Title and Approval Page (A1)

Quality Assurance Project Plan for Silver Lake Water Quality Monitoring Program

Revision: 1
Lead Organization: Central Plymouth County Water District Commission
Partner Organization(s): ESS Group, Inc., Kimberly Groff Consulting
January 18, 2022

Joanne Zygmunt – Chair, Central Plymouth County Water District Commission	Date
Jack O'Leary – Commissioner, Central Plymouth County Water District Commission	Date
Mark Sotir – Commissioner, Central Plymouth County Water District Commission	Date
Kimberly Groff – Project Liaison	Date
Matt Ladewig - ESS Project Manager	Date
Jeff Hershberger – ESS QA Officer	Date
Nora Conlon – EPA Region 1 Quality Assurance Reviewer	Date
Suzanne Flint – MassDEP Environmental Analyst	Date

This QAPP was developed in accordance with the following guidance documents: EPA Guidance for Quality Assurance Project Plans (EPA QA/G-5) (EPA, 2002), EPA New England Environmental Data Review Program Guidance (EPA, 2018), and EPA New England QAPP Guidance for Projects Using Secondary Data (EPA, 2009).

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Distribution List (A3)

Organization	Contact(s)	Email Address(es)
Central Plymouth County Water District Commission	**Joanne Zygmunt** Chair, CPCWDC **Jack O'Leary** Commissioner, CPCWDC **Mark Sotir** Commissioner, CPCWDC **Frank Basler** Administrator, CPCWDC (508) 830-9104	jzygmunt@plymouthcountyma.gov joleary@plymouthcountyma.gov msotir@plymouthcountma.gov fbasler@plymouthcountyma.gov
Kimberly Groff Consulting	**Kimberly Groff** Consultant (508) 932-5528	kimberlygroffma@gmail.com
ESS Group, Inc.	**Matt Ladewig** Senior Water Resources Scientist (401) 330-1204 **Jeff Hershberger** Senior Hydrogeologist (781) 419-7702	mladewig@essgroup.com jhershberger@essgroup.com
Massachusetts Department of Environmental Protection	**Suzanne Flint** Environmental Scientist (508) 767-2859	Suzanne.flint@state.ma.gov
US Environmental Protection Agency	**Nora Conlon** Quality Assurance Officer (617) 918-8335	conlon.nora@epa.gov

Section A: Project Management

The following section provides information regarding the background of the Silver Lake Water Quality Monitoring Program, the tasks involved in completing the project, and the names and responsibilities of key project team members.

A4: Project Task and Organization

See **Table 1** for a list of the specific members from each organization and **Figure 1** for an organizational chart.

Table 1. Project Participants

Name	Title	Organization	Primary Responsibility
Joanne Zygmunt	Chair	CPCWDC	Project Oversight
Jack O'Leary	Commissioner	CPCWDC	Project Oversight
Mark Sotir	Commissioner	CPCWDC	Project Oversight
Frank Basler	County Administrator	County of Plymouth	Project Administration
Kimberly Groff	Consultant	Kimberly Groff Consulting	Project Liaison
Matt Ladewig	Senior Water Resources Scientist	ESS Group, Inc.	Project Manager
Jeff Hershberger	Senior Hydrogeologist	ESS Group, Inc.	Project QA Officer

Central Plymouth County Water District Commission

The Central Plymouth County Water District Commission (CPCWDC; The Commission) oversees water supply resources within the Central Plymouth County Water District. The Commission works with partners to ensure safe, sustainable drinking water supplies, ecological health, and recreational enjoyment.

The Silver Lake Water Quality Monitoring Program was initiated by the Commission, who is responsible for funding and overseeing the project. The three-person volunteer Commission receives staff support from Plymouth County.

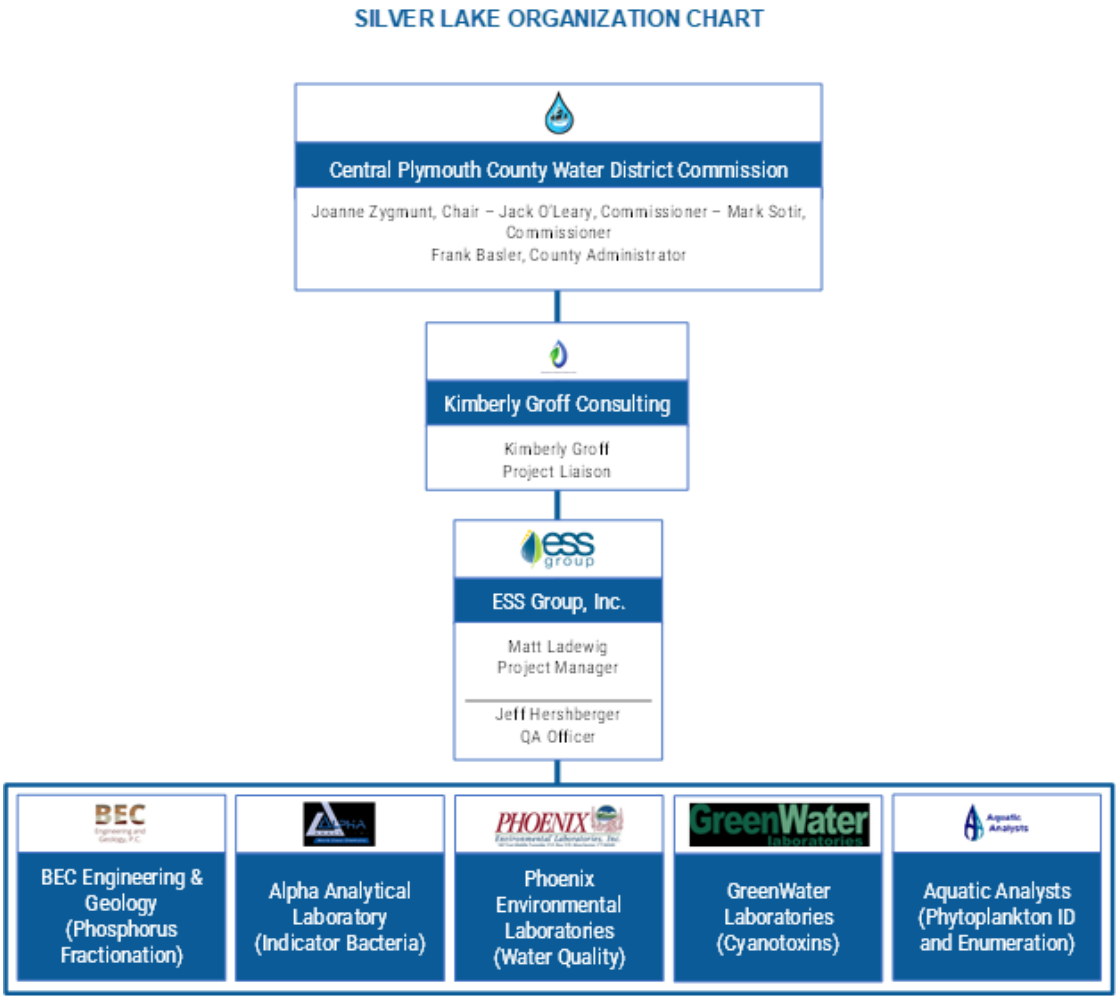


Figure.1. Project Organizational Chart

Kimberly Groff Consulting

Kimberly Groff Consulting will act as project liaison between the CPCWDC and ESS. In this role, Dr. Groff will assist the Commission in overseeing the project and reviewing ESS's project deliverables. She will also serve as the day-to-day project point of contact for ESS.

ESS Group, Inc.

ESS Group, Inc. (ESS) is the prime environmental contractor responsible for the design and implementation of this project under contract with the CPCWDC. ESS Project Manager Matt Ladewig will serve as the contractor's primary point of contact for the project, overseeing implementation of the field program, management of laboratory subcontractors, and development of project deliverables. ESS QA Officer Jeff Hershberger will be responsible for quality assurance/quality control review of field and laboratory-generated data and ESS deliverables.

A5: Problem Definition and Background

Silver Lake is a designated Class A waterbody and Outstanding Resource Water (ORW) located in the towns of Halifax, Plympton, Pembroke, and Kingston, Massachusetts. In addition to serving as the primary source water reservoir for the City of Brockton and connected drinking distribution systems, Silver Lake constitutes the headwater source of the Jones River.

Concerns have arisen regarding potential water quality impacts to Silver Lake from watershed sources and water diversions. From the north, surface water is sourced from Tubbs Meadow Brook, with connections to Furnace Pond (via seasonal diversion) and other waterbodies. From the west, water is seasonally diverted to Silver Lake from East Monponsett Pond, which induces backflow from West Monponsett Pond. A draft Phosphorus Total Maximum Daily Load (TMDL) has been developed for the Monponsett Ponds (MassDEP, 2021).

Silver Lake is now proposed for listing in the Draft 2018/2020 Integrated List of Waters by the Massachusetts Department of Environmental Protection (MassDEP). The proposed impairments include Fish Passage Barrier, Flow Regime Modification, and Dissolved Oxygen. Once the 2018/2020 Integrated List is approved by the EPA, the Dissolved Oxygen impairment would require a TMDL to be developed for the waterbody.

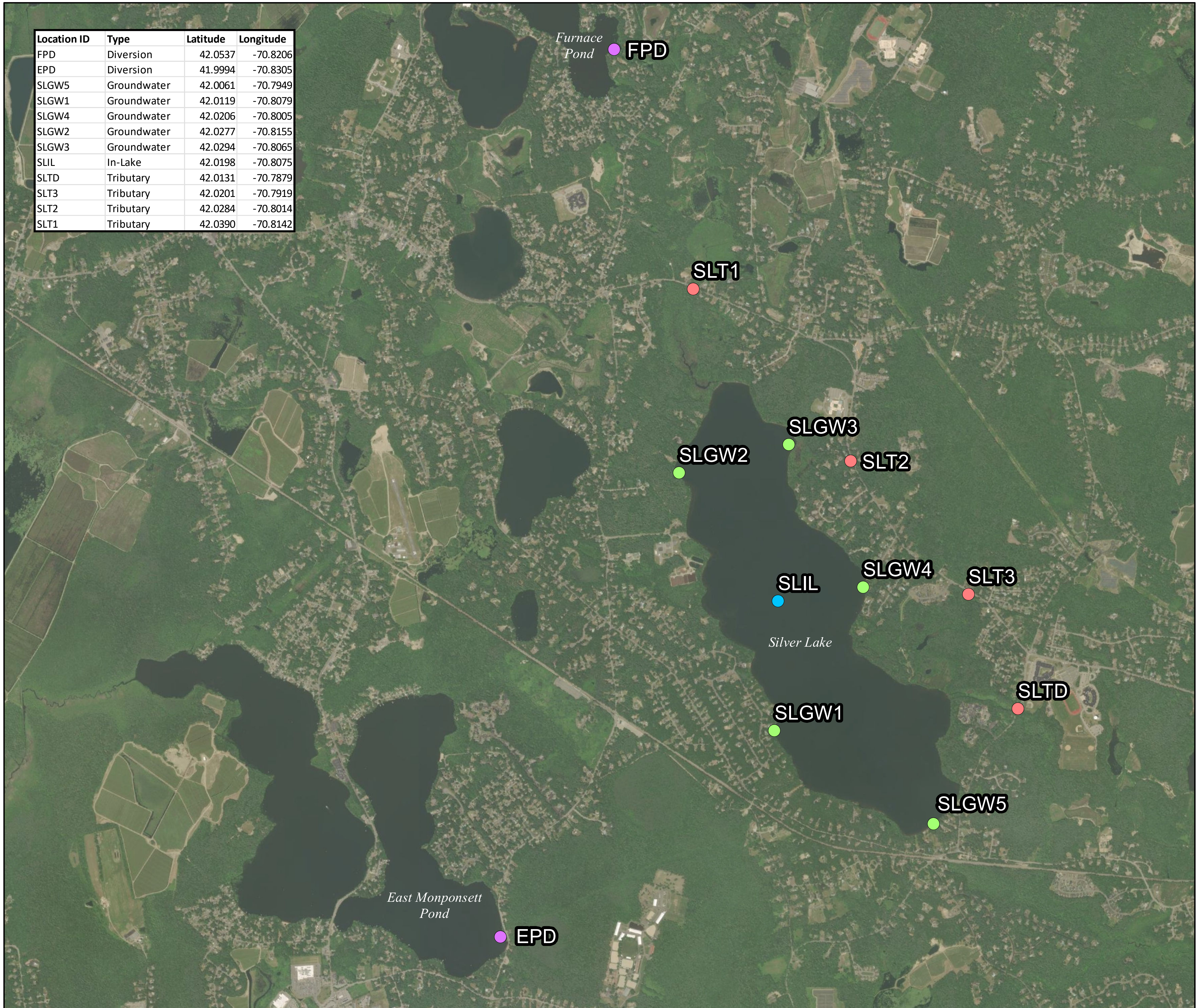
Project Description

The Silver Lake Water Quality Monitoring project seeks to investigate water quality conditions within the geographic area of interest, which includes Silver Lake, its watershed, and interbasin diversion sources (**Figure 2**).

The overall goals of this Project are as follows:

1. Collect water quality data to help inform community management decisions to address water quality and quantity issues in Silver Lake and connected water bodies; and
2. Develop a baseline understanding of current water quality and continue to develop solutions-oriented relationships with the City of Brockton's Water Division and the public.

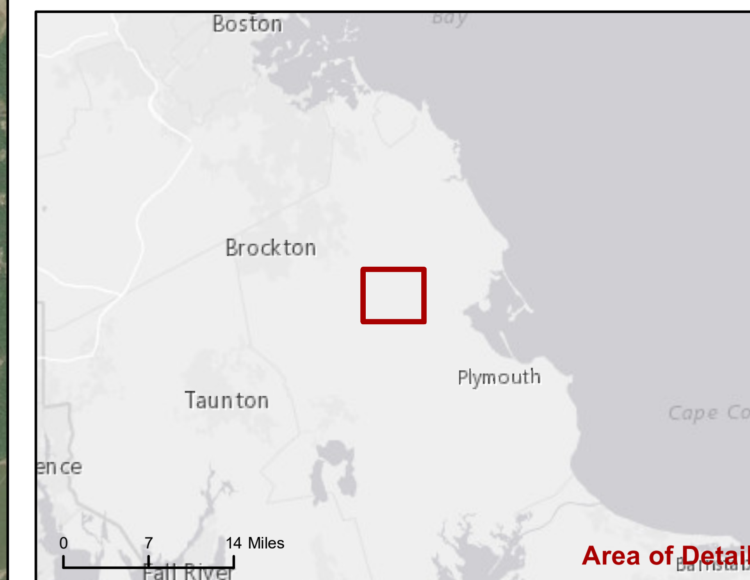
To support these goals, ESS will collect and analyze detailed water quality, physical, hydrologic, and biological data in the area of interest.



Location ID	Type	Latitude	Longitude
FPD	Diversion	42.0537	-70.8206
EPD	Diversion	41.9994	-70.8305
SLGW5	Groundwater	42.0061	-70.7949
SLGW1	Groundwater	42.0119	-70.8079
SLGW4	Groundwater	42.0206	-70.8005
SLGW2	Groundwater	42.0277	-70.8155
SLGW3	Groundwater	42.0294	-70.8065
SLIL	In-Lake	42.0198	-70.8075
SLTD	Tributary	42.0131	-70.7879
SLT3	Tributary	42.0201	-70.7919
SLT2	Tributary	42.0284	-70.8014
SLT1	Tributary	42.0390	-70.8142

Legend

- Sample Location with Diversion
- Groundwater Sample Location
- In-Lake Sample Location
- Tributary Sample Location



0 550 1,100 2,200 Feet

Source:
1) ESRI, World Imagery, 2019
2) ESS, Field Survey, September 2021

Water Quality Sample Locations

Silver Lake Water Quality Monitoring Program
Central Plymouth County Water District Commission

ess group
environmental consulting & engineering services

Figure 2

Drawing Date: 1/17/2022 Path: ...C663-000 Central Plymouth County Water District Commission- Silver Lake Water Quality Monitoring Program\04 GRAPHICS\GIS\MXD\C663_FigXX_WQSampleLocationsV05.mxd

A6: Project Task Descriptions

An overview of project tasks and schedule is presented in **Table 2** and **Figure 3**.

Table 2. Project Tasks and Schedule

Task	Deliverable	Timeline	Relevant Details/Comments
1. Administration and Reporting	Detailed Project Plan	By July 2021	Completed
2. Develop Sampling and Analysis Plan and Associated QC Documents	Sampling and Analysis Plan (SAP)	SAP by August 2021	SAP finalized in January 2022 following extended public comment period.
	Approved/Approvable QAPP	QAPP by February 2022	Assumes review by EPA and MassDEP
3 Public Input Plan	Public Input Plan (PIP)	PIP by August 2021	Completed
	Informational Leaflets	Informational leaflets twice over project period	Timing of distribution to be determined
	Agendas, presentations, and notes for SAP Review Meeting and Public Listening Session	SAP Review Meeting by August 2021	SAP Review Meeting completed in August 2021.
		Public Listening Session following completion of field program	Public Listening Session will include a presentation of project findings and opportunity for public comment.
4. Implementation of Sampling and Analysis Plan	Data deliverables, tabular summaries, figures, and accompanying narrative to be included in Technical Memorandum	Technical Memorandum following completion of field program	Entire field program is included in this task. The field program will extend from September 2021 through October 2022.

Prepared on 1/17/2022

CPCWDC - Silver Lake
Detailed Project Schedule: June 2021 to June 2022

Task	2021 June Late	July Early	July Late	August Early	August Late	September Early	September Late	October Early	October Late	November Early	November Late	December Early	December Late	2022 January Early	January Late	February Early	February Late	March Early	March Late	April Early	April Late	May Early	May Late	June Early	June Late
1 Administration and Reporting																									
Project Kick-off																									
Project Detail																									
Status Updates/Meetings								Canceled							Timing of additional meetings TBD										
2. SAP and QAPP																									
Draft SAP																									
Revised SAP (v1)																									
Revised SAP (v2)																									
Final SAP																									
Draft QAPP																									
Revised QAPP																									
Final QAPP																Depends on EPA & DEP review time									
3. Public Input Plan																									
Draft PIP																									
Final PIP																									
SAP Review Meeting																									
Draft Launch Informational Leaflet															TBD										
Final Launch Informational Leaflet															TBD										
Public Listening Session																									
Draft Project Informational Leaflet																									
Final Project Informational Leaflet																									
4. SAP Implementation																									
Bathymetric, Aquatic Plant, and Benthic Surveys																									
In-Lake Water Column & Quality Sampling																									
In-Lake Bacteria Testing (approved under Task 4)																									
In-Lake Cyanotoxin Testing (approved under Task 4)								Contingent on diversion activity																	
Upstream and Downstream Monitoring																									
Groundwater Assessment																									
Draft Technical Memorandum																									
Revised Final Technical Memorandum																									
5. Internal P-Loading Analysis and Water Quality Modeling																									
Sediment Coring and Phosphorus Fractionation																									
Water Quality Model																									

	Planned Event
	Completed Event
	Planned Deliverable
	Completed Deliverable

Prepared on 1/17/2022

CPCWDC - Silver Lake
Detailed Project Schedule: July 2022 to June 2023

Task	2022												2023											
	July		August		September		October		November		December		January		February		March		April		May		June	
	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
1 Administration and Reporting																								
Project Kick-off																								
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5. Internal P-Loading Analysis and Water Quality Modeling																								
Sediment Coring and Phosphorus Fractionation																								
Water Quality Model																								

Planned Event
Completed Event
Planned Deliverable
Completed Deliverable

Task 1: Administration and Reporting

This task consists of project kick-off and ongoing coordination between the primary project partners, including the CPCWDC, Kimberly Groff, and ESS's Project Manager. Additionally, this task includes the development of a detailed project plan (Project Detail) by ESS showing key events, deliverables, and milestones over the duration of the project.

Task 2: Development of Sampling and Analysis Plan and Associated QC Documents

To facilitate the collection of high quality data that address the primary goals of the Silver Lake Water Quality Monitoring Program, the Project Team will develop the following documents:

1. Sampling and Analysis Plan (SAP) – The SAP provides the framework for field components of the program. In general, the SAP documents the procedural and analytical requirements for collection of sampling data to characterize Silver Lake. The SAP was designed to identify and investigate key water quality drivers in Silver Lake; as such, it identified the key parameters to be measured or sampled and presented an overview of the means and schedule for doing so. The SAP also defined the geographic area for data collection, including Silver Lake, its natural tributaries and outlet, water diversion sources, and shallow groundwater.

   ESS was responsible for preparation of the Draft SAP. Kimberly Groff Consulting and the CPCWDC provided input on the Draft SAP that was incorporated into the Draft Final (Revised) SAP. Additionally, ESS presented the Draft Final SAP at a public meeting on August 9, 2021. The CPCWDC allowed for public comment on the Draft Final SAP at this meeting, followed by a written comment period that extended to September 30, 2021. The SAP was further revised, and a QAPP was developed, to address the comments received.

   The Final SAP describing the full monitoring program incorporated revisions, such as the inclusion of public comments, and will be approved by the CPCWDC in early 2022 after comment and review..

2. Quality Assurance Project Plan (QAPP) – Although the Silver Lake Water Quality Monitoring Program has not received federal funding to date, the development of an EPA-approvable QAPP is highly desirable, given the need for high quality data to inform decisions regarding the management of a primary source water reservoir. The QAPP will also be submitted to MassDEP for review and approval.

   This QAPP describes the quality management system and procedures, as well as the roles and responsibilities of the Project Team. It also provides an

overview of the project goals, data quality objectives, and quality assurance related to data collected and used as part of the project.

ESS is responsible for development of the Draft QAPP, which will be submitted to Kimberly Groff Consulting and the CPCWDC for review and comment. ESS will incorporate comments and edits received into a Revised Draft QAPP for submission to EPA and MassDEP. Upon receiving comments from EPA and MassDEP, ESS will revise the QAPP and distribute it to signatory parties for sign-off. ESS will maintain this final version of the QAPP as part of the project documentation and distribute it electronically to designated recipients.

At the conclusion of the project, ESS will include an assessment of quality assurance/quality control measures implemented, their outcomes, and impacts on the usability of project data.

Task 3: Public Input Plan

The Public Input Plan seeks to ensure engagement in the monitoring project and anticipated results among the Silver Lake communities and interested stakeholders. The Public Input Plan was designed to acknowledge community concerns early in the project development phase, allowing community members to provide suggestions, communicate concerns, and raise questions as the SAP was developed and as the Project is implemented. Once sampling is completed, results will be interpreted and shared with the public. The Public Input Plan was designed to provide transparency and knowledge to the community via direct outreach, a public presentation, a public listening session, and publicly accessible deliverables.

ESS develops technical content for presentation and discussion at public meetings and, together with Kimberly Groff Consulting, assists the CPCWDC in receiving, organizing, and tracking public comments and feedback on the Silver Lake Water Quality Monitoring Program. The CPCWDC conducts direct outreach to stakeholders and posts meeting announcements, recorded video of remote meetings, meeting minutes, and key Project deliverables on their website (https://www.centralplymouthcountywater.org/) and affiliated webpages on the Plymouth County website (https://www.plymouthcountyma.gov/).

Task 4: Implementation of Sampling and Analysis Plan

Field data will be collected as part of the Silver Lake Water Quality Monitoring Program in the following categories:

A. Bathymetric, Aquatic Plant, and Benthic Surveys

B. In-Lake Water Column & Quality Sampling

C. Upstream and Downstream Monitoring

D. Groundwater Assessment

E. Sediment Coring and Phosphorus Fractionation

The approach for documenting each of these is detailed in the following sections:

A. Bathymetric, Aquatic Plant, and Benthic Surveys

Bathymetric, aquatic plant, and benthic surveys of Silver Lake will be completed and used to create detailed maps of water depth, plant growth, and area of the lake impacted by anoxia. Please refer to Section B1 for explanation of the rationale and goals for the bathymetric, aquatic plant, and benthic surveys. Further task details are provided for each component in the following sections:

Bathymetry

Bathymetry will be measured using an echosounder in deep, open waters and a calibrated sounding line in shallower waters where plant growth is dense. Lake bathymetry will be tied to the same vertical control as the prior survey completed by Coler and Colantonio (2003) and used to create an updated contour map of the lake. This will allow for comparison with the prior survey and the targeting of specific locations for additional survey effort, if needed.

The bathymetry survey will include at least 350 survey locations. The locations will be distributed using a gridded survey approach. This method is similar to point-intercept survey methods, in that it uses a pre-determined sampling interval to ensure adequate coverage of the entire water body. The primary difference is that, whereas point-intercept survey methods require navigation to a specific point (i.e., the intersection of each grid line), the gridded survey only requires navigation to each cell. This ensures adequate coverage of survey data throughout the lake while providing field crews with flexibility select the exact location and number of points within each cell based on observed field conditions.

The field data and geographic coordinates for each data point will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Aquatic Plants

Aquatic plant growth will be assessed at each survey location using macrophyte throw-rakes and direct observation from the boat. Rooted and floating aquatic plants will be field identified to the lowest practicable taxonomic level (typically genus/species). However, specimens that cannot be readily field-identified to the target level will be returned to the office and identified under a high-powered dissecting microscope. Please refer to the attached SOP in Appendix A for additional details on field collection and identification of aquatic plants.

Aquatic plant data collected will include community composition, vegetative cover (percent of bottom) and biovolume (measure of vegetative growth in the water column). Data collected from the aquatic plant survey will be used to generate maps

of plant cover and biovolume for the lake, as well as the locations of any aquatic invasive species encountered.

The aquatic plant surveys will include at least 350 survey locations. As with the bathymetry survey, the plant survey locations will be distributed using a gridded survey approach.

The field data and geographic coordinates for each data point will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Macroinvertebrates

Due to their relatively long lifespan (typically months) and wide range of sensitivity to water quality conditions, benthic macroinvertebrates are one of the most useful organisms for inferring longer term water quality conditions in surface waters. At Silver Lake, macroinvertebrate samples will be used to supplement active measurements of dissolved oxygen to help understand and map areas of the lake affected by substantial periods of seasonal anoxia (i.e., areas lacking dissolved oxygen). It is anticipated that bottom sediments that experience extended periods of anoxia will either be devoid of macroinvertebrates or dominated by taxa that are tolerant low dissolved oxygen concentrations.

Benthic macroinvertebrate samples will be collected along a transect perpendicular to the long axis of the lake, allowing the collection of samples from both shallow and deep environments within the lake. A total of seven samples will be collected, each from a different depth (approximately 5 ft, 15 ft, 25 ft, 35 ft, 45 ft, 55 ft, and 65 ft, to be modified as needed if direct dissolved oxygen readings suggest depletion at shallower depths).

ESS will use a 6" x 6" Ekman grab sampler to collect macroinvertebrate samples. This will allow the data to be used to estimate densities of each organism in the sample locations.

Samples will be field-sieved using a 0.5-mm mesh bucket sieve and preserved in 75% ethanol.

Macroinvertebrates will be sorted from each sediment sample in its entirety, then identified and enumerated by a Society for Freshwater Science (SFS) certified taxonomist under a high-power dissecting and/or compound microscope. The target level for macroinvertebrate identification will be genus/species for most organisms. Please refer to the ESS Group Standard Operating Guidelines for Freshwater Macroinvertebrate Sorting in Appendix A for further details on laboratory analysis of macroinvertebrates.

The geographic coordinates for each data point will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

B. In-Lake water Column & Quality Sampling

In-lake sampling of water quality will be used to establish the current baseline conditions in Silver Lake. Sampling will begin in late summer 2021 and extend into 2022, exclusive of months when ice cover is present (currently anticipated to be January and February). The duration of the monitoring program will continue until October 2022.

To ensure acquisition of the most useful and complete dataset over a short period of time, the in-lake monitoring program will include both continuous data logging and collection of discrete water quality samples as part of the field program.

