program Sample Processing for a detailed explanation of how the field samples are processed.

Samples collected: Depth integrated samples collected at the vertical sampling points will be composited in the churn splitter. Sub-samples will then be dispensed from the churn splitter and used to generate:

One 500 ml HDPE bottle of whole water for Suspended Sediment Concentration analysis at the USGS Sediment Lab in Kentucky (during storm events and routine monthly samples that are impacted by high flow). Each quarter one sand/fine particle size analysis is done from the same bottle for each of the primary stations.

One TSS whole water bottle 20ml to 950 ml (volume dependent on turbidity)

One HDPE 2 quart nutrient sample bottle that will be field processed into:
- Two 25mm GF/F 0.7 micron Particulate Carbon/Particulate Nitrogen filters
- Two 47mm GF/F 0.7 micron Particulate Phosphorus filters
- Two 8 ounce HDPE bottles of filtrate for dissolved nutrient analysis

SAMPLE QA/QC

Duplicate Stream Samples are collected at a minimum of one (1) per water year (WY) per station. A total of twenty four (24) duplicates are collected per WY.

One (1) Deionized (DI) Water Blank is processed per each station per WY. A total of 20 DI Blanks are collected per WY.

Duplicate Sample

Twenty four duplicate samples will be processed per WY. Each sample site has a minimum of 1 duplicate sample per WY. Sixty percent (60%) of the duplicates will be collected as routine samples. The remaining forty percent (40%) will be collected during storm sampling events. This breakdown of duplicate sampling mimics the percentages that each site is sampled per WY. The duplicate will be drawn from the same churn splitter as the original sample and processed identically. See Churn Splitter Sub-Sampling Procedure and Non-tidal Network

Program Sample Processing for a detailed explanation of these processes. Results will be reviewed as part of MD DNR duplicate data set.

DI Blanks: Processed and Unprocessed

One DI water equipment blank is processed and submitted for each sample site, once per WY. Sixty percent (60%) of the DI blanks will be collected as routine samples. The remaining forty percent (40%) will be collected during storm sampling events. This breakdown of DI Blank sampling mimics the percentages that each site is sampled per WY. **Procedure:** Rinse the appropriate sample bottles with DI water; use the type of sample bottle (nozzle bottle or weighted bottle) that will be used at the station you are currently visiting, under the current conditions. Rinse the appropriately- sized churn splitter (for the current station & conditions) thoroughly with DI water. Fill the sampling bottle with DI water and pour into the rinsed churn splitter. Actively churn the DI water sample according to the Churn Splitter Sub-Sampling Procedure. Collect one bottle for SSC (only if doing a DI Blank during Storm Event Sampling), one bottle for TSS and one 2 quart bottle to be processed for particulate and dissolved nutrients. Process this bottle in the same way that stream samples are processed. Generate filtrate, PC/PN filters and PP filters. Also submit an unprocessed blank to be analyzed for dissolved nutrients. This whole water sample is taken from the supply of DI water that is used for the processed DI water blank. The unprocessed sample should be submitted in two 8oz. bottles, labeled “whole water, 1 of 2 and 2 of 2”. Ice samples and ship with regular stream samples. Results will be reviewed by the Field Office Chief. Problems will be discussed with the Project Officer and potential solutions explored.

Routine Sampling Frequency

Both primary and supplemental stations are sampled once per month on a predetermined schedule, these are Routine Samples (RS).

If high discharge occurs during routine monthly sampling, samples are collected on the scheduled date using procedures for storm event sampling, including a SSC sample (primary stations only). These samples are to be counted as routine, monthly samples and designated as sample type “Routine, Storm-impacted” (RI).

Storm Sampling Frequency

Eight storm event samples per station are required each year, with an attempt to collect 2 storm events per quarter.

A storm event has a rising discharge (cfs) that is a significant change above the average daily discharge with a minimum of 2 times the pre-storm discharge value. The storm event triggers are site specific and are usually a five- to ten-fold increase over the average daily discharge. This guidance may change seasonally to account for low flow periods and site specific guidelines have been implemented. Samples are collected at any point in the hydrograph, i.e., rising or falling limb, or at peak discharge. Two samples may be collected during a single storm event, however these samples must be collected on different days. This also applies to storm sampling following the collection of a routine, storm-impacted sample.

Non-tidal Network Program Sampling Procedure

1. You will need to record the time at the beginning and end of the sample collection period on the field sheet. Before all gages were online, gage readings were obtained in the field, before and after sampling. Now all gages are on-line so the data reporter can now add in this information when reviewing the data sheets. If you need assistance in figuring out whether the velocity is such that isokinetic sampling is warranted, check the current gage height against the stage vs. velocity table in the field pack to determine if maximum velocity expected is greater than or equal to 1.5 ft/sec. **If YES,** an isokinetic composite sample **MUST** be collected. If the maximum velocity is under 1.5 ft/sec or over 7.0 ft/sec a non-isokinetic composite sample is collected.

2. On the downstream side of the bridge, set up cones on the road to block off enough room so that you feel safe, but do not block traffic. Wear your orange safety vest.

3. Measure stream width by placing the measuring tape along the bridge from stream bank to stream bank. Measure from left to right looking downstream for **total width**. Subtract any areas with eddies, obstructions or no flow to get the **sampled width**. Establish the number of increments (transects) you will sample by using the table on page 3. After you determine the number of increments (transects) that will be sampled, use one of the formulas below to determine the location of each vertical sample.

   Here is a formula to determine the number of transects and the location of verticals using **total stream width**:

   - Stream Width / number of transects = transect length
   - 1st vertical = Transect length / 2
   - 2nd vertical = Transect length + 1st vertical
   - 3rd vertical = Transect length + 2nd vertical
   - 4th vertical = Transect length + 3rd vertical
   - 5th vertical = Transect length + 4th vertical

   For example, if the stream is 60 feet wide and it is divided into 3 transects, the 1st sample should be taken at 10 feet from the left bank (while facing downstream), the 2nd at 30 feet, the 3rd at 50 feet. Record vertical locations in the comment section of the field sheet.

   If any subtractions have been made **only from the right side** of the stream, use the **sampled stream width** in the formula above.

   If any subtractions have been made **from the left side of the stream**, here is a formula to determine the number of transects and the location of verticals using **sampled stream width**:

   - Sampled Stream Width / number of transects = transect length
   - 1st vertical = Transect length / 2 + the length of the subtraction from left side

- 2nd vertical = Transect length + 1st vertical
- 3rd vertical = Transect length + 2nd vertical
- 4th vertical = Transect length + 3rd vertical
- 5th vertical = Transect length + 4th vertical

For example, if the total width of the stream is 60 feet wide with a 6 foot eddy from 0 – 6 feet, then the sampled width is 54 feet. When it is divided into 3 transects, the 1st sample should be taken at 15 feet from the left bank (while facing downstream), the 2nd at 33 feet, the 3rd at 51 feet. Record vertical locations in the comment section of the field sheet.

4. Once you have established a location for each vertical, record Hydrolab or YSI readings for each vertical by immersing the sonde in the stream directly (if equipment and stream velocity are suitable) or by collecting a rinsed bucket at each vertical. You must note on the field sheet if the readings were taken from bucket samples. Refer to Instrument Sampling Procedures for a detailed explanation of this process. Review these readings. The stream is well mixed if no set of readings for any one parameter differs by 20 %. If the stream is well mixed, record the median value for each parameter on the field sheet and begin collecting samples. If stream is not well mixed, increase the numbers of verticals by at least two and repeat steps 3 & 4.

5. Based on the stream velocity and sampler choices available, choose a sampler to use. Rinse the sampler collection bottle and all whole water sample bottles three times with stream water (directly in stream or from a freshly collected bucket of stream water). Rinse the churn splitter with 2 to 4 L of stream water. Run a liter of water through the spigot. Wear gloves while rinsing.

1) **DH-95** (bronze, plastic coated isokinetic sampler)

A. Follow instructions 1 – 5 under Non-tidal Network Program Sampling Procedure.

B. Connect the sampler to a hanger bar and the hanger bar to the suspension cable on the crane or the bridge board.

C. Decide who will be the clean hands person (who handles the sample) and who will be the dirty hands person (who handles the equipment). The clean hands person must wear gloves during the sampling. They will only touch the sample bottle and churn splitter.

D. Select the largest diameter nozzle size that the transit rate and depth will allow. The largest size (5/16” diameter) nozzle is the most commonly used, and seems to be appropriate for most situations. Screw the selected nozzle into a clean cap and bottle configuration.

E. Lift the bungee and place the bottle configuration into the sampler cavity. The bungee should fit over the neck of the bottle and hold it in place. Rotate the bottle until the air vent hole is vertical. Visually check the nozzle and air vent hole for obstructions.

F. If the stream looks deeper and faster in one area, establish your transit rate at that spot before starting to sample. Remember, if the sampler collection bottle is over-filled (more than ¾ full) at any of the verticals using the transit rate you establish initially, you must

discard all sample water in the churn splitter and begin sampling over again. The sampler collection bottle must still be bubbling when it reaches the surface. If the collection bottle is less than 40% filled after the first up/down pass, you can lower and raise it again before you empty it into the churn splitter. Remember total amount collected must be ¾ or less of the liter bottle. **If you decide to do multiple dips, you must do the same number of dips at each vertical sampled.**

G. Once you establish your transit rate and number of dips, you are ready to collect the first vertical. Lower the DH-95 sampler until the tail makes contact with the water surface. Record the number from the reel in the "Cable reading - start" line on the field sheet.

H. Wait until the sampler orients towards the flow before lowering the sampler into the water column. Once it has aligned, begin to lower the sampler at a fixed rate, when you feel it touch the bottom, quickly note the number on the reel and reverse directions to raise the sampler to the surface. Record the number from the reel in the "Cable reading - end" line on the field sheet. Repeat, if doing multiple dips.

I. Raise the collected sample. The clean hands person should then retrieve the bottle from the sampler and empty it into the churn splitter. Gently swirl the bottle to ensure that all sediment has been transferred to the churn splitter. The churn should stay inside a clean plastic bag in between each vertical.

J. Move the sampler to the next vertical location. Repeat steps G through I for each vertical.

K. Continue this process until the verticals for all transects have been completed. The churn splitter must contain enough water to process all the samples. If necessary you can return to each vertical location and collect an additional sample if more water is needed. Remember, the churn splitter cannot be overfilled!!! If it does overfill, you must empty the churn splitter and begin the sampling over again.

L. Follow the instructions under Churn Splitter Sub-Sampling Procedure at the end of this section.

2) WBH-96 (Weighted bottle, hand held, non-isokinetic sampler)

A. Follow instructions 1 – 5 under Non-tidal Network Program Sampling Procedure.

B. Put the plastic liter size collection bottle in the sampler and place the elastic around the neck of the bottle making sure it is secure.

C. Decide who will be the clean hands person (who handles the sample) and who is the dirty hands person (who handles the equipment). The clean hands person must wear rubber gloves during the sampling. They will only touch the sample bottle and churn splitter.

D. If the stream looks deeper and faster in one area, begin there. Remember, if the sample collection bottle is over-filled (past the neck of the bottle) you must discard the sample

water and begin again. The sample collection bottle must still be bubbling when it reaches the surface.

E. Lower the sampler until the bottom of the sampler is touching the water surface. Begin lowering the sampler and when you feel it touch the bottom, automatically begin to raise the sampler to the surface.

F. Raise the collected sample. The dirty hands person should swirl and dump the sample into the churn while the clean hands person opens the churn. The churn should stay inside a clean plastic bag in between each vertical.

G. Continue this process until the verticals for all transects have been completed. The churn splitter must contain enough water to process all the samples. If necessary you can return to each vertical location and collect an additional sample if more water is needed. Remember, the churn splitter cannot be overfilled!!! If it does overfill, you must empty the churn splitter and begin the sampling over again.

H. Follow the instructions under Churn Splitter Sub-Sampling Procedure at the end of this section.

3) DH-81 (Hand held wading sampler, optional isokinetic/ non-isokinetic)

If stream velocity is ≥1.5 ft/s and considered safely wadeable, you may use the DH-81 as an isokinetic sampler by using the appropriate nozzle (usually 5/16”). If flows are less than 1.5 ft/s the DH-81 may be used without the nozzle to obtain a grab sample.

A. Select the area of stream that you will be sampling and secure the tape measure across the stream. Measure from left to right looking downstream. Establish the number of increments (transects) you will be sampling and the location of where you will be collecting your vertical sample based on the width of the stream.

B. Once you have established a location for each vertical, record Hydrolab readings for each vertical. Have one person on the stream bank recording the numbers while the other person handles the Hydrolab. See Instrument Sampling Procedures for a detailed explanation of the process.

C. Rinse the DH-81, including bottle, nozzle (if needed), cap and the churn splitter in the stream. Make sure you are downstream of the sample area to ensure that you do not stir up the streambed prior to sampling.

D. Assemble the DH-81 by screwing the cap onto the liter sample bottle and attach the nozzle to the cap (if the steam velocity is under 1.5 ft/sec you can sample without the nozzle). Secure the DH-81 to the wading rod by snapping it into place over the cap.

E. Decide who will be the clean hands person (who handles the sample) and who is the dirty hands person (who handles the equipment). The clean hands person must wear rubber gloves during the sampling. They will only touch the sample bottle and churn splitter.

F. Enter the stream down river of the sampling location and walk up to the sampling location in the centroid (maximum) of the steam flow. Raise and lower the sampler at a constant rate such that the sample bottle is ½ - ¾ full when breaking the surface.

G. **If the sample bottle is too full pour out the sample and speed up your transit rate or use a smaller nozzle or a combination of both until the sample bottle fills ½ - ¾ when the sampler is raised out of the water column. Likewise, if the sample is not full enough, pour out the sample and use a larger nozzle or slow your transit rate to increase sample volume.**

H. Empty the collected sample into the churn splitter. Move to the next vertical and repeat the collection process. The churn should stay inside a clean plastic bag in between each vertical

I. Repeat the sample collection process until there is sufficient volume to fill the 4 or 8 Liter churn splitter.

J. Follow the instructions under Churn Splitter Sub-Sampling Procedure at the end of this section.

4) DH-59 (Brass, hand-held isokinetic sampler with a fixed nozzle.)
As of December 2005, the DH-59 has not been used. Refer to a previous revision for instructions regarding the use of the DH-59.

5) Bucket Sampling Note: If D.O. and Temp are read from a bucket sample YOU MUST Enter a B in the G/L box associated with D.O. so that these values are deleted from data sent to CBP.

Bucket samples are taken from bridges. A sample may be collected to provide stream rinse water for sampling equipment and whole water bottles. If in-situ readings cannot be made by immersing Hydrolab directly in the stream you can collect a bucket from each vertical point for readings. See note above.