Please refer to Section B1 for further details on the rationale and goals for in-lake water quality monitoring

Continuous Data Logging.

The continuous data logging portion of the field program will include deployment of monitoring arrays at the deepest location in the lake (approximately 70 to 75 feet deep). The datalogger arrays will be used to detect differences in key parameters (primarily temperature and chlorophyll, although water level will also be measured) over time. One array will be suspended approximately five feet from the lake surface and the second array will be located within five feet of the sediment-water interface. The chlorophyll a datalogger is currently only planned to be deployed in the surface monitoring array. The depth of the surface datalogger array will be tied to a surface buoy so that it is able to move up and down with changes in water level and remain at the same relative depth. However, the bottom datalogger will maintained at a fixed location to effectively represent conditions in the deepest portion of the lake hypolimnion.

Loggers will be programmed to collect readings at hourly intervals. Data will be downloaded during each site visit. The logger arrays will be removed prior to the onset of ice cover in December and redeployed once Silver Lake is ice-free in the spring.

The data loggers will be deployed in late summer 2021 and will continue recording data until October 2022, exclusive of the winter months. Depending on the findings of early deployments and the data collected, additional dataloggers may be added to the array to collect data at key locations in the water column. It is also possible that other types of dataloggers would be deployed to monitor dissolved oxygen, pH, or other parameters of interest. Should these additional dataloggers be added to the array, they will generally be deployed in a similar manner as the existing arrays to the extent possible and reasonable given manufacturer recommendations.

The geographic coordinates for the data logger array will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Discrete Water Quality Sampling.

To complement and supplement the continuous data logging program, multiple rounds of discrete in-lake water quality samples will be collected from late summer 2021 and extend into 2022, exclusive of months when ice cover is present (currently anticipated to be January and February). The duration of the monitoring program in 2022 is expected to continue until October 2022, for a total of no less than twelve sampling events.

During these events, samples will be collected from the same in-lake location as the datalogger array. Samples will be collected near the surface, at mid-depth, and near the bottom of Silver Lake. Additionally, water quality profiles will be measured in situ within the water column.

The geographic coordinates for each sampling location will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Water quality samples will be sent to the laboratory for analysis of the following:

- Total Phosphorus
- Total Soluble Phosphorus
- Total Nitrogen (nitrite-N+nitrate-N and Total Kjeldahl N)
- Alkalinity
- Chlorophyll a – surface only
- Algal (Phytoplankton) Enumeration and ID – surface only
- *E. coli* – surface only
- Cyanotoxins (Microcystins/Nodularins) – surface only

Additionally, the following parameters will be field measured:

- pH
- Secchi Disk Transparency
- Apparent Color
- Turbidity
- Water Temperature – full vertical profile at 1 m increments
- Specific Conductance – full vertical profile at 1 m increments
- Dissolved Oxygen – full vertical profile at 1 m increments

Since there is concern regarding documented impairments in East Monponsett Pond and Furnace Pond and the potential for these to impact Silver Lake through inter-basin water transfer, surface samples will also be collected from these diversions

concurrent with a subset of the in-lake sampling events at Silver Lake. The timing of these sampling events will be targeted during periods of active diversion, as conditions allow. Samples will be collected in the ponds near active diversion structure so that they are representative of water quality in the source waters themselves. The same water quality analytes and field-measured parameters will be collected for these locations as the in-lake location in Silver Lake.

C. Upstream and Downstream Monitoring

Upstream and downstream monitoring will be used to improve understanding of the hydrologic and nutrient budgets for Silver Lake and the seasonal distribution of these contributions from surface water sources. Survey timing is for late summer/early fall 2021 and extending into 2022, inclusive of the winter months. The duration of the monitoring program in 2022 is expected to continue until October 2022.

To ensure acquisition of the most useful and complete dataset over a short period of time, the upstream and downstream monitoring field program will include continuous data logging, direct measurement of discharge, and collection of discrete water quality samples.

Please refer to Section B1 for further details on the rationale and goals for upstream and downstream water quality monitoring.

Continuous Data Logging.

The continuous data logging portion of the field program will include deployment of four water level loggers, including one each at Tubbs Meadow Brook, Little Brook, and Mirage Brook (tributary inlets) and one downstream (outlet from Forge Pond). Additionally, since the water level loggers will be sealed (unvented), a fifth pressure logger will be deployed in a discreet shoreline location to allow for continuous atmospheric pressure correction. The deployed loggers will also continuously monitor temperature over the course of the study. Water level loggers will be programmed to collect readings at hourly intervals. Data will be downloaded during each site visit.

The geographic coordinates for each logger location will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Discrete Water Quality and Discharge Sampling.

To complement and supplement the continuous data logging program, monthly rounds of discrete upstream and downstream water quality and discharge measurement will be completed, for a total of thirteen sampling events. At least one round of sampling will be collected during wet weather conditions to capture the impact of stormwater runoff.

The geographic coordinates for each upstream and downstream sampling location will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Water quality samples will be sent to the laboratory for analysis of the following:

- Total Phosphorus
- Total Soluble Phosphorus
- Total Nitrogen (includes nitrite-N+nitrate-N and TKN)

Additionally, the following parameters will be field measured:

- Stream Discharge
- pH
- Apparent Color
- Turbidity
- Specific Conductance
- Temperature
- Dissolved Oxygen

The discharge measurements collected in each stream monitoring location will be used to develop stage-discharge rating curves. These curves will, in turn, be used to convert logger water levels into a continuous discharge record for the period of study.

D. Groundwater Assessment

Groundwater seepage sampling will be used to evaluate the influence of groundwater inflows on water quality in Silver Lake. The assessment will serve as an evaluation of the influence of groundwater inputs to Silver Lake but is not intended to be a comprehensive inventory of all groundwater sources. Survey timing for two sampling events is anticipated for April 2022 and October 2022.

Direct groundwater seepage has the potential to be a major source of pollutants to surface water bodies, particularly those with densely developed shorelines. Measuring the quantity and quality of these groundwater inputs can be important for understanding why an aquatic system is no longer meeting its water quality goals. A seepage survey measures the quantity and quality of groundwater entering the lake along the immediate shorelines where groundwater seepage is highest and typically the most influenced by human behaviors and activities. This assessment may suggest potential pollutant sources and opportunities for further monitoring and research.

To measure the seepage rate, ten seepage meters will be deployed along five shoreline segments of Silver Lake. Two of these shoreline segments will be located downgradient of nearby developed areas and two will be located adjacent to natural

or less-developed areas. Two meters will be deployed along each shoreline segment to capture the local variability in groundwater movement and allow for calculation of an average seepage rate.

On the same day, shallow porewater samples will be collected from at least two locations along each of the four shoreline segments. Samples will be collected using a littoral interstitial porewater (LIP) sampler, which acts as a small-scale well that extracts groundwater from sediments for water quality testing. Samples will be measured in the field for temperature, pH, and specific conductance and compared to surface water quality measurements to ensure that groundwater is being obtained by the LIP sampler. A total of four composite groundwater quality samples will be collected in Silver Lake; one from each shoreline segment.

The geographic coordinates for each groundwater sampling location will be recorded using a Differential Global Positioning System (DGPS) in the NAD83 Massachusetts State Plane Coordinate system.

Laboratory analysis will be conducted for the following at each shoreline sampling segment:

- Total Soluble Phosphorus
- Ammonia
- Nitrate-Nitrogen

Please refer to Section B1 for further details on the rationale and goals for the groundwater assessment.

E. Sediment Coring and Phosphorus Fractionation

Internal recycling is a potentially critical source of phosphorus loading in lakes that experience dissolved oxygen depletion from deep waters. The collection and analysis of sediment core samples will be used to determine the potential impact of internal nutrient recycling on water quality within Silver Lake. This will be accomplished through a sediment phosphorus fractionation analysis that quantifies the different forms of phosphorus present in the top sediment layers.

This work is planned for April 2022 to avoid sampling during periods of strong thermal stratification, when phosphorus release into the water column would be expected to be at its maximum.

Under this approach, a gravity corer or similar device will be deployed from a boat to collect undisturbed and uncontaminated cores of 10 cm to 25 cm (depending on penetration and recovery depths achieved) for use in evaluating maximum phosphorus release rates. Collected cores will be extruded through the top of the upright corer and a sharp, clean blade will be used to section the material into 2-4 cm section.

Eight sediment cores will be collected and sliced into multiple 2-4 cm sections (for an anticipated total of up to 40 sections). Cores will be collected from locations distributed across a range of depths and geographic positions to be identified using the bathymetric, benthic, and water quality data collected under other tasks.

These core samples will be sent to a specialty phosphorus fractionation laboratory and analyzed for the following specialty parameters:

- Iron-bound phosphorus
- Aluminum-bound phosphorus
- Calcium-bound phosphorus
- Organically-bound phosphorus
- Percent water
- Loss on ignition-organic carbon content
- Density

These data will help to define the spatial variability of phosphorus across the lake bottom, as well as vertically within the bottom sediment. This will help to provide some indication of how deep into the sediment phosphorus release occurs, as well as provide critical information for dosing if nutrient inactivation is advanced as a desired future management approach.

Please refer to Section B1 for further details on the rationale and goals for sediment coring and phosphorus fractionation.

Project Reporting

At the conclusion of the Silver Lake Water Quality Monitoring field program, data, results, and recommendations will be presented at a public listening session and shared via an informational leaflet. This information will also be documented in the project Technical Memorandum, which will serve as a final report.

ESS will develop the content for the public listening session and Technical Memorandum, as well as supporting informational leaflets. Kimberly Groff Consulting and the CPCWDC will be responsible for reviewing this content. The CPCWDC will post project deliverables on its website.

A7: Quality Objectives and Criteria

Description of Data Acceptance

The data quality objectives (DQOs) for this project will be met if the data collected can be used to assess or determine the value or change in value of each of the water and sediment quality measures.

DQOs can be described in terms of the following performance criteria: precision, accuracy and bias, representativeness, comparability, and completeness.

Precision:

Water Quality. Water quality laboratory sample and field measurement results will be sufficiently precise to ensure reliable detection of each analyte or parameter within its expected environmental range.

Field duplicates will be collected at a 10% rate and submitted as blind duplicates (i.e., not labeled as "duplicate") for quality control purposes. Laboratory duplicates will be tested in accordance with the requirements of the laboratory SOPs and/or Quality Manual/Quality Assurance Manual.

Precision will be measured using Relative Percent Difference (RPD) between duplicates. The precision objective is presented in **Table 3**.

Sediment Cores. Sediment laboratory sample results will be sufficiently precise to ensure reliable detection of each analyte within its expected environmental range. Laboratory duplicates will be tested at a 5% rate.

Other Data. The Project Manager will check other data to ensure that they are of sufficient precision to meet data quality objectives.

Accuracy and Bias:

Water Quality. Water quality laboratory sample and field measurement results will be sufficiently accurate and unbiased to ensure results that reflect the true value of the analyte or parameter being measured.

Where applicable, laboratory accuracy will be measured using Percent Recovery of one or more of the following quality control samples: laboratory control samples (LCS) and matrix spikes (MS). Method blanks and other checks on accuracy may also be used. The accuracy objective for this project is presented in **Table 3**.

Sediment Cores. Sediment laboratory sample results will be sufficiently accurate and unbiased to ensure results that reflect the true value of the analyte or parameter being measured.

Table 3. Laboratory Performance Objectives

Analyte	Units	Laboratory Minimum Detection Limit	Expected Range	Precision Objective	Accuracy Objective
Phosphorus (Total and Total Soluble)	mg/L	0.003	0.003 – 0.1	±20%	±15%

Analyte	Units	Laboratory Minimum Detection Limit	Expected Range	Precision Objective	Accuracy Objective
Total Kjeldahl Nitrogen	mg/L	0.1	0.2-2.0	±20%	±15%
Nitrate-Nitrogen	mg/L	0.01	0.02-1.0	±20%	±10%
Nitrite-Nitrogen	mg/L	0.01	0.005-0.02	±20%	±10%
Ammonia-Nitrogen	mg/L	0.05	0.05-0.5	±20%	±10%
Alkalinity	mg/L	5.00	2.5-25.0	±20%	±15%
E. coli	MPN/100 mL	1	0-2,420+	±30%	N/A
Chlorophyll a	mg/m^3	2	1-150	±35%	N/A
Cyanotoxins (Adda Microcystins / Nodularins)	ppb	0.15	0.1-15	±15%	±40%
Phosphorus (Sediment - Wet)	mg/cm^3	0.002	0.002-0.25	±20%	±15%

Other Data. The Project Manager will check other data to ensure that they are not unreasonably biased and are of sufficient accuracy to meet data quality objectives.

Representativeness:

Water Quality. Water quality samples will be collected at stations selected to be representative of incoming flows to Silver Lake, diversions to Silver Lake, outgoing flows from Silver Lake, and Silver Lake proper.

Sample results are expected to be representative provided that samples are collected in accordance with relevant SOPs and analytical laboratory sampling protocols, including use of appropriate containers, collection of sufficient volume, use of preservatives as directed, and adherence to sample hold times. Deviations from the established protocols will be noted on the chain-of-custody form or field books by field personnel and included in data reporting.

Diversion samples should be targeted for periods of active diversion to be considered most representative of incoming water quality to Silver Lake. This is particularly true for the diversion from East Monponsett Pond, as water quality of the diverted water

may be affected by backflows from West Monponsett Pond. Samples collected outside of active diversion periods should be noted as such.

Sediment. Sediment samples will be collected at stations selected to be representative of the different areas in the lake. This will include sediments in the deepest parts of Silver Lake, which are expected to experience the longest period of seasonal anoxia, as well as sediments from shallower waters that are anoxic for shorter periods. Given the size of Silver Lake, the sediment sampling event will also include collection of cores that are representative of northern and southern portions of the lake, which receive inflows from different watershed and diversion sources.

Additionally, sediment cores will be considered representative of surficial sediments if the recovered core is undisturbed and at least 15 cm deep. If core recovery is less than 15 cm, a second core will be attempted at a location that is offset from the first by at least two meters. If the recovery of the second core is also less than 15 cm, the field crew will contact the Project Manager to consider alternative sampling locations or approaches to obtain a representative core.

Other Data. The Project Manager will check other data to ensure that they are adequately representative of the targeted measure and will meet data quality objectives.

Comparability:

Water Quality. Water quality samples collected from different monitoring locations and events will be sufficiently comparable provided that samples are collected, stored, and transported in accordance with relevant SOPs and the laboratory sampling protocols. Deviations from the established protocols will be noted by field personnel and included in data reporting.

Sediment. Sediment samples collected from different monitoring locations will be sufficiently comparable provided that samples are collected, stored, and transported in accordance with relevant field collection procedures and the laboratory analytical protocols. Deviations from the established protocols will be noted by field personnel and included in data reporting.

Other Data. The Project Manager will check other data to ensure that they are sufficiently comparable to meet data quality objectives.

Completeness:

Water Quality. Data will be considered complete if water quality samples are collected from each of the proposed stations during each anticipated sampling event and analyzed for the intended analytes. If data cannot be obtained for an event (e.g., due to lack of access or inadequate flow), this will be reported to the Project Manager in a timely fashion to determine whether this is likely to affect completeness of the dataset in a substantive way. If the effect is determined to be substantive, the Project

Manager will consider alternatives to improve completeness of the dataset, potentially including but not limited to additional sampling events.

Sediment. Sediment data will be considered complete if cores are collected from each of the proposed stations during the collection event. If data cannot be obtained as planned, this will be reported to the Project Manager in a timely fashion to determine whether it is likely to affect completeness of the dataset If the effect is determined to be substantive, the Project Manager will consider alternatives to improve completeness of the dataset.

Other Data. The Project Manager will check other data to ensure that they are sufficiently complete to meet data quality objectives.

A8: Special Trainings and Certification

No special training or certification courses were specifically attended in preparation for this project.

ESS personnel involved with this project have received education and training in necessary field methods, including water quality sampling and measurement, hydrologic investigations, taxonomic identification, and GIS analysis. Knowledge and skills have been acquired from previous academic study, routine participation at conferences, and in-house training associated with similar projects throughout New England. Additional in-house training will be provided for ESS field staff as necessary to meet project requirements.

At a minimum, all personnel involved in field sample collection or data analysis will be provided with a copy of this QAPP and attachments (once the QAPP is finalized).

Analytical laboratories that are responsible for producing microbiology or chemistry data for tests listed under MassDEP's laboratory certification program will maintain certification through MassDEP, or equivalent agency in other states, for analysis of water samples, as applicable to the analytes processed under this QAPP.

A9: Documentation and Records

ESS will maintain the current approved version of the QAPP. Should any change to the QAPP be necessary, the current version of the QAPP will be saved in the Project Manager's electronic archive folder for the project before any changes are made, and the updated version will be assigned the next consecutive version number. The Project Manager will distribute the new version of the QAPP to the individuals listed on the Title and Approvals page in Section A1 of this QAPP for approval before distributing the new version of the QAPP to the individuals identified on the Distribution List in Section A3.

The following data will be included as part of the Technical Memorandum:

- Chain-of-custody forms, laboratory analytical reports, and electronic data deliverables for samples analyzed in the laboratory
- Tabular data for field-measured parameters
- Continuous datalogger outputs
- Rating curves developed for each discharge monitoring station
- GIS data created to support development of map figures

The Project Manager will review analytical reports for accuracy and completeness and contact the party responsible for completing the form or report in the event that any omissions or suspected inaccuracies are found. In the event that any corrections are made, they will be written on the form, dated and initialed by the party responsible for completing the form, or a new report will be issued with a new version number and date. All project data will be retained in the Project Manager's electronic files for this project for a period of at least three years after the end of the project.

Section B: Data Generation and Acquisition

The description of data generation and acquisition contained in this QAPP was developed with guidance from EPA's Requirements for Quality Assurance Project Plans (QA/R-5).

B1: Sampling Process Design (Experimental Design)

Rationale for Design.

Bathymetric and Aquatic Plant Mapping. The focus of this mapping effort will be aquatic plants, which can impact water quality conditions in Silver Lake through a wide variety of means including but not necessarily limited to the following:

- Greater amplitude of diel cycles in dissolved oxygen due to daytime photosynthesis and ongoing respiration by plants
- Contribution to taste and odor issues by potentially hosting epiphytic growths of cyanobacteria, algae, actinomycetes, or other taste and odor compound-producing organisms
- Interruption of free exchange of atmospheric gases across the air-water interface (in the case of species with floating or emergent leaves)
- Alteration of redox potential in sediments, which may alter biochemical reactions and processes
- Recycling of nutrients from the sediments
- Seasonal senescence of aquatic plant biomass, resulting in increased biological oxygen demand and sedimentation over time
- Leaching of dissolved organic carbon from plant tissues
- Alteration of water circulation patterns and currents
- Potential to result in higher turbidity or suspended solids in raw water when plant matter is abundant near water intakes

The mapping of aquatic plants is typically accompanied by measurements of water depths, both as a practical matter for collecting adequate field observations (e.g., greater lengths of rope for plant rake tosses in deeper water) as well as to provide context regarding the maximum depth at which plant growth is observed. Although a bathymetric map of Silver Lake was created by Coler and Colantonio (2003), the aquatic plant mapping effort allows for opportunistic collection of bathymetric data and the subsequent update to the existing bathymetry map.

The bathymetry and aquatic plant mapping survey at Silver Lake will include at least 350 survey locations each at which bathymetry, plant coverage, and plant composition are recorded. The number of survey locations and distribution of these location using a gridded survey approach will support representative coverage of Silver Lake.

This gridded survey method uses a pre-determined sampling interval to ensure coverage of the entire water body, while only requiring navigation to each cell in the field. This provides field crews with flexibility to select the exact location and number of points within each cell based on observed field conditions. For example, the field crew may elect to add more positions to capture variability in areas observed to be characterized by rapidly changing water depths and aquatic plant growth.

Aquatic plant growth will be observed using direct observations from the surface (in shallow water only) and plant rake tosses (in both shallow and deeper water). At least two plant rake tosses will be made in opposite directions at each location.

Each aquatic plant species observed will be identified to the lowest practicable taxonomic level (typically genus species) and assigned a percent cover. Additionally, overall aquatic plant growth will be categorized by percent cover and biovolume.

Cover and biovolume are related but distinct metrics used to track aquatic plant distribution and density. Cover is defined as the areal coverage of aquatic plants in plan view (i.e., two dimensions), whereas biovolume is defined as the portion of the water column occupied by aquatic plant biomass. Percent cover can be higher than biovolume at a location but not lower. Both metrics will be visually scored using a five-point scale, as follows:

- 0 = 0% (no plants)
- 1 = 1%-25% cover or biovolume
- 2 = 26%-50% cover or biovolume
- 3 = 51%-75% cover or biovolume
- 4 = 76%-100% cover or biovolume

This scale was chosen because it provides enough categories to allow for meaningful differentiation between areas of higher and lower amounts of plant growth while remaining reliably distinguishable by a trained professional field observer.

One round of mapping will be completed during the period of peak overall growth of the aquatic plant community in late summer to early fall. Although other aquatic plants, such as invasive curly-leaf pondweed (*Potamogeton crispus*) – not yet documented at Silver Lake – may reach peak development outside of this period, the majority of aquatic plant species are anticipated to be at or near peak biomass development during this seasonal window. Therefore, the late summer to early fall period is likely to be the optimal time for obtaining an estimate of maximum aquatic plant cover and biovolume.

Benthic Survey. It is anticipated that the benthic macroinvertebrate assemblage will serve as a *supplemental* measure of hypoxic or anoxic conditions at Silver Lake. Certain benthic taxa (e.g., *Chironomus* spp.) are known to be tolerant of these conditions and are likely to become the dominant macroinvertebrate taxon where dissolved oxygen concentrations are limiting to the survival of many other taxa. Furthermore, where anoxic conditions persist for extended periods, benthic macroinvertebrates may be absent altogether.

For this program, benthic macroinvertebrate samples will be collected along a transect perpendicular to the long axis of the lake, allowing the collection of samples from both shallow and deep environments within the lake. A total of seven samples will be collected with the same quantitative sampling equipment, each from a different depth, to ensure representative coverage of Silver Lake macroinvertebrates. Soft substrates (i.e., fine sand, silt, or organic muck) will be targeted for all samples to minimize the effective of grain size on the assemblage collected.

One round of samples will be collected during the period of hypothesized peak seasonal anoxia at depth (i.e., late summer to early fall). The deepest sample will be collected from sediments known to be anoxic based on field measurements of dissolved oxygen. The shallowest sample will be collected from substrates known to be well-oxygenated at the sediment-water interface based on field measurements of dissolved oxygen. The other five samples will be collected between the upper and lower samples to capture the gradient between the shallow and deep samples.

In-Lake Water Quality. Two monitoring arrays of continuous data loggers will be deployed in the deepest location of Silver Lake. Deployment at the deepest location will ensure representative data from the surface to the most extreme depth of the lake. The continuous loggers will provide a record of select parameters in Silver Lake over the duration of the program, with the exception of potentially ice-impacted periods.

The data loggers will record data in 1-hour intervals, which allows for collection of representative data around-the-clock while reducing tedious data processing and management associated with shorter time intervals. This methodology is anticipated to be sufficient for the purposes of this study and consistent with what has been collected to date.

Monthly discrete sampling events will also be completed to provide data for parameters not measured by the loggers and for comparison with the continuous data collected by the loggers. The duration of the monitoring program in 2022 is expected to continue until October 2022, for a total of no less than twelve sampling events.

During these events, samples will be collected from the same in-lake location as the datalogger array. Samples will be collected near the surface, at mid-depth, and near the bottom of Silver Lake. Additionally, water quality profiles will be measured in situ within the water column to provide a detailed profile of dissolved oxygen and

temperature, which are considered critical to understanding the potential for internal recycling and providing context for other physicochemical and biological processes in Silver Lake.

Additionally, discrete surface water quality samples will be taken within surrounding waterbodies to assess water quality of diversions and flows to Silver Lake. This includes Furnace Pond and East Monponsett Pond. These measurements will be performed when diversion to Silver Lake is active to assess the quality of water being diverted toward Silver Lake.

Upstream and Downstream Discharge and Water Quality. Water quality sampling and discharge measurements will occur in three Silver Lake tributaries and the outlet. This will provide insight on the composition of water directly entering and leaving Silver Lake via the surface.

Although water quality sampling near the mouth of each tributary inlet would be ideal for quantifying loading into the lake, this does not appear to be practical based on field reconnaissance. Each of the stream channels are poorly defined near the Silver Lake shoreline due to past alterations or natural conditions that cause ponding or backwater effects for a substantial length upstream. In contrast, the configurations of each of the tributaries near the Route 27 (School Street) road crossing is excellent for collecting all of the data targeted by the Silver Lake Water Quality Monitoring Program. Although this will necessarily mean losing some of the potential input into each stream channel that occurs downstream of Route 27, the impact is considered to be minimal on Tubbs Meadow Brook (SLT1) and Mirage Brook (SLT3), where many of the potential sources of pollutants are located upstream of the crossing. The impact likely to be more pronounced on Little Brook (SLT2), where much of the developed watershed land is located downstream of the crossing. However, Little Brook is the smallest of the three tributaries and receives stormwater inputs from Route 27. Therefore, the overall effect on the outcome of this study is unlikely to be significant.

A water level logger will be deployed at each tributary/outlet location to provide continuous water level data. Loggers will be deployed in stream reaches with active flow and located away from impoundments.

In addition to the continuous data loggers, monthly discrete water quality sampling events will be completed. Stream discharge will also be measured on at least fourteen occasions over the course of the study to develop stage-discharge rating curves. These curves will, in turn, be used to convert logger water levels into a continuous discharge record for the period of study. This will also allow for the estimation of surface water contaminant loads from surface tributaries into Silver Lake and out of the lake into downstream waters.

Groundwater Seepage. Groundwater seepage will be investigated to assess contaminants and water quality vulnerability sourcing from groundwater inputs to Silver Lake. The assessment will serve as a qualitative evaluation of the influence of

groundwater inputs to Silver Lake, but is not intended to be a comprehensive assessment of all groundwater sources. Ten seepage meters will be deployed along five key shoreline segments of Silver Lake, including three in the northern half of the lake and two in the southern half. Three of these shoreline segments will be located downgradient of nearby developed areas and two will be located adjacent to natural or less-developed areas. Two meters will be deployed along each shoreline segment to adequately capture the local variability in groundwater movement. These ten seepage meter sampling locations will provide distributed and varied representation of groundwater inflow to Silver Lake.

Additionally, shallow porewater water quality samples will be collected from each of the five shoreline segments using a LIP sampler. A total of five composite groundwater quality samples will be collected in Silver Lake; one from each shoreline segment. These water quality samples, coupled with the seepage volume, will provide an estimate of groundwater influence on Silver Lake's water quality.

Groundwater seepage sampling is planned to occur in April 2022 and October 2022.

Sediment. Internal recycling is a potentially critical source of phosphorus loading in lakes that experience dissolved oxygen depletion from deep waters. The collection and analysis of sediment core samples will be used to determine the potential impact of internal nutrient recycling on water quality within Silver Lake. This will be accomplished through a sediment phosphorus fractionation analysis that quantifies the different forms of phosphorus present in the top sediment layers. Multiple cores will be collected from sediments in different parts of the lake and samples will be extruded from each core and analyzed to provide a vertical profile of phosphorus fractions in the sediments.

Types and Number of Samples Required.

Bathymetry and Aquatic Plant Mapping. No direct sampling is required for bathymetry or aquatic plant mapping. Both surveys will be completed in the field with no need for sample collection. If the there is uncertainty regarding the identification of an aquatic plant in the field, it will be placed in a container with water and transported to the ESS office for further identification. These samples will be collected on an as-needed basis.

Benthic Survey. Seven benthic samples will be collected during the benthic survey. Samples will be field sieved using a 0.5-mm bucket sieve and preserved in labeled, 1-quart HDPE plastic bottles with 75% ethanol. Samples will be processed in-house by ESS in a manner consistent with SOPs (Appendix B).