A. Select the appropriate length of rope for the bridge from which you will be sampling and secure tightly to bucket.

B. Chose a vertical sampling location to sample.

C. Lower the bucket to the water.

E. Tip the bucket and fill with enough water to rinse the bucket (at least a few inches).

F. Depending if it is a high or low 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. Rinse three times.

G. Fill the bucket.

H. 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.

I. Immediately carry the bucket back to the van. Rinse equipment or go to J.

J. If using for in-situ readings immerse the Hydrolab sonde in the bucket, swirl at 1 ft/sec. Record readings. Repeat for all verticals. Remember to record a "B" in G/L box for D.O.

Churn Splitter Sub-Sampling Procedure

The following steps are to be completed for filling of all sample bottles from the churn splitter:

A. Set the churn splitter in an area where the spigot is easily accessed to dispense water.

B. One person should churn the sample, while the other person fills the sample bottles from the churn splitter.

C. Churn the sample at 9 inches per second.

D. Do not break the water surface with the wand while churning.

E. Churn the sample a minimum of 10 times before dispensing water.

F. Continue to churn the sample until all the sample bottles are filled. Samples cannot be dispensed if the water level is at or below the spigot.

G. Dispense whole water sediment related samples first. Dispense:
   a. SSC (storms only)
   b. TSS
   c. Nutrient Filtration Bottle

Note: After filtering for PP if the sample volume originally chosen for the TSS sample is too small or too large, discard and dispense a TSS bottle with a better volume.

H. Process sample bottles filled as per the instructions under Non-tidal Network Program Sample Processing.

Churn Splitter Cleaning Procedure

After all samples are collected and processed empty churn splitter and rinse well with DI water. Rinse sampler collection bottle with DI. If any of the sample collection equipment needs to be reused before it can be cleaned at the office, follow procedure below.

A. Soak equipment in 10% Liquinox Solution for 20 – 30 mins. If churn splitter is being cleaned fill it with Liquinox and add collector bottle and nozzles. Let sit while completing station or while driving to next station. There is a small cup for soaking just nozzles in the field tub.

B. After soaking, scrub with the brush provided and rinse completely with tap water. Rinse three times with DI. Air dry and store in clean baggies or use again.

Non-tidal Network Program Sample Processing

A. Laboratory Supplies

Pads

a) PC/PN
The pads used for PC/PN samples come directly from MDH. The PC/PN pads are pre-combusted (490 ^{o}C), 25mm Whatman GF/F glass fiber filters – pore size 0.7 µm. Two PC/PN pads are used per sample.

b) PP
The pads used for PP samples are 47mm Whatman GF/F glass fiber filters - pore size 0.7 µm. Two PP pads are used per sample.

B. Particulate sample filtration, processing and storage

1. **Particulate Carbon/ Particulate Nitrogen (PC/PN)**
   a) To generate PC/PN filters first clean two 25mm bells 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.
   b) Place a pre-combusted 25 mm GF/F filter (pore size = 0.7 µm) on each filter frit. Always use clean forceps when handling the filter pads.
   c) Using the two quart whole water sample drawn from the churn splitter **(please see Section D- Churn Splitter Sub-Sampling Procedure)** mix sample thoroughly by agitating and shaking the sample bottle vigorously, then rinse graduated cylinder three times with sample.
   d) 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 inches of Hg is good).
   e) Filter 10-200 ml through each filter. Filter enough sample to leave noticeable color on the filter pad.
   f) Make sure filter is sucked dry and the **same volume is filtered for both pads**.
   g) 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.

   h) Using forceps, fold each filter in half.
   i) Place both filters in a foil square labeled with date, station, sample layer, PC/PN, and volume filtered. Be sure that the pads are not overlapping in the foil square to keep them from freezing together.
   j) 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. Place the folded foil in a zip-lock bag and put it in a cooler on ice.
   k) Upon return to the Field Office, place the foils in their appropriate zip-lock bag in the sample freezer and place the bag in the MDH bin. Put the completed volume sheet in the bag with the foils.

2. **Particulate Phosphorus**

a) To generate PP filters, clean two 47mm bells 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. The filters used are two Whatman 47 mm GF/F filters.
b) Using the two quart whole water drawn from the churn splitter filter 50 ml of sample through each filter pad.
c) Use the filtrate as an equipment rinse and discard.
d) Then filter enough additional (another 50 - 750 ml) to leave a noticeable color on the filter pad.
e) Record the **total** volume filtered through each 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.
f) Use this filtrate to fill up the container for the dissolved parameter analysis. See section C (Filtered dissolved nutrient sample collection) below.
g) After collecting filtrate, make sure filter is sucked dry.
h) Rinse the filter pad using at least three - 10 ml rinses of DI water sucking the pad dry after each rinse.
i) Using forceps, fold each filter in half.
j) Place both filters in a foil square labeled with date, PP, station, sample layer, 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.
k) Fold the foil square as described in step B.1.i., above. Place foil square in zip-lock bag or pad container, and put in the cooler on ice until you return to the field office.
l) Upon return to the Field Office, place the foils in their appropriate zip-lock bag in the sample freezer and place bag in the MDH bin. Put the completed MDH volume sheet in bag along with the foil squares. Frozen samples are delivered Friday of sampling week so the Lab can analyze them within 28 days of collection.

C. Dissolved nutrient sample filtration, collection and storage

NOTE: The filtrate collected for this sample must come from the PP filtration set-up. If you cannot get enough water through these pads to fill the filtrate sample bottle, then use more GF/F filters to get enough filtrate. The filtrate may not come from pads that are pre-combusted (PC/PN).

The following steps are to be completed for collection of all filtrate:
a) Run 50 ml of sample water through the filter.
b) Use this 50 ml of filtrate to rinse the flask and then discard.
c) Run more sample water through the filter and collect in the flask.
d) Label two 8 oz HDPE bottles with “NTN”, station, date, “Filtrate” and “1 of 2” on the first bottle and “2 of 2” on the second bottle.
e) Rinse the two labeled 8 oz HDPE bottles and caps three times with filtrate.

f) Fill the bottles with filtrate and replace cap. If sample will be frozen before delivery to the lab do not fill more than ¾ full.

g) Store the bottle on ice in a cooler. Deliver to the courier or directly to MDH at the end of the field day. If you miss the courier filtrate sample bottles may be frozen and delivered on Friday directly to MDH. If freezing the filtrate sample, copy the regular MDH Lab Sheet and place the copy in a zip-lock bag with the filtrate bottle. Lab must analyze unfrozen sample within 24 hrs of collection; frozen sample within 28 days.

D. Total Suspended Solids (TSS) collection and storage

a) Choose the appropriate size sample bottle.** Label with "NTN", Station Id, date and "TSS ONLY". Rinse cap and bottle three times with sample water. Bottle may be rinsed from a Rinse Only bucket of water.

b) Fill bottle with sample from the churn splitter. The TSS bottle should be filled before the nutrient bottle. Follow the Churn Splitter Sub-Sample Procedures. Because MDH will be sampling the entire amount we send them, you cannot dump any water out once you fill the container.

c) Ice sample. Deliver directly or send by courier to the MDH within 72 hrs. An original completed MDH Lab Sheet must accompany the sample.

Note: If you are sampling on a weekend, or miss the courier, the holding time for TSS samples is one week. The sample can be sent by courier the day after you collected it OR delivered directly to the Lab within 72 hours of collection.

** Note: MDH will be using all the water in the TSS sample bottle to generate the TSS filter. DO NOT SEND THEM TOO MUCH WATER. The MDH uses 47 mm filters of larger pore size for TSS. Fill the TSS Sample Bottle with the same amount of water or slightly more than you expect to use for the PP filters.

REMEMBER the Lab will need to rinse their TSS filters THREE TIMES. There are 60 ml, 8 oz. (237 ml), 16 oz. (473 ml) and 1 qt. (947 ml) bottles in the field tub to use for the TSS sample. The 60 bottles are graduated. The 8 oz,16 oz and 1 qt bottles can be filled to any estimated volume.

E. Suspended Sediment Concentration (SSC) collection and storage

a) Label 500 ml plastic Nalgene bottle with station location and date.

b) Fill bottle to shoulder from churn splitter. Follow the Churn Splitter Sub-Sample Procedure instructions. No water can be dumped from the filled Nalgene bottle. **This sample must be the first sample taken from the churn splitter**

c) Sample does not need to be iced but should be kept in the dark. Place in the box labeled "Sediment Samples" when you return to the office. Samples will be shipped at least quarterly to the USGS Sediment Lab in Kentucky. Note: Each year (Oct-Sept.) all samples must be shipped by September 30th.

Instrument Calibration Procedures

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

B. When large adjustments to the dissolved oxygen (DO) value were necessary during calibration, the DO should be checked prior to taking the instrument in the field. Instrument calibrations may be also be rechecked in the calibration lab any time the field readings seem suspect.

C. Instruments are post-calibrated (checked for their accuracy) 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.

I. Hydrolab Series 5 Instruments

a. Set up a calibration log book for each instrument with make, model, serial numbers and first-in-service date. Assign a letter for DNR use as required. Calibrations are best done in the field office instrument lab which is kept at a stable temperature of 20-25°C.

b. Calibrate instruments on Friday for use the next week. If possible, calibrate instrument within 24 hours of first field deployment. After one to four days of field deployment, post-calibrate instruments after last use to determine if calibration of any parameter drifted. If possible, post-calibrate instrument within 24 hours after last field deployment.

c. Calibrate specific conductance sensor with standards generated by the field office from dry KCl and deionized water with specific conductance equal to 0 µS/cm. Standards are 147, 292, 718, 1413, 2767, 6668, 12900, 24820 and 58640 microSiemens/cm (µS/cm) (microSiemens/cm is equivalent to micromhos/cm at 25°C). Respective concentrations are 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 molar KCl. Calibrate specific conductance sensors of Series 5 instruments following a two point linear protocol. Calibrate the zero point with the sensor dry and the slope with one of the above standards. The most common standard to use for NTN is 292.

d. Calibrate pH sensor with premixed standards of pH 4.00, pH 7.00 and pH 10.00 purchased from Fisher Scientific. Standards are color coded (red for pH 4.00, yellow for pH 7.00 and blue for pH 10.00) and certified as accurate at 25°C (pH 4.00 ± 0.01, pH 7.00 ± 0.01, pH 10.00 ± 0.02) and used before their labeled expiration dates. Calibrate pH sensor with these standards using a two point linear protocol. First, calibrate the zero point with pH 7.00 standard buffer. Then, calibrate slope with either pH 4.00 or 10.00 standard buffer. The slope buffer is selected so that pH measurements anticipated during field deployment are between the zero point and slope buffer values. The pH value of each buffer is adjusted for instrument temperature before calibration. A pH calibration value

vs. temperature table (pH Calibration Table) is supplied by the buffer manufacturer for each standard buffer. This value is the pH calibration point.

e. Calibrate the optical dissolved oxygen sensor (LDO) using a 1 point percent saturation protocol in the common standard of air saturated water. The volume of water must have a specific conductance less than 100 µS/cm. Determine the oxygen saturation calibration point in air-saturated water from theoretical DO saturation tables using the temperature from the instrument and local barometric pressure from a standard Fortin Mercury Barometer. A specific notation on the field data sheet shows that Hydrolab instruments are equipped with LDO sensors.

f. Temperature sensor is calibrated by the manufacturer and cannot be adjusted by the user.

g. Calibrate depth sensor by submerging it to a known depth at the field sampling station and calibrating to this known depth.

h. Record all calibration and post-calibration information (e.g. barometric pressure, calibration values and instrument readings), maintenance procedures and repairs in the instrument specific calibration log book. An example of this log is included.

i. During calibration, post-calibration and field deployment, record in the calibration log book any unusual circumstances that may affect instrument readings.

II. YSI Series 6 Instruments

a. Set up a calibration log book for each instrument with make, model, serial numbers and first-in-service date. Assign a letter for DNR use as required. Calibrations are best done in the field office instrument lab which is kept at a stable temperature of 20-25°C.

b. Calibrate instruments on Friday for use the next week. If possible, calibrate instrument within 24 hours of first field deployment. After one to four days of field deployment, post-calibrate instruments after last use to determine if calibration of any parameter drifted. If possible, post-calibrate instrument within 24 hours after last field deployment.

c. Calibrate specific conductance sensor with standards generated by the field office from dry KCl and deionized water with specific conductance equal to 0 µS/cm. Standards are 147, 292, 718, 1413, 2767, 6668, 12900, 24820 and 58640 microSiemens/cm (µS/cm) (microSiemens/cm is equivalent to micromhos/cm at 25°C). Respective concentrations are 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 molar KCl. Calibrate specific conductance sensor following a two point linear protocol with one of the above standards as the slope standard. The zero point is factory calibrated and cannot be adjusted by the user. The most common standard to use for NTN is 292.

d. Calibrate pH sensor with premixed standards of pH 4.00, pH 7.00 and pH 10.00

purchased from Fisher Scientific. Standards are color coded (red for pH 4.00, yellow for pH 7.00 and blue for pH 10.00) and certified as accurate at 25°C (pH 4.00 ± 0.01, pH 7.00 ± 0.01, pH 10.00 ± 0.02) when used before their labeled expiration dates. Calibrate pH sensor with these standards using a two point linear protocol. First, calibrate the zero point with pH 7.00 standard buffer. Then, calibrate slope with either pH 4.00 or 10.00 standard buffer. The slope buffer is selected so that pH measurements anticipated during field deployment are between the zero point and slope buffer values. The pH value of each buffer is adjusted for instrument temperature before calibration. A pH calibration value vs. temperature table (pH Calibration Table) is supplied by the buffer manufacturer for each standard buffer. This value is the pH calibration point.

e. Calibrate the optical dissolved oxygen sensor (ROX) using a 1 point percent saturation protocol in the common standard of air saturated water. The volume of water must have a specific conductance less than 100 µS/cm. Check and calibrate, if necessary, the YSI 650 MDS Display Unit barometer to local barometric pressure in mm Hg as measured from a standard Fortner Mercury Barometer. Determine the oxygen saturation calibration point in air-saturated water from theoretical DO saturation tables using the temperature from the instrument and local barometric pressure from a standard Fortin Mercury Barometer or display unit barometer. Note in the calibration if the display unit barometer was calibrated and reading used to calibrate the dissolved oxygen sensor. A specific notation on the field data sheet shows that YSI instruments are equipped with ROX sensors.

f. Temperature sensor is calibrated by the manufacturer and cannot be adjusted by the user.

g. Calibrate depth sensor by submerging to a known depth at the field sampling station and calibrating to this known depth.

h. Record all calibration and post-calibration information (e.g. barometric pressure, calibration values and instrument readings), maintenance procedures and repairs in the instrument specific calibration log book. An example of this log is included.

i. During calibration, post-calibration and field deployment, record in the calibration log book any unusual circumstances which may affect the instrument readings.