In-Lake Water Quality. In-lake water quality samples at Silver Lake will be analyzed by the laboratories indicated for the analytes identified in **Table 4**.

Table 4. Anticipated In-Lake Sample Collection and Analysis

Analyte / Parameter	Sampling Position(s) at SLIL	Min Number of Visits	Number of Samples per Visit	Min Total Number of Samples	Laboratory
Total Phosphorus	Surface, Mid-depth, Bottom	12	3	36	Phoenix
Total Soluble Phosphorus	Surface, Mid-depth, Bottom	12	3	36	Phoenix
Total Kjeldahl Nitrogen	Surface, Mid-depth, Bottom	12	3	36	Phoenix
Nitrate-Nitrogen	Surface, Mid-depth, Bottom	12	3	36	Phoenix
Nitrite-Nitrogen	Surface, Mid-depth, Bottom	12	3	36	Phoenix
Alkalinity	Surface, Mid-depth, Bottom	12	3	36	Phoenix
Chlorophyll a	Surface	12	1	12	Alpha
Macroinvertebrate ID and Enumeration	Sediment	1	7	7	ESS
Algal ID and Enumeration	Surface	10	1	10	Aquatic Analysts
E. coli	Surface	10	1	10	Alpha
Cyanotoxins (Adda Microcystins / Nodularins)	Surface	2	1	2	GreenWater
Phosphorus (Sediment)	Sediment	1	Up to 8 cores/ 40 samples	6 cores/ 24 samples	BEC

Additionally, several water quality parameters will be measured in the field, as identified in **Table 5.**

Table 5. Anticipated Measurement of In-Lake Water Quality Field Parameters

Analyte / Parameter	Sampling Position(s) at SLIL	Min Number of Visits	Number of Measurements per Visit	Min Total Number of Measurements
pH	Surface, Mid-depth, Bottom	12	3	36
Secchi Disk Transparency	Surface	12	1	12
Apparent Color	Surface, Mid-depth, Bottom	12	3	36
Turbidity	Surface, Mid-depth, Bottom	12	3	36
Water Temperature	Every meter	12	22	264
Specific Conductance	Every meter	12	22	264
Dissolved Oxygen	Every meter	12	22	264

In addition, in-lake water quality samples will be collected at the two water diversions into Silver Lake, at East Monponsett Pond and Furnace Pond. In-lake water quality samples at these diversions will be analyzed for the analytes and parameters as identified in **Table 6**.

Table 6. Anticipated Diversion Sample Collection and Analysis

Analyte / Parameter	Sampling Locations	Number of Visits	Number of Samples per Location per Visit	Total Number of Samples	Laboratory
Total Phosphorus	2	3	1	6	Phoenix
Total Soluble Phosphorus	2	3	1	6	Phoenix
Total Kjeldahl Nitrogen	2	3	1	6	Phoenix

Analyte / Parameter	Sampling Locations	Number of Visits	Number of Samples per Location per Visit	Total Number of Samples	Laboratory
Nitrate-Nitrogen	2	3	1	6	Phoenix
Nitrite-Nitrogen	2	3	1	6	Phoenix
Alkalinity	2	3	1	6	Phoenix
Chlorophyll a	2	3	1	6	Alpha
Algal ID and Enumeration	2	3	1	6	Aquatic Analysts
E. coli	2	3	1	6	Alpha
Cyanotoxins (Adda Microcystins / Nodularins)	2	2	1	4	GreenWater

Upstream and Downstream Water Quality and Discharge. Upstream and downstream will be analyzed for analytes identified in **Table 7**.

Table 7. Anticipated Upstream and Downstream Collection and Analysis

Analyte / Parameter	Number of Sampling Locations	Min Number of Visits	Number of Samples per Location per Visit	Min Total Number of Samples	Laboratory
Total Phosphorus	4	14	1	56	Phoenix
Total Soluble Phosphorus	4	14	1	56	Phoenix
Total Kjeldahl Nitrogen	4	14	1	56	Phoenix
Nitrate-Nitrogen	4	14	1	56	Phoenix
Nitrite-Nitrogen	4	14	1	56	Phoenix

Additionally, several water quality parameters will be measured in the field, as shown in **Table 8**.

Table 8. Anticipated Measurement of Upstream and Downstream Water Quality Field Parameters

Analyte / Parameter	Number of Locations	Min Number of Visits	Number of Measurements per Location per Visit	Min Total Number of Measurements
pH	4	14	1	56
Apparent Color	4	14	1	56
Turbidity	4	14	1	56
Water Temperature	4	14	1	56
Specific Conductance	4	14	1	56

Groundwater Seepage. Groundwater water quality will be analyzed for analytes and parameters identified in **Table 9**.

Table 9. Anticipated Measurement of Water Quality in Groundwater

Analyte / Parameter	Number of Locations	Min Number of Visits	Number of Samples / Measurements per Location per Visit	Min Total Samples / Measurements	Lab
Total Soluble Phosphorus	5	2	1 composite sample	10	Phoenix
Ammonia	5	2	1 composite sample	10	Phoenix
Nitrate-Nitrogen	5	2	1 composite sample	10	Phoenix
pH	5	2	2*	20	Field
Specific Conductance	5	2	2*	20	Field
Water Temperature	5	2	2*	20	Field

*Not composite samples. Will measure from each discrete LIP point.

B2: Sampling Methods

Water Quality. Field sampling of water quality and collection of discharge and seepage measurements will be conducted in a manner consistent with SOPs (**Appendix A)**.

Laboratory sample containers, preservation methods, and hold times that are applicable to the analytes being sampled as part of the Silver Lake Water Quality Monitoring Program are summarized in **Table 10**. Additionally, laboratory analysis will be conducted in a manner consistent with the SOPs provided (**Appendix B**).

Table 10. Laboratory Requirements for Collected Samples

Analyte	Container	Hold Time	Preservative*	Comments
Total Phosphorus	250 mL plastic	28 days	H_2SO_4	
Total Soluble Phosphorus	250 mL plastic	28 days	H_2SO_4	Field-filter with 45-micron filter. If field filtering is not possible, collect in unpreserved bottle and submit to laboratory as soon as possible
Total Kjeldahl Nitrogen	250 mL plastic	28 days	H_2SO_4	
Nitrate-Nitrogen	250 mL plastic	48 hours	None	
Nitrite-Nitrogen	250 mL plastic	48 hours	None	
Ammonia-Nitrogen	500 mL plastic	28 days	H_2SO_4	
Alkalinity	100 mL plastic	14 days	None	
Chlorophyll a	1 L opaque plastic	24 hours	None	Collect 2 bottles per sample for improved lab QC analysis
Macroinvertebrate ID and Enumeration	1 Q plastic	Unspecified	75% ethanol	Sieve through 0.5-mm mesh before preserving

Analyte	Container	Hold Time	Preservative*	Comments
Algal ID and Enumeration	125 mL opaque plastic	Unspecified	Lugol's Solution	Avoid exposure to light
E. coli	125 mL sterile	8 hours	None	24 hour holding time for non-regulatory samples Collect 2 bottles per sample for improved lab QC analysis
Cyanotoxins (Adda Microcystins / Nodularins)	125 mL plastic	48 hours	None	Freeze at -20°C within 48 hours of collection to extend hold time to 90 days
Phosphorus (Sediment)	4 oz glass jar	7 days	None	

*All samples should be held at 4°C, unless otherwise specifically noted.

B3: Sample Handling and Custody

Water Quality. Water quality samples will be collected in accordance with relevant procedures or SOPs for water quality field sampling and handling (**Appendix A)**. Sample collection personnel will wear previously unused nitrile gloves when collecting and handling samples. Bacteria samples will be collected using aseptic techniques to avoid contamination of the sample matrix or containers.

Sample collection personnel will use permanent marker to label all sample bottles with project ID, sample ID, date and time sampled, analyses requested, and initials of the sampler, at a minimum. Samples will be placed on ice or in a dedicated refrigerator to maintain sample temperature between 0°C and 4°C until transfer to the laboratory's custody. Cyanotoxin samples may be frozen at -20°C and shipped via overnight courier to extend hold time. Samples will be transferred to the laboratory prior to exceeding the analytical hold time.

All samples transferred to separate laboratory custody will be accompanied by a completed chain-of-custody (COC) form. Personnel transferring samples to the laboratory will sign the COC and indicate the date and time of sample transfer.

B4: Analytical Methods

Laboratory analytical methods are summarized in **Table 11**. SOPs for each method are provided in **Appendix B**.

Table 11. Anticipated Laboratory Analytical Methods

Analyte	Matrix	Laboratory	Analytical Method(s)
Phosphorus (Total and Total Soluble)	Water	Phoenix	SM4500PE
Total Kjeldahl Nitrogen	Water	Phoenix	351.1
Nitrate-Nitrogen	Water	Phoenix	353.2
Nitrite-Nitrogen	Water	Phoenix	353.2
Ammonia-Nitrogen	Water	Phoenix	350.1
Alkalinity	Water	Phoenix	SM2320B
Chlorophyll a	Water	Alpha	SM10200H
E. coli	Water	Alpha	SM9223B
Cyanotoxins (Adda Microcystins/Nodularins)	Water	GreenWater	ELISA
Phosphorus	Sediment	BEC	Sequential fractionation method*

*See description of method in **Appendix B**

B5: Quality Control

Laboratory analytical SOPs and quality control information relevant to each analyte are provided in **Appendix B**.

Additionally, field duplicates will be collected for water quality samples, field water quality measurements, seepage measurements, and discharge measurements at an overall 10% rate. These field duplicates will be used expressly for quantifying the RPD between samples collected or measurements taken at the same location. To avoid biasing the dataset, duplicate sample data will not be included in the final data analyses.

The minimum field duplicate sampling rate for each laboratory analyte is summarized in **Table 12**.

Table 12. Analytical Sample Field Duplicates

Analyte	Minimum # Field Duplicate Samples*
Total Phosphorus	10
Total Soluble Phosphorus	11
Total Kjeldahl Nitrogen	10
Nitrate-Nitrogen	11
Nitrite-Nitrogen	10
Ammonia-Nitrogen	1
Alkalinity	5
Chlorophyll a	2
E. coli	2
Cyanotoxins (Adda Microcystins/Nodularins)	1
Phosphorus (Sediment)	2

*Assumes field program starting in September 2021 and extending through October 2022.

B6: Instrument/Equipment Testing, Inspection and Maintenance

Laboratory analytical equipment testing, inspection, and maintenance protocols are provided in **Appendix B**.

Field instrumentation will be cleaned, stored, and serviced in accordance with manufacturer recommendations.

B7: Instrument/Equipment Calibration and Frequency

Laboratory analytical equipment calibration protocols are provided in **Appendix B**.

Field instrumentation will be calibrated and/or checked in accordance with manufacturer recommendations.

B8: Inspection/Acceptance of Supplies and Consumables

Sample collection personnel will complete a bottle order request form with each analytical laboratory to help ensure that the number, type, and condition of sample containers are appropriate for the analyses to be conducted. Additionally, sample collection personnel will also be responsible for visually inspecting sample containers and verifying their cleanliness, integrity, and consistency with this QAPP before

collecting samples for laboratory analysis. Sample containers that are visibly damaged, soiled, or inappropriate for the required analysis will not be used for sample storage.

B9: Non-Direct Measurements (i.e., secondary data)

Geospatial data is the main type of secondary data that will be used to support the Silver Lake Water Quality Monitoring Program. To ensure that the geospatial data are of sufficient quality to support the analyses identified in this QAPP, data will be obtained through official government sources or otherwise be of a quality meeting government standards for accuracy as documented in metadata or other supporting documentation.

Publicly available geospatial information, including high resolution orthophotography, will be obtained to support this assessment of Silver Lake, its watershed, and the sources of flow diversions to it. Geospatial imagery and other data files may also be used to develop map figures of the Project area. This information will be obtained through MassGIS, Massachusetts's official geospatial data hub. Geospatial data available through MassGIS meet accepted metadata standards (https://www.mass.gov/massgis-standards-and-best-practices).

Other secondary data sources may include water quality, water elevation, and/or discharge rates provided or shared by municipal, state or federal agencies. It is not currently anticipated that these data will be used as the primary basis for conclusions or recommendations in Project reporting. Rather, these data will be used to guide or characterized time periods in the sampling program (e.g., identify periods of active flow diversion), for comparison with data specifically collected as part of the Silver Lake Water Quality Monitoring Program, or to provide longer term environmental context.

B10: Data Management

Field Data Collection and Records

Field notes, forms, and other documentation (e.g., photographs) will be saved for each round of field sampling or survey. A copy of each will be retained by the Project Manager for at least three years following project completion in the electronic records for the project.

Information to be recorded for each laboratory analytical sample will include the following: the individual performing the sample collection, date and time of sample collection, and laboratory analysis requested. This information will be recorded on a laboratory COC form (**Appendix B**). All original COC forms will accompany samples for laboratory analysis and a copy of each will be retained by the Project Manager for at least three years following project completion in the electronic records for the project.

Raw water level datalogger files will be transferred from each logger to a laptop computer, tablet, or other media storage device during each field visit. These raw files will be transferred to a permanent storage location upon return to the office and maintained in their original version, in addition to any corrected files incorporating atmospheric corrections or other data processing techniques. A copy of each will be retained by the Project Manager for at least three years following project completion in the electronic records for the project.

Analytical Laboratory Data

All electronic data deliverables, laboratory reports, case narratives, QC reports, email correspondence, or other information generated by an analytical laboratory will be retained by the Project Manager for at least three years following project completion in the electronic records for the project. This will include preliminary or draft data, in addition to any revisions.

Section C: Assessment and Oversight

This section addresses the activities for assessing the effectiveness of the implementation of the quality assurance and quality control activities. The purpose of the assessment is to ensure that QAPP is implemented as described.

C1: Assessments and Response Actions

The Project Manager will provide oversight for field data collection to ensure that protocols described in this QAPP are being followed.

The Project Manager will review each batch of reported data to ensure that appropriate protocols are being followed and data are within the accepted range for each parameter or analyte. Any "outlier" data discovered will be identified and potential sources of error will be assessed to determine possible causes and avoid or minimize those going forward.

The Project Manager may also request data audits or reviews to assess conformance and compliance to the QAPP.

C2: Reports to Management

The Project Manager will provide regular updates to Kimberly Groff Consulting and the CPCWDC related to project progress and results observed, including but not limited to chemical analyses. These updates will include information results of data quality assessments, as well as recommendations for resolving any issues associated with data integrity.

Section D: Data Validation and Usability

This section addresses the QA activities that occur after the data collection of the project has been completed. Implementation of these elements ensures that the data conform to the specified criteria and achieve the project objectives.

D1: Data Review, Verification, and Validation

Water Quality. Laboratories will use the control limits set forth in Section B6 to accept analytical data generated under conditions that meet the control limits for instrument precision and accuracy and reject or qualify analytical data generated under conditions that do not meet the control limits for instrument precision and accuracy.

The Project Manager or Quality Assurance Officer will review analytical reports to verify and validate the completeness and reasonableness of laboratory results using the record of samples submitted for analysis on the completed COC forms and the anticipated environmental range of values for the selected parameter and sample matrix.

Data will be considered complete when analytical values are reported for all analyses requested for all samples submitted to the laboratory. Data will be considered reasonable when all values measured for the sample and all values generated by the same analytical procedure fall within the expected range of values for that sample or that parameter.

Data that do not meet completeness or reasonableness criteria will be evaluated for rejection (if unusable) or qualification (if usable) and flagged accordingly.

Other Data. Other data will be reviewed by the Project Manager or Quality Assurance Officer to verify that the values or observations reported are consistent with the expected range for the Project Study Area and that the data are complete and otherwise reasonable.

Data that do not meet completeness or reasonableness criteria will be evaluated for rejection (if unusable) or qualification (if usable) and flagged accordingly.

D2: Verification and Validation of Methods

Water Quality. The Project Manager or Quality Assurance Officer will be responsible for verifying that all water quality samples collected are completely and accurately represented on COC forms prior to transfer to the laboratory for analysis. If there are data gaps or inaccuracies on COC forms, the forms will be corrected immediately upon noticing the error and the revised information will be communicated to the laboratory.

Laboratory technicians are responsible for validating instrument calibration by measuring the calibration standards for the selected parameter after accepting a new

calibration and recalibrating if the measurements do not meet established control limits for accuracy and precision.

Analytical data provided by the laboratories will also be reviewed and validated by the Project Manager or Quality Assurance Officer to provide information on whether data are acceptable for use in developing project conclusions and deliverables. Results of the verification and validation processes will be addressed in project deliverables. The Project Manager will be responsible for final review of deliverables for completeness and adherence to method and quality control requirements.

Other Data. Other data will be reviewed by the Project Manager or Quality Assurance Officer to verify that the values or observations reported are consistent with the expected range for Silver Lake and that the data are complete and otherwise reasonable.

Data that do not meet completeness or reasonableness criteria will be evaluated for rejection (if unusable) or qualification (if usable) and flagged accordingly.

D3: Reconciliation with User Requirements

Deliverables will include results of quality assurance activities and/or quality control outcomes. Results will be accompanied by an assessment of the impact of quality control anomalies or departures from assumptions, if any, on the ultimate usability of the data. Data qualifiers will also be described so that users are aware of these limitations.

References

Environmental Protection Agency. 2001. EPA Requirements for Quality Assurance Project Plans. EPA QA/R-5.

Environmental Protection Agency. EPA R-5 Checklist for Review of Quality Assurance Project Plans.

Environmental Protection Agency. 2010. New England Quality Assurance Project Plan Program Guidance. EQAQAPP2005PG2.

MassDEP. 2021. Draft Final West and East Monponsett Pond System Total Maximum Daily Loads for Total Phosphorus (CN 446.1).

Appendix A – Field Sampling SOPs and Data Forms

STANDARD OPERATING GUIDELINES FOR THE CREATION OF AN AQUATIC PLANT MAP

1.0 INTRODUCTION

This Standard Operating Guideline (SOG) provides basic instructions for the mapping of aquatic plants present within standing waterbodies. The methods outlined below are intended to, (1) standardize plant mapping techniques used by ESS Group, Inc. (ESS) field personnel; and (2) standardize recording of field data to assure the creation of an accurate plant map.

2.0 REQUIRED MATERIALS

The following materials may be necessary for the creation of a plant map:

- Boat
- Long handled grappling rake (8-10 feet)
- Throw grappling rake (minimum of 75 ft of rope)
- Viewing scope
- Aquatic plant taxonomic keys and/or field guides
- Field map on water resistant paper
- Water resistant field notebook
- Small, sealable plastic bags (e.g., Whirl-pak or Ziploc) or jars
- Indelible marker
- GPS unit or tablet with GPS capability
- Underwater camera
- Echosounder (handheld or transom-mounted as appropriate to the vessel)
- Cooler
- Ice

3.0 METHODS

4.1 Aquatic Plant Survey and Sample Collection

Depending on the survey approach (point-intercept, point-transect, etc.) a project-specific survey plan will be developed prior to field collection of aquatic plant data. This plan will identify survey locations or areas to target based on size, shape, and bathymetry of the waterbody, as well as the project goals.

During the survey, the boat will be driven along each transect, to each point, or through each area identified in the survey plan. At the identified locations, a detailed survey of the aquatic plants will be carried out in the immediate area. The number of points surveyed will depend on the bathymetry and plant diversity in the survey area, with the aim of characterizing the composition, cover and biovolume of plant beds. Each point sampled will be numbered and recorded on the site map or using a GPS, in order to link plant survey data with location information.

At each survey point, a grappling rake will be used to sample aquatic plants from the waterbody for closer identification. Each plant present within each sample will be identified *in situ* (using keys if necessary) and recorded in the species list for the waterbody. Some surveys may require estimation of the level of growth (cover and/or biovolume) separately for one or more target species. In these instances, the code system specified in Section 4.2 should also be applied to the individual target species.

If identification of certain plants is not possible in the field, a generous sample of these plants will be stored with a little water in a plastic bag or jar clearly labeled in indelible ink. All such samples will be stored in a cooler to preserve the quality of the samples, and transported back to the lab for identification using a hand lens or dissecting microscope, if necessary. Unknown plants will be assigned a sequential code number (U1, U2, etc.) to use as a temporary species identification code. Tentatively identified plants should also be flagged with a note or comment that clearly indicates the need for lab confirmation. Once identified, these codes should be updated in the project database to reflect the final species determination.

4.2 Assessment of Percentage Plant Cover and Percentage Plant Biomass

At each survey point, ESS field personnel will estimate the percentage plant cover (i.e. the percentage of the bottom covered by plants, which is a factor of plant density). Estimates of plant cover may be made from the surface, if plants are adequately visible. Otherwise, a viewscope, underwater camera, or sampling rakes may be necessary to estimate plant cover. A simple code system will be used whereby percentage "ranges" are assigned an integer: i.e. 0 = 0%; 1 = 1%-25%; 2 = 26%-50%; 3 = 51%-75%; 4 = 76%-100%. At each survey point the estimation of plant cover will be recorded. Estimations of plant cover should be made by the same field staff member for the duration of the day's survey work for consistency.

In addition to plant cover, biovolume will be estimated by ESS field personnel at each survey point. The percentage of biovolume represents that percentage of the water column that is occupied by plants; biovolume is a factor of water depth, plant height, and plant density. As noted above, a simple code system will be used to assign integers as estimations of percent biovolume. At each survey point the estimation of biovolume will be recorded with the associated transect and point number in the field notebook. Estimations of plant biovolume should be made by the same field staff member for consistency.

4.3 Creation of Plant Maps

- Download GPS data and apply differential correction, if real-time kinematic collection was not used and sub-meter horizontal accuracy is needed to meet project objectives.
- If vegetation survey data were recorded on a field map, associate the data with the measurement point locations recorded on the GPS.
- Using GIS, create maps that illustrate the distribution of target plants, percentage cover and percentage biomass of aquatic plants, as appropriate. Maps may be point-based or interpolated (polygon-based), depending on the objectives of the project.

5.0 QUALITY CONTROL

Unidentified plants (unknowns) or those whose taxonomic identification requires additional review for confirmation will be sampled *in situ* and transported back to the lab in plastic bags. Identification checks with other plant keys and consultations with ESS plant experts will be made to confirm species identification. In some cases, assistance from outside experts or genetic lab methods (cryptic species) may be required to confirm taxonomic identification. This will be determined on a case-by-case project basis.

Plant mapping figures should be checked by a second staff person before finalizing for inclusion in any project deliverables.

6.0 DOCUMENTATION

All observed and sampled plants will be recorded by ESS personnel in field notebooks, maps, and/or on a GPS unit. Any unanticipated site-specific information, which requires ESS personnel to deviate from the

above SOG or project-specific requirements will be recorded. Documentation for recorded data must include a minimum of the following:

- Name or initials of person collecting the samples
- Sample identification/station location
- Date and time of sample collection
- Environmental conditions (e.g. wind, weather)
- Comments/observations
- Photographic documentation of unusual or unidentified species.

Photographic documentation of observed general conditions is also desirable.

GIS data may also require addition of metadata prior to finalizing the project geodatabase.

GUIDELINES FOR MEASUREMENT OF SPECIFIC CONDUCTANCE

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine calibration and operation of a variety of specific conductance meters. This SOG document also addresses estimation of total dissolved solids (TDS) and salinity by direct measurement of specific conductance (specific methods and capabilities for these parameters are outlined in the manufacturer's individual instrument manuals). This SOG is designed to be consistent with EPA Method 120.1 and Standard Method 2510 B which address specific conductance measurements of drinking, surface, and saline waters, domestic and industrial wastes, and acid rain.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory analyses.

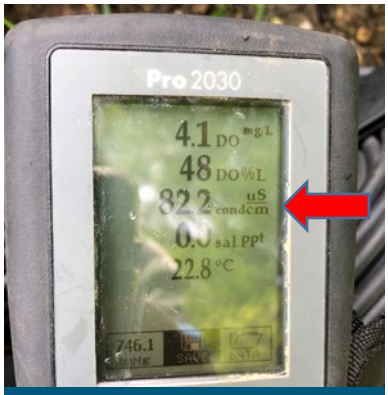
2.0 RESPONSIBILITIES

- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.
- The analyst is responsible for verifying that the specific conductance meter is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.

3.0 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Specific conductance meter
- Specific conductance meter manufacturer's instruction manual
- Deionized water
- Conductivity standard at concentration that approximates anticipated range of sample concentrations
- Lint-free tissues
- Calibration sheets or logbook
- Laboratory or field data sheets or logbooks



Handheld YSI 2030 meter, red arrow showing where the specific conductance is measured

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

- Specific conductance measurements should be taken in situ or soon after sample collection since temperature changes, precipitation reactions, and absorption of carbon from the air can affect the specific conductance. If specific conductance measurements cannot be taken immediately (within 24 hours), samples should be filtered through a 0.45 µm filter, stored at 4°C and analyzed within 28 days.
- Report results as specific conductance, µmhos/cm or µS/cm at 25°C.

- Secondary standards may be purchased as a solution from commercial vendors. These standards should not be used after their expiration dates as provided by the manufacturer. An expiration date of one year from date of purchase should be used if the expiration date is worn or missing.

4.2. Calibration and Measurement Procedures

- The specific conductance meter must be calibrated (or the calibration checked) before any analyses are performed.
- Set up the instrument according to the manufacturer's instructions.
- Rinse the probe with deionized water prior to use. If necessary, dry with a lint-free tissue or cloth.
- Follow the manufacturer's recommendations for appropriate calibration receptacle and depth of immersion.
- Record the stabilized specific conductance reading of the standard and the temperature. Enter the calibration mode (according to manufacturer's instructions) and change the value on the primary display to match the value of the calibration standard. The meter may be adjusted to ±20% from the default setting. If the measurement differs by more than ±20%, the probe should be cleaned, serviced, or replaced as needed.
- An additional check may be performed, if required by the project plan, by placing the probe into an additional standard. This standard should be from a different source than the standard used for the initial calibration. This standard should read within 5% of the true value.

Use of YSI handheld device and probe

- Verify the calibration at least once a month or whenever the instrument has been moved from freshwater to saltwater environments or vice versa. Recalibrate or service the instrument, as needed, if the check value is not within 15% of the true (calibration standard) value.

4.3. Troubleshooting Information

If there are any performance problems with any of the specific conductance meters which result in inability to achieve the acceptance criteria presented in Section 5.0 or the project-specific acceptance criteria, consult the appropriate section of the meter instruction manual for troubleshooting procedures. If the problem persists, consult the manufacturer's customer service department immediately for further guidance.

4.4. Maintenance

- Instrument maintenance should be performed according to the procedures and frequencies required by the manufacturer.
- The probe must be stored and maintained according to the manufacturer's instructions.

5.0 QUALITY CONTROL

- The meter must be calibrated (or the calibration checked) before sampling, and will not be used for sample determinations of specific conductance unless the initial check standard value is within 5% of the true value.

- Duplicate measurements of a single sample will be performed at the frequency specified in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within 10%.

6.0 DOCUMENTATION

- Meter calibration, temperature check, and maintenance information will be recorded in a calibration log. Specific conductance data may be recorded on the appropriate laboratory or field data sheets or logbooks.
- Calibration documentation should be maintained in a thorough and consistent manner. At a minimum, the following information should be recorded:
    - Date and time of calibration
    - Person performing the measurement
    - Instrument identification number/model
    - Expiration dates and batch numbers for all standards
    - Reading for standard before and after meter adjustment
    - Readings for all continuing calibration checks
    - Temperature of standards (corrected for any difference with reference thermometer)
    - Comments
- Documentation for recorded data must include a minimum of the following:
    - Date and time of analysis
    - Person performing the measurement
    - Sample identification/station location
    - Temperature (corrected for any difference with reference thermometer) and conductance of sample (including units and duplicate measurements).
    - Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform specific conductance measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

Certain state certification programs require that specific conductance measurements be taken in the field by, or in the presence of, personnel that are qualified under the certification program.

GUIDELINES FOR MEASUREMENT OF DISSOLVED OXYGEN

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine measurement of dissolved oxygen using a polarographic sensor-equipped dissolved oxygen meter with a digital read-out (e.g., YSI Pro2030 Dissolved Oxygen, Conductivity, Salinity Instrument). Measurements are made in accordance with methods that address dissolved oxygen measurement of drinking, surface, and saline waters, and domestic and industrial wastes.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory measurements.

2.0 RESPONSIBILITIES

- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.
- The analyst is responsible for verifying that the dissolved oxygen measuring device is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.

Handheld YSI 2030 meter, Red arrow showing were the dissolved oxygen is measured

3.0 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Dissolved oxygen meter with digital read-out device
- Manufacturer's instruction manual for the instrument
- Manufacturer’s recommended operating solution and replacement membranes or caps
- Laboratory or field data sheets or logbooks

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

To achieve accurate dissolved oxygen measurements, samples should be analyzed in situ. Measurements in flowing waters should be made in relatively turbulent free areas. Measurements in standing waters may require gentle probe agitation to create water movement around the probe (check instrument manual to confirm).

4.2. Calibration and Measurement Procedures

To accurately calibrate some dissolved oxygen meters, it may be necessary to know the altitude of the region in which you are located and the approximate salinity of the water you will be analyzing. Fresh water has a salinity of approximately zero. Seawater has an approximate salinity of 35 practical salinity units

(psu). If uncertain, measure salinity first with an appropriate device. The instructions below are applicable to the YSI Model 55; for other instruments, consult the instruction manual.

- Ensure that the sponge inside the instrument's calibration chamber is wet then insert the probe into the chamber. Turn the instrument on and wait for readings to stabilize (as long as 15 minutes, depending on the model).
- To calibrate, enter the calibration menu by pressing and releasing both the up and down arrow keys at the same time. Enter the altitude (in hundreds of feet) at the prompt by using the arrow keys to increase or decrease the altitude (example: 12 = 1,200 feet). Press enter when correct altitude is shown.
- The meter should display CAL in the lower left of the display with the calibration value in the lower right of the display and the current D.O. reading (before calibration) should be on the main display. Once the D.O. reading is stable, press ENTER. Enter the salinity at the prompt by using the arrow keys. Press ENTER when finished and the instrument will return to normal operation.
- Calibration should be performed at a temperature within ± 10°C of the sample temperature. Recalibrate every 15 samples and whenever the unit is turned on.
- If calibration is out of range, erratic readings occur, bubbles appear, or if the membrane becomes damaged, wrinkled, or fouled refill the membrane solution and/or replace the membrane, per the manufacturer's manual.

Use of YSI handheld device and probe

- Avoid contact with environments containing substances that may attack the probe materials (e.g. acids, caustics, and strong solvents).

4.3. Troubleshooting Information

If there are any performance problems with the dissolved oxygen-measuring device, consult the appropriate section of the instruction manual for the checkout and self-test procedures. If the problem persists, consult the manufacturer's customer service department immediately for further instructions.

4.4. Maintenance

Instrument maintenance for meter-type dissolved oxygen measuring devices should be performed according to the procedures and frequencies required by the manufacturer. Rinsing the probe with distilled or deionized water and preventing exposure of the membrane to drying is typically all that is required on a day-to-day basis.

5.0 QUALITY CONTROL

Duplicate measurements of a single sample should be performed at the frequency specified in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within ± 0.2 mg/L.

6.0 DOCUMENTATION

All dissolved oxygen meter calibration, checks, and maintenance information will be recorded in a calibration logbook. Dissolved oxygen data may be recorded on the appropriate field data sheets or field books.

- Calibration documentation must be maintained in a thorough and consistent manner. At a minimum, the following information must be recorded:
    - Date and time of calibration
    - Person performing the measurement
    - Instrument identification number/model
    - Readings for all continuing calibration checks
    - Comments
- Documentation for recorded data must include a minimum of the following:
    - Date and time of analysis
    - Person performing the measurement
    - Sample identification/station location
    - Dissolved oxygen, both in mg/L and percent saturation and temperature of sample (including units and duplicate measurements)
    - Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform dissolved oxygen measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

Certain state certification programs require that dissolved oxygen measurements in the field be taken by, or in the presence of, personnel that are qualified under the certification program.

GUIDELINES FOR MEASUREMENT OF PH

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine calibration and operation of a variety of pH field pens. Although these meters may measure additional parameters (e.g., temperature, specific conductance, etc.), this SOG addresses pH measurement only (other capabilities are outlined in the appropriate SOG and manufacturer's individual instrument manuals). This SOG is designed specifically for the measurement of pH in accordance with EPA Method 150.1 and Standard Method 4500-H B which address electrometric pH measurements of drinking, surface, and saline waters, domestic and industrial wastes, and acid rain.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory analyses.

2.0 RESPONSIBILITIES

- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.
- The analyst is responsible for verifying that the pH meter is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.

3.0 REQUIRED MATERIALS

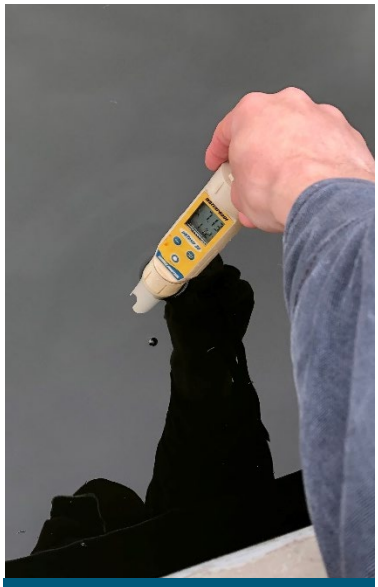
The following materials are necessary for this procedure:

- pH meter
- pH meter manufacturer's instruction manual
- Deionized or distilled water
- 4.0, 7.0, and 10.0 buffer solutions
- Lint-free tissues
- Mild detergent
- Manufacturer’s recommended storage solution
- Manufacturer’s recommended cleaning solution
- Field data sheet or logbook
- Calibration sheet or logbook

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

- To achieve accurate pH measurements, samples should be analyzed immediately in the field, or as soon as possible after collection. Sample should be measured *in situ* or collected in plastic or glass containers.
- As temperature can affect the pH measurements obtained, both the pH and the temperature of the sample must be recorded, unless the meter is capable of automatic temperature correction (ATC).
- Primary standard buffer salts available from NIST can be purchased and are necessary for situations where extreme accuracy is required. Secondary standard buffers may be purchased as a solution from commercial vendors and are recommended for routine use. Buffers should not be used after their expiration dates as provided by the manufacturer. An expiration date of one year should be used if the manufacturer does not supply an expiration date or if the buffers are prepared from pH powder pillows, etc.
- Keep the probe elevated off the bottom to avoid disturbing sediments. Allow readings to fully stabilize before recording the pH measurement. This may take several minutes, especially if the pH is drastically different from the last reading or the bulb has been allowed to dry out between readings.
- Rinse the electrode with deionized or distilled water between samples and wipe gently, if needed, with a lint-free tissue. If a more thorough cleaning is required, use a mild detergent (e.g., dish soap) or the manufacturer's recommended cleaning solution.
- Store the probe in the manufacturer's recommended storage solution or, if this is not available, tap water. Do not use distilled or deionized water for storage purposes.



Handheld pH meter

4.2. Calibration and Measurement Procedures

- The pH meter should be checked weekly before any analyses are performed. Otherwise, the meter should be checked or calibrated at the frequency specified in the project plan.
- Calibration should include a minimum of one point but ideally, a two point calibration that brackets the expected pH of the samples to be measured is desirable. Calibration measurements should be recorded in the calibration logbook.
- Choose either 7.0 and 10.0 (high range) or 4.0 and 7.0 (low range) buffers, whichever will bracket the expected sample range. Pour each buffer into a clean glass beaker. The volume should be sufficient to fully submerse the pH bulb and thermistor. If the pH is being measured in a laboratory, place the beaker on the magnetic stirrer and place the stirring bar in the beaker. Measure and record the temperatures of the buffers using a calibrated thermometer or automatic temperature compensation (ATC).
- Follow the manufacturer's calibration instructions.
- Once calibration is complete, discard the buffer and rinse the beaker (and stirring bar, if used) thoroughly with distilled or deionized water.

- An additional check may be performed, if required by the project plan, by placing the electrode into an additional buffer solution. This buffer should be from a different source than the buffers used for the initial calibration. This buffer should read within ±0.2 pH units of the buffer's true pH value.
- Recalibrate the instrument if any of the following apply:
  - the check value varies more than 0.2 pH units from the true value
  - the expected pH of the sampled water body is outside the current calibration range
  - readings are erratic or do not stabilize
  - the instrument has just been cleaned or otherwise disturbed for maintenance

4.3. Troubleshooting Information

If there are any instrument performance problems that result in the inability to achieve the acceptance criteria presented in Section 5.0, consult the appropriate section of the meter instruction manual for troubleshooting procedures. If the problem persists, consult the manufacturer's customer service department immediately for further guidance.

4.4. Maintenance

- Instrument maintenance should be performed according to the procedures and frequencies required by the manufacturer.
- The electrode should be stored and maintained according to the manufacturer's instructions.

5.0 QUALITY CONTROL

- Duplicate measurements of a single sample will be performed at the frequency specified in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within ±0.2 pH units.

6.0 DOCUMENTATION

- All pH meter calibration, temperature check, and maintenance information will be recorded in a calibration logbook.
- pH data may be recorded on the appropriate laboratory or field data sheets or logbooks.
- Calibration documentation must be maintained in a thorough and consistent manner. At a minimum, the following information must be recorded:
  - Date and time of calibration
  - Person performing the measurement
  - Instrument identification number/model
  - Expiration dates and batch numbers for all buffer solutions
  - Reading for pH 7.0 buffer before and after meter adjustment
  - Reading for pH 4.0 or 10.0 buffer before and after meter adjustment
  - Readings for all continuing calibration checks
  - Temperature of buffers (corrected for any difference with reference thermometer), including units
  - Comments

- Documentation for recorded data must include a minimum of the following:
    - Date and time of analysis
    - Person performing the measurement
    - Sample identification/station location
    - Temperature and pH of sample (including units and duplicate measurements)
    - Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform pH measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

GUIDELINES FOR MEASURING STREAMFLOW

1.0 INTRODUCTION

These guidelines provide instructions for the field measurement of flow rate in bodies of running water.

Descriptions of two field techniques are provided.

The first, called the time of travel method, is simple and does not require expensive or specialized equipment. This is most appropriate for rapid streamflow assessments where order of magnitude accuracy is acceptable or water depth is too low for the accurate measurement using a velocity meter.

The second method requires the use of a current meter, which is the preferred method where discharge measurements are being used to develop at-a-station rating curves and water depth is sufficient for measurement.

Additionally, these guidelines provide This method of calculating streamflow involves determining the cross-sectional area of the stream and measuring the average time it takes for a neutrally buoyant object to travel a known distance.

2.0 REQUIRED MATERIALS

The following materials are necessary for the measuring streamflow:

- Measuring stick to measure stream depth (folding stick is recommended)
- Flexible tape measure (longer than the width of the stream)
- Field data sheet, logbook, or tablet with electronic data sheet

If using a velocity meter, the following additional materials are also required:

- Swoffer Model 2100 current velocity meter (or similar)
- Calibrated wading rod

If using the time of travel method, the following additional materials are also required.

- A neutrally buoyant float
- Stopwatch (built-in app on most smartphones)
- Net (to catch the float)

3.0 METHODS

3.1 Choosing a Cross Section

Measuring stream depth using a folding yard stick.

- Select an appropriate stream cross section. The location selected should be straight (no bends), and free of obstructions. Unobstructed runs are ideal.
- Identify the left and right banks of the stream. When working in streams, left and right are relative to the mean flow direction. Therefore, the left bank will be to one's left when facing downstream but to one's right when facing upstream.
- To assure consistency of measurements and allow for easier comparison of data across time, flow should be measured at the same cross section of the stream during all visits. Include descriptions of site landmarks in field notes, and/or take photos of measurement locations. If site conditions allow, install permanent cross section markers, such as stakes or rebar.
- If a staff gauge is present near the stream measurement location, record the staff gauge depth during each visit.

3.2. Divide the Channel into Subsections

- Establish a transect by stretching the measuring tape across the stream, perpendicular to the channel axis. Secure each end of the tape to the stream banks so that the tape is taut.
- Take a minimum of four photographs, including one each facing upstream, the left bank, downstream, and the right bank.
- Starting with the left edge of water, measure width and stream depth at no less than three locations (stations) within the steam channel. This is the minimum number of stations and most streams will require more than three measurements to accurately calculate discharge.
- The area between each vertical station represents a channel subsection.

3.3. Measuring Velocity

3.3.1 Time-of-Travel Method

- To measure travel time, time how long it takes for a neutrally buoyant object (a float) to travel a known distance. Suitable objects should float, but sit very low in the water to minimize influence from wind, and can be untethered or tethered (methods adapted from EPA, 2012a described below).

- Suitable floats include:
    - citrus fruits or pieces of citrus peel
    - cheese puffs
    - small sponge rubber balls
    - small sticks or bits of vegetation
- Always face upstream when taking velocity measurements. Stand far enough downstream that stream velocity is not affected in the location being measured.
- Surface velocity is generally greater than depth-averaged velocity, so a correction factor (0.8 for rocky-bottom streams, 0.9 for muddy-bottom streams) is applied to float travel times (see Section 3.3, EPA 2012b)
- Untethered floats should be biodegradable, or a second person equipped with a net should be stationed downstream of the sampling reach to retrieve the float(s).
- Hold the measuring stick above the water surface, perpendicular to the cross section. Release the untethered float somewhat upstream of the end of the measuring stick to allow the float to reach full flow velocity. Using a stopwatch, time how long it takes for the float to travel a known distance (3 ft is recommended for most streams but longer distances may be appropriate where velocity is high). Repeat this process three times to obtain an average time to travel at one station before proceeding to the next station.

3.3.2 Depth-Averaged Current Meter Method

- Set the current meter to average measurements over at least a three second period. Longer periods may be used if appropriate to conditions.
- Always face upstream when taking velocity measurements. Stand far enough downstream that stream velocity is not affected in the location being measured.
- Carefully place the wading rod in the flow until the base is firmly on the stream bottom.
- Orient the current meter perpendicular to the cross section transect.
- Ensure that the wading rod is straight up and down (not angled).
- Hold the wading rod steady while adjusting the calibrated height of current meter to match the measured depth. This will allow collection of measurements that are reflective of depth-averaged velocity.
- Once at least three seconds have passed, view the reading from the current meter. Allow at least three readings to occur before recording. This will prevent erroneous data due to averaging of measurements from the set up process.

3.4. Calculating Flow

- The following equation is used to calculate flow using the time-of-travel method):

$$Q = (ACL)/T$$

  - Q = stream discharge
  - A = cross sectional area
  - L = distance traveled by the float
  - C = correction factor (0.8 for rough streambeds, 0.9 for smooth streambeds)
  - T = average time of travel (seconds)

The following equation is used to calculate flow using the depth-averaged current meter method:

$$Q = AV$$

  - Q = stream discharge
  - A = cross sectional area
  - V = velocity at 60% depth

4.0 DOCUMENTATION

Record streamflow data on field sheets, field notebooks, or electronic tablets. Any unanticipated site-specific information, which requires deviation from the above guidelines should also be recorded. In addition to recording the required discharge data, field notes for streamflow measurement should include a minimum of the following:

- Name or initials of person conducting the measurement
- Discharge measurement method used
- Site ID or name
- Date and time of streamflow measurement
- Environmental conditions (wind, temperature, etc.)
- Other relevant observations about site conditions
- Photographic evidence of streamflow and site conditions is also useful for verification of relative stream stage and flow from different visits, as well as any environmental factors that may have influenced data collection.

5.0 REFERENCES

EPA, 2012a. Water: Monitoring and Assessment. 5.1 Stream Flow. United States Environmental Protection Agency. Office of Water. EPA 841-B-97-003. Accessed January 27, 2020 at https://archive.epa.gov/water/archive/web/html/vms51.html

EPA, 2012b. SESD Operating Procedure, Hydrologic Studies. Effective Date November 1, 2012. United States Environmental Protection Agency. Office of Water. SESDPROC-501-R3. Accessed January 27, 2020 at https://www.epa.gov/sites/production/files/2015-06/documents/Hydrological-Studies.pdf

GUIDELINES FOR COLLECTION OF SURFACE WATER SAMPLES

1.0 INTRODUCTION

These guidelines provide basic instructions for the routine acquisition of surface water from lakes, ponds, and streams. The methods outlined below are intended to (1) standardize water sample collection methods; (2) ensure that samples delivered to the laboratory represent field conditions as accurately as possible; (3) assure proper documentation of sample collection; and (4) minimize cross contamination between sampling sites.

2.0 REQUIRED MATERIALS

The following materials are necessary for the acquisition of surface water samples:

- Nitrile gloves
- Labeled sample bottles provided by contracted laboratory (appropriately sanitized and containing the necessary preservative for desired analyses, see Table 1.0 for examples)
- Field data sheets or logbooks, including list of sites or locations to be sampled, and pencil
- Cooler with ice packs for sample storage
- Integrated depth sampler (if collecting algae sample)
- Secchi disk (if collecting algae samples)
- Laboratory Chain of Custody

Table 1.0 Example Container Types, Preservative Requirements, and Hold Times for Water Quality Samples.

Analysis	Bottle Type	Preservative	Hold Time
Total Phosphorus	plastic	H_2SO_4	28 days
Dissolved Phosphorus	plastic	As Is	analyze immediately*
Total Suspended Solids (TSS)	plastic	As Is	7 days
Nitrate/Nitrite	plastic	As Is	48 hrs
Total Kjeldahl Nitrogen (TKN)	plastic	H_2SO_4	28 days
Metals - Total	plastic	HNO_3	6 months**
Metals - Dissolved	plastic	As Is	6 months**
Algae	opaque plastic	Lugol's iodine	>1 year
Chlorophyll-a	opaque plastic	As Is	analyze immediately
Bacteria	sterile plastic	As Is	6 hrs

* = 24 hrs with field filtration, ** = 28 days for mercury

3.0 METHODS

3.1 General Sampling Instructions

- Testing methods, sample containers, preservation techniques, and sample volumes should be selected in consultation with the laboratory to ensure that samples obtained will provide the desired results.
- Hold times vary considerably between different analytes and must be taken into consideration when planning field sampling efforts and lab courier pickups to assure the validity of analytical results.
- Field filtration of certain samples (dissolved phosphorus) is recommended. The laboratory can supply syringes and filters for use in the field.

Using a pre-cleaned (as is) bottle to fill a sample bottle containing preservative.

- In general, surface water samples should be collected via direct grab methods.
- Sample collection should precede the measurement of physical field parameters (including pH, apparent color, turbidity, conductivity, and dissolved oxygen) in order to minimize the risk of sediment disturbance and/or sample contamination.
- Clean rubber gloves should be worn at each sampling location. When sampling multiple sites on the same day, gloves may be rinsed in the immediate area of the waterbody to be sampled (downstream at flowing sites).
- Approximately 1-inch of air space should be left when filling sample bottles (except for dissolved oxygen, alkalinity, and BOD samples), so that bottles may be shaken (if needed) before analyses (EPA, 1997; Simpson 1991).
- Sample containers with preservatives should not be used to collect water samples. If using containers with preservatives, a pre-cleaned container of similar type (an as is bottle) should be used to collect and subsequently transfer the sample to the preserved container.
- Ensure that all sample bottles are correctly and completely labeled before storage. Sample bottles should be stored in a cooler with ice packs (it is best to avoid ice, as meltwater could potentially contaminate samples) or in a refrigerator until they are submitted to a lab courier.

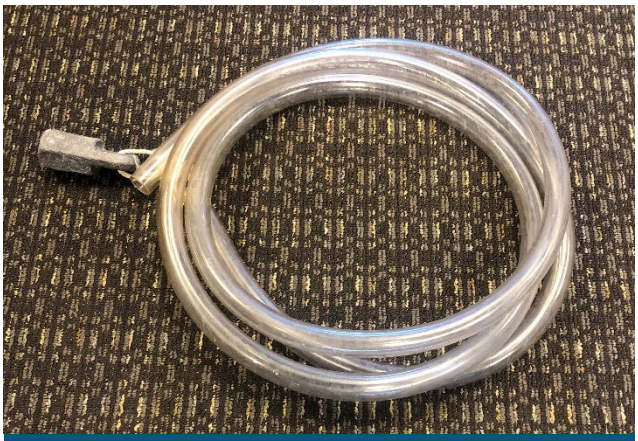
3.1.1. Lake and Pond Sampling

- Grab samples from lakes and ponds should be collected at approximately 8 to 12 inches beneath the water surface or mid-way between the surface and the bottom if the waterbody is shallow (EPA 1997). Samples should not be collected in close proximity to the lake shoreline or submerged obstacles.

- To collect water samples, hold an as is bottle near the base, remove the lid, and plunge it into the water with the opening facing downward. Invert the bottle and allow it to fill before bringing it to the surface. Decant sufficient water from the bottle to allow for the required headspace and replace the cover, or carefully pour the contents into a bottle containing preservative. Repeat the above process to refill the as is bottle as many times as necessary.

Algae Samples

- Algae samples should be stored in opaque bottles with a small amount of Lugol's iodine for preservative (~1-2 drops in a 250 mL bottle). Algal taxonomy labs can provide opaque plastic bottles, but standard plastic as is bottles covered in aluminum foil can also be used.



Integrated depth sampler for collection of algae samples.

- Algae samples should be collected using an integrated depth sampler. An integrated depth sampler consists of a length of tubing (~1in diameter, at least 2 m long) with a weight attached to one end. Sample collection procedures using the depth sampler should proceeds as follows (procedure adapted from EPA 2012):

    - Determine the euphotic zone:

        - Lower the secchi disk over the shaded side of the boat until it disappears. Lower the disk a bit further, then slowly raise the disk until it reappears. Record the reappearance depth. The euphotic zone is calculated by multiplying the reappearance depth by 2.

    - Holding onto the non-weighted end of the sampler, lower the tube into the water column. Rinse the sampler by submerging it three times.

    - Lower the sampler so that it is submerged to the depth of the euphotic zone, or fully submerged if the euphotic zone is deeper than the length of the sampler. Cover the opening at the non-weighted end with a gloved thumb.

- Lift the sampler completely out of the water and cover the opening at the weighted end with a gloved thumb (both ends should be covered). Repeatedly lift each end of the sampler to mix the water sample within the tube.
- Fill the algae sample bottle with the required volume of water from the sampler (the bottle will contain Lugol's solution as preservative so be careful not to over-fill).
- Unlike samples for most other analytes, preserved algae samples can be stored at room temperature before submission to a lab.

3.1.2. Stream Sampling

- Samples should be collected from the center of small streams (i.e., 10-20 feet wide with a maximum depth of less than 2 feet), and at a location where water depth is 2-3 feet in larger streams.
- Always approach a sampling location from downstream, traveling so as to minimize the disturbance of bottom sediments and upstream waters.
- Stand downstream of the desired sampling location, hold the sample bottle near its base and plunge it below the water surface with the opening (mouth) downward. The opening of sample bottles should always be directed away from the sampler in an upstream direction.
- To inform investigations about nutrient inputs, stream flow should be measured whenever water quality samples are collected (see Guidelines for Measuring Stream Flow)

4.0 DOCUMENTATION

Report surface water field data on sheets or in notebooks. Any unanticipated site-specific information, which requires deviation from the above guidelines, should be recorded. Field notes for surface water sampling should include a minimum of the following:

- Name or initials of person collecting the samples
- Sample identification/station location
- Date and time of sample collection
- Environmental conditions (e.g. wind, weather)
- Other comments or observations about water quality and site conditions (e.g. visible algae bloom, dead fish nearby, sample has noticeable odor or color, etc.)

Photographic evidence of any notable conditions is also desirable.

5.0 REFERENCES

EPA, 2012. 2012 National Lakes Assessment Field Operations Manual. Version 1.0, May 15, 2012. United States Environmental Protection Agency. Office of Water. EPA-841-B-11-003. Accessed January 22, 2020 at https://www.epa.gov/sites/production/files/2013-11/documents/nla2012_fieldoperationsmanual_120517_final_combinedqrg.pdf

GUIDELINES FOR MEASURING GROUNDWATER SEEPAGE QUANTITY AND QUALITY

1.0 INTRODUCTION

These Standard Operating Guidelines (SOG) provide basic instructions for the routine measurement of groundwater seepage quality and quantity. These standard methods describe the proper installation of seepage meters and the operation of Littoral Interstitial Porewater (LIP) samplers.

2.0 REQUIRED MATERIALS

The following materials are necessary for the seepage meter installation procedure:

- Seepage meters of known diameter
- Plastic tubing with one hole stopper
- Seepage bags with one hole stoppers and plastic clamps
- 250 mL graduated cylinder
- Field book or data sheets

The following materials are necessary for the collection of groundwater samples for analysis:

- Hand pump
- 2-1 L filter flasks with stoppers and tubing
- Littoral Interstitial Porewater (LIP) sampler
- Sample bottles with labels

3.0 METHODS

3.1 Seepage Meter Installation

- Initially, representative segments of the shoreline, where seepage meters will be positioned, are selected based on topography and housing density. Such segments may also be assigned to shoreline locations based on specific project objectives.

- ESS personnel shall estimate seepage quantity by installing two seepage meters per defined shoreline segment and measuring the change in volume in the attached seepage bag over time. Change in volume multiplied by a conversion factor relating the allotted seepage time (i.e., fraction of the day for which the seepage meter was running) and then adjusting to unit area (square meter), yields the liters of inseepage (positive value) or outseepage (negative value) per square meter per day.

- Seepage meters shall be firmly embedded in the substrate to depth of greater than 4 inches. Inserting seepage meters to this preferred depth will ensure that volumetric changes observed in the attached seepage bags are truly representative of groundwater flows and will increase the likelihood that seepage meters will not be disrupted by strong currents or wave action.

- At each designated shoreline location (segments pre-determined by project plan), one seepage meter should be placed at a relatively shallow depth and one at a deeper depth in order to capture ground water flows that may be occurring in different strata.

- Seepage meters must be allowed to equilibrate for a minimum of 5 minutes before the system is “closed” by the attachment of the seepage bags.

- The seepage bag should be filled with an appropriate pre-measured volume of water. In most instances 250 mL will be appropriate. The pre-determined volume of water is necessary since this volume is compared to the volume obtained after sufficient time has elapsed to quantify the change in volume (either positive or negative).

- Seepage bags are to be secured in place with as little disturbance of the seepage meter as possible. The best approach is to slowly twist the seepage bag's rubber stopper into the hole of the seepage meter.

- Prior to use, seepage bags must be air dried in order to ensure that all residual water is removed from bags and therefore will not confound the change in volume measurements. Additionally, each bag and associated stopper must be visually inspected and air pressure tested prior to each use to ensure that no leakage can occur.

3.2. Groundwater Sampling Using Littoral Interstitial Porewater Sampler

- Groundwater seepage quality can be collected through sampling with a Littoral Interstitial Porewater (LIP) sampler. A hand pump, attached to a 250 ml HDPE plastic flask, creates a low-pressure vacuum causing water to flow from the LIP sampler into the attached plastic flask. To avoid accidental contact of the extracted water with the hand pump, a second plastic flask should be connected in-line using additional tubing.

- Porewater should be extracted from a minimum of three locations in each segment and composited using equal volumes from each location.

- Samples collected may be tested in the field for parameters such as, temperature, conductivity, and pH, and/or transferred into labeled bottles and sent to a laboratory for the other analyses.