Instrument Sampling Procedures

Submerge the sonde directly in the stream to obtain the required readings. If flow is too swift or your meter is not practical for in-situ readings obtain readings from a bucket grab. Add B in G/L box for DO and note “Bucket Readings” in “Comments” on Field Sheet.

1. Remove plastic storage cup from the meter, check all sensors before deployment to make sure they look OK.

2. Protect probes by installing probe guard.

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A. Sampling in the bucket: (Remember “B” in DO G/L box

a) Follow steps 1 and 2 above.
b) Swirl the meter in the bucket until the readings stabilize.
c) Record readings on the field sheet.
d) Remove the meter from the bucket, and rinse probes with de-ionized water before replacing plastic storage cup.

B. Sampling from the bridge:

a. Follow steps 1 and 2 above.
b. Lower the meter over the bridge at the first vertical. Position the probes at mid-depth for the vertical.
c. Wait for the readings to stabilize and record them on the field sheet.
d. Carefully raise the meter back up and move to the next vertical.
e. Repeat steps c through e until all the verticals have been sampled.
g. Remove guard. Rinse probes with DI. Replace storage cup.

C. Sampling while wading:

a) Follow steps 1 and 2 above.
b) Place the meter directly in the water at the first vertical. Position the probes at mid-depth for the vertical.
d) Wait for the readings to stabilize and record them on the field sheet.
e) Repeat steps c and d until all the verticals have been sampled.
f) Remove guard. Rinse probes with DI. Replace storage cup.

Log of Significant Changes for the Non-Tidal Network Program

Date Initiated	Procedural Changes
January 1, 2005	The non-tidal network sampling program for base flow and storm events was initiated. Samples were analyzed by the Maryland Department of Health and Mental Hygiene using whole water analyses and concurrently by the Chesapeake Biological Laboratory using filtered water analyses. Both laboratories analyzed samples through June 2005.
July 1, 2005	The Maryland Department of Health and Mental Hygiene laboratory initiated filtered water analyses for all laboratory parameters except total suspended solids. Due to concerns about potential methods and laboratory change effects, data for January through June 2005 were not delivered to the Chesapeake Bay Program. Data collected from July 1, 2005 onward have been submitted to the Bay Program data base. As of July 2005 the program consisted of the following ten stations: ANT0047, BEL0053, DER0015, GEO0009, GWN0115, MON0546, NPA0165, TF1.2, TUK0181, and WIL0013. The stations were sampled during base flow and storm events and were classified as primary stations in the non-tidal network.
October 1, 2005	Three stations (GUN0258, PXT0972, and CAC0148) were added to the network, but only sampled during base flow conditions. These stations were classified as secondary stations in the non-tidal network.
November 1, 2005	Discontinued use of the DH-59. After this point, all samples were collected with one of the following: DH-95, DH-81 or WBH-96.
January 1, 2007	GUN0258, PXT0972, and CAC0148 were upgraded to primary sites and sampled during base flow and storm event conditions.
July 1, 2008	Began collecting Routine Impacted samples. Until July 2008 all sites that were influenced by storms were collected as storm event samples. All routine stations were sampled under “baseflow”. If a storm occurred during a scheduled baseflow run, a storm sample was collected and the baseflow (routine) was rescheduled for collection another day.
July 1, 2009	The Department of Health and Mental Hygiene Laboratory switched from using a 24 millimeter diameter Whatman Grade 934-AH: 1.5 µm filter pad to an 47 mm diameter 1.5 µm Environmental Express filter pad for the analysis of total suspended solids.
March 25, 2010	The Department of Health and Mental Hygiene Laboratory changed analysis methods for ammonia from the phenate method to the salicylate method. Differences in the analytical results for the two methods were assessed in the Data Analysis Issues Tracking System report #049 which showed that the salicylate method yielded slightly higher results than the phenate method ($p<0.0001$). DHMH switched back to the phenate method November 5, 2010.

Log of Significant Changes for the Non-Tidal Network Program (continued)

Date Initiated	Procedural Changes
October 17, 2011	Sampling was initiated at MKB0016 on Manokin Branch.
October 24, 2011	Sampling was initiated at MGN0062 on Morgan Creek.
October 27, 2011	Sampling was initiated at WCK0001 on Wheel Creek.
April 2012	Three stations were added in the spring of 2012 using funding from the 2010 Chesapeake and Atlantic Coastal Bays Trust Fund. These stations include CVA0046, WIL0065, and at USGS gage number 01636500 near Millville, WV.
April 2012	Sampling initiated at CVA0046 & WIL0065
October 2014	Assumed responsibility for sampling six stations in Maryland previously sampled by PA DEP (CON0180, TOC0037, TOW0030, SID0015, LIC0042, and ANT0366).
July 2015	DNR stopped sampling at Trust Fund stations (WIL0065, CVA0046, and USGS gage number 01636500 near Millville, WV). USGS in Baltimore agreed to fund sampling at Millville, WV for one year.
July 2018	Maryland Department of Health & Mental Hygiene (DHMH) changes name to Maryland Department of Health (MDH)
October 2018	Station CON0180 dropped from sampling.

Appendix B

Cross Reference Sheet
Documentation and Procedures

Cross Reference Sheet Procedures

The following documentation outlines the conventions for filling out the cross reference sheet. (Note: Although this sheet is labeled "Progress Report," it is actually the Cross Reference Sheet) The cross reference sheet is sent along with field sheets from the field office to the DNR Tawes Building, so that the DNR data management staff knows what data to expect in the form of field sheets and laboratory data.

The cross reference sheet includes the name of the program, the sampling month and year, the name of the field office representative who originates the sheet, the station name, the sampling day, the sampling depth, the sequence number, layer code, and columns for tracking laboratory sheets and if a sediment sample was collected for analysis at the USGS sediment laboratory. There is also a comment line to explain missing samples, stations, field abnormalities, sampling intervals, or potential data problems.

1. The name of the program
2. The sampling month and year
3. The name of the Field Office representative who originates the sheet
4. The station name
5. The sampling day
6. The sequence number
7. The sampling depth
8. The layer code
9. Column for tracking receipt of laboratory data
10. Column for USGS sediment sample taken
11. Column for comments

An example Cross Reference Sheet (labeled "Progress Report/Cross Reference Sheet") follows.

Maryland Department of Natural Resources MANTA
Chesapeake Bay Water Quality Monitoring Progress
Report - Non-tidal Network Baseflow Sampling

Station	Day	Sequence Number	Sample Depth	Layer code	DHMH	USGS sediment	Comments
GEOOO09	26	0707801	Start 0.0 End 0.2	VH	0185	N/S	26 feet total. 3 verticals @4.4, 13 and 21.7 feet. Waded at gage. Water very dark. Scattered thunderstorms last evening. Water depths (M): I (0.4),2 (0.3), 3 (0.2)
ANT0047	25	0707802	Start 0.0 End 0.4	VH	0179	N/S	92 feet total. 7 verticals @ 8 (eddy - 6.6), 19.6,32.8,45.9,57 (eddy - 59), 72.2 and 82 (shallow and rocks at 85.2ft) feet from the left. Sampled from the bridge. Meter K's DO membrane had a hole in it after this station, and was not able to be post- calibrated. Water depths (M): I (0.4),2 (0.7), 3 (1.0),4 (1.1), 5 (0.9),6 (0.4), 7 (0.3)
MON0546	25	0707803	Start 0.0 End 0.2	VH	0181	N/S	79 feet total. 5 verticals@ 7.9, 23.7, 39.5, 55.3 and 71.1feet from the left. Sampled from the bridge. Non-isokinetic sample. Extremely low water level. Lots of algae on rocks. Meter K's DO membrane had a hole in it after doing the Antietam station, and was not able to be post- calibrated. Water depths (M): 1 (0.2),2 (0.4), 3 (0.4),4 (0.5), 5 (0.2)
TUKOl81	2	0707804	Start 0.0 End 0.9	VH	0005	N/S	96 feet total width. 5 verticals @ 9.6, 28.8, 48, 67.2 and 86.4ft from the left. Sampled from the bridge. Non-isokinetic sample. Rinsed PC/PN pads with 200m I of D I before filtering sample. Water depths (M): I (0.7),2 (1.6), 3 (2.7),4 (1.8), 5 (2.0).

Appendix C

Corrective Action Form for the Maryland Department of Health

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 D

Standard Operating Procedures for Maryland Department of Health

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

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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

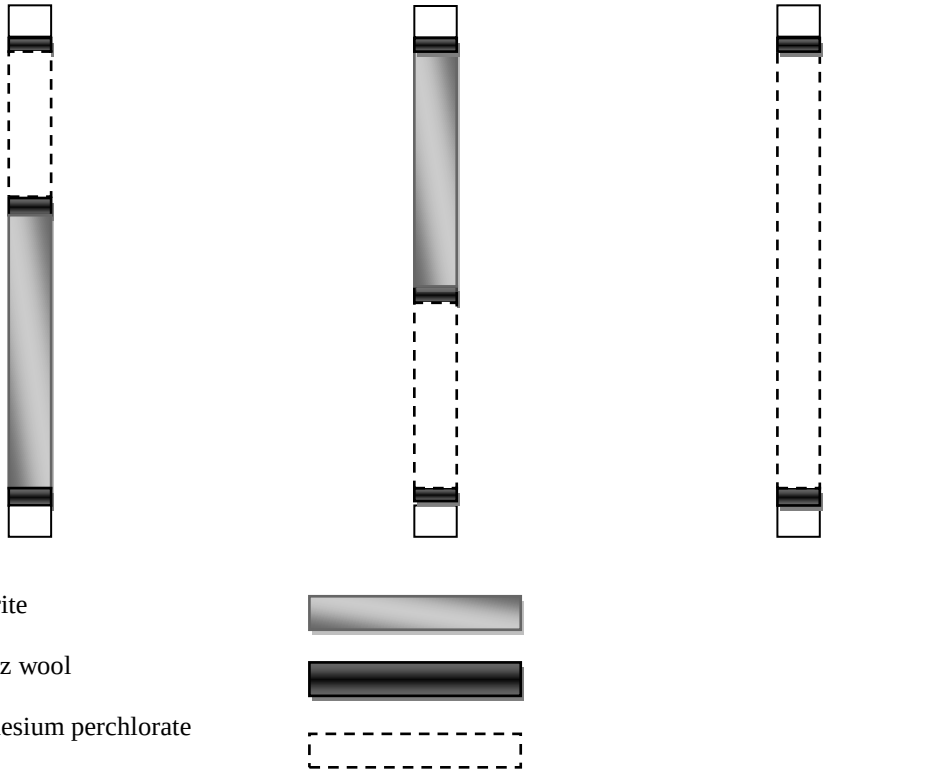
Exeter Method 440

CHN Mode Helium & Oxygen Scrubbers

CHN Mode CO_2 Trap

(This end up)

CHN Mode H_2O trap



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

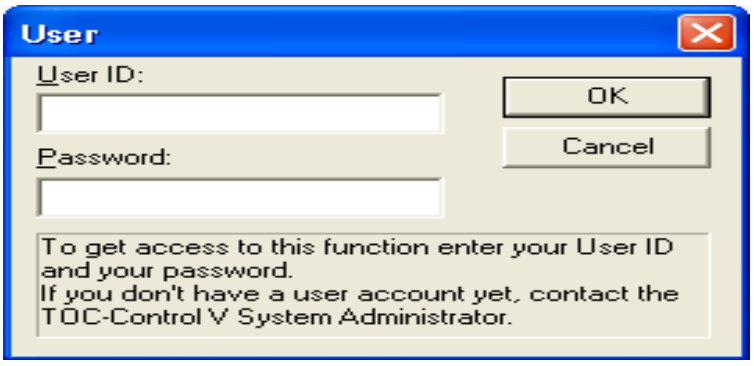
9.1 Analysis Flow

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.

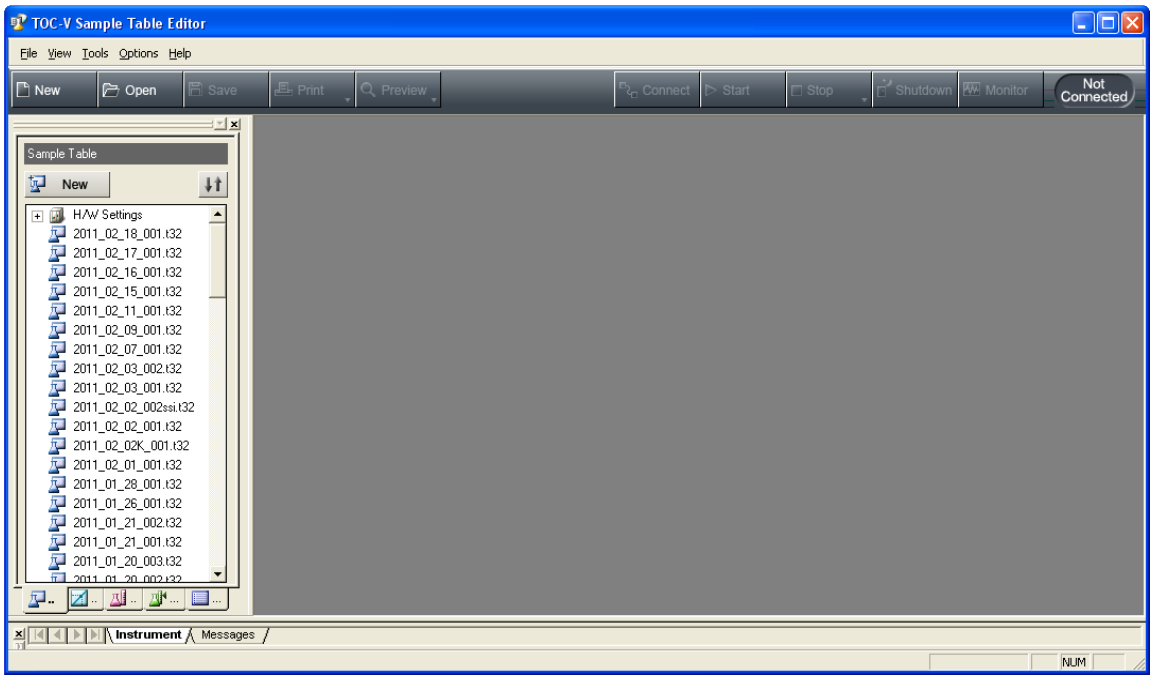
9.1.2 Switch on TOC-V_{cph} or $_{TOC}$-L_{CPHCPN} Analyzer, which undergoes the initialization sequence.