4.0 DOCUMENTATION

Record data on field sheets, field notebooks, or electronic tablets. Any unanticipated site-specific information, which requires deviation from the above guidelines should also be recorded. Documentation should include a minimum of the following:

- Name or initials of person conducting the measurement
- Date
- Site ID or name
- Size of seepage meter (diameter)
- Time of seepage meter installation
- Time of seepage meter retrieval
- Volume of water added to seepage meter bag at installation
- Volume of water remaining in seepage meter bag at retrieval
- Results of in-lake and extracted groundwater field parameter measurements (temperature, pH, and specific conductance at a minimum)
- Environmental conditions (wind, temperature, etc.) and other relevant observations about site conditions
- Photographic evidence of conditions

GUIDELINES FOR THE MEASUREMENT OF TURBIDITY

1.0 INTRODUCTION

1.1 Purpose and Applicability

These Standard Operating Guidelines (SOG) provide basic instructions for routine measurement of turbidity using a nephelometric turbidity meter with a digital read-out device (e.g., LaMotte 2020we Turbidimeter). Measurements are made in accordance with EPA Method 180.1 that addresses nephelometeric turbidity measurement of drinking, surface, and saline waters, and domestic and industrial wastes.

1.2 Quality Assurance Planning Considerations

The end use of the data will determine the quality assurance requirements that are necessary to produce data of acceptable quality. These quality assurance requirements will be defined in the site-specific workplan or Quality Assurance Project Plan (QAPP) (hereafter referred to as the project plan) or laboratory Quality Assurance Manual (QAM) and may include duplicate or replicate measurements or confirmatory measurements.

2.0 RESPONSIBILITIES

- The analyst is responsible for verifying that the turbidity measuring device is in proper operating condition prior to use and for implementing the calibration and measurement procedures in accordance with this SOG and the project plan.
- The project manager is responsible for ensuring that project-specific requirements are communicated to the project team and for providing the materials, resources, and guidance necessary to perform the measurements in accordance with this SOG and the project plan.

3.0 REQUIRED MATERIALS

The following materials are necessary for this procedure:

- Turbidity meter with digital read-out device
- Manufacturer's instruction manual for the instrument
- Turbidity tubes/cuvettes
- Mild detergent
- Lint-free cloth
- Distilled water
- Nephelometric Turbidity Unit (NTU) calibration standards
- Laboratory or field data sheets or logbooks

Example of a sample cuvette

4.0 METHODS

4.1 Sample Handling, Preservation, and General Measurement Procedures

To achieve accurate turbidity measurements, samples should be analyzed immediately upon collection (preferably within 15 minutes). Samples should be collected in glass or plastic containers.

4.2. Calibration and Measurement Procedures

- Select a turbidity standard in the range of the samples to be tested (typically 0.00 NTU, 1.00 NTU or 10.0 NTU) or as recommended by the manufacturer. Fill a turbidity tube or cuvette with the standard, cap, and wipe the tube with the clean lint-free cloth.

- Place the sample into the turbidity meter such that the indexing arrow or line on the turbidity tube is aligned with the indexing arrow or notch on the meter face. Close the lid and press the “READ” button. If the displayed value is not the same as the value of the standard (within 10%), continue with the calibration procedure.
- Follow the calibration procedures outlined by the manufacturer’s manual.
- Verify the calibration every 15 samples and at the end of the day. Recalibrate the instrument if the check value varies more than 10% from the true value.
- Rinse cuvettes with deionized or distilled water and wiped gently with a lint-free tissue between sample analysis.
- Recalibrate the instrument with the appropriate NTU standard if the standard is not of the same order of magnitude as the samples being tested.
- The meter must be re-calibrated following any maintenance activities and prior to the next use.
- Record the turbidity reading to the nearest 0.01 NTU for measurements less than 11 NTU and to the nearest 0.1 for measurements greater than 11 NTU but less than 110 NTU. For values greater than 110 NTU record to the nearest 1 NTU.

Turbidity meter

4.3. Troubleshooting Information

If there are any performance problems with any of the meter-type turbidity measuring devices, consult the appropriate section of the meter instruction manual for the checkout and self-test procedures. If the problem persists, consult the manufacturer's customer service department immediately for further instructions.

4.4. Maintenance

Instrument maintenance for meter-type turbidity measuring devices should be performed according to the procedures and frequencies required by the manufacturer.

5.0 QUALITY CONTROL

The turbidity measuring tubes will, at a minimum, be checked against NTU calibration standards at the frequency stated in Section 4.2. This verification procedure will be performed in accordance with the manufacturer’s manual.

Duplicate measurements of a single sample will be performed at the frequency required in the project plan. In the absence of project-specific criteria, duplicate measurements should agree within + 5% for readings below 10 NTU and + 10% for readings above 100 NTU.

6.0 DOCUMENTATION

All turbidity meter calibration, checks, and maintenance information will be recorded on the daily calibration sheet or logbook. Turbidity data may be recorded on the appropriate laboratory or field data sheets or logbooks.

Calibration documentation must be maintained in a thorough and consistent manner. At a minimum, the following information must be recorded:

- Date and time of calibration
- Person performing the measurement
- Instrument identification number/model
- Expiration dates and batch numbers for all standard solutions
- Reading for each standard before and after meter adjustment
- Readings for all continuing calibration checks
- Comments

Documentation for recorded data must include a minimum of the following:

- Date and time of analysis
- Person performing the measurement
- Sample identification/station location
- Turbidity of sample (including units and any duplicate measurements)
- Comments

7.0 TRAINING/QUALIFICATIONS

To properly perform turbidity measurements, the analyst must be familiar with the calibration and measurement techniques stated in this SOG. The analyst must also be experienced in the operation of the meter.

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GUIDELINES FOR USE OF WATER LEVEL DATALOGGERS IN SURFACE WATER

1.0 INTRODUCTION

These guidelines provide basic instructions for programming, deploying and downloading data from electronic dataloggers which are used for long-term water level monitoring and monitoring of other water quality parameters (e.g., temperature, conductivity). The methods outlined below are intended to (1) standardize datalogger monitoring of water levels; (2) ensure that data collected represent field conditions as accurately as possible; (3) provide guidance for the secure transfer and storage of data; and (4) assure proper field measurements and documentation.

2.0 REQUIRED MATERIALS

The following materials are necessary:

- Datalogger or Barologger
- Rebar, metal fence post or similar
- Sledgehammer or post driver
- Computer, laptop or tablet
- Communications device
- Datalogger software
- PVC housing and hose clamps
- Braided nylon twine or wire
- Water level monitoring device (electronic water level meter, plunker, etc.)
- Tools

3.0 PROGRAMMING THE DATALOGGER

The following general procedure is followed to edit the datalogger information and program the datalogger to collect readings. Always refer to the manufacturer's instructions for datalogger programming, deployment and data downloading and correction instructions.

If the datalogger is unvented, a second datalogger or Barologger will also need to be deployed to collect barometric pressure readings to support the barometric pressure correction of the data downloaded from the unvented datalogger.

- Connect the datalogger or Barologger to the computer, laptop or tablet using the supplied communications device.
- Select the appropriate COM or USB port for the communications device.
- Open the logger programming or launching page to allow for entering the logger details and programming the device for frequency of readings.
- Check the datalogger battery life and available memory to verify that it is acceptable for the intended use. This information can also be used to determine the frequency of data downloads that will be necessary.
- Rename the datalogger using the Site location.

- Program the datalogger to collect readings for the selected parameters at the specified interval.
    - The additional datalogger or Barologger should be programmed to collect readings at the same interval.
- Disconnect the datalogger from the communications device using the software program to disengage or remove from the communications device.

4.0 DEPLOYING THE DATALOGGER

The following general procedure should be used to deploy the datalogger for collection of readings.

- An appropriate location for the installation of the datalogger should be selected within a deep portion of the water body to allow for fluctuations in the water level.
    - An additional datalogger or Barologger should be mounted in the air in an inconspicuous location such as a tree or attached to a building.
- In surface water, the datalogger should be deployed within a PVC housing, if possible, to help to dampen any fluctuations (e.g., wave action or turbulence) in the surface water surface.
- The PVC housing should be secured using hose clamps to a mounting post (typically either rebar, metal fence post or similar) that can be driven into the substrate beneath the water body.
- Prior to deployment, collect a depth to water measurement from the top of the mounting post (rebar, metal fence post or similar) and an approximate depth to the bottom of the PVC housing.
- Deploy the datalogger by placing inside the PVC housing, attaching either braided nylon twine or wire to the datalogger, and threading through the top of the PVC cap to allow for retrieval of the datalogger without disturbing the position of the PVC housing.

5.0 DOWNLOADING DATA

The following general procedure should be followed when downloading the data.

- Soon after deployment (typically within two weeks, or as soon as possible given project constraints), a verification download should be performed to verify that the datalogger was programmed correctly and is collecting data at the appropriate interval.
- Subsequent downloads can be scheduled as necessary taking into consideration the frequency of readings and the available memory and battery life of the datalogger.
- Prior to removing the datalogger to download the data, collect a depth to water measurement from the top of the mounting post or other acceptable measuring point.
- Remove the datalogger and connect to the communications device.
- Connect the communications device to the computer, laptop or tablet and open the software.
- Navigate to the data download page and proceed with downloading the collected data.

  - Certain datalogger software packages will require that the datalogging process be stopped and the datalogger will have to be reprogrammed prior to redeployment.

- If the datalogger is unvented, correct the downloaded data using the program supplied by the Vendor using the data collected from the additional datalogger or Barologger.

6.0 STREAMFLOW MEASUREMENTS

In order to convert the hydraulic head (pressure) measurements from the datalogger to streamflow, a series of streamflow measurements needs to be collected at varying stream stages or flow stages to support the development of a rating curve.

REFER TO THE GUIDELINES FOR MEASURING STREAMFLOW SOP FOR DETAILS ON THE COLLECTION OF STREAMFLOW MEASUREMENTS AND DEVELOPMENT OF A RATING CURVE.

7.0 DOCUMENTATION

Maintain field notes for the datalogger deployment and data download events including the collected field measurements that will be used to QC the data collected by the datalogger and convert the collected measurements to elevation, if applicable.

Photodocumentation of the datalogger deployment and setup should also be maintained as well as any observations during the field visits.

8.0 REFERENCES

Select references to Vendors that supply dataloggers are provided below.

Solinst, Levelogger Series Software User Guide, https://www.solinst.com/products/dataloggers-and-telemetry/3001-levelogger-series/operating-instructions/user-guide/3001-user-guide.php

In-Situ, Rugged Troll 100 and 200 and Rugged Troll BaroTroll Instruments, https://in-situ.com/pub/media/support/documents/Rugged_TROLL_Manual.pdf

Onset, HOBO U20L Water Level Logger (U20L-0x) User's Manual, https://www.onsetcomp.com/support/manuals/u20l_17153/

GUIDELINES FOR USE OF CYCLOPS-7 DATA LOGGER

1.0 INTRODUCTION

These guidelines provide basic instructions for programming, deploying and downloading data from Cyclops-7 data logger which is used for long-term monitoring of Chlorophyll-a and temperature. The methods outlined below are intended to (1) standardize datalogger monitoring; (2) ensure that data collected represent field conditions as accurately as possible; (3) provide guidance for the secure transfer and storage of data; and (4) assure proper field measurements and documentation.

2.0 REQUIRED MATERIALS

The following materials are necessary:

- Cyclops-7 Data Logger
- Computer with java script installed
- USB to micro-USB adapter
- Stainless steel shackle and zip-tie
- Turner Designs Rhodamine Dye, 400 ppb, 1L (P/N: 6500-120)
- Distilled water
- 1L Beaker

3.0 PROGRAMMING AND CALIBRATING THE DATALOGGER

The following general procedure is followed to edit the Cyclops-7 information and program the datalogger to collect readings. Always refer to the manufacturer's instructions for datalogger programming, deployment and data downloading and correction instructions.

- Plug cyclops into computer that contains Java Script.
- Open Cyc7Control.jar located here Y:\EEP General\Cyclops and other Sensors
- Adjust collection rate to desired frequency (EX: 1reading/60 min)
- Click "Connect"
- Use distilled water for baseline reading
- Transfer sensor to Rhodamine Dye solution
- Enter 400 and ppb into their respective boxes.
- Click "Calibrate"
- Follow prompts until calibration is complete.
- Disconnect the logger

4.0 DEPLOYING THE DATALOGGER

The following general procedure should be used to deploy the Cyclops-7 for collection of readings.

- Unscrew the waterproof housing and flip control switch from "halt" to "record".
- The logger should be shackled to a vinyl coated steel cable using a stainless-steel shackle locked with a zip-tie to prevent the loss of the logger.

5.0 DOWNLOADING DATA

The following general procedure should be followed when downloading the data.

- Upon retrieval of the logger, unscrew the waterproof housing and flip the control switch from “record” to “halt”.
- Plug Cyclops into a computer and transfer data to an appropriate file.
- If redeploying the cyclops, flip control switch to “record” before reattaching the waterproof housing.

6.0 DOCUMENTATION

Maintain field notes for the Cyclops-7 deployment. Always note deployment time and download time.

Photo documentation of the cyclops deployment and setup should also be maintained as well as any observations during the field visits.

7.0 REFERENCES

Rhodamine dye for calibration: https://www.turnerdesigns.com/product-page/400-ppb-rhodamine-wt

Date:
Personnel:
Weather:
Objective:
Measurements Make Sense (Y/N):
 # Photos:

Waterbody
Silver Lake

YSI Profile at Deep Hole - SLIL

Depth (m)	DO (mg/L)	DO (%)	Spec. Cond. (µS/cm)	Temp (C)	Total Depth (ft)	Secchi Depth (m)
0.5						
1.0						
2.0						
3.0						
4.0						
5.0						
6.0						
7.0						
8.0						
9.0						
10.0						
11.0						
12.0						
13.0						
14.0						
15.0						
16.0						
17.0						
18.0						
19.0						
20.0						
21.0						
22.0						

Lab Samples and Non-YSI WQ Data

Sample ID	Sample Depth	Sample Collection Time	Turb (NTU)	Apparent Color (PCU)	pH	Check & Download Loggers	Duplicate Sample @ 10% Rate
	Surface						
	Mid						
	Bottom						

Collected samples tested for:

Alpha: Chlorophyll-a and E. coli (two bottles per sample, collected surface only) - SAME DAY SHORT HOLD
Aquatic Analysts: Phytoplankton (surface only)
Phoenix: Total Phos, Soluble Phos, Total Nitrogen (nitrate, nitrite, TKN), Alkalinity - NEXT DAY SHORT HOLD
GreenWater Labs: Microcystins via ELISA - NEXT DAY SHORT HOLD

Check After Completing Each Location
1. Do these readings make sense? Is the order of magnitude for each element correct?
2. Did I use the right units? If I collected using different units, did I clearly note this in the table?
3. Did I collect and appropriately label all required sample bottles?
4. Did I field filter soluble phosphorus into an H_2SO_4-preserved bottle? If field filtering was not possible, did I collect soluble phosphorus in an as-is bottle?
5. Do I need to take a duplicate field measurement or sample? Target is 10% duplication (once every 10 samples).

Date:
Personnel:
Weather:
Objective:
Measurements Make Sense (Y/N):
 # Photos:

Diversions Sampled (Circle)
Furnace Pond East Monsponsett Pond

YSI Reading or Profile for:

Depth (m)	DO (mg/L)	DO (%)	Spec. Cond. (µS/cm)	Temp (C)	Total Depth (ft)	Secchi Depth (m)
0.5						
1.0						
1.5						
2.0						
2.5						
3.0						
3.5						
4.0						
4.5						
5.0						

YSI Reading or Profile for:

Depth (m)	DO (mg/L)	DO (%)	Spec. Cond. (µS/cm)	Temp (C)	Total Depth (ft)	Secchi Depth (m)
0.5						
1.0						
1.5						
2.0						
2.5						
3.0						
3.5						
4.0						
4.5						
5.0						

Lab Samples and Non-YSI WQ Data

Sample ID	Sample Depth	Sample Collection Time	Turb (NTU)	Apparent Color (PCU)	pH	Duplicate Sample @ 10% Rate

Collected samples tested for:

Alpha: Chlorophyll-a and E. coli (two bottles per sample, collected surface only) - SAME DAY SHORT HOLD
Aquatic Analysts: Phytoplankton
Phoenix: Total Phos, Soluble Phos, Total Nitrogen (nitrate, nitrite, TKN), Alkalinity - NEXT DAY SHORT HOLD
GreenWater Labs: Microcystins via ELISA - NEXT DAY SHORT HOLD

Check After Completing Each Location
1. Do these readings make sense? Is the order of magnitude for each element correct?
2. Did I use the right units? If I collected using different units, did I clearly note this in the table?
3. Did I collect and appropriately label all required sample bottles?
4. Did I field filter soluble phosphorus into an H2SO4-preserved bottle? If field filtering was not possible, did I collect soluble phosphorus in an as-is bottle?
5. Do I need to take a duplicate field measurement or sample? Target is 10% duplication (once every 10 samples).

Date:
Personnel:
Weather:
Objective:
Duplicate Measurements or Samples - collect at 10% rate (Y/N):
Measurements Make Sense (Y/N):
 # Photos:

Instream WQ sampling to be conducted monthly

Site #	Site Name	Sample Collection Time	DO (mg/L)	DO (%)	Sp. Cond. (µS/cm)	Temp (C)	Turb (NTU)	pH	Apparent Color (PCU)	Discharge Measured	Logger Downloaded
SLT1	Tubbs Meadow Brook										
SLT2	Little Brook										
SLT3	Mirage Brook										
SLTD	Jones River										

Instream Lab Analytes):
Phoenix: Total Phos, Soluble Phos, Total Nitrogen - NEXT DAY SHORT HOLD (nitrite & nitrate)

Observations of Interest:

Check After Completing Each Location

1. Do these readings make sense? Is the order of magnitude for each element correct?

2. Did I use the right units? If I collected using different units, did I clearly note this in the table?

3. Did I collect and appropriately label all required sample bottles?

4. Did I field filter soluble phosphorus into an H2SO4-preserved bottle? If field filtering was not possible, did I collect soluble phosphorus in an as-is bottle?

4. Do I need to take a duplicate field measurement or sample? Target is 10% duplication (once every 10 samples).

Date:
Personnel:
Weather:
Objective:
Duplicate Cross Section - collect at 10% rate (Y/N):
Measurements Make Sense (Y/N):
 # Photos Taken:

Site #	Site Name	Time	Total width of stream (ft)	Depth to bottom of logger (ft)

Distance from Bank (ft)	Stream Depth (ft)*	Velocity from Flow Meter (ft/s)	Or, if too shallow Time to travel _ ft (s)	Comments (e.g. REB, eddy, etc.)

*Depth for the first and last measurement should be 0.

Site #	Site Name	Time	Total width of stream (ft)	Depth to bottom of logger (ft)

Distance from Bank (ft)	Stream Depth (ft)*	Velocity from Flow Meter (ft/s)	Or, if too shallow Time to travel _ ft (s)	Comments (e.g. REB, eddy, etc.)

*Depth for the first and last measurement should be 0.

Check After Completing Each Cross Section

1. Do these readings make sense? Is the order of magnitude for each element correct?
2. Did I use the right units? If I collected using different units, did I clearly note this in the table?
3. Did I take enough measurments to capture changes in velocity and depth across the channel? Target is 10 points across channel, when conditions allow.
4. Do I need to do a duplicate cross section flow measurement here? Target is 10% duplication (once every 10 cross sections).
5. Did I take photos of the logger as well as looking upstream and downstream from the cross section?

Date:
Personnel:
Weather:
Objective:
Measurements Make Sense (Y/N):
 # Photos:

Seepage Samplig
Silver Lake

Seepage Meter Location ID	Seepage Meter Diameter	Water Depth (cm)	Time In	Time Out	Volume In (mL)	Volume Out (mL)

Lab Samples and Field WQ Data

Sample ID	Time	Surface or Groundwater?	Temperature (C)	Spec. Cond. (µS/cm)	pH (SU)	Duplicate Sample @ 10% Rate

Collected groundwater samples tested for:

Phoenix: Soluble Phos, Nitrate, Ammonia - NEXT DAY SHORT HOLD

Notes:

Check After Completing Each Location
1. Do these readings make sense? Is the order of magnitude for each element correct?
2. Did I use the right units? If I collected using different units, did I clearly note this in the table?
3. Did I collect and appropriately label all required sample bottles?
4. Did I field filter soluble phosphorus into an H2SO4-preserved bottle? If field filtering was not possible, did I collect soluble phosphorus in an as-is bottle?
5. Do I need to take a duplicate field measurement or sample? Target is 10% duplication (once every 10 samples).

Appendix B – Laboratory SOPs

Chlorophyll A

References: **SM 10200 H,** Standard Methods for the Examination of Water and Wastewater, 18th Edition

1. Scope and Application

Matrices: Waters

Definitions: Refer to Alpha Analytical Quality Manual.

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass. All green plants contain chlorophyll *a*, which constitutes approximately 1 to 2% of the dry weight of planktonic algae. Other pigments that occur in phytoplankton include chlorophylls *b* and *c*, xanthophylls, phycobilins, and carotenes. The important chlorophyll degradation products found in the aquatic environment are the chlorophyllides, pheophorbides, and pheophytins. The presence or absence of the various photosynthetic pigments is used, among other features, to separate the major algal groups. This method describes the spectrophotometric method for determining chlorophyll *a* in phytoplankton.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method by completing the record of training.

2. Summary of Method

Chlorophyll pigment is extracted from the plankton concentrate using aqueous acetone and the Optical Density (Optical Density (OD) = Absorbance) of the extract is determined with a spectrophotometer. The ease with which the chlorophylls are removed from the cells varies considerably with different algae. To achieve consistently the complete extraction of the pigments, the cells are disrupted with a tissue grinder. All work is conducted in subdued light to avoid degradation.

2.1 Method Modifications from Reference

None.

3. Reporting Limits

The Reported Detection Limit is 2 mg/m^3.

4. Interferences

4.1 Turbidity: Turbidity is removed by filtration of the extract through a 1.0µm solvent-r resistant disposable filter.

4.2 Light Exposure: Light exposure to the sample will degrade the chlorophyll. Therefore collection and analysis are to be conducted in subdued lighting conditions.

5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

NOTE: When using the tissue grinder, be sure to hold the glass cuvette on the sides and not on the bottom. This is to ensure that the grinder will not injure the chemist, should the cuvette accidentally break.

6. Sample Collection, Preservation, Shipping and Handling

6.1 Sample Collection

Samples are collected in dark glass or dark plastic containers. Containers may be covered with aluminum foil to minimize degradation of the sample by light.

6.2 Sample Preservation

None.

6.3 Sample Shipping

No specific requirements.

6.4 Sample Handling

Samples are filtered as soon as possible after sample collection. Filters are stored in the dark, refrigerated at 4ºC for no more than 3 days.

7. Equipment and Supplies

7.1 Glass Cuvettes: 1 cm and 2.5 cm

7.2 Aluminum Foil

7.3 Tissue Grinder

7.4 Glass Fiber Filters: Gelman, P/N 61631, 47mm

7.5 Filtration Equipment: Vacuum pump; membrane filter funnel.

7.6 Forceps: Smooth-tipped, without corrugations on the inner sides of the tips.

7.7 Pipets: 1mL, glass

7.8 10mL Solvent-resistant syringe: with 1.0µm PTFE filter membrane.

7.9 Centrifuge

7.10 Centrifuge tubes: 15mL , graduated, screwcap

7.11 Genesys 5 Spectrophotometer, UV: with a narrow band-width (0.5 to 2.0nm)

7.12 Amber Glass Jars: 250mL, with covers

7.13 Filter discs: 1.0um pore size, solvent resistant

7.14 10mL syringe: Solvent resistant

7.15 Membrane Filter Funnels: 0.45 µm filter

7.16 Refrigerator: 4 °C

8. Reagents and Standards

8.1 Saturated Magnesium carbonate solution: Add 1g fine powdered $MgCO_3$ to 100mL DI. Undissolved material is not uncommon.

8.2 Acetone: Reagent grade BP 56 ºC.

8.3 90% Aqueous Acetone/10% $MgCO_3$ working solution: 90 parts acetone (Section 8.2): 10 parts saturated $MgCO_3$ solution (Section 8.1). Store in a 250mL glass amber jar.

8.4 $MgCO_3$: Powder form, Lab grade (USP-FCC)

9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1 Blank

The Blank consists of 10mL of 90% Aqueous Acetone/10% $MgCO_3$ working solution in a 15mL centrifuge tube covered with aluminum foil. It is steeped for at least 2 hours at 4ºC alongside of the samples and is filtered through a 1.0µm solvent-resistant disposable filter.

The Spectrophotometer is zeroed at OD750 with the Blank.

9.2 Laboratory Control Sample (LCS)

Not Applicable

9.3 Initial Calibration Verification (ICV)

Not Applicable

9.4 Continuing Calibration Verification (CCV)

Not Applicable

9.5 Matrix Spike

Not Applicable

9.6 Laboratory Duplicate

A sample is analyzed in duplicate (if sample volume is available) once per batch of 20 samples or less

9.7 Method-specific Quality Control Samples

None.

9.8 Method Sequence

- Filter the sample onto a glass fiber filter.
- Wrap the filter in foil, secure in airtight bag, and place in 4 C refrigerator (if not immediately grinding).
- Grind the glass fiber filters in a tissue grinder with 90% Aqueous Acetone/10% $MgCO_3$ working solution.
- Steep the samples and Blank for 2 hours at 4ºC.
- Clarify the sample extract and Blank by centrifuging and filtering.
- Zero the Spectrophotometer at 750, 664, 647, and 630 respectively, with Blank
- Determine OD's of the samples
- Perform calculations.

10. Procedure

10.1 Set-up

Perform all analysis in subdued light, with all containers covered in aluminum foil to avoid degradation of the chlorophyll.

10.1.1 Sample Filtration/Preparation

Concentrate the chlorophyll by filtering the whole volume of sample onto a glass-fiber filter as soon as possible after sample collection.

The sample container should be gently swirled before each portion poured to ensure the sample aliquots are homogeneous. If the sample has too much sedimentary material present, and the filtering process becomes sluggish, do not add further sample volume to the filtering unit. Make sure to note correct volume of sample filtered in the Micro logbook.

If the sample is to be analyzed immediately, record in the laboratory notebook the sample ID, date and time of filtration, sample volume filtered, and the chemist's initials. Also record the lot # of the glass-fiber filters and the filter funnels used. Proceed to Section 10.3.1.

Otherwise, if the sample is not to be analyzed at this time, the filter is carefully (to avoid piercing of the filter) placed onto a piece of aluminum foil and folded in half so the chlorophyll extract is folded onto itself. (This is to avoid the chlorophyll adhering to the aluminum foil.) The folded filter/foil is stored in a sealed plastic bag at 4ºC until pigment extraction takes place (not to exceed 3 days). Note on the plastic bag the sample ID, date and time of filtration, sample volume filtered, and the initials of the chemist.

10.2 Initial Calibration

The spectrophotometer is zeroed at each wavelength with a Blank sample. The Blank sample is prepared as in Section 9.1.

10.3 Equipment Operation and Sample Processing

If the filter containing the chlorophyll has been stored in the refrigerator, transfer the following information into the laboratory notebook from the plastic bag in which the filter has been stored: the date and time of filtration, sample volume filtered, and the chemist's initials.

10.3.1 Pigment Extraction

Using forceps, carefully fold the glass-fiber filter containing the extract and place it in a 2.5cm glass cuvette that is covered with aluminum foil. Cover the filter with 5mL of 90% Aqueous Acetone/10% $MgCO_3$ working solution and place onto the tissue grinder. Grind at 500rpm for 1 minute. (Note: Whenever the 90% Aqueous Acetone/10% MgCO3 solution is used, the bottle it is contained within should be gently swirled first to ensure all undissolved material in the solution is uniformly dispersed.)

Transfer the slurry to a screwcap centrifuge tube that is covered with aluminum foil. Into the cuvette, rinse the grinder with a few mLs of 90% Aqueous Acetone/10% $MgCO_3$ working solution, and swirl to combine any leftover slurry. Add the rinse to the extraction slurry in the centrifuge tube. If there is still remaining slurry in the cuvette, use a 1mL glass pipette to transfer to the centrifuge tube. Adjust the total volume to a constant level

of 10mL with 90% Aqueous Acetone/10% $MgCO_3$ working solution. (Avoid excessive dilution of pigments.) Cover the centrifuge tube and swirl to ensure all of the slurry is mixed with the Aqueous Acetone.

If additional samples are to be ground, rinse the tissue grinder with Acetone (Section 8.2) and allow to dry before each sample.

Steep in darkness for at least 2 hours at 4ºC, not to exceed 2 ½ hours. Record the times in the laboratory notebook.

Place the 15mL centrifuge tube that contains the sample into a 50mL centrifuge tube that has a KimWipe in the bottom. (The KimWipe is used to minimize the movement of the smaller centrifuge tube within the larger centrifuge tube.)

Clarify the extract by centrifuging in the closed centrifuge tube for 20 minutes at 2800rpm. Then filter the supernate through a 1.0µm solvent-resistant disposable filter. (Note: Make sure to not over-centrifuge, since acetone will break down integrity of centrifuge tube over time).

Decant the clarified extract into a clean, calibrated 15mL screwcap centrifuge tube covered with aluminum foil.

10.3.2 Spectrophotometric Determination of Chlorophyll *A* – Trichromatic Method

Transfer the Blank sample into a DI-rinsed 1cm glass cuvette. Also, transfer the clarified extract(s) into separate DI-rinsed 1cm glass cuvette(s).

Zero the Spectrophotometer with the Blank sample at 750nm. Read the Optical Density (OD) of the samples at 750nm. Record OD in the laboratory notebook.

Zero the Spectrophotometer with the Blank sample at 664nm. Read the Optical Density (OD) of the samples at 664nm. Record OD in the laboratory notebook. (The OD664 should be between 0.1 and 1.0, therefore prepare dilutions with 90% Aqueous Acetone/10% $MgCO_3$ working solutions as necessary to provide an OD within this range.)

Zero the Spectrophotometer with the Blank sample at 647nm. Read the Optical Density (OD) of the samples at 647nm. Record OD in the laboratory notebook.

Zero the Spectrophotometer with the Blank sample at 630nm. Read the Optical Density (OD) of the samples at 630nm. Record OD in the laboratory notebook.

10.4 Continuing Calibration

Not Applicable.

10.5 Preventive Maintenance

An instrument service company calibrates the Spectrophotometer-UV on an semi-annual basis. Certificates are kept on file.

11. Calculations

11.1 Calculations are automatically performed on an Excel Spreadsheet for the Chlorophyll A analysis. Input the following information into the Spreadsheet:

- Extract Volume (mL), as determined in the second paragraph of Section 10.3.1.
- Volume of Sample (L), as determined prior to filtration in Section 10.1.1
- OD 750
- OD 664
- OD 647
- OD 630

11.2 The actual calculation performed on the Spreadsheet is as follows:

The OD750 is a correction for turbidity. Therefore, subtract the 750nm OD value from the readings at the other wavelengths ***before*** using them in the equations below.

Using the ***corrected*** values, calculate Chlorophyll *a* in the extract as follows:

$$C_a \text{ (mg/L)} = (11.85 \times OD664 \times \text{dilution factor}) - (1.54 \times OD647) - (0.08 \times OD630)$$

After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

$$\text{Chlorophyll } a, \text{ mg/m}^3 = \frac{C_a \times \text{extract volume, mL}}{\text{Volume of sample, L}}$$

- The *extract volume* is determined in the second paragraph of Section 10.3.1.
- The *volume of sample* is determined prior to filtration in Section 10.1.1.

12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Holding time exceedance and improper preservation are noted on the nonconformance report form.

Perform routine preventative maintenance following manufacturer's specification. Record all maintenance in the instrument logbook.

Review of blanks for acceptable performance occurs for each batch of samples. Record any trends or unusual performance on a nonconformance action form.

13. Method Performance

13.1 Method Detection Limit Study (MDL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

The laboratory follows the procedure to determine the MDL, LOD, and/or LOQ as outlined in Alpha SOP/1732. These studies performed by the laboratory are maintained on file for review.

Not applicable to this method.

13.2 Demonstration of Capability Studies

Refer to Alpha SOP/1734 and 1739 for further information regarding IDC/DOC Generation.

Not applicable to this method.

13.2.1 Initial (IDC)

The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

13.2.2 Continuing (DOC)

The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

14. Pollution Prevention and Waste Management

Refer to Alpha's Chemical Hygiene Plan and Waste Management and Disposal SOP for further pollution prevention and waste management information.

15. Referenced Documents

Chemical Hygiene Plan

SOP/1732 MDL/LOD/LOQ Generation

SOP/1734, 1739 IDC/DOC Generation

SOP/1728 Waste Management and Disposal SOP

16. Attachments

None.

Total Coliform, Fecal Coliform, E.Coli, and Enterococcus by Quantification Methods (Quanti-Tray and Quanti-Tray2000)

Reference Method No.: **SM 9223 B.** Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF. Standard Methods Online.

1. Scope and Application

Matrices: The chromogenic substrate coliform test is recommended for the analysis of drinking and clean source water samples and non-potable water samples.

Definitions: Refer to Alpha Analytical Quality Manual.

The Enterolert method works in a similar way to that of the Colilert/Colilert-18 methods, except that it works with a different substrate. The nutrient-indicator for enterococci is 4-methyl-unbelliferyl B-D-glucoside. When enterococci utilize their B-glucosidase enzyme to metabolize this indicator, it causes the sample to fluoresce. Enterolert can detect enterococci such as E. faecium and E. faecalis in fresh and marine water. This procedure can detect enterococci at 1cfu per 100 ml sample within 24 hours.

The Idexx Quanti-Tray procedure enhances the current Colilert/Colilert-18 & Enterolert presence/absence methods with the ability to quantify the results. It also provides a more efficient, automated alternative to membrane filtration methods for bacterial enumeration. The Idexx Quanti-Tray System consists of a motor-driven, heated roller instrument (the Sealer) designed to seal Idexx Quanti-Trays. By combining Idexx's technologies, the presence/absence test methods utilized in combination with the Quanti-Tray System produce counts as accurate as membrane filtration without the additional time burden of counting colonies.

This method is restricted to use by or under the supervision of analysts experienced in microbiological analysis and in the interpretation of microbiological data. Each analyst must demonstrate the ability to generate acceptable results with this method by completing the record of training.

2. Summary of Methods

Colilert-18 for Total Coliform and E. Coli: 100 ml of drinking water (or a dilution of non-drinking water) sample is mixed with chromogenic substrate, poured into a Quanti-Tray, and incubated for 18-22 hours at 35 C +/-0.5 C. Positive wells in the tray are counted upon removal from incubator. The results are as follows: Less or no yellow color than that of the comparator signifies negative for total coliform & E.coli presence; yellow color equal to or greater than the comparator signifies positive for total coliform; yellow color and fluorescence (inspection under UV light at 366nm) equal to or greater than the comparator signifies positive for total coliform & E.coli presence.

Colilert-18 for Fecal Coliform: Same procedure as for Total Coliform and E. Coli, except that the prepared Quanti-Trays are incubated for 18-22 hours at 44.5 C +/- 0.2 C. Positive (yellow) wells in the tray are counted after removal from the incubator.

Enterolert: 100 ml of drinking water (or a dilution of non-drinking water) sample is mixed with chromogenic substrate, poured into a Quanti-Tray, and incubated for 24 hours at 41 C +/-0.5 C. Positive wells in the tray are counted upon removal from incubator. The results are as follows: Lack of fluorescence under UV light at 366nm signifies negative for enterococci. Blue fluorescence signifies positive for enterococci.

2.1 Method Modifications from Reference

The Quanti-Tray method is an addition to the original presence/absence methods, whereby samples can be quantified.

3. Detection Limits

Results are reported as Most Probable Number (or 1mpn/100ml sample), based on the Idexx MPN table provided with the Idexx trays. There are two tables, one for the low level tray (51-well) and one for the high level tray (97-well). Refer to Table 1 and Table 2.

4. Interferences

4.1 Instrumental

4.1.1 Improper incubator temperature may inhibit growth.

4.1.2 Improper sealing of Quanti-Tray prior to incubation may enhance or hinder bacterial growth in the tray.

4.2 Parameters

4.2.1 One interference common in this analysis is the negative interference due to the presence of bacteriacidal concentrations of chlorine or other halogen. This interference is countered in the sampling and preservation step by using sodium thiosulfate at a great enough concentration to counter the chlorine.

4.2.2 Another interference is the positive interference due to the use of non-sterile sample containers or the use of non-aseptic sampling technique allowing the contamination of the sample. This interference is countered by ensuring the use of properly sterilized containers. Non-sterile sampling technique is countered via education of the samplers.

4.2.3 Water samples containing humic or other material may be colored. If there is background color, compare inoculated vessels to a control vessel containing only water sample.

4.2.4 Strict attention must be paid to the length of the incubation period.

4.2.5 For Enterolert, if marine water samples are analyzed, the sample must be diluted a minimum of tenfold with sterile fresh water prior to analysis.

4.2.6 If reagent is added to sample vessel and immediately turned a flash of blue color, halt analysis and notify manager/client. Method suggests high Chlorine content (matrix interference).

4.2.7 If analysis concludes from incubation and the resulting liquid in the sample vessel is visibly black in color, the analysis is halted and a manager/client is notified. Method suggests high metals content (matrix interference).

5. Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material data handling sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

Any personnel utilizing the Idexx Sealer instrument should be familiar with the user manual and should have proper laboratory Quanti-Tray method training prior to use.

6. Sample Collection, Preservation, and Handling

6.1 Sample Collection

6.1.1 Samples for microbiological examination are collected in pre-sterilized specimen cups.

6.1.2 The volume of sample collected should be enough to carry out all tests required, preferably no less than 100mL. There must be a minimum volume of 100ml available for these analyses. Any additional volume (ie. additional containers of sample), permits QC to be analyzed in association with methods analyzed.

6.1.2.1 If less than 100ml volume is submitted, notify the Project Manager who will contact the client for any further instructions.

If the client cannot be reached before hold time is exceeded, set samples with dilutions, which will increase the MDL/RL information. Narrate any required dilutions necessary for limited sample volume.

6.2 Sample Preservation

For the collection of samples having residual chlorine, pre-sterilized specimen cups containing sodium thiosulfate ($Na_2S_2O_3$) are used. $Na_2S_2O_3$ is added to neutralize any residual halogen and prevent continuation of bacteriacidal action during sample transit. The analysis will therefore indicate more accurately the true microbial content of the water at the time of sampling.

6.3 Sample Shipping

No special requirements

6.4 Sample Handling

6.4.1 Start microbial examination of water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 1 hour after collection, use an iced cooler for storage during transport to the Laboratory.

6.4.2 Refrigerate samples upon receipt in the laboratory at a temperature below 4ºC, but do not freeze.

6.4.3 Initiate analysis as soon as possible after collection to minimize changes in bacterial population. The holding time for non-potable and potable water is 8 hours from time of collection.

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7. Equipment and Supplies

7.1 Incubators: 35 ± 0.5ºC , 41 ± 0.5ºC, and 44.5 +/- 0.2 C

7.2 UV Lamp: Wavelength 366nm

7.3 100mL analysis Vessels: Sterile, non-fluorescent, containing sodium thiosulfate. Volume is verified per lot, and recorded on the Filter Funnel and Colilert Container form 18026.

7.4 Autoclavable Bottles: 500ml wide mouth, with caps

7.5 Autoclave Tape

7.6 Autoclave: For use at 121 °C, 20psi

7.7 Autoclave Disposal Bags

7.8 Residual Chlorine Strips: 0.5mg/L Residual Chlorine sensitivity

7.9 pH Paper Strips: Range 0 – 14 pH units

7.10 Inoculation Loops: Sterile, 10 µL size

7.11 Idexx Quanti-Tray Sealer (model x2)

7.12 Idexx Quanti-Trays: 51-well (Quanti-Tray) & 97-well (Quanti-Tray2000)

7.13 10ml and 1ml sterile, disposable, polystyrene pipettes, cotton plugged.

8. Standards and Reagents

8.1 Substrate

8.1.1 **Colilert reagent,** (Colilert-18 method), single doses for 100mL samples.

8.1.2 **Enterolert reagent**, single doses for 100ml samples.

Each lot of substrate is checked for viability using the organisms as defined in Section 8.5. Results are recorded on Form 18023. Analyze a Blank with each set of new reagent.

Corrective Action:

If viability results are not as indicated, do not use the lot of Substrate. Run a second test of the same lot of Substrate to confirm, and if the results are still failing manufacturer's criteria, then DO NOT USE, and contact the manufacturer.

8.2 Color Comparator

8.2.1 Colilert-18: Obtained from the same manufacturer as the substrate (same comparator for Colilert & Colilert-18).

8.2.2 Enterolert: Compare color to negative control (method Blank sec 9.1) per manufacturer instructions.

8.3 Sterile DI

Dispense DI into 500mL autoclavable bottles and cap loosely. (note: Place a clean strip of autoclave tape on each bottle. Label each bottle with a batch number and autoclave for 30 minutes at 121ºC and 20 psi.

Once bottles have cooled, tighten the caps and store in the refrigerator (1 – 4 °C) until ready to use.

Prior to use, however, the following positive/negative check must be completed for each batch of sterile DI. This will confirm that the DI is indeed sterile and ready for use.

- Take 1 bottle of TSB (Section 8.4) and label it as the positive/negative check for the particular batch number.

- Transfer 50mL of the DI into the bottle of TSB using 10ml pipette.
- Incubate the bottle at 35 ± 0.5ºC for 24 hours.
- Examine bottle for gas and/or turbidity. If the bottle is clear, re-incubate for another 24 hours and reexamine. The bottle should remain clear, indicating that there is no growth and the DI batch is sterile.
- Note the results in the Microbiology Dilution Water logbook (Form No.: 18012).
- If the results are not as stated above, then there is a problem that needs to be corrected. The problem could lie in several different areas:

  1. Verify that the batch of TSB that was used was tested prior to use (Section 8.4).
  2. Verify that the temperature of the incubator was within the proper range.
  3. The DI may not be sterile. Test the DI batch again and if the results are similar, then do not use the batch of DI.

8.4 Tryptic Soy Broth (TSB) x2 conc, glass bottle:

Commercially prepared media from Lakewood Biochemical Co. Inc, Catalog #B40162.

Store at room temperature. Expires upon manufacturer's specified date.

Take 1 bottle of TSB to run sterility check for each Lot of the media. No media should be used until it has been checked.

Incubate the bottle of TSB at 35 ±0.5ºC for 48 ±2 hours.

Examine bottles for turbidity. The liquid should be clear and if so it may be used.

If the liquid is not clear, then there is a problem. The TSB sterility check should be reset using a new bottle of the same Lot. After incubation, if the liquid is clear then the media is acceptable for use. If the results fail again, then discard that entire Lot of media, and test a bottle from a new Lot.

8.5 Nutrient Broth, 500mL bottle by Teknova (product cat #N1260) 0.8% Nutrient Broth (8g/L formulation)

Purchased commercially prepared: VWR, cat # 76062-100

Store in the refrigerator at 1-4 °C. Use as needed and allow to warm to room temperature before use. Expires upon manufacturer's specified date.

The laboratory must verify that the media is valid for use:

Check each Lot of Nutrient Broth prior to use: Pour 30ml of broth into one sterile bacteria cup with volume line markings. Incubate 35 ±0.5ºC for 48 ±2 hours; remove and check for turbidity/growth. If broth remained clear, then that Lot is approved for use.

If the broth does not remain clear, then the media should be retested with a new aliquot of the broth. A second failure of the media signifies that the media is not valid for lab use, and a new lot of media must be ordered (and subsequently validated prior to lab use).

8.6 Control Bacteria

8.6.1 Colilert Reagent:

A Quanticult Kit is used for the verification of Colilert/Colilert-18 substrates. This kit contains E. Coli (EC), Klebsiella pneumoniae (KP), and Pseudomonas aeruginosa (PA). Follow instructions below to rehydrate and incubate (Sec 8.6.3) . Use to verify each new

lot of Colilert or Colilert-18 Substrate (Section 8.1). Record results in the Colilert Reagent Check Log (Form 18023). Expected results will be:

EC = Positive for total coliform and **positive** for E. Coli and/or Fecal Coliform

KP = Positive for total coliform and **negative** for E. Coli.

PA = Negative for total coliform and **negative** for E. Coli and /or Fecal Coliform

Blank = Sterile DI, for each batch of reagent.

8.6.2 Enterolert Reagent:

For Enterolert substrate (Section 8.1) verification, use the following:

Follow instructions below to rehydrate and incubate (Sec 8.6.3) . Use to verify each new lot of Enterolert Reagent. Record results in the Reagent Check Log (Form 18023). Expected results will be:

Enterococcus faecium (ATCC#35667) as the positive control (ie. fluorescence)

Serratia marcescens (gram -) (ATCC#43862) as the negative control (ie. no fluorescence)

Aerococcus viridans (gram +) (ATCC#10400) as the negative control (ie. no fluorescence)

If the results Fail the manufacturer's criteria, then do not use the lot of Enterolert Reagent. Run a second test of the same lot of Enterolert Reagent to confirm, and if the results are still failing manufacturer's criteria, then DO NOT USE, and contact the manufacturer.

8.6.3 Culture (Micro-organism) Preparation

Remove the Organism Kit from the freezer. Allow to warm to room temperature.

Prepare one 100ml colilert vessel for each organism listed above, plus one vessel for the Blank. Fill each vessel with 100ml sterile DI.

Once warmed, open each vial, aseptically transfer the colored disc within to the coordinating labeled vessel and cap.

Swirl each vessel, then let sit for approximately 15 minutes, or until the disc is entirely dissolved.

Once dissolved, invert the vessel ten times to mix, then incubate per the methods:

for 22 hours at 35 +/- 0.5 C for Coliert-18,

for 24 hours at 41 +/- 0.5 C for Enterolert

Record the final results in the laboratory notebook.

If any organisms do not pass the criteria with the reagent, the test must be rerun with a new organism kit, and the tested reagent lot must not be used in the lab until verified.

If the reagent lot fails a second test, it should be removed from the lab, not used, and a new lot of reagent should be purchased to test.

9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method

Note: One batch is defined as 10 or less samples, plus one Blank and one Duplicate.

9.1 Method Blank

Negative Check - Use 100mL of sterile DI in a 100mL sterile, non-fluorescent vessel with one dose of Colilert-18/Enterolert reagent. Analyze one Method Blank with each batch of samples per method.

Corrective action:

If the Method Blank is positive and all associated samples within the batch are negative, then the batch can be reported with the appropriate narrative, and no client contact is necessary.

If the Method Blank is positive, but the associated sample concentrations are more than 10x the concentration of the Method Blank, then the batch can be reported with the appropriate narrative.

If the Method Blank is positive, but the associated sample concentrations are less than 10x the concentration of the Method Blank, then the client must be contacted, and the samples will be narrated and qualified.

9.2 Seed

Positive Check - The seed is a sample of secondary effluent. This is obtained every other day by an Alpha courier at a local Wastewater Treatment Plant.

This is the known positive check. Add 1ml of seed to a 100ml sterile, non-fluorescent vessel, fill to 100ml line-marking using unsterilized DI, and add one dose of Colilert/Colilert-18/Enterolert reagent. Analyze one seed with each batch of samples per method.

Corrective Action:

If the seed fails during the analysis, the data is considered unreportable, and the Project Manager is notified, who will contact the Client.

9.3 Laboratory Control Sample (LCS)

Not Applicable.

9.4 Initial Calibration Verification (ICV)

Not Applicable

9.5 Continuing Calibration Verification (CCV)

Not Applicable

9.6 Matrix Spike

Not Applicable

9.7 Laboratory Duplicate

A duplicate is analyzed daily for each microbiology analysis, if available.

9.8 Method-specific Quality Control Samples

None.

9.9 Method Sequence

- Sample Preparation:
  1. Blank
  2. Seed

3. Samples and duplicate sample, if applicable.

- Check samples for Free Chlorine and pH.
- Add appropriate substrate/reagent to the sample aliquot and mix. Transfer to the appropriate Quantitray size.
- Total Coliform/E.Coli: Sample incubation at 35 +/- 0.5 C 18-22 hours (Colilert-18). **OR**
- Enterococcus: Sample incubation at 41 +/- 0.5 C for 24-28 hours (Enterolert). **OR**
- Fecal Coliform: Sample incubation at 44.5 +/- 0.2 C (Colilert-18)
- Sample inspection and counting the wells for each Analyte:
  - Total Coliform will show yellow color change.
  - E.coli will show florescence under the UV lamp.
  - Fecal coliform will show yellow color change.
  - Enterococcus will show florescence under UV lamp.
- Compare sample color to Color Comparator, if needed.
- Report positive results to Client Services Department
  1. Client is notified within 24-hours.

10. Procedure

10.1 Equipment Set-up

10.1.1 Laboratory Notebook: The lab notebook must contain the following information:

- Date
- Name of test
- Method used Quanti-tray with Colilert-18/Enterolert
- Client Name
- pH result
- Cl^- result + or –
- Lot# of Colilert-18/Enterolert reagent used
- Any pre-dilutions
- Quanti-Tray size used for each sample analysis: 51-well or 97-well
- Prep time
- Time in/temp of the incubator
- Time out of the incubator
- Analyst's initials

10.1.2 Sample Vessels: Use one vessel per sample, and another for a Blank. If sample volume permits, use one vessel for the Duplicate. Label each vessel with the sample number for which it will be used.

10.2 Initial Calibration

Not Applicable

10.3 Equipment Operation and Sample Processing

10.3.1 Turn on the Idexx Quanti-Tray Sealer to warm up for minimum of 10 minutes prior to use.

10.3.2 Add one dose of Colilert-18 or Enterolert reagent to 100mL, non-fluorescent vessel. Shake the sample vigorously and pour into the appropriate vessel up to the 100mL mark. Cap and mix thoroughly. (Reagent will not dissolve completely.)

10.3.2.1 DILUTIONS:
If any samples require a dilution, the sample is diluted into 99ml sterile DI bottles.

10X DILUTION = Take a sterile 10ml pipette and remove 9ml of DI from the 99ml bottle. Then take 10ml of sample and transfer this from the sampling container to the 99ml dilution bottle. Shake well and then pour into the analysis vessel.

100X DILUTION = Take a sterile 1ml pipette and transfer 1ml of sample from the sampling container to the 99ml dilution bottle. Shake well and then pour into the analysis vessel.

10.3.3 Check samples for presence of chlorine

The presence of chlorine is checked by using Free Chlorine test strips (Section 7.8). These strips reveal if there is some level of Free Chlorine present in the sample.

Dip the test strip into the sample container AFTER analysis has been set up and record the results, according to the manufacturer's information, in the laboratory notebook (Section 10.1.1).

If the sample is positive for Free Chlorine, this is considered a variance from the method and must be written into a laboratory note and submitted with the reported data.

10.3.4 Check pH of samples

The pH of the sample is checked by using the pH paper strips (Section 7.9). These strips reveal the pH by comparison to a color-coded chart.

Dip the paper strip into the sample container AFTER analysis has been set up and compare the strip color pattern to the coded chart on the box of pH paper strips. Record results in the laboratory notebook (Section 10.1.1).

10.3.5 Transfer of Sample to Quanti Tray

After the sample and analysis-specific substrate has been added to the vessel (Section 10.3.2), the sample is transferred to the Quanti-Tray as follows:

- Select the 51-well trays for samples expected to have lower bacterial concentrations, such as the Blank. Select the 97-well trays for samples expected to have higher bacterial concentrations, such as the Seed, and any samples requiring a preliminary dilution.

- Label corresponding Quanti-Trays with sample numbers from the vessels using a permanent ink source; do not puncture the foil of the tray.

- Begin by holding a tray with the tab opening facing upward, and with the wells of the tray turned to the hand's palm side. Gently pull the foil away from the tray (ie. away from the palm); do not touch the inner tray. Pour the already well-shaken reagent mix from the vessel into the tray; do not make contact with the foil tab or tray while doing this.

- Allow all foam to settle. Tap the tray gently, on the plastic side, 2 to 3 times to dislodge any remaining air bubbles.

10.3.6 Sealing the Tray

Place the tray onto the corresponding rubber tray carrier of the Sealer with the well-side facing down. Be careful not to tip the tray too much while doing this so that sample is spilled.

Allow the Sealer to seal the Quanti-Tray per the Sealer's instructions.

When the tray is removed from the Sealer, ensure that all wells (no more than 2 wells can be empty) have sample fluid within them. If more than 2 wells are empty, the sample must be reset into a vessel and Quanti-Tray (if volume permits) because the first tray is invalid for further analysis

10.3.7 Incubation

Place the sealed tray in the appropriate incubator with the wells-side down (no more than 8 trays per stack) for the method desired time/temp.

10.3.8 Calculating Results

When tray is removed after the proper incubation time, count all positive wells and refer to the MPN table provided by Idexx for sample results. See Table 1 and Table 2 and refer to Section 11.

10.4 Continuing Calibration

Not Applicable

10.5 Preventive Maintenance

Incubators are calibrated on an annual basis by an instrument service company. These records are kept on file.

Temperatures of the water baths are recorded twice daily (with a minimum of 4 hours between each reading). Adjustments are made as necessary.

The stability and uniformity of temperature distribution of any incubator must be established prior to use. (Work Instructions 2432)

11. Data Evaluation, Calculations and Reporting

11.1 Calculations

11.1.1 Record in the laboratory notebook the counted results from each sample tray.

For the 51-well tray (Quanti-Tray), the maximum counting range is 200. Check results on MPN table for Quanti-Tray.

For the 97-well tray (Quanti-Tray2000), the maximum counting range is 2419. Check results on MPN table for Quanti-Tray2000.

11.1.2 Determine the Range of Logs (ROL) as specified in Alpha's Quality Manual, Sections 5.9 and 5.13. Refer to Work Instructions WI/2434 for ROL Calculation procedure.

11.2 Reporting Sample Results

Every effort is made to notify the Client within a 24-hour period of any findings other than negative.

The Microbiology Manager or Microbiology analyst notifies the Project Management Department of the sample number(s), Client name, analysis, and sample results. The Project Management Department contacts the Client.

12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Holding time exceedance, and improper preservation are noted on the nonconformance report form.

Review of blank response for acceptable performance occurs for each batch of samples. Record any trends or unusual performance on a nonconformance action form.

Refer to Sections 9.1 and 9.2 for corrective actions for failed Method Blank and Seed, respectively.

13. Method Performance

13.1 Detection Limit Study (DL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

The laboratory follows the procedure to determine the DL, LOD, and/or LOQ as outlined in Alpha SOP ID 1732. These studies performed by the laboratory are maintained on file for review.

This procedure does not apply to this method.

13.2 Demonstration of Capability Studies

Refer to Alpha SOP ID 1739 for further information regarding IDC/DOC Generation.

13.2.1 Initial (IDC)

The analyst must make an initial, one-time, demonstration of the ability to generate acceptable accuracy and precision with this method, prior to the processing of any samples.

13.2.2 Continuing (DOC)

The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

14. Pollution Prevention and Waste Management

All sample waste which is generated before, during, and after analysis is collected in a temporary waste collection zone (a box lined with an autoclave bag). When this becomes full, the waste is taken to the Hazardous Waste Room adjacent to the Loading Dock, and placed in the Fiber Drum labeled Medical Waste. .

Refer to Alpha's Chemical Hygiene Plan and Hazardous Waste Management and Disposal SOP for further pollution prevention and waste management information.