9.1.3 Turn on the computer.

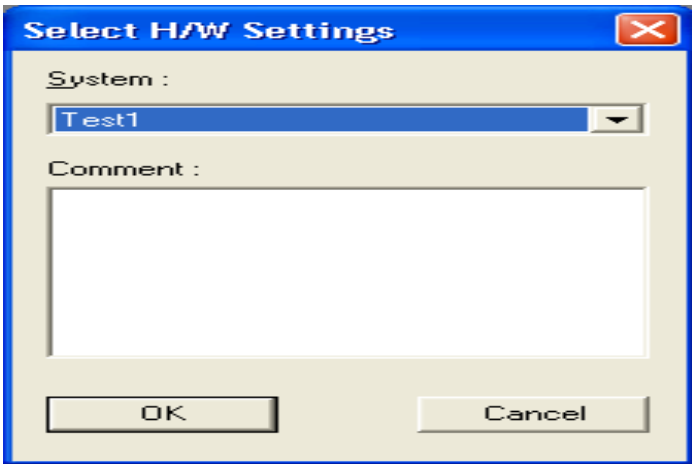
9.1.4 Double click on TOC Sample Table Editor icon on the Monitor. The "User" window is displayed.



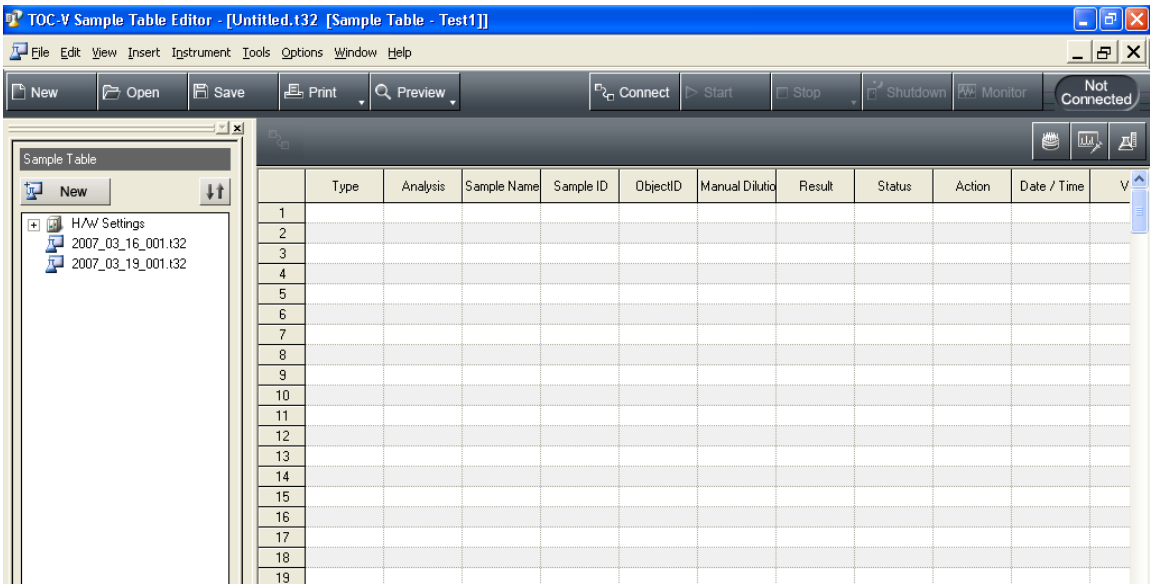
9.1.5 Click OK on the User. A TOC Sample Table Editor is opened.



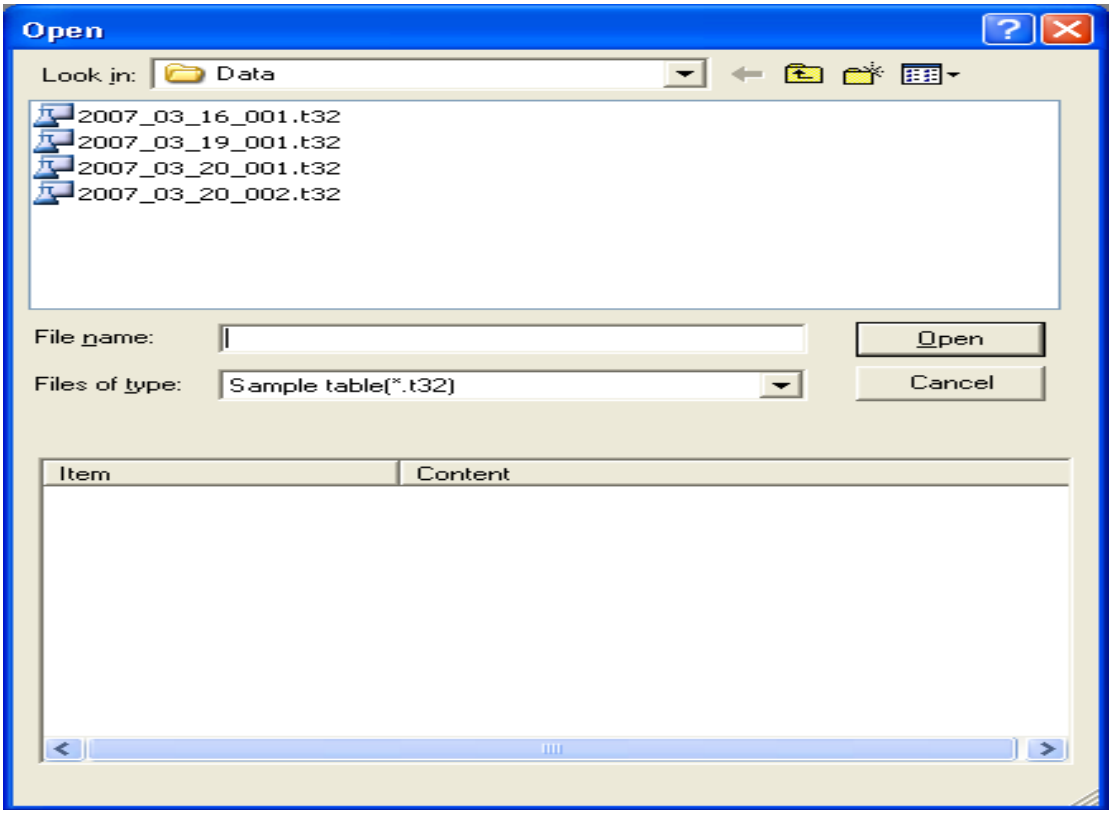
9.1.6 Click "New" on Sample Table Editor, The"Select H/W Settings" window is displayed.



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

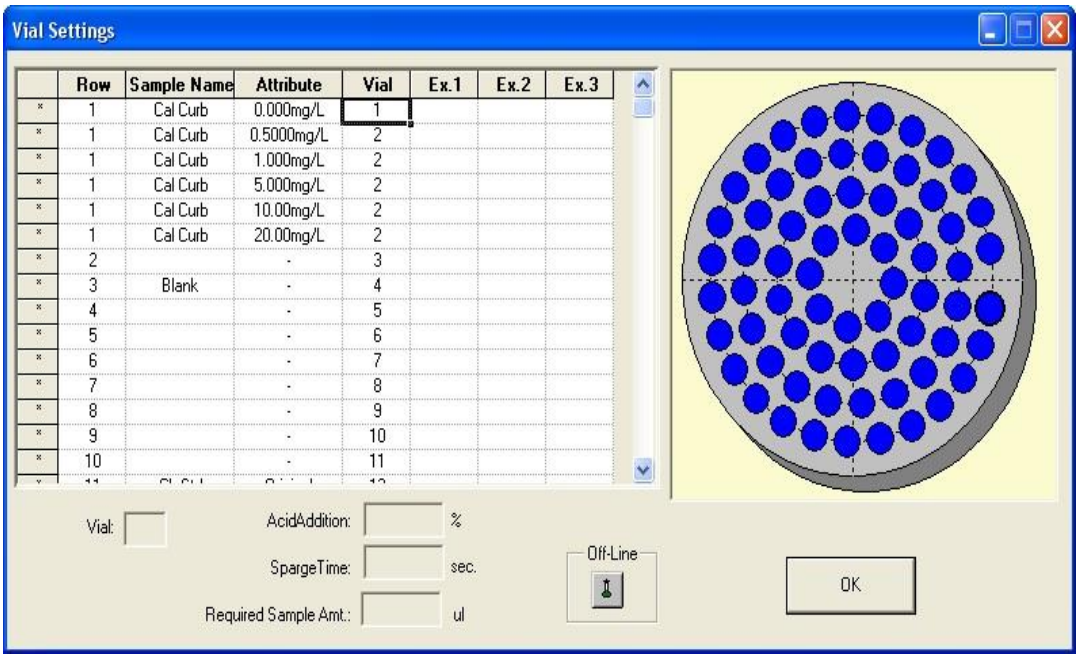


9.1.8 On TOC Sample Table Editor, select File, Import Schedule File in the menu Bar. The Open dialog is displayed.



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

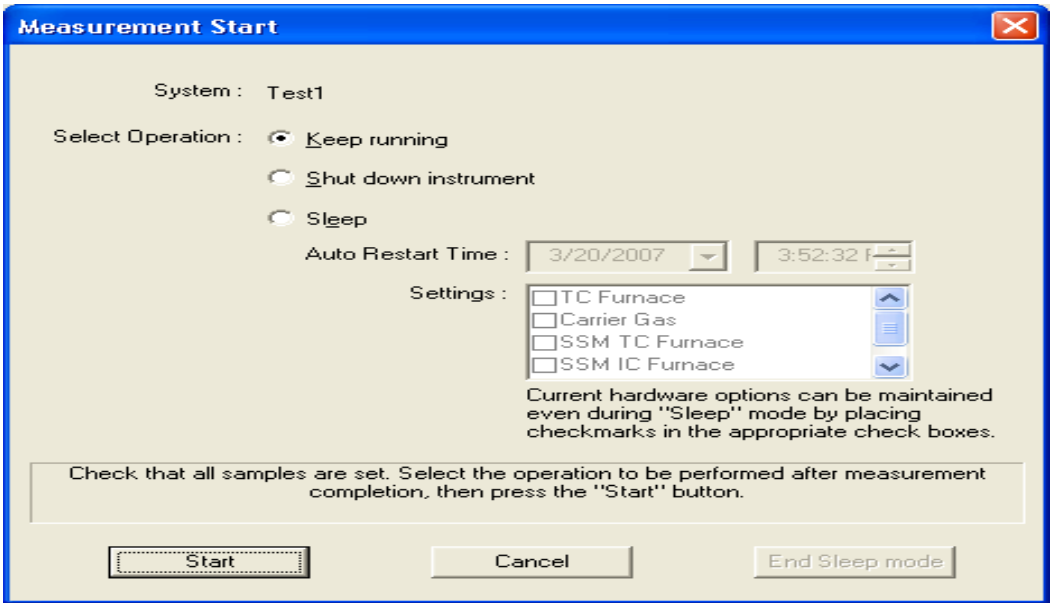
replicates and spikes for every ten samples. A blank, and a check standard are analyzed after every 10 samples. A blank, a check standard and a QC should also be included at the end of each run.

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

MDH- Laboratories Administration
DIVISION OF ENVIRONMENTAL SCIENCES

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

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

3.1 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.

3.2 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.

3.3 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 ^{o}C, 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 ^{o}C prior to analysis is required to minimize microbiological decomposition of solids. The holding time is 7 days at 4 ^{o}C. 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

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

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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.

6.1.2 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.

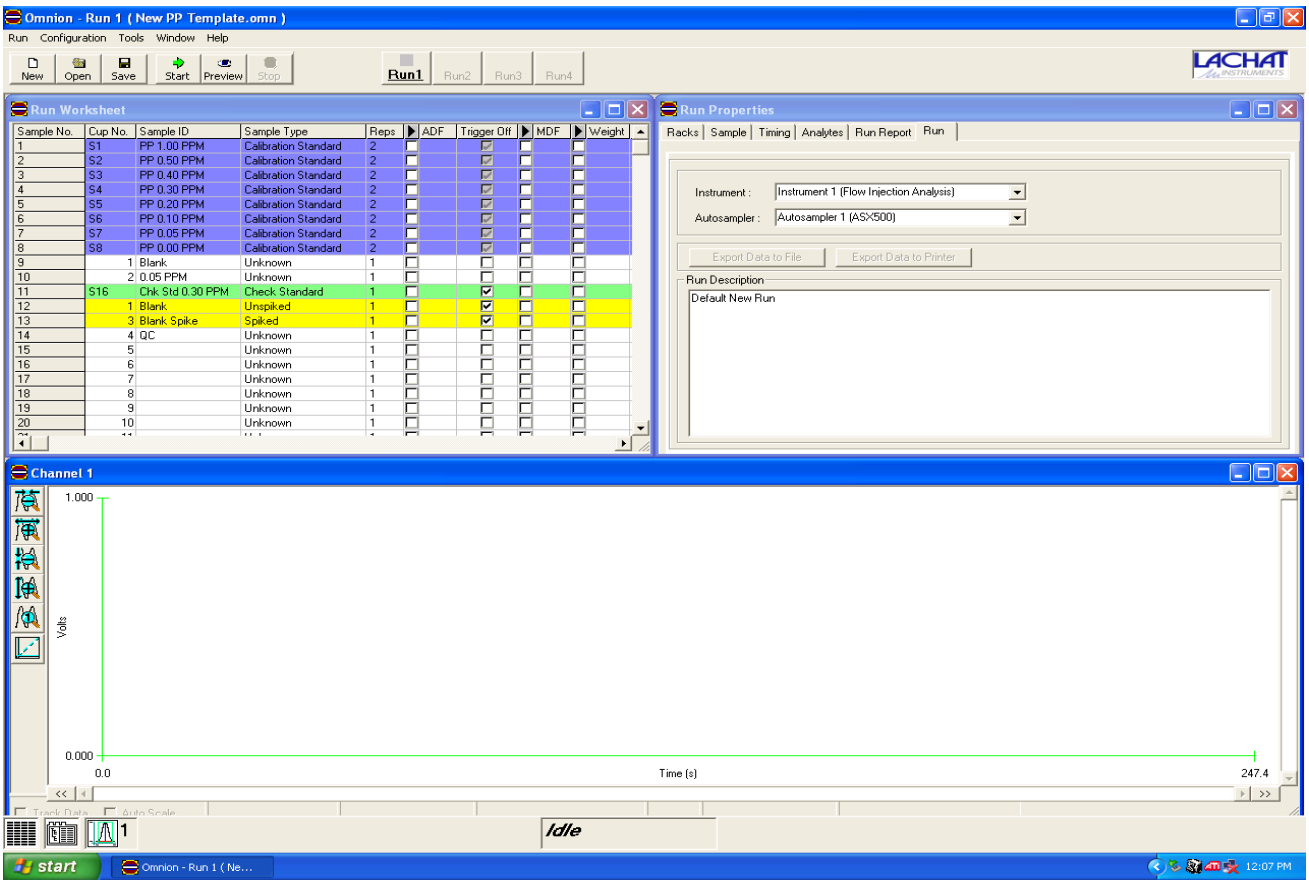
9.1.6 Transfer the filtrate to auto sampler tubes following the order of the run worksheet.