15. Referenced Documents

2121 Chemical Hygiene Plan

1732 Detection Limit (DL), Limit of Detection (LOD) & Limit of Quantitation (LOQ) SOP

1739 Demonstration of Capability (DOC) Generation SOP

1728 Hazardous Waste Management and Disposal SOP

2434 Range of Logs Calculation and Acceptance Criteria Work Instruction

18010 Microbiology Filter Funnel Log Form

18012 Microbiology Dilution Water Log Form

18015 Microbiology Media pH and Control Check Log Form

18023 Colilert Reagent Check Form

16. Attachments

Table 1: 51-Well Quanti Tray MPN Table

Table 2: Quanti Tray/2000 MPN Table

Table 1: 51-Well Quanti Tray MPN

No. of wells giving positive reaction per 100 mL sample	Most Probable Number (MPN)	95% Confidence Limits	
		Lower	Upper
0	<1	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

Table 2: Quanti Tray/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.8	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.8	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	68.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.8	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.8	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.8	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	455.2

Quanti-Tray and Defined Substrate Technology are trademarks or registered trademarks of IDEXX Laboratories, Inc. In the United States and/or other countries. Covered by U.S. Patent Numbers 4,925,788; 5,429,933; 5,518,892. Other patents pending.

Table 2: Quanti Tray/2000 MPN Table (continued)

# Large Wells Positive	# Small Wells Positive																									
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.8	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.8	95.8	97.8	99.8	101.8	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.8	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.8	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	868.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.8	>2419.8

Aquatic Analysts

Algae Analytical and Quality Assurance Procedures

January 15, 2021

Sample Handling

Sample Collection and Preservation

Phytoplankton are collected by filling bottles with natural water samples. Samples are collected at either discrete depths, or integrated through the photic zone of lakes. A volume of 125 mL is sufficient for most samples.

These samples are preserved with 1% Lugol's solution immediately after collection. Refrigeration is not necessary, and holding times are a year or more.

Sample Tracking

All samples received in the laboratory are immediately logged into a Sample Receipt Log. All samples are stored in a dedicated area until they are processed. After samples are processed and analyzed and data reports have been submitted to clients, samples are placed in storage for at least one year.

Sample Preparation

Permanent microscope slides are prepared from each sample by filtering an appropriate aliquot of the sample through a 0.45 micrometer membrane filter (APHA Standard Methods, 1992, 10200.D.2; McNabb, 1960). A section is cut out and placed on a glass slide with immersion oil added to make the filter transparent, followed by placing a cover slip on top, with nail polish applied to the periphery for permanency. A benefit to this method is that samples can be archived indefinitely; we have nearly 35,000 slides archived.

Microscopic Analyses

Algae Identifications

Aquatic Analysts has an extensive library of algae literature, including journal reprints, standard reference books, and internet reference sites. We also maintain files, notes, and photographs of algae we've encountered during the past 38 years of identifying algae. Most algae are identified by cross-referencing several taxonomic sources.

Enumeration

Algal units (defined as discrete particles - either cells, colonies, or filaments) are counted along a measured transect of the microscope slide with a Zeiss standard microscope (1000X, phase contrast). Only those algae that were believed to be alive at the time of collection (intact chloroplast) are counted. A minimum of 100 algal units are counted. (Standard Methods, 1992, 10200.F.2.c.).

Biovolume Estimates

Average biovolume estimates of each species are obtained from calculations of microscopic measurements of each alga. The number of cells per colony is recorded during sample analysis to arrive at biovolume per unit-alga. Average biovolumes for algae are stored in a computer, and measurements are verified for each sample analyzed.

Data Analyses and Reports

Sample Reports

Results of sample and data analyses are provided to the client in electronic format. Deliverables include individual sample reports, data summaries, database file, and combined species lists.

Individual sample reports include sample identification, a trophic state index, total sample density, total sample biovolume, and a list of algae species with their absolute and relative densities and biovolumes. All data are reported in Excel format.

Data summaries include sample identification, total density, total biovolume, the trophic state index, and the top 5 most common algae species (codes) and their relative densities. The summary format allows for easy calculations and graphs of algae sample data.

Database files include information for each species from each sample within a sample set. Information includes sample ID, species names and codes, densities and biovolumes, taxonomic group, and any notes on each sample.

Combined species lists of all species within related groups of samples allow greater sensitivity in comparing different lakes, sites, dates, or depth. Algae species are compiled according to their relative densities.

Trophic State Index

A Trophic State Index based upon phytoplankton biovolume has been developed from a data set of several hundred lakes located throughout the Pacific Northwest (Sweet, 1986, Report to EPA). The index was derived in a similar fashion as Carlson (1977) derived indices for Secchi depth, chlorophyll concentration, and total phosphorus concentration. The biovolume index ranges from 1 for ultraoligotrophic lakes to 100 for hypereutrophic lakes. Values agree well with Carlson's indices.

The index is defined as:

TSI (biovolume) = (Log-base 2 (B+1)) * 5

Where B is the phytoplankton biovolume in cubic micrometers per milliliter divided by 1000.

TSI values below 20 are generally considered to be ultraoligotrophic, values from 20-35 are oligotrophic, 35-50 mesotrophic, 50-65 eutrophic, and above 65 is hypereutrophic.

Quality Assurance

Microscope Calibration

Aquatic Analysts use a Zeiss Standard phase-contrast microscope primarily with a 1000X magnification for identification and enumeration of algal samples. The diameter of the field of view at 1000X magnification is 0.182 mm. The effective area of a filter is 201 millimeters square.

Algae are enumerated along a measured transect, measured accurately to 0.1 mm with a stage micrometer. The algal densities are calculated from the area observed (transect length times diameter of field of view), the effective filter area, and the volume of sample filtered.

The microscope was calibrated using a standard concentration of latex spheres provided by EPA (Cincinnati, OH). The concentration of these spheres was 12,075 per milliliter. Duplicate preparations of the standard spheres were analyzed; the average result was

11,700 spheres per milliliter (96.9 percent). The computer program used to calculate algae densities compensates for this 3.1% error.

Replicates

Replicate algae samples are analyzed at the client's request. We encourage blind replicates for approximately 10% of all samples collected. Replicates are assessed for algae abundance (relative mean difference of densities) and species composition (similarity indices, species lists).

Independent Analyses

Aquatic Analysts has participated in the analyses of split algae samples on several occasions, with general agreement between samples in terms of algae density and algae species compositions. On occasion, we also contract independent algae analysts for second opinions on some difficult to identify algae species.

Internal Data Verification

A custom computer program handles all calculations and data analyses. Final sample reports are compared with laboratory bench sheets before releasing data.

Data summaries, tables of similarity indices, abundance graphs, and combined species lists are searched for inconsistencies, outliers, and interrupted patterns that may indicate possible errors.

Archives

Aquatic Analysts maintains an herbarium of all microscope slides analyzed (over 35,000 to date). These may be reviewed if questions arise after data are reported. In addition, all computer data (sample tracking data, raw count data, final reported data, data analyses, narrative reports) are archived on CD's in permanent storage.

Sequential fractionation of phosphorus in sediment

To assess phosphorus in sediment a sequential chemical extraction can be used, commonly called phosphorus (P) fractionation. Different pools, with varying solubility and reactivity, are examined. This method description is developed from Psenner & Pucsko (1988) and Hupfer *et al.* (1995, 2009).

The pools are arbitrarily defined as in figure 1.

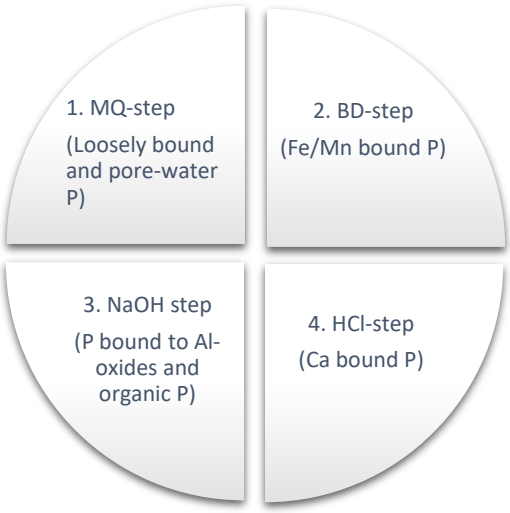

Figure 1. P pools generally extracted using the method described herein.

Quantification range: 0-800 µg/l

Equipment

Scale:
Spectrophotometer:
Autoclave:
Centrifuge:

Appendix 1: Chemical and solution preparation
Appendix 2: Phosphorus extraction with spectrophotometer
Appendix 3: Dry weights and loss on ignition
Appendix 4: Methodological background and references
Appendix 5: Adaptations of the method
Appendix 6: Quality assurance

Edited by: SS, O A-N, EL
Approved by:

Procedure

General advice

- For the pipettes, never turn the pipette upside down and be careful tilting it when it's full, to avoid contaminaton inside of the pipette. If the pipette is spraying a lot, sometimes the pipette tip needs to be changed if it's old or adjust it so the tip sits firmly on the pipette.
- Be careful not to contaminate any of the glass/plastic containers with detergent. Use only acid wash (HCl) and/or high purity (MQ) water for cleaning.
- It is preferred to use the same glass/plastic containers for the same solutions. If a container is contaminated, wash it with acid.
- Do not put the spatula inside the chemical containers when weighing chemicals for making the reagents. Never pour exessive chemicals back into the container but dispose according to safety protocols.
- Make sure that all working places, scales, sink and fume hood is clean and tidy at the end of the day. Don't leave traces of sediment and chemicals should be stored properly.
- When pipetting with a new or dry pippett tip, fill it up once and discard the solution. Up to 10% of the volume is lost as the pippett tip becomes "wet" with solution the first time it is used.
- Check the calibration of the pipettors monthly by pipetting water at 20 C (ca. room temperature) and weighing in a weigh boat.
- **You are responsible for your own safety, use gloves, safety glasses and lab coat accordingly. Be especially careful when handling $K_2S_2O_8$ and strong acids/bases.**

General routine

Days 1-3 describe the steps of the fractionation procedure. Basically what you do each day for three days

Appendix 1
Describes how to make the chemicals used in the fractionation procedure and for making reagents so the samples can be read on the spectrophotometer

Appendix 2
Describes how to analyze the samples on the spectrophotometer

Appendix 3
Describes how to analyze dry weight and loss on ignition

The description of days 1 to 3 and appendix 1 and 2 are all used during the fractionation procedure. Appendix three is used for dry weight and loss on ignition which can be done any time during the fractionation or before/after.

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Day 1

- Add 100 to 150 mg (up to 200 mg for 0-1cm layer)[1] of wet sediment to 15 ml centrifuge tubes. Mix sediment well before putting the subsample in the tube. If sample is very wet, a pipette can be used, otherwise use a regular spatula. Rinse the pipette between each sample, and change rinsing water between each sediment core or sampling site. After rinsing with water, pipette and discard two samples (one if sample is limited) so that any remaining water from rinsing is removed. Then pipette the third sample into the centrifuge tube.

1. MQ-step
- Add 10ml of double de-ionized (MQ) water to the centrifuge tubes, shake to make sure all the particles end up in solution and wait 2 hours.
- Add 5 ml of MQ to 3 vials to be used for blanks in the analysis
- Make the BD solution during the 2 hours (**appendix 1**)
- Centrifuge the tubes at 3000 rpm for 10 minutes and pipette 5 ml of the supernatant from each tube into scintillation vials.
- Take remaining **5 ml** of supernatant out carefully **without** disturbing the sediment, pour the solution out by tilting the centrifuge tubes **once**.
- Add 5 ml of MQ water to the scintillation vials with MQ extract to make 10 ml total.
-Cover the vials with parafilm and place in a cupboard.

2. BD-step
- Add 10 ml $Na_2SO_3/NaHCO_3$ (BD) to the centrifuge tubes, mix sediment with spatula, shake carefully (avoid forcing air into the solution) to make sure all the particles end up in solution and wait 1 hour.
- Add 2 ml of stock BD solution to 3 vials to be used as blanks in the analysis
- Make 0.1 M NaOH during the hour (**appendix 1**).
- Centrifuge the tubes at 3000 rpm for 10 minutes and pipette **2 ml** of the supernatant from each tube to scintillation vials.
- Take remaining 8 ml of supernatant out carefully **without** disturbing the sediment, pour the solution out by tilting the centrifuge tubes **once**.
- Let BD-samples in scintillation vials sit over night at 4 C in a refrigerator. Do not cover the BD-samples to allow the samples to oxidize, which happens over night.

3. NaOH-step
- Add 10 ml NaOH (0.1 M) to the centrifuge tubes, mix sediment with spatula, shake to make sure all the particles end up in solution and wait 15-18 (16 hours is typical) hours. Whichever time is chosen must be consistent for all samples from the same lake. The samples can be stored at room temperature with parafilm on top.

[1] The amount is depending on the watercontent and the detection limit of the standard curve

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Day 2

- Take out BD vials from the fridge and cover with parafilm. Store in a cupboard. If you have more than one tray, you can stack them and have parafilm on only the top/uppermost tray.
- Make $K_2S_2O_8$ (**appendix 1**)
- Make HCl solution (0.5 M) (**appendix 1**)
- Add 2 ml of NaOH (0.1 M) to 3 scintillation vials and 3 digestion tubes to be used as blanks in the analysis.
- Centrifuge tubes with NaOH for 10 minutes at 3000 rpm and follow table 1.

Table 1. Distribution order of chemicals. (All volumes should add up to 10 ml).

Reagent	**$NaOH_{rp}$ (scint. vials)**	**$NaOH_{nrp}$ (digestion tubes)**
Supernatant	1 ml	1 ml
HCl (0.5 M)	0.25 ml	0.25 ml
$K_2S_2O_8$	-	2 ml
MQ-water	8.75 ml	6.75 ml

- Place caps on digestion tubes and put in autoclave for 30 mins at 120 C (Liquid B waste program)
-If the samples are very yellow, take an additional 2 mL out of the tubes to use as color blinds (talk to Brian about this for help)
- Take remaining 6 ml of supernatant out carefully **without** disturbing the sediment, pour the solution out by tilting the centrifuge tubes **once**.
- Cover the NaOH-sample scintillation vials with parafilm and store at room temperature if not analysing them the same day.
-Remove digestion tubes with NaOH from the autoclave and let stand over night to cool.

4. HCl step
-Add 10 ml of HCl (0.5 M) to the centrifuge tubes, mix sediment with spatula, shake to make sure all the particles end up in solution, and wait 16-24 hours. The samples can be stored in room temperature.

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Day 3

- Make NaOH (**2 M**) if needed (**appendix 1**)
- Pour some P-standard solution into the beaker labelled “low P” to let the solution get to room temperature. This can be done by covering with parafilm and waiting (best solution), or heating carefully on a hot plate if you need to do it quickly.
- Centrifuge tubes with HCl at 3000 rpm for 10 minutes and pipette 2 ml of the supernatant to scintillation vials.
- Add 2 ml of HCl (0.5 M) to 3 scintiallation vials to be used as blanks in the analysis
- Add 0.5 ml NaOH (**2 M)** to all HCl sample scintillation vials, including blanks.
- Add 7.5 ml MQ to make 10 ml.

- Add 8 ml MQ water to the BD samples (from step 2) to make 10 ml.

- Analyze MQ-samples, BD-samples, $NaOH_{rp}$, $NaOH_{nrp}$ (from autoclave), and HCl-samples using the spectrophotometer according to “Measuring sediment P extractions on the spectrophotometer” (**appendix 2**) for methods.

-Clean out all vials and centrifuge tubes 3 rinses of de-ionized water and one rinse MQ water.

-Analyze dry weights and loss on ignition (**appendix 3**). This can also be started during days 1 and 2 when you have time.

Appendix 1 - Chemicals and solutions

For fractionation

BD-solution (Na_2SO_3/$NaHCO_3$)
NaOH (0.1 M)
NaOH (2 M)
$K_2S_2O_8$
HCl (0.5 M)

Preparation

BD-solution (500 ml, 40-45 samples)

Lasting quality: Make new for each fractionation
Storage: Room temperature
Ingredients:

- $Na_2S_2O_4$ (solid)
- $NaHCO_3$ (solid)
- MQ-water

Procedure:

- Take a 500 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Take a new weighing boat for 4.6 g of $NaHCO_3$
- Pour the $NaHCO_3$ into the 500 ml flask
- Weigh 8.7 g $Na_2S_2O_4$
- Pour the $Na_2S_2O_4$ into the same flask
- Fill the flask with MQ-water up to the line to make 500 ml
- Put a cap on the flask and turn it (don't shake) to make sure the chemicals are dissolved completely.
- The solution volume might be reduced, fill up with more MQ-water to the line again.

NaOH (0.1 M)

Ingredients:

- NaOH-concentrated (solid)
- MQ-water

Procedure:

- Take a 1000 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 4 g NaOH
- Pour the NaOH in the flask
- Fill the flask with MQ-water to the line

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- Put a cap on the flask and turn it to make sure the solution is mixed
- The solution volume might be reduced, fill up with more MQ-water to the line again.

NaOH (2 M)
Lasting quality: approx. 6 months
Storage: Room temperature
Procedure:

- Take a 250 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 20 g NaOH
- Pour the NaOH into the flask
- Fill the flask with MQ-water to the line
- Put a cap on the flask and turn it to make sure the solution is mixed.
- The solution volume might be reduced, fill up with more MQ-water to the line again.

$K_2S_2O_8$ (Potassium persulfate)
Lasting quality: 2 weeks
Storage: Room temperature
Ingredients:

- $K_2S_2O_8$ (solid)
- MQ-water

Procedure:

- Take a 250 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 12.5 g $K_2S_2O_8$
- Fill the flask with MQ-water to the line to make 250 ml
- Put a magnetic stir bar in the flask
- Put the flask on a heater and carefully heat the solution until all chemicals are dissolved
- Either pour the solution into a dark flask, or cover the flask with aluminum foil to keep it from light

HCl (0.5 M)
Lasting quality: approx. 6 months
Storage: Room temperature
Ingredients:

- HCl concentrated (liquid)

- MQ-water

Procedure:

- **Note**: **Always be careful when using concentrated acids, use protective gloves, lab coat and glasses**
- Mix the solution in a fume hood
- Use a 1 L flast
- Add MQ water (about 500 mL)
- Pour 40 ml HCl in a measuring cylinderPour the HCl from the measuring cylinder into the volumetric flask
- Then fill up with MQ-water in the 1 L flask to the line
- Put on a cap and turn the flask to make sure the solution is mixed
- Rinse the outside of the bottle with MQ-water, and wash the used measuring glass while still wearing gloves to make sure no acid gets in contact with skin

For spectrophotometer process

Ascorbic acid
Ammonium molybdate
KSb
H_2SO_4 (2.5 M)
Reagent-solution (the combination of the above 4 chemicals)
P-standard solution
Comment: The amount of these solutions depends on how many samples that will be analyzed

Ascorbic acid

Lasting quality: Make fresh for each analysis occasion
Storage: Room temperature
Ingredients:

- Ascorbic acid (solid)
- MQ-water

Procedure:

- Take a 100 ml volumetric flask (or 200 mL)
- Put a weighing boat on the scale and tare the scale
- Weigh 1.75 g ascorbic acid (3.5 g for 200 mL flask)
- Pour the ascorbic acid into the flask, rinse the weighing boat with MQ-water and pour the water in the flask to make sure all chemicals are transferred from the weighing boat to the flask
- Fill the flask with MQ-water to the line
- Put on a cap and turn the bottle, make sure the chemicals are solved
- Check volume, if reduced add more MQ water to the line

Ammonium molybdate

Lasting quality: Make fresh for each analysis occasion
Storage: Room temperature
Ingredients:

- Ammonium molybdate (solid)
- MQ-water

Procedure:

- Take a 50 ml volumetric flask (or 100 mL for more reagent)
- Put a weighing boat on the scale and tare the scale
- Weigh 2.0 g ammonium molybdate (or 4 g for 200 mL flask)
- Fill the flask with MQ-water to the line
- Put on a cap and turn the bottle, make sure the chemicals are solved
- Check volume, if reduced add more MQ water to the line

KSb (Sodium antimony)

Lasting quality: 1 month
Storage: Fridge, dark (sensitive to light)
Ingredients:

- KSb ($K(SbO)C_4H_4O_6$-$0.5H_2O$) (solid)
- MQ-water

Procedure:

- Take a 250 ml volumetric flask
- Put a weighing boat on the scale and tare the scale
- Weigh 0.6855 g KSb
- Pour the KSb into the flask, rinse the weigh boat with MQ-water and pour the water in the flask to make sure all chemicals are transferred from the weighing boat to the flask
- Fill the flask with MQ-water to the line to make 250 ml
- Put on a cap and turn the bottle, make sure the chemicals are solved
- Check volume, if reduced add more MQ water to the line
- Pour into and store in a dark, non-clear glass bottle.

H_2SO_4 (2.5 M)

Lasting quality: approx. 6 months
Storage: Room temperature
Ingredients:

- H_2SO_4 concentrated (liquid)
- MQ-water

Procedure:

- **Note**: **Always be careful when using concentrated acids, use protective gloves, lab coat and glasses. H_2SO_4 is corrosive and gets heated when in contact with water**
- Make a water bath to prepare the solution in
- Measure and pour 140 ml H_2SO_4 in a measuring cylinder
- Pour some MQ-water into the volumetric flask (ca. 500 mL)
- Pour the H_2SO_4 from the measuring cylinder into the volumetric flask slowly and let the acid react with the MQ-water without shaking (the flask will get heated). Place stopper in the flask
- Put a 1000 ml volumetric flask in the water bath
- When the flask is starting to cool, slowly turn flask upside down and then back up (with stopper in!) one time. Check temperature and wait till cool

again. Repeat until flask does not get hot (you can also see the acid reacting with the water).
- Carefully add more MQ-water into the volumetric flask up to the line and let the acid and MQ-water react. Repeat previous step.
- Rinse the outside of the bottle with MQ-water, and wash the used measuring glass while still wearing gloves to make sure no acid gets in contact with skin

Reagent solution

Lasting quality: 4 hours
Storage: Room temperature
Ingredients:

- H_2SO_4 (2.5 M)
- Ammonium molybdate
- KSb
- Ascorbic acid

Procedure:

- ***Note: The following amounts are examples depending on how many samples you are analyzing, see the sheet with different volumes based on number of samples to be measured***
- Use a 500 ml beaker and add the following, in order:
- Measure 125 ml H_2SO_4 in a measuring cylinder
- Measure 37.5 ml ammonium molybdate in a measuring cylinder
- Measure 12.5 ml KSb in a measuring cylinder
- Measure 75 ml ascorbic acid in a measuring cylinder
- Add the ingredients to the beaker in the same order they are measured: H_2SO_4, ammonium molybdate, KSb and last ascorbic acid.

P-standard solution (from liquid solution) (1 mg/L)

Lasting quality: Approx. 6 months
Storage: Fridge
Ingredients:

- Phosphate Standard solution (1000 mg/L PO_4)
- MQ-water

Procedure:

- Pour some of the stock standard solution (usually in a plastic beaker in the fridge) in a plastic beaker (marked high P in red) and leave it in room temperature for at least one hour, to get the solution to room temperature
- Use either a glass pipette or the regular pipette (with tip marked high P).
- Take 3 ml stock solution in the pipette and waste it

- Pipette 3 ml stock solution in a 1000 ml glass bottle marked LP
- Fill up the rest of the bottle to the line with MQ-water.
- This will in reality give a concentration of 0,978 mg/L, not 1 mg/L
- Make a standard curve and measure on the spectrophotometer to make sure the concentration is right

P-standard solution (from dry chemical) (1 mg PO_4-P/L):

Lasting quality: Approx. 6 months
Storage: Fridge
Ingredients:

- KH_2PO_4 (dried at 105 ^{0}C for 24 hours, allow to cool in a desiccator before using)
- MQ-water

Procedure:

- Weigh 0.4392 KH_2PO_4
- Dissolve it in MQ-water in a 1000 ml glass bottle
- Add 1 mL chloroform to prevent bacterial growth
- Fill up the rest of the bottle to the line with MQ-water.
- Make a standard curve and measure on the spectrophotometer to make sure the concentration is right (see below).

Appendix 2 - Measuring sediment P extractions on the spectrophotometer - Ascorbic acid- ammonium molybdate method

- Make the reagent using ascorbic acid, ammonium molybdate, KSb, H2SO4 (2.5 M) and MQ-water. See appendix 1 for how to make the reagent solutions.

- Turn on the spectrophotometer and set the wavelength to 880 nm. Blank the machine by putting MQ water in the cuvette, and placing the cuvette in the machine. Press the auto-zero button.

- Make 6 **standards** with following amounts (table below) of P-standard and then fill to 10 ml with MQ water. See **appendix** 1 for how to make and make the stock the P-standard solution.

Add ml of P standard	**MQ water**	**Concentration P (µg/L)**	Approximate absorbance value
0	10	0	0
0.5	9.5	50	30
1	9	100	60
2	8	200	120
4	6	400	240
8	2	800	480

-Note, the above concentrations are for 1 mg/L P standard. If the P standard in different, the concentrations above will be different. But still add the same amount of standard to each vile as shown above.
- Mix the reagent solution with the pipette tip and then add 2 ml of reagent to the standards **only** and wait 15 mins to make sure you get blue colour. The higher concentration of the standard, the darker blue. If no blue color, unexpectedly low blue color, or the color “runs away” (i.e. turns too blue), remake reagent solution and try again.
-Read the MQ water sample occasionally while you are waiting for the reagent to turn blue in the samples to make sure the machine is not drifting. If it does, zero it again.
- If the standards look ok after 15 mins (have turned blue) measure the standards and then rinse the cuvette carefully with MQ-water until the signal is around zero. If the standards look ok (see approximate absorbance in above table) and the values of the standard curve seem correct, add 2 ml of reagent to the first tray with samples and blanks. Shake the tray a little bit to help mix the samples and reagent, and wait at least 15 mins before starting to measure on the spec if the samples are at room temperature. If they are cold wait 30 mins.

- Start each sample series by measuring the three blanks in that series followed by the samples.
- Check the cuvette between samples to make sure there is no dirt/water on the cuvette.
- Measure your samples. If there is a large difference in blue colour between samples, put some sample in the cuvette to rinse it, then pour it out and put more in for measurement.
-Add reagent to each tray in the same pace that you read them, so each tray sits with reagent for at least 15 mins.

- Measure a standard (similar to your sample values) every 20 samples to make sure the machine isn't drifting.
- When done, pour out the samples and rinse the scintillation vials 2 times with deionized water
-Rinse the cuvette with MQ water at least three times, wipe with chemwipe to clean any smudges and store upside down (using an empty tray on top to keep the vials from falling out) to dry.

Appendix 3 - Dry weights and loss on ignition

In order to calculate P concentrations and P mass, quantification of dry weight and density of the sediment samples is needed.

Dry weight (DW) determination using freeze drier

Material: Freeze dryer, freezer, furnace, plastic scintillation vials with caps, spatula, scale (accuracy: 0.1µg)

1. Label all scintillation vials
2. Weigh the first empty scintillation vial without cap, record that value.
3. Homogenize the wet sample with a spatula and transfer at least 7-8g to the scintillation vial, record the new weight. If other analyses will be done (like metals, TOC, TN, etc.) make sure to find out how much dry sample is needed. Often you will need to add more wet sample if other analyses will be done.
4. Continue for all samples
5. Place caps on samples tightly
6. Freeze the samples in (-20°C or colder) for at least 24 hours.
7. Run the frozen samples in the freeze drier for 4 days, make sure the lids are loosely placed (unscrew cap ½ turn) to allow for vapor to escape container.
8. Transfer the freeze dried samples to a oven (60°C for 24h) to get rid of residual moisture.
9. Weigh the scintillation vial with dry sample (without cap) and note the value. Place the cap back on the vial tightly.