9.2 Instrument set-up and sample analysis

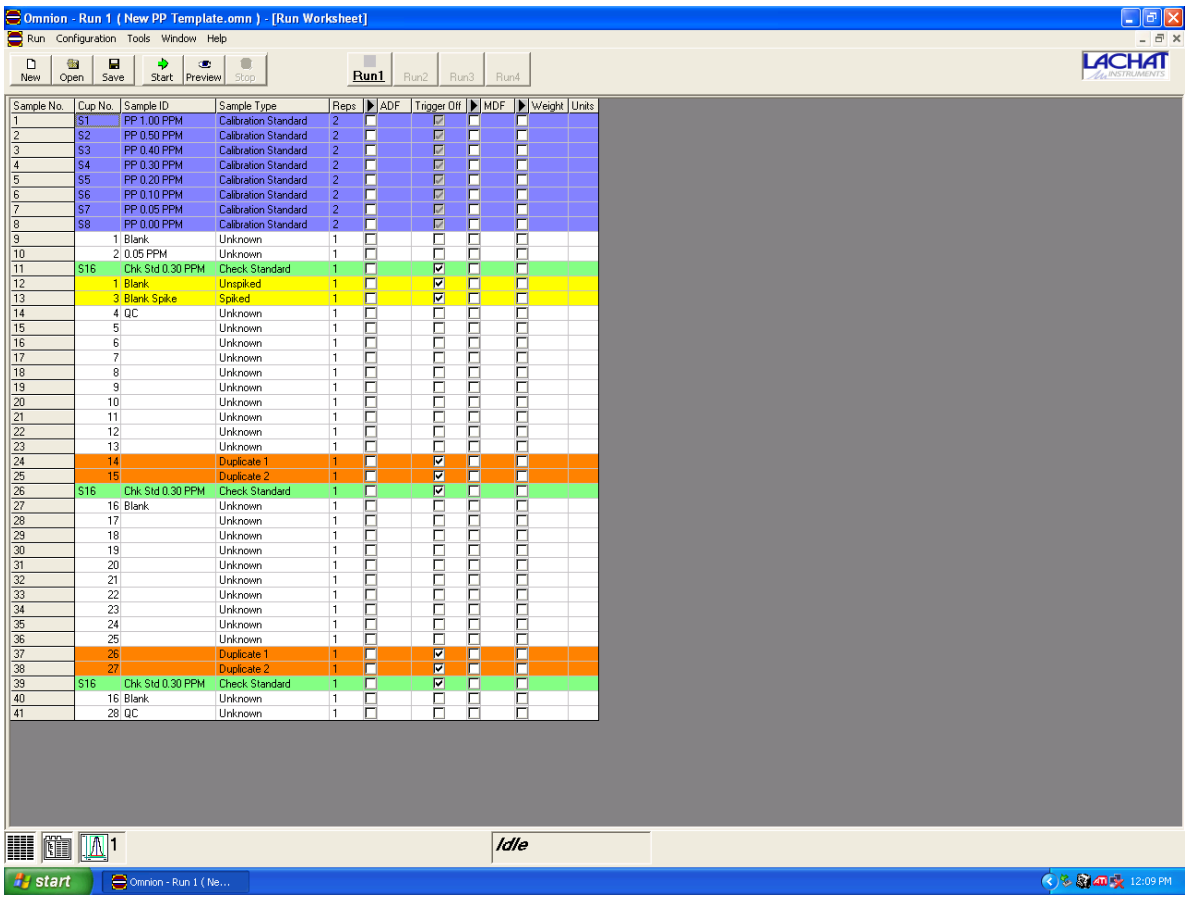
9.2.1 Set up manifold as described in the method.

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.



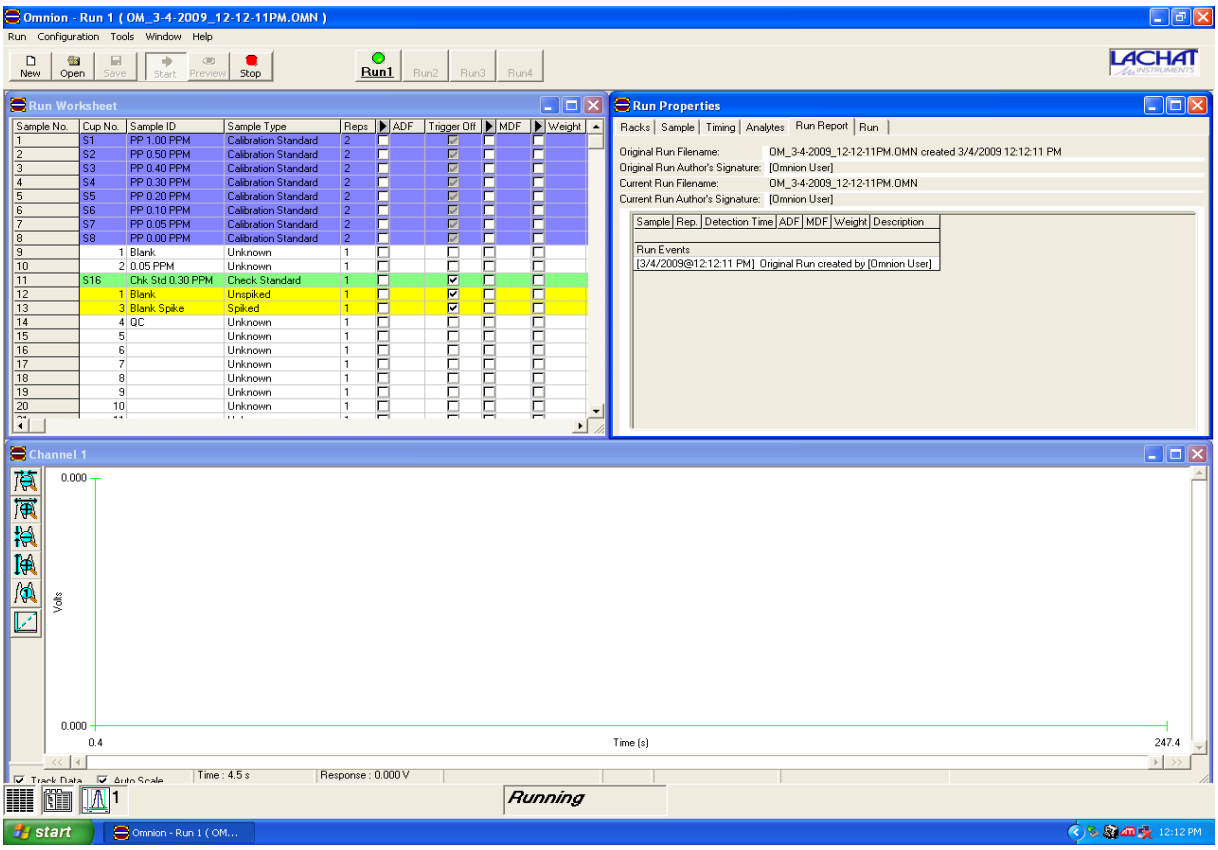
Sample No.	Cup No.	Sample ID	Sample Type	Reps	ADF	Trigger Off	MDF	Weight	Units
1	S1	PP 1.00 PPM	Calibration Standard	2					
2	S2	PP 0.50 PPM	Calibration Standard	2					
3	S3	PP 0.40 PPM	Calibration Standard	2					
4	S4	PP 0.30 PPM	Calibration Standard	2					
5	S5	PP 0.20 PPM	Calibration Standard	2					
6	S6	PP 0.10 PPM	Calibration Standard	2					
7	S7	PP 0.05 PPM	Calibration Standard	2					
8	S8	PP 0.00 PPM	Calibration Standard	2					
9	1	Blank	Unknown	1					
10	2	0.05 PPM	Unknown	1					
11	S16	Chk Std 0.30 PPM	Check Standard	1					
12	1	Blank	Unspiked	1					
13	3	Blank Spike	Spiked	1					
14	4	QC	Unknown	1					
15	5		Unknown	1					
16	6		Unknown	1					
17	7		Unknown	1					
18	8		Unknown	1					
19	9		Unknown	1					
20	10		Unknown	1					
21	11		Unknown	1					
22	12		Unknown	1					
23	13		Unknown	1					
24	14		Duplicate 1	1					
25	15		Duplicate 2	1					
26	S16	Chk Std 0.30 PPM	Check Standard	1					
27	16	Blank	Unknown	1					
28	17		Unknown	1					
29	18		Unknown	1					
30	19		Unknown	1					
31	20		Unknown	1					
32	21		Unknown	1					
33	22		Unknown	1					
34	23		Unknown	1					
35	24		Unknown	1					
36	25		Unknown	1					
37	26		Duplicate 1	1					
38	27		Duplicate 2	1					
39	S16	Chk Std 0.30 PPM	Check Standard	1					
40	16	Blank	Unknown	1					
41	28	QC	Unknown	1					

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!	###
			#DIV/0!	###				#DIV/0!	###
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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 0.05 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

TABLE OF CONTENTS

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.

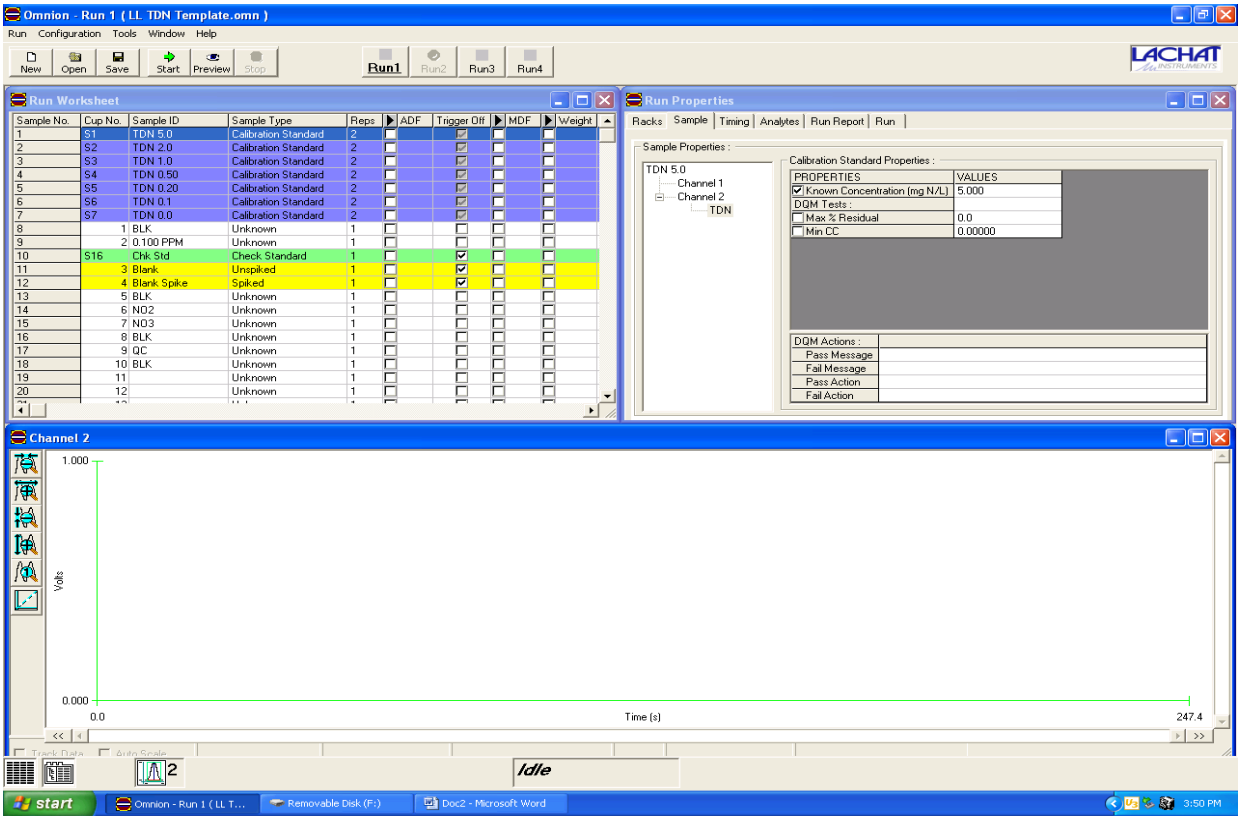
9.2.7 Get the data reviewed by a designated scientist, and then, report the results on the Analysis Request Forms.

9.3 Instrument set-up and sample analysis

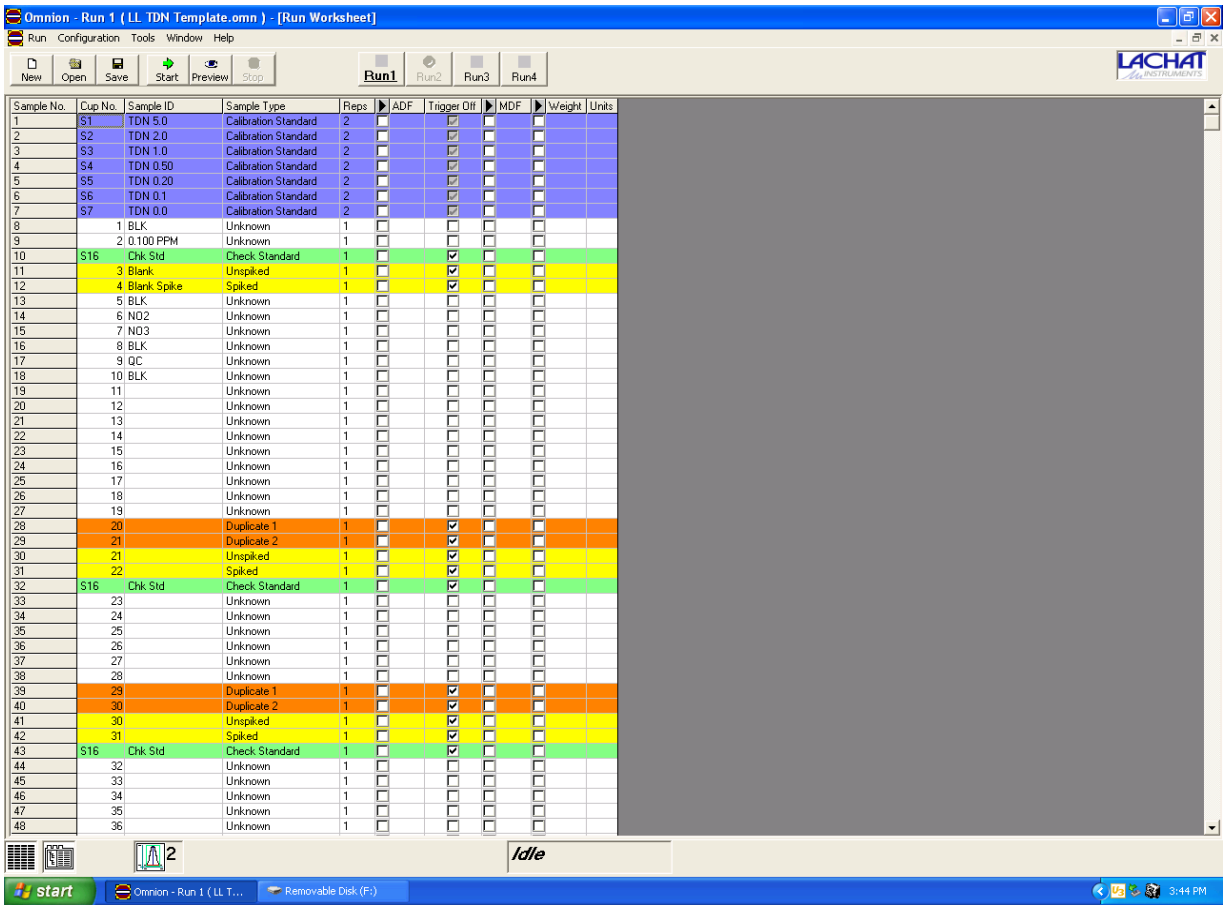
9.3.1 Set up manifold as in the diagram.

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.

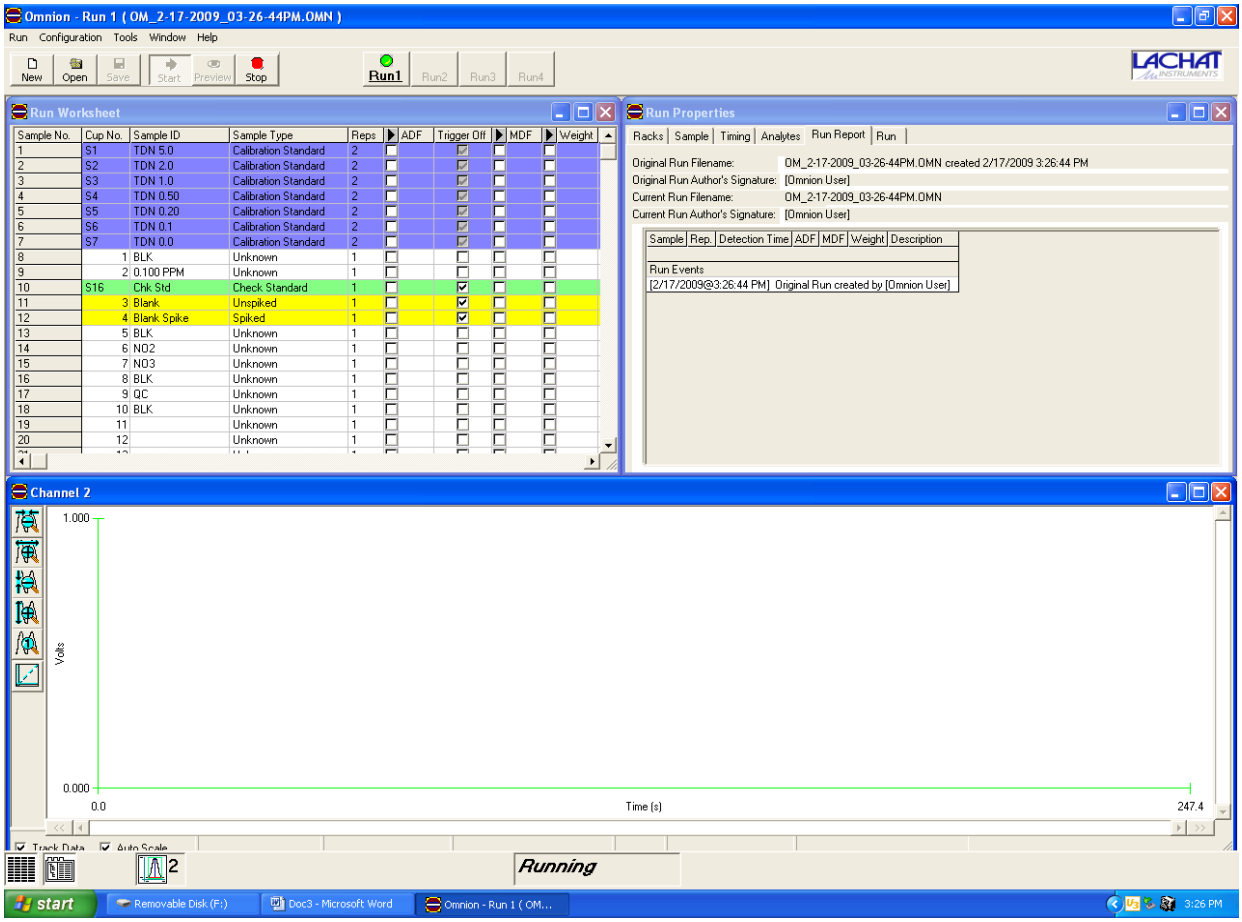


9.3.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

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.

_____ 6 _____

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

7 _____ 8 9

Supervisor's Signature and Date 10

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	
Oxidizing Reagent			

External QC

Identification = _____

True Value = TDN /TDP ppm

Range = TDN ppm

Range = TDP ppm

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

TABLE OF CONTENTS

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.

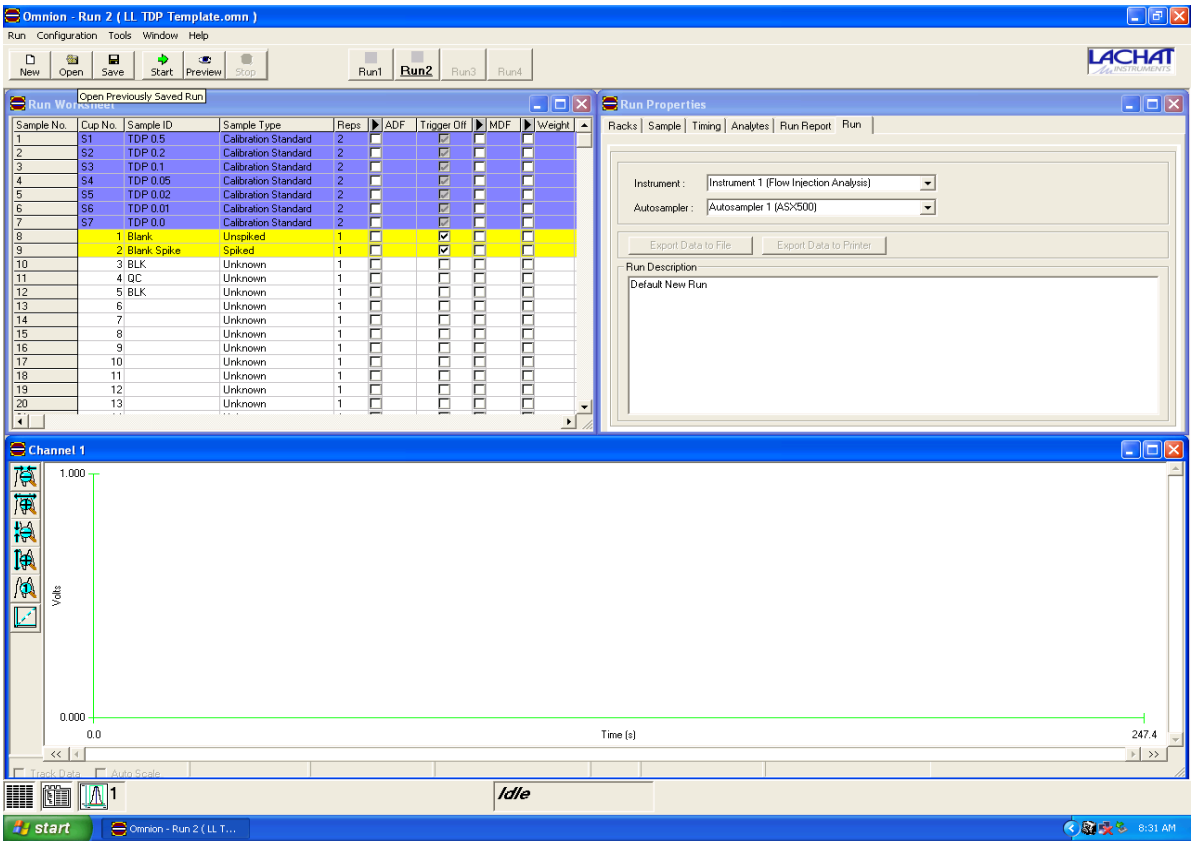
9.2.12 Click on **"Start"** tab to begin the analysis.

9.3 Instrument set-up and sample analysis

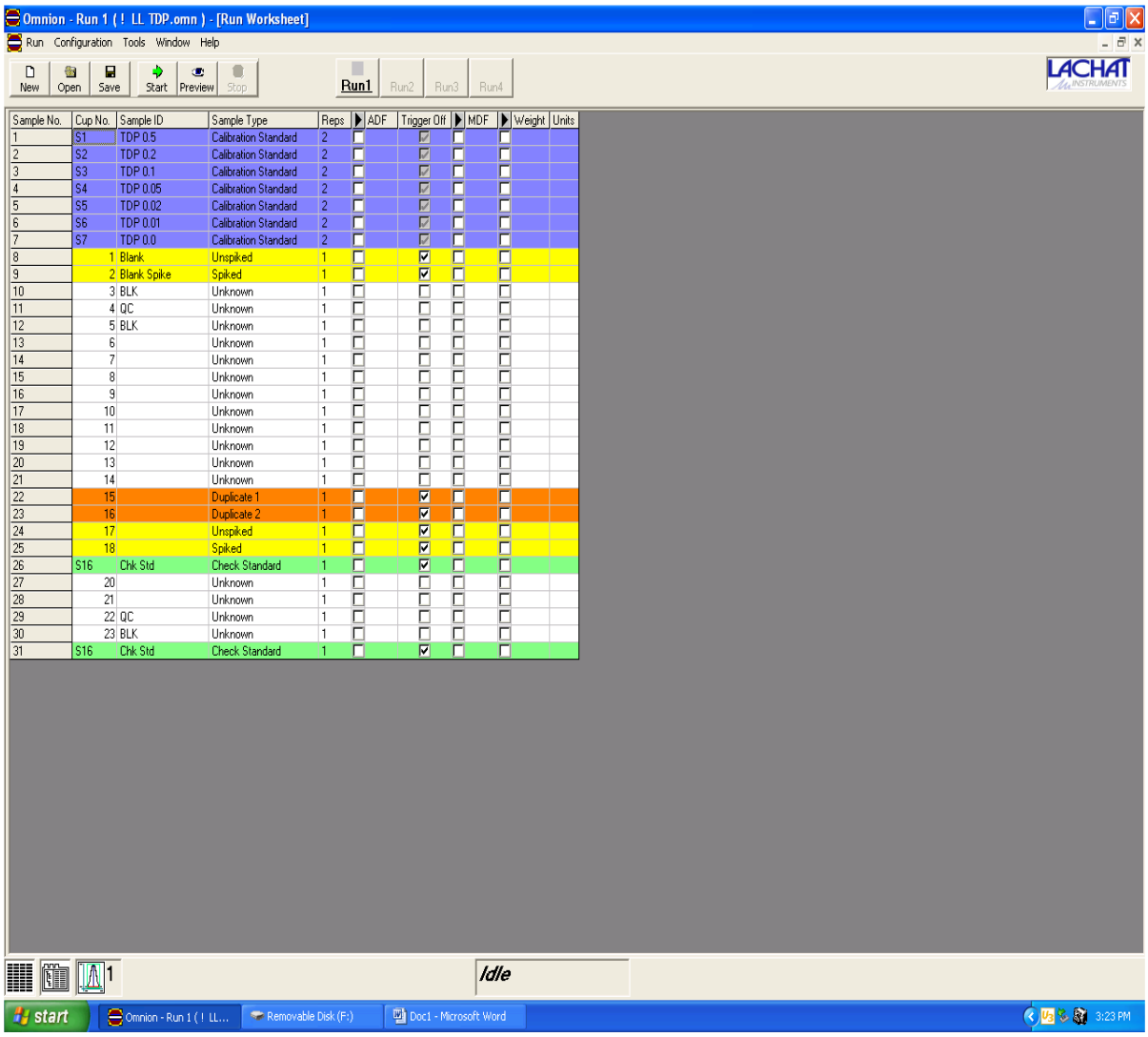
9.3.1 Set up manifold as in the diagram.

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	☐	☑	☐	☐	

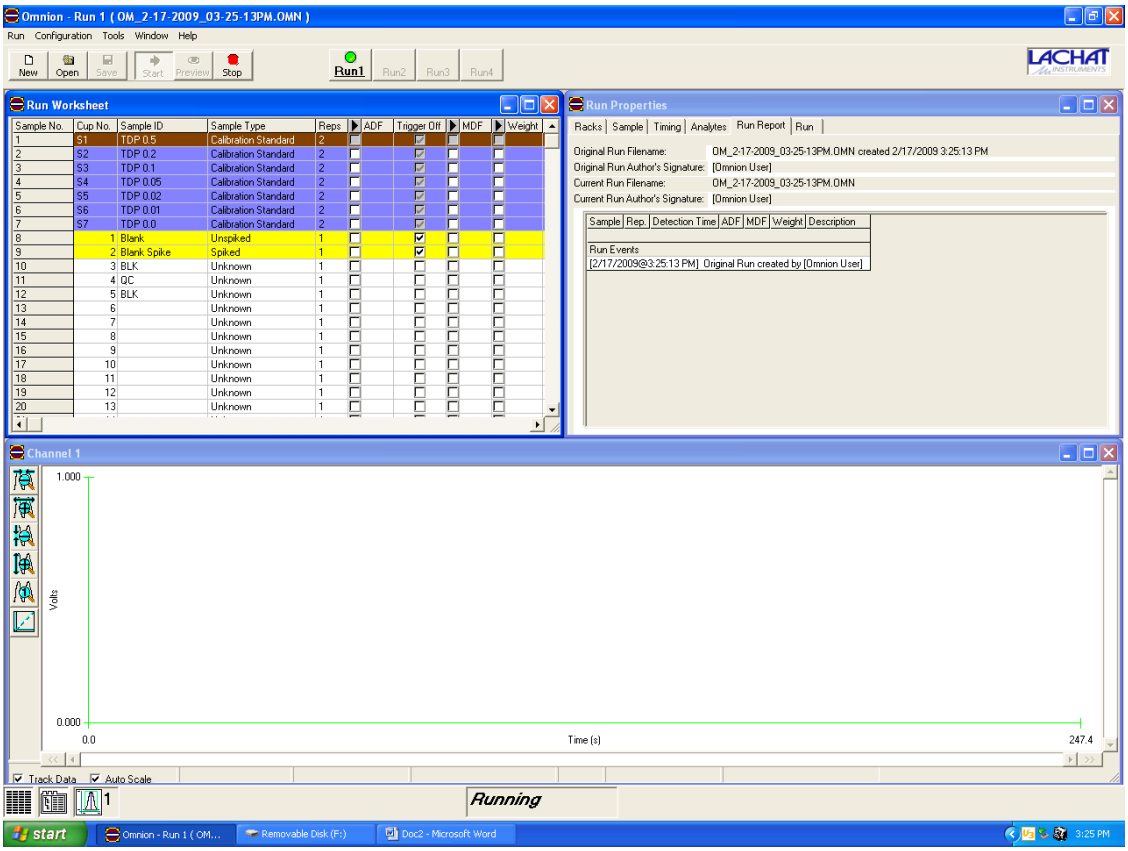
9.3.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

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. ≥ 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.