Calculations:

$$Water\ content = \frac{Mass(wet\ sample) - Mass(Dry\ sample)}{Mass(Wet\ sample)} \times 100$$

$$Dry\ weight\ (\%) = 100 - Water\ content$$

Loss on ignition (LOI) on freeze dried sediment

Material: Muffle oven, crucibles, spatula, scale (accuracy: 0.1µg)

1. Make sure crucibles are clean. If unsure, wipe with paper towels.
2. Place the empty crucible on the scale and note that value, also make sure the crucible is numbered and also record that to keep track of the sample.
3. Homogenize the freeze dried sample directly in the scintillation vials using a spatula to crush the any sediment clusters, wipe off spatula with paper towel between samples.
4. Pour approximately 1g of dry sample in the crucible and note the new value. Less can be used for samples with very little sediment (minimum 200 mg).

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Approved by:

5. Gently transfer all samples to the muffle oven and burn the sediment for at least 2h in 550°C. Move slowly when transporting, because the dry sediment can be blown out of the crucibles quite easily.
6. Let the samples cool down to room temperature in the oven and measure the weight of the crucibles with burnt sample. Crucibles can be placed in a desiccator if they are not weighed immediately.

Calculation:

$$LOI(\%\ of\ Dry\ weight) = \frac{Mass(Dry\ sample) - Mass(Ignited\ sample)}{Mass(Dry\ sample)} \times 100$$

Appendix 4 – Description of sequential phosphorus fractionation method

To assess phosphorus in sediment a sequential chemical extraction can be used, commonly called phosphorus (P) fractionation. Different pools of P, with varying solubility and reactivity, that are separated and examined. Typically the procedure begins with dilute extractants, first removing loosely bound/pore water P, and then proceeding stepwise towards stronger extractants that dissolved/release more strongly bound forms (Lukkari *et al*., 2007).

In the late 1950s Chang & Jackson (1957) proposed a sequential extraction technique for P fractionation on soil. The scheme was later adapted to sediment by Williams *et al.* (1967, 1976). In the following decades the procedure was altered in different ways. For example, it was adapted for analysis of calcareous sediment by Hieltes & Lijklema (1980), Golterman (1977, 1982) wanted to further separate the bioavailable forms of P and developed a method using chelating agents in the procedure, and Ruttenberg (1992) focused on marine sediment using the SEDEX method. Psenner & Pucsko (1985, 1988) developed their P fractionation scheme in which they divided the different P forms into loosely sorbed P, redox sensitive (Fe/Mn bound P), ligand exchangeable P (Al bound and organic P), Ca bound P, and refractory P.

Due to lack of a harmonized procedure for determining forms of P in sediment, the European Commission (through the Standards, Measurements and testing – SMT) developed the SMT-protocol. This extraction procedure has been evaluated by different authors, e.g. Ruban *et al.* (2000) and Pardo *et al.* 2013.

However, the scheme by Psenner & Pucsko (1988) has not been abandoned, but further used, evaluated and adapted by e.g. Jensen & Thamdrup (1993) for marine sediment, Hupfer *et al.* (1995, 2009), Paludan & Jensen for sediment rich in humic material, and Jan *et al.* (2012, 2015) who came up with a strategy to better quantify P easily affected by low redox conditions. The fractionation protocol by Psenner & Pucsko (1984, 1988), and adapted by Hupfer *et al.* (1995, 2009), are used in this method description (Table 1).

Table 1. The differences in methods by Psenner & Pucsko to Hupfer et al (1995, 2009).

Psenner & Pucsko, 1985	a. H_2O (water soluble P), 10 min, centrifuge 3000 laps/10 min b. BD 0.11 mol/l (reductant soluble P), 40°C water bath, 30 min, centrifuge, wash with BD solution again, centrifuge. c. NaOH 1 mol/l (Fe and Al bound P), room temperature, 16 h, centrifuge, wash with NaOH, centrifuge. d. HCl 0.5 mol/l (Ca bound P), room temperature, 16 h, centrifuge, wash with HCl, centrifuge. e. NaOH 1 mol/l (refractory P), 85°C water bath, 24 h, centrifuge, wash with

	NaOH, centrifuge.
Hupfer *et al.* 1995, Hupfer *et al.* 2009	All analyses are conducted at room temperature. a. Changed H_2O for deoxygenated NH_4Cl (1M), extraction time 30 min. b. Changed reaction time to 1 h. c. Added a step for determining NRP from the NaOH-step (persulfate and autoclave). d. No change. e. Last step emitted.
This document	a. MQ water, room temperature, 2 h. b. BD 0.11 M, pH 7.2, room temperature. 50 min. c. NaOH (1 M), room temperature, 16 h (one set of samples NRP, addition of persulfate and autoclaved) d. HCl (0.5 M), room temperature, 16 h e. Last step omitted.

References

Chang, S. C. and M. L. Jackson. (1957). Fractionation of soil phosphorus. *Soil Science*, 84, pp 133–144.

Golterman, H. L., 1977. Sediments as a source of phosphate for algal growth. In: Golterman, H. L. (Ed.) Interactions between Sediments and Freshwater, pp. 286-293. Junk, The Hague.

Golterman, H. L. (1982). Differential extraction of sediment phosphates with NTA solutions. *Hydrobiologia*, 91, pp 683–687.

Hieltjes, A. H. M. and L. Lijklema. (1980). Fractionation of inorganic phosphates in calcareous sediments. *Journal of Environmental Quality*, 9, pp 405–407.

Hupfer, M., Gächter, R. & Giovanoli, R. (1995). Transformation of phosphorus species in settling seston and during early sediment diagenesis. *Aquatic Sciences-Research Across Boundaries*, 57(4), pp 305–324.

Hupfer, M., Zak, D., Roβberg, R., Herzog, C. & Pöthig, R. (2009). Evaluation of a well-established sequential phosphorus fractionation technique for use in calcite-rich lake sediments: identification and prevention of artifacts due to apatite formation. *Limnology and Oceanography: Methods*, 7(6), pp 399–410.

Jan, J., Borovec, J., Kopáček, J. & Hejzlar, J. (2013). What do results of common sequential fractionation and single-step extractions tell us about P binding with Fe and Al compounds in non-calcareous sediments? *Water Research*, 47(2), pp 547–557.

Jan, J., Borovec, J., Kopáček, J. & Hejzlar, J. (2015). Assessment of phosphorus associated with Fe and Al (hydr)oxides in sediments and soils. *Journal of Soils and Sediments*, 15(7), pp 1620–1629.

Jensen, H. S. & Thamdrup, B. (1993). Iron-bound phosphorus in marine sediments as measured by bicarbonate-dithionite extraction., 1993. pp 47–59. Springer.

Lijklema, L. (1980). Interaction of orthophosphate with iron (III) and aluminum hydroxides. *Environmental Science & Technology*, 14(5), pp 537–541.

Lukkari, K., Hartikainen, H. & Leivuori, M. (2007). Fractionation of sediment phosphorus revisited. I: Fractionation steps and their biogeochemical basis. *Limnology and Oceanography: Methods*, 5(12), pp 433–444.

Paludan, C. and Jensen, H. (1995) Sequential extraction of phosphorus in freshwater wetland and lake sediment: Significance of humic acids, Wetlands, 15 (4), pp 365-373.

Pardo, P., López-Sánchez, J. F. & Rauret, G. (2003). Relationships between phosphorus fractionation and major components in sediments using the SMT harmonised extraction procedure. *Analytical and Bioanalytical Chemistry*, 376(2), pp 248–254.

Psenner, R., R. Pucsko, and M. Sager. (1984). Die fractionerung organisher und anorganisher Phosphorverbindungen von Sedimenten. *Archiv fur Hydrobiologie—Supplementband*, 70, pp 111–155.

Psenner, R. and R. Pucsko. (1988). Phosphorus fractionation: advantages and limits of the method for study of sediment P orgins and interactions. *Archiv fur Hydrobiologie Beiheft—Ergebnisse der Limnologie,* 30, pp 43–59.

Ruban, V., LópezSánchez, J. F., Pardo, P., Rauret, G., Muntau, H. & Quevauviller, P. (2001). Development of a harmonised phosphorus extraction procedure and certification of a sediment reference material. *Journal of Environmental Monitoring*, 3(1), pp 121–125.

Ruttenberg K. C. *(*1992*).* Development of a sequential extraction method for different forms of phosphorus in marine sediments*, Limnology and Oceanography, 37 (7), pp 1460-1482*

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and Global Dynamics. *National Center for Atmospheric Research, Boulder Colorado*, 80307, pp 31–66.

Williams, J. D. H., J. K. Syers a T. W. Walter, 1967. Fractionation of soil inorganicphosphate by a modification of Chang and Jackson procedure. Soil Sci. Sot. Amer. Proc. 3 1: 736-739.

Williams, J. D. H., Jaquet, J. M. & Thomas, R. L., 1976. Forms of phosphorus in the surficial sediments of Lake Erie. J. Fish.Res. Bd Can. 33: 413-429.

Appendix 5 - Adaptations of the method

Yellow samples in MQ-step

When encountering strong yellow colour in the supernatant (before reagent added) it might be useful to quantify this background signal.

-Take an extra sub sample batch of MQ supernatant (4 ml) and read the samples in the spectrophotometer (880nm) without adding any reagent. Subtract this absorbance value from the MQ-samples analysed according to the ascorbic acid – ammonium molybdate method.

Brown/yellow samples in NaOHrp-step

During the extraction with NaOH there is a possibility that precipitates and colouring of the samples may occur. Often this occurs when analysing sediment samples from humic lakes.

-Quantify the background signal (brown colour) according to the description in “Yellow samples in MQ-step”.

If precipitate is present it might disturb the light beam in the spectrophotometer and thus overestimate the absorbance.

-Take an extra sub sample batch of NaOH supernatant (2 ml), add the reagent and then centrifuge the samples for 10 minutes at 3000rpm. Further read the samples on the spectrophotometer at 880nm.

Appendix 6 - Quality assurance

Reference material

In order to monitor the quality of the complete procedure it is needed to use a “reference sediment”, this is treated as any other sample in the batch. The reference sediment is created by pooling residual freeze dried sample and homogenizing it thoroughly. All data from different runs of the reference sediment should be saved in a database to create the possibility to target any errors in the method handling.

Replicated samples

It is recommended to replicate samples to assess the measurement error for the method, especially when the method handles low P concentrations.

Revised January 6, 2015

STANDARD OPERATING GUIDELINES FOR FRESHWATER MACROINVERTEBRATE SORTING

1.0 INTRODUCTION

The following guidelines are to be used for freshwater macroinvertebrate sample sorting. The laboratory analysis procedures outlined below specify critical techniques and quality assurance and quality control procedures.

2.0 MATERIALS AND EQUIPMENT

- 70% ethanol (or other project-specific preservative) for storage
- Squirt bottles
- Sieve with screen size of 0.5 mm (500 µm), or as otherwise required by project
- Sieve tray
- Containers for retaining sample debris
- Specimen vials with caps or stoppers
- Two pairs of forceps – fine or ultrafine gauge
- Dissecting microscope or other magnification source
- Light source – gooseneck fiber optic lamp preferred
- Laboratory (petri) dishes
- Personal protective equipment: nitrile gloves and safety glasses or goggles
- Sample labels
- Pencil or archival pen
- Log-in sheet for samples
- Laboratory bench sheets
- Laptop (for electronic data entry)
- QA/QC logbook (optional)

3.0 GUIDELINES FOR MACROINVERTEBRATE SAMPLE SORTING

Summary of Requirements:

- Samples will be entered into a project log-in sheet upon arrival and inspection at the facility where sorting occurs.
- Samples will be rinsed through a sieve to remove preservatives and fine sediments.
- Organisms will be preserved and stored in appropriately labeled vials or jars.

Specific Requirements:

1. Consult the chain-of-custody to confirm that all samples have arrived. Each sample should be recorded on a sample log-in sheet. The log-in sheet should be reviewed and annotated, as necessary, to verify that all samples are in proper condition for processing.
2. Sample processing begins by rinsing the sample material in a 500-µm mesh (or other specified mesh size) sieve to remove preservative and fine sediments. A sieve tray should be placed under the sieve to capture all rinseate. Take care to ensure that direct flow of water does not impinge and damage organisms against the mesh screen. Large organic material (whole leaves, twigs. algal or macrophyte mats, etc.) not removed in the field may be carefully rinsed, visually inspected, and discarded once organisms have been removed and placed in the sieve.
3. After rinsing, the sample will be transferred incrementally into laboratory dishes and immersed in water for sorting.

4. The sample should be carefully sorted under a dissecting microscope or other magnifying device sufficient to pick out organisms 500 μm and larger. All organisms should be sorted from sample debris.
5. The sorted organisms should be placed into glass vials and preserved in 70% ethanol. The vials will be labeled inside and out with the sample identifier or lot number, date, sorter name and other information, as required by project. If more than one vial is needed, each will be labeled separately and numbered (e.g., 1 of 2, 2 of 2). Some projects will require sorting into multiple vials (by taxonomic group) while others do not. Confirm project-specific requirements with the project manager.
6. Sorted residue will be retained in a separate container and preserved in 70% ethanol. The container will be labeled inside and out as "sorted residue." The label should also include the sample identifier or lot number, date, sorter name and other information, as required by project.
7. The sorter will fill out the laboratory bench sheet or electronic data sheet, noting the number of organisms removed. The sorter will also record the date of sorting, if applicable, on a log-in sheet as documentation of progress and status of completion of the sample lot.
8. Any sample material that is released to the client or to an outside laboratory must be accompanied by a signed chain-of-custody form. Copies of all chains-of-custody should be retained on file, as needed.

4.0 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

The project Quality Assurance Officer will perform a random quality check on a minimum of 10% of the samples analyzed. The quality check will include re-sorting of the material designated as "sorted residue" and calculating sorting efficiency by dividing the number or organisms found in the quality check by the total number of organisms sorted. Check with the project manager for details on the required minimum sorting efficiency. A sorting efficiency of 95% is required for some projects. For example, samples sorted for the New Hampshire Department of Environmental Services are required to meet or exceed this threshold.

If sorting efficiency is less than the designated threshold, the Quality Assurance Officer should determine why the sorting efficiency did not meet the required threshold for the sample in question. Once the reason for the error has been identified, the Quality Assurance Officer should advise the sorter responsible for the error and provide guidance for corrective action. This may include assisting the sorter in recognizing missed organisms, re-sorting of additional samples or other remedial action, as required by the project or nature of the error.

Records of QA/QC checks performed on a particular sample should be indicated on the bench sheet or in a QA/QC logbook. These should include a description of the remedial action.

5.0 QUALIFICATIONS

In-house training with an experienced aquatic macroinvertebrate taxonomist is required for all staff responsible for sorting.

The Quality Assurance Officer must be familiar with the protocols stated in this SOG and with freshwater macroinvertebrate taxonomy.

All staff should be familiar with project-specific guidance documents. For freshwater macroinvertebrate projects with the New Hampshire Department of Environmental Services, refer to Davies and Tsomides (2002), unless otherwise directed.

6.0 REFERENCES

Davies, S. P. and L. Tsomides. 2002. Methods for Biological Sampling and Analysis of Maine's Rivers and Streams. Maine Department of Environmental Protection, Bureau of Land and Water Quality, Division of Environmental Assessment. DEP LW0387-B2002.

ESS GROUP, INC.

STANDARD OPERATING GUIDELINES

FOR FRESHWATER MACROINVERTEBRATE IDENTIFICATION

1.0 INTRODUCTION

The following Standard Operating Guidelines (SOGs) are used by ESS Group, Inc. (ESS) for identification of freshwater macroinvertebrate samples. The laboratory analysis procedures outlined below are specific with respect to critical techniques and quality assurance and quality control procedures. Procedures for macroinvertebrate sample collection and processing (sorting) are available under a separate set of SOGs.

2.0 REQUIRED MATERIALS

The following materials are necessary or useful for this procedure:

Equipment

- Chain-of-custody sheets
- Sample log-in sheets
- Laboratory bench sheets
- Laboratory analysis log
- Denatured 70% - 80% ethanol for storage of specimens
- Forceps – very fine or superfine gauge
- Shell vials or specimen vials with screw-caps
- Cotton (if shell vials used)
- Glass jars or Nalgene bottles with caps (if shell vials used)
- Sample labels (internal and external)
- Archival ink pen for labeling
- Standard laboratory bench sheets for identification
- Dissecting microscope for organism identification
- Compound microscope for slide-mounted organism identification
- Fiber optics light source for dissecting microscope
- Petri dishes
- Glass slides
- Glass cover slips (size and grade may vary depending on specimen size and type)
- Slide case
- CMC- 9/10 mounting medium

3.0 TAXONOMY

- Taxonomic determinations (to the genus/species level or the lowest practical taxonomic level based on organism condition or taxonomic key availability) and counts for all organisms within each sample will be determined through the use of a dissecting microscope (up to 45X magnification), a fiber optic lamp, standard dissecting tools, and appropriate taxonomic keys. Depending on the level of identification desired, non-biting midges (Diptera: Chironomidae) and aquatic worms (Oligochaeta) may need to be mounted on slides and identified using a compound microscope with magnification up to 1,000X.
- For archiving purposes, samples (grouped by station and date) will be preserved with denatured 70%-80% ethanol and placed in tightly capped and labeled vials. The inside of each vial will be labeled with the site identifier (code and full site name), date collected, the taxonomic determination of the specimens in the vial (if appropriate), the first initial and last name of the taxonomist and any other information, as may be required by specific project. The outside of each vial (or jar, if shell vials are used) will be labeled with the sample station code, date collected and "75% ethanol" as an indication of preservative.
- Aquatic worms (Oligochaeta) and non-biting midge (Chironomidae) larvae and pupae may be mounted on slides using an appropriate medium (e.g., Euporal, CMC-9, CMC-10) in accordance with the methods outlined by Epler (2001). Slides will be labeled with the site identifier (code and full site name), date collected, taxonomic group (e.g. Oligochaeta or Chironomidae), the first initial and last name of the taxonomist and any other information, as required by project. Slides may be archived in a slide case, which should be labeled on the outside with the project name, date and total number of slides. Oligochaetes that are too large to be mounted (e.g. certain Lumbriculidae) will be preserved in denatured 70% ethanol and stored in appropriately labeled vials.Each taxon found in a sample will be recorded and enumerated on a laboratory bench sheet for subsequent data entry and reporting. Any difficulties encountered during identification (e.g. missing organism parts, degraded condition, etc.) that result in a taxonomic determination coarser than the target level will be noted on these sheets.
- Each taxon found in a sample will be recorded and enumerated on a laboratory bench sheet for subsequent data entry and reporting. Any difficulties encountered during identification (e.g. missing organism parts, degraded condition, etc.) that result in a taxonomic determination coarser than the target level will be noted on these sheets.

4.0 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Following the initial identification by an aquatic macroinvertebrate taxonomist, a second staff member trained in aquatic macroinvertebrate identification will act as the QC officer, randomly checking a minimum of 10% of the samples (or at least one sample, if there are fewer than ten samples). The purpose of this check will be to validate the identifications made on the individuals comprising the sample, as well as to examine for errors in sample labeling and transcription of names and enumerations onto laboratory bench sheets. The results of the random QC checks will be recorded on the respective laboratory bench sheets, dated and initialed by the QC officer. If systematic errors in identification, labeling or transcription are noted, the QC officer will bring specific problems to the attention of the original taxonomist for correction throughout the dataset.

A reference collection of all taxa identified during each project may be maintained if required by the project. These specimens will be labeled and preserved in 70% - 80% ethanol (or slide mounted, where appropriate) and stored for future reference.

Taxonomic determinations may be confirmed with other regional experts, if deemed necessary. Specimens submitted during any QA checks must be accompanied by a chain-of-custody form. Following a QA check, labels on externally validated samples will be updated to reflect the name of the taxonomist performing the confirmatory identification as well as the date of validation.

Once the taxonomy is complete, data may be entered into the project database by project personnel. The QC officer will check the data at a minimum of 5% of the project sites and review any calculations in order to ensure a satisfactory level of accuracy in data entry. Any systematic errors in data entry or calculation of metrics will be brought to the attention of the project data entry personnel for correction throughout the dataset.

Records of the results of QA/QC checks described above will be maintained in a laboratory analysis log for each project.

5.0 QUALIFICATIONS

To properly conduct the taxonomic identification of aquatic macroinvertebrates, the taxonomist and QC officer must be familiar with the protocols stated in this SOG, have confidence in the appropriate use of aquatic macroinvertebrate keys and be familiar with the organisms of the area in question.

Staff responsible for slide mounting of Chironomidae and Oligochaeta must be familiar with the protocols stated in this SOG and be proficient in the methods outlined by Epler (2001).

In-house training with an experienced aquatic macroinvertebrate taxonomist is required for all staff responsible for entering taxonomic data into a project database. The staff member responsible for data entry must be familiar with the structure of the database and nature of the calculated metrics in order to ensure accuracy of the data and any associated calculations.

6.0 REFERENCES

Epler, J.H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. Special Publication SJ2001-SP13. North Carolina Department of Environment and Natural Resources, Raleigh, NC and St. Johns River Water Management District, Palatka FL. 526 pp.

Microcystins/Nodularins Analysis by Adda ELISA Standard Operating Procedure

Print ID#	**SOP50**	Title:	**Microcystins/Nodularins Analysis by Adda ELISA**	
SOP#	**50.9**	Version:	**10**	
Effective Date	**2/20/2010**	Revision Date	**3/18/2021**	
Prepared by	**Amanda Foss**	Date Prepared	**2/15/2010**	
Approved by	**Mark Aubel**	Date Approved	**3/24/2021**	**Page 1 of 25**

1. Purpose

The purpose of the "Microcystins/Nodularins analysis by Adda ELISA SOP" is to describe the method and requirements for the analysis of Microcystins/Nodularins using the Adda ELISA at GreenWater Laboratories.

2. Scope

The "Microcystins/Nodularins analysis by Adda ELISA SOP" applies to water samples and extracted material submitted to GreenWater Laboratories for analysis of total Microcystins/Nodularins by ELISA.

3. Summary of Method

The "Microcystins/Nodularins analysis by Adda ELISA SOP" is used to screen samples (water and extracted material) for the presence of microcystins and nodularins. The assay is sensitive to a wide array of microcystin/nodularin (MC/NOD) congeners with varying cross-reactivity. The reporting limit for MCs/NODs using this assay is 0.3 ng/mL (ppb) for general reporting and Method 546 samples, with project specific method detection limits described. Samples are analyzed, in duplicate wells, with an Abraxis Adda microcystin/nodularin Enzyme Linked Immunosorbent Assay (ELISA). A calibration curve is prepared using a certified reference standard of MC-LR or the provided kit standards. The curve non-linear R^2 value, lab fortified matrix (LFSM), lab fortified blank (LFB), blank and other QC data are utilized to validate assay results. Samples are diluted, as required, to remain in range of the standard curve.

4. Definitions

a. ELISA = enzyme linked immunosorbent assay

b. MC-LR = microcystin-LR

c. REAGENT WATER – Per Method 546 = 100 mg/L Sodium Thiosulfate in DI water. Purified water that does not contain any measurable quantity of microcystins, nodularins, or interfering compounds at or above one-half of the MRL.

d. PRIMARY DILUTION STANDARD (PDS) - A solution of MC-LR in methanol prepared from the MC-LR Stock Standard Solution. The PDS solutions are used to fortify QC samples (LFB, LFSM, and LFSMD).

e. QUALITY CONTROL SAMPLE (QCS) - A solution containing MC-LR at a known concentration that is obtained from a source different from the source of calibration standards at or near the EC50 (ca 0.75-1.00 ng/mL). The purpose of the QCS is to verify the accuracy of the primary calibration standards.

f. STOCK STANDARD SOLUTION - A concentrated standard in methanol that is prepared in the laboratory from purified MC-LR or that is purchased from a commercial source with a certificate of analysis

g. LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water to which a known quantity of MC-LR is added (1 ng/mL – ppb). The LFB is lysed and filtered to match the analytical procedure for field samples. The LFB is used during the IDC to verify method performance for precision and accuracy. The LFB is also a required QC element with each Analysis Batch. The results of the LFB verify method performance in the absence of sample matrix.

h. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – An aliquot of a field sample to which a known quantity (1ppb) of MC-LR is added. The purpose of the LFSM is to determine whether the sample matrix contributes bias to the analytical results.

i. LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) - A second aliquot of the field sample used to prepare the LFSM that is fortified and assayed in the same Analysis Batch as the LFSM. The LFSMD is used to verify method precision in both ambient water and drinking water matrixes.

j. LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water that is lysed and filtered to match the sample processing procedure. The LRB is used to determine if microcystins or other interferences are introduced from the sample containers, sample processing equipment, or the reagents used in the assay.

k. LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) - The single-laboratory LCMRL is the lowest spiking concentration such that the probability of spike recovery in the 50 to 150% range is at least 99% (U.S. EPA, 2004 and U.S. EPA, 2010).

l. LOW-RANGE CALIBRATION VERIFICATION (Low-CV or Low CCC) - The Low-CV is a calibration standard with a concentration equal to, or less than, the MRL. The purpose of the Low-CV is to confirm the accuracy of the calibration at concentrations near the MRL. 3

m. MINIMUM REPORTING LEVEL (MRL) - The minimum concentration that can be reported by a laboratory as a quantified value for total microcystins and nodularins in a sample following analysis

5. Interferences

a. General Considerations

i. The accuracy of the ELISA procedure is dependent upon analyst technique, the accuracy of pipetted volumes, and consistent incubation periods across the wells of each plate.

b. Assay Drift

i. Assay drift refers to systematic rather than random imprecision in measured analyte concentrations, the magnitude of which depends on the sample position within the plate. A possible cause for assay drift is slight differences in incubation times as reagents are added sequentially across the plate. Identical control samples distributed throughout the plate are necessary to detect assay drift. This method includes QC measures to evaluate assay drift. During the IDC, laboratories must assay five LRBs distributed across the plate and each Analysis Batch must include two LRBs placed on opposite sides of 5 the plate. Because the LRBs give absorbance values near the upper plateau of the calibration curve, the calculated LRB concentrations are sensitive to slight changes in the measured absorbance. If the distributed LRBs pass the QC limit of one-half the MRL, then plate drift is minimal and under control.

c. Sample Matrix Effects in Ambient & Finished Water

i. Salts and other matrix co-contaminants frequently cause an increase in non-specific binding, resulting in >130% LFSM recoveries. This can be mitigated using solid phase extraction or dilution. Confer with the Quality Manager to develop an approach to address samples with high LFSM returns and/or salt content

d. Cross Contamination

i. This method covers the analysis of samples collected from both ambient water and drinking water. To avoid cross contamination, use plastic, disposable syringes and do not reuse vials/materials used in sample preparation without written approval from the quality manager.

e. Interpretation of Results

i. The results reported by this method represent total microcystins and nodularins based on Adda ELISA calibrated with MC-LR. Nodularin and other variants (e.g. MC-LA) are known to react >100% and are over-estimated with this method, and to react < 0.2% to ADMAdda and DMAdda containing MCs/NODs.

6. References

a. Vendor Documentation for ELISA procedure (http://www.abraxiskits.com/moreinfo/PN520011USER1.pdf)

b. Foss and Aubel. 2015. Using the MMPB technique to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS). Toxicon. doi:10.1016/j.toxicon.2015.07.332.

c. Ohio EPA Total (Extracellular and Intracellular) Microcystins - ADDA by ELISA Analytical Methodology. Version 2.0 - January 2015http://www.epa.state.oh.us/Portals/28/documents/habs/HAB_Analytical_Methodology.pdf

d. US EPA Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay

e. GreenWater Laboratory Quality Manual

f. National Wetland Condition Assessment 2021. Laboratory Operations Manual. Version 1.0, March 2021

7. Associated SOPS

a. GreenWater Laboratory General Safety SOP#1

b. Sample Receipt, Log, Handling and Disposal