6.1.3 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.

6.1.4 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.

6.2.2 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.

6.2.3 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^{o}C. Use this standard as spiking solution.

6.2.4 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.

6.2.5 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.

6.2.6 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 –20ºC 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.

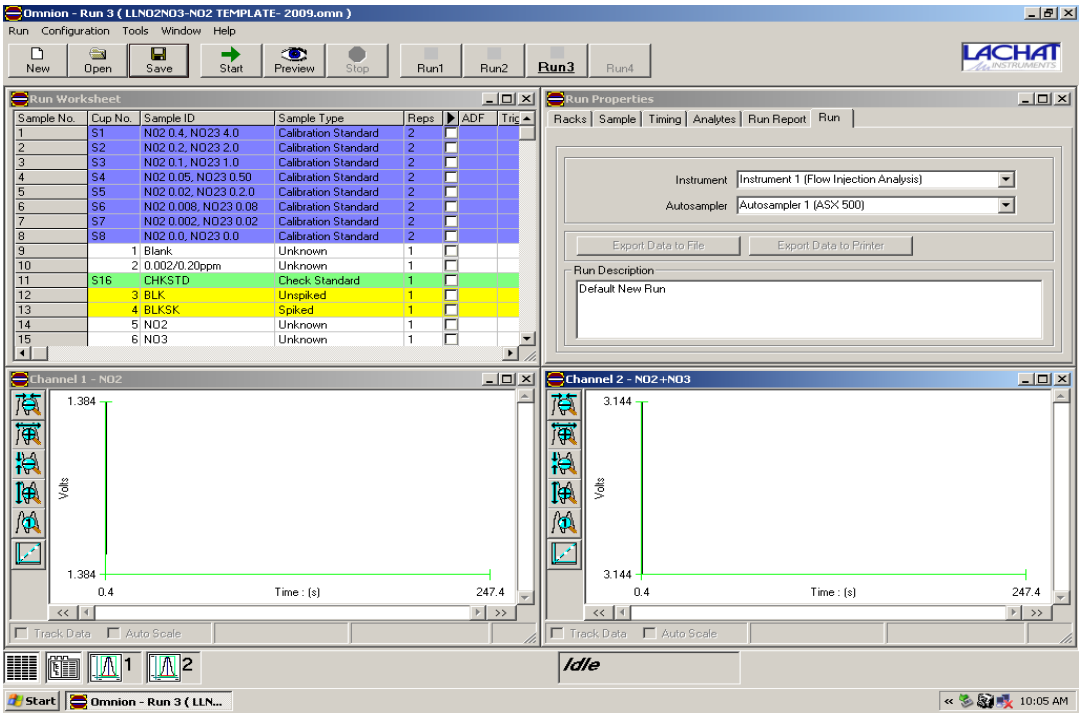
9.1.3 To prevent bubble formation, degas all reagents with helium, except those specified not to. Use helium at 140 Pa (20lb/in2)

9.2 Instrument calibration and sample analysis

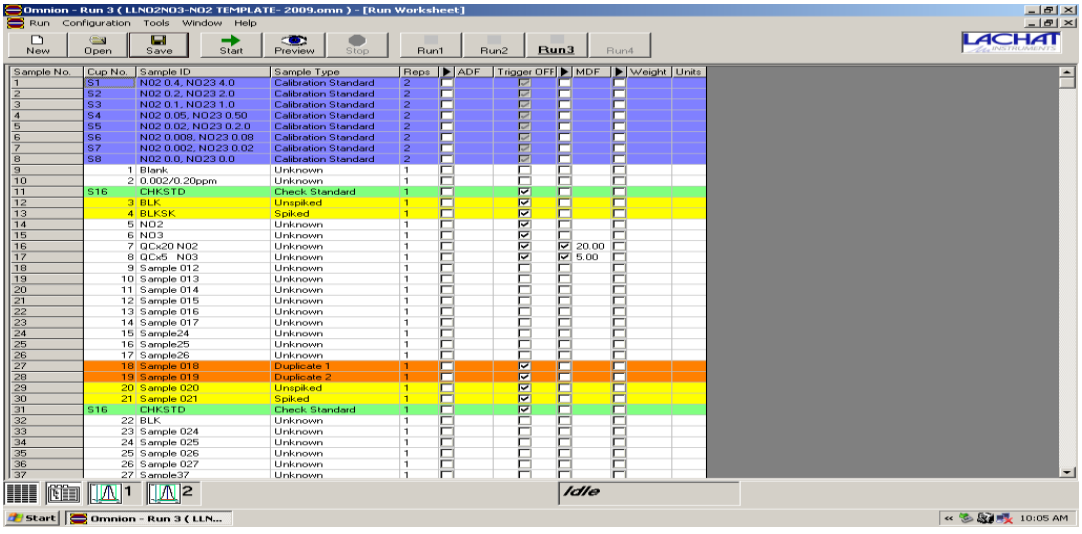
9.2.1 Set up manifold as in the method's manifold diagram.

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.



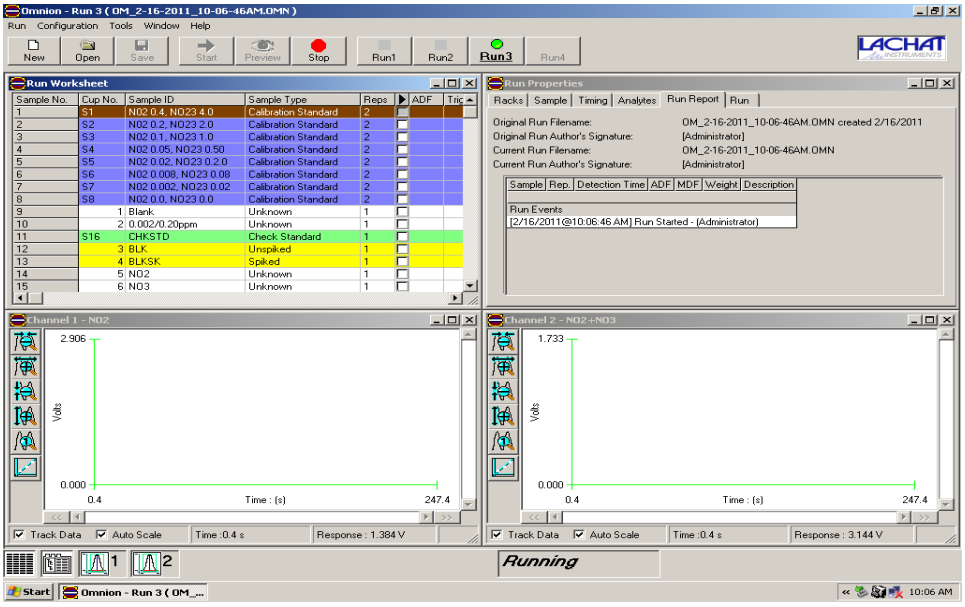
9.2.5 Enter all the sample, sample spike, QC and standard information in the run worksheet and print out the list.

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

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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.

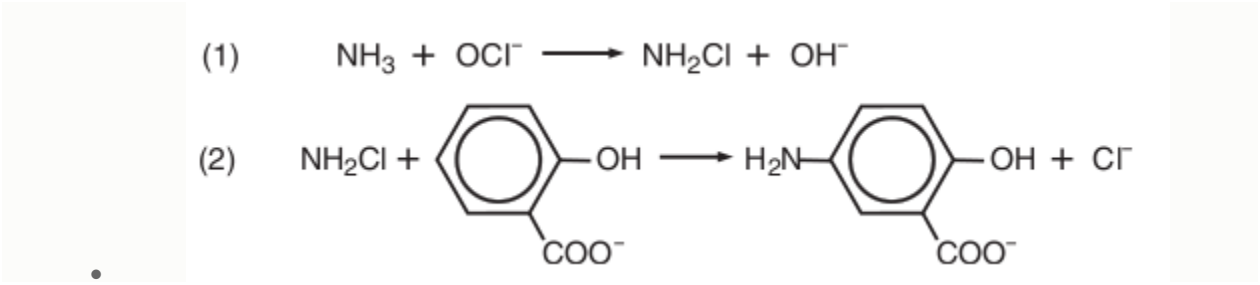


Fig 1: Ammonia compounds are initially combined with hypochlorite to form monochloramine (1),which then reacts with salicylate to form 5-aminosalicylate (2).

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.

5.1.1.1 XYZ Auto sampler ASX-520 series with sample, standard and dilution racks

5.1.1.2 Manifold or reaction unit

5.1.1.3 Multichannel Reagent Pump RP-100 series

5.1.1.4 Colorimetric Detector

5.1.1.4.1 Flowcell, 10 mm, 80uL, glass flow cell

5.1.1.4.2 660 nm interference filter

5.1.1.5 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.

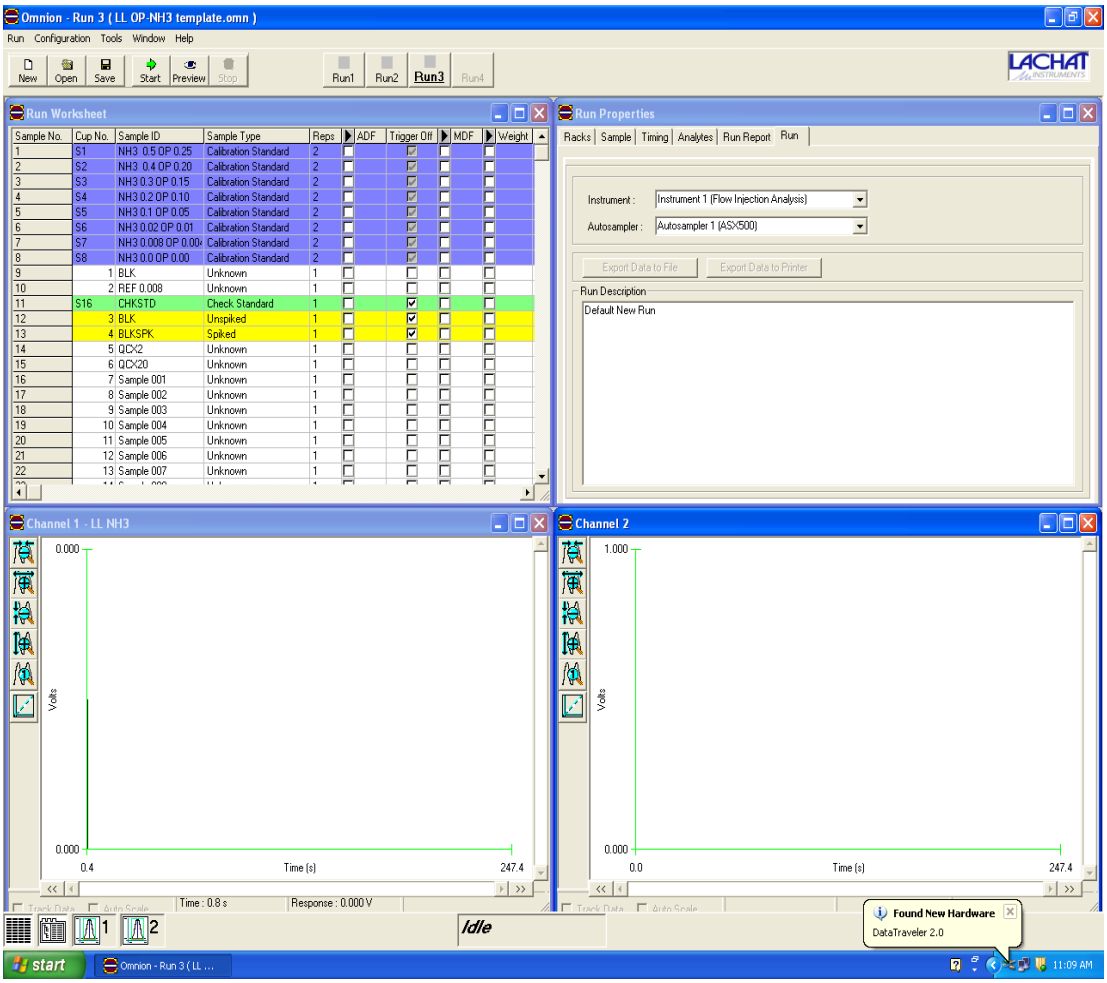
9.1.4 To prevent bubble formation, degas all reagents with helium for one minute. Use Helium at 140 kPa (20lb/in2) through a helium degassing tube or a pipette.

9.2 Instrument set-up and sample analysis

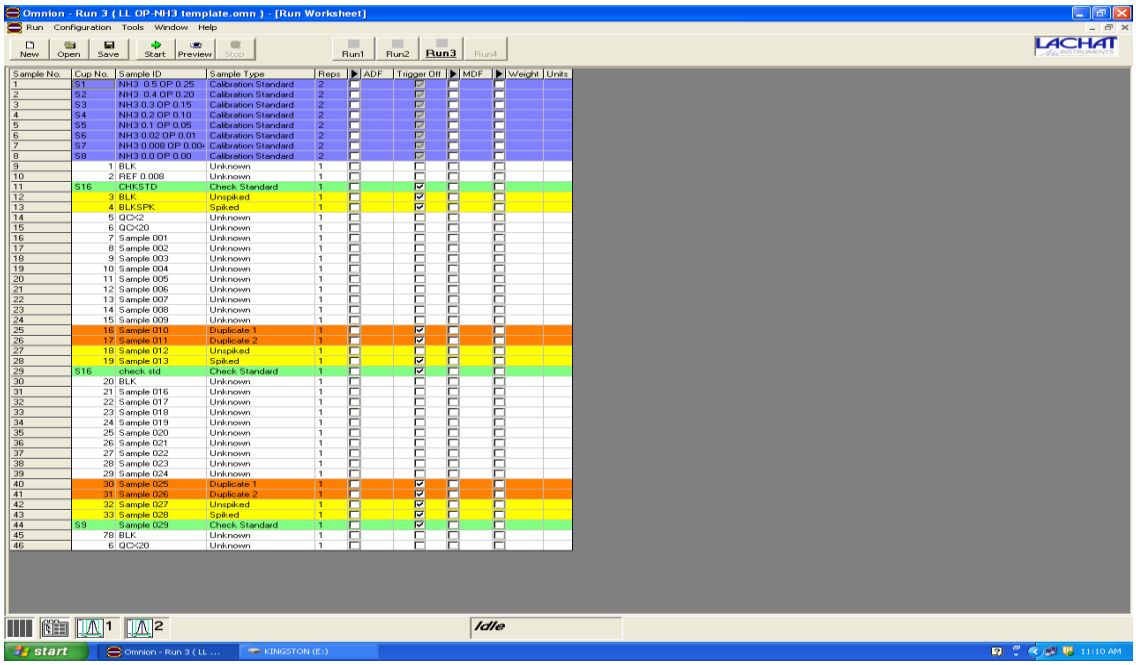
9.2.1 Set up manifold as in the diagram.

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.

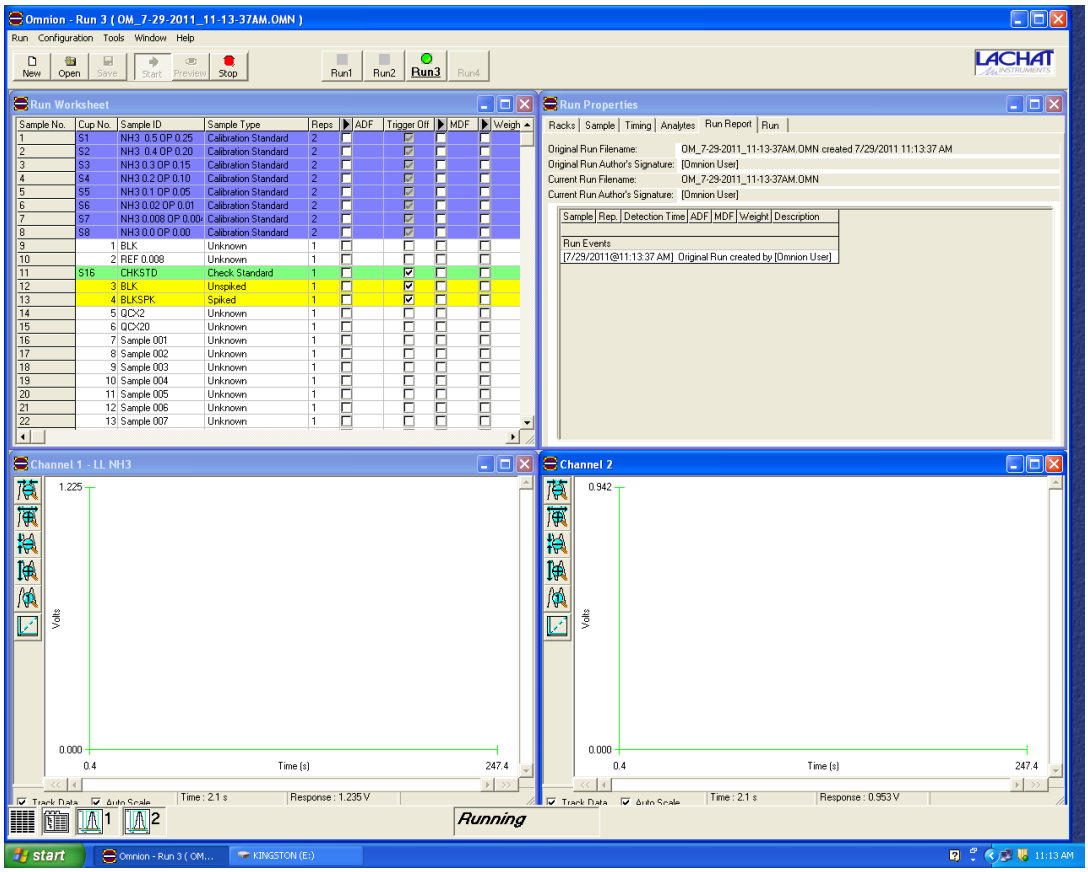


9.3.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

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:

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

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

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

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 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	External QC	
Buffer				Identification =	
Salicylate Nitroprusside				True Value =	ppm
Sodium Hypochlorite				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

5.2.1.1 Manifold or reaction unit

5.2.1.2 Multichannel Reagent Pump RP-100 series

5.2.1.3 Colorimetric Detector

5.2.1.3.1 Flowcell, 10 mm, 80uL, glass flow cell

5.2.1.3.2 880 nm interference filter

5.2.1.4 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.

6.2.2 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.

6.2.3 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.

6.2.4 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.

6.2.5 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.

6.2.6 Carrier – Use DI water for carrier degassed for one minute.

6.3 Standards

6.2.7 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.

6.2.8 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.

6.2.9 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.

6.2.10 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.

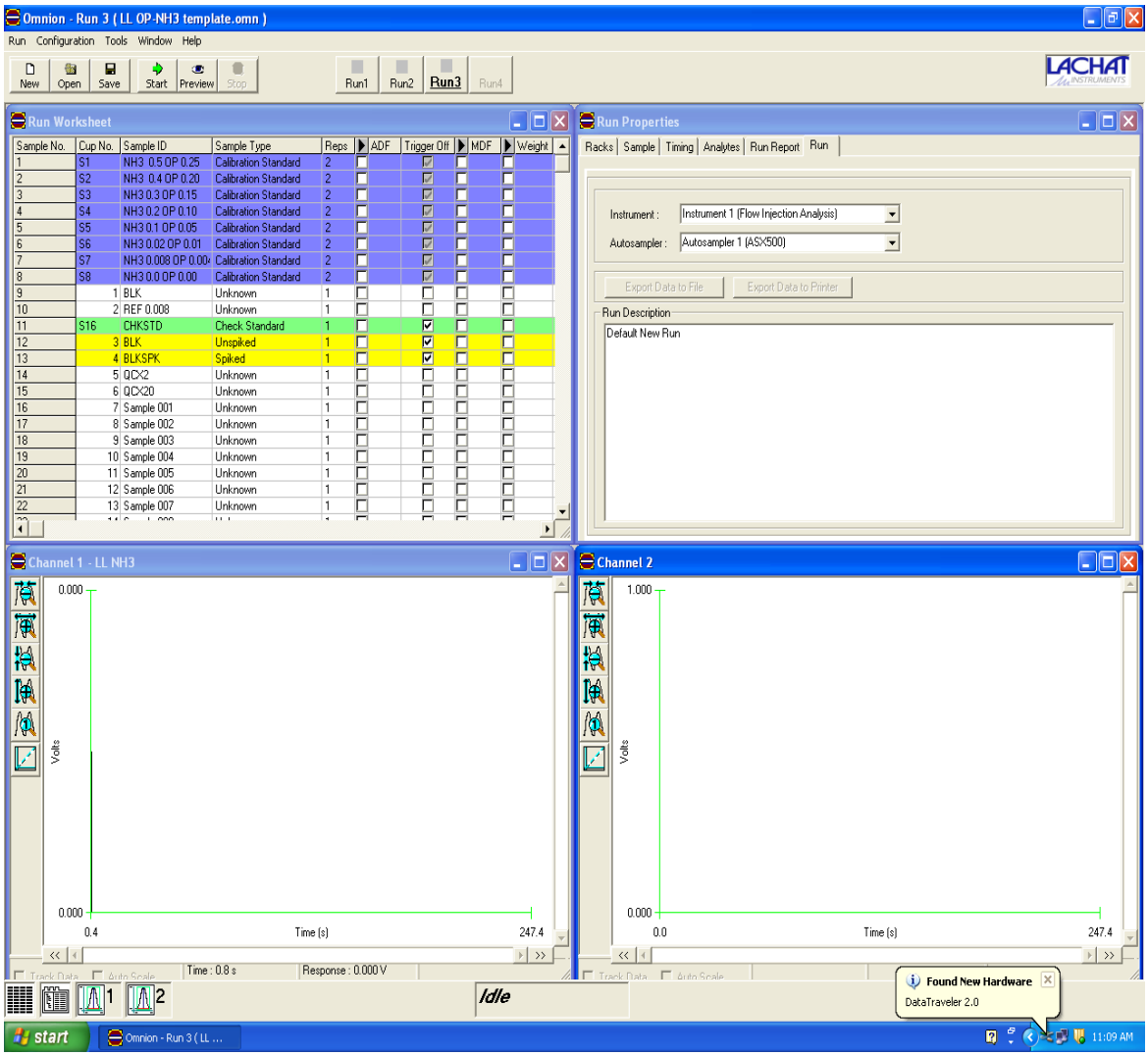
9.1.2 To prevent bubble formation, degas all reagents, except those specified by the method with helium. Use He gas at 140 kPa ($20 lb/in^2$) through a helium degassing tube or a pipette for 1.5 minutes.

9.2 Instrument set-up and sample analysis

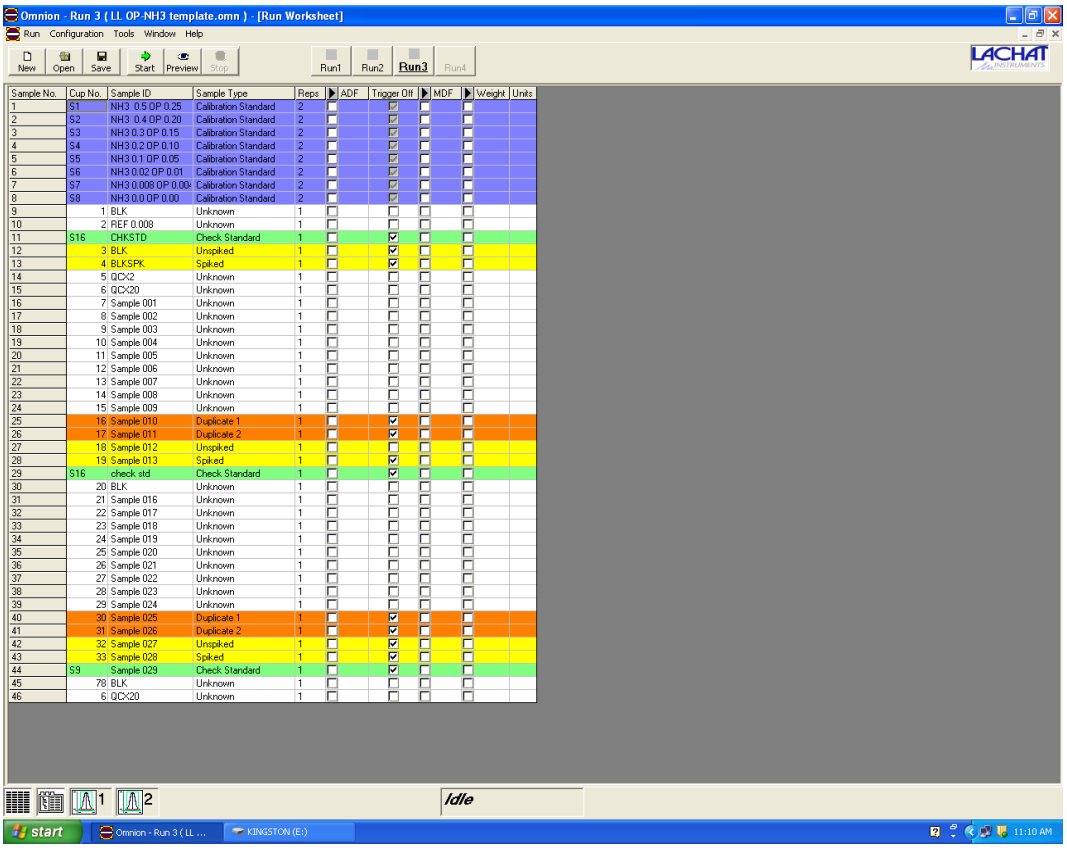
9.2.1 Set up manifold as in the diagram.

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.

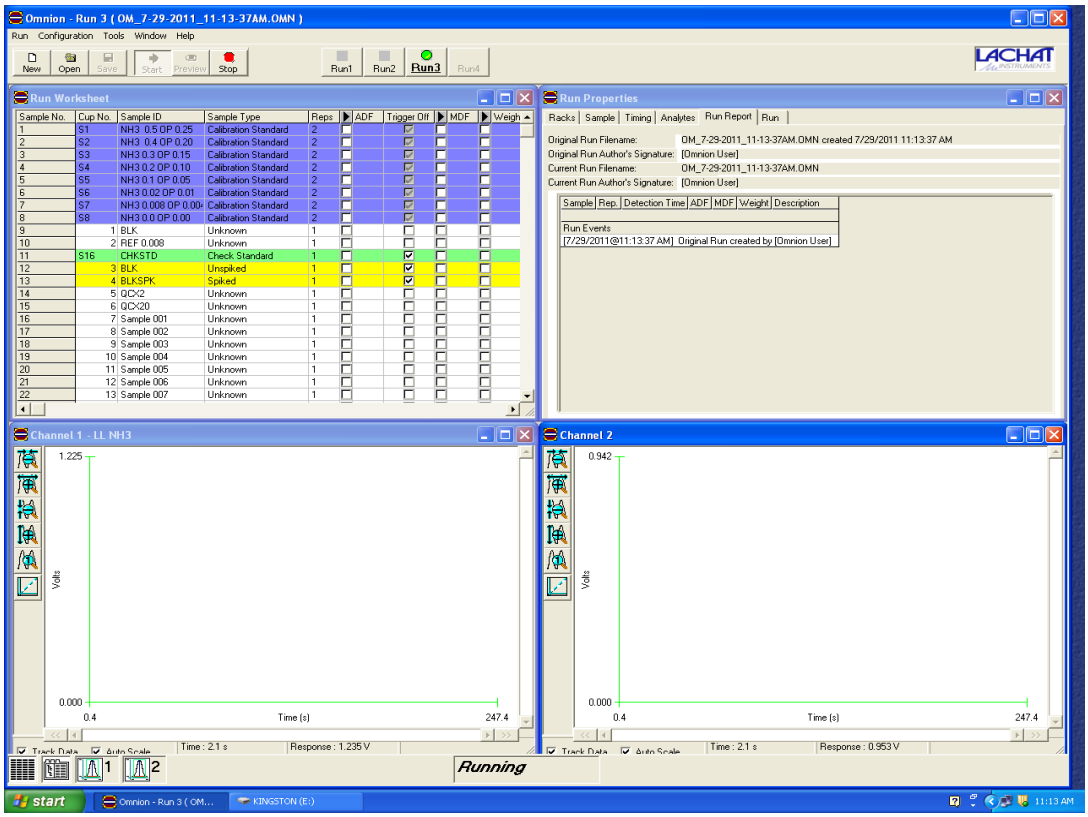


9.2.5 Print a copy of this worksheet by first double clicking on **"Run"** icon and then selecting **"Export Worksheet Data"**.

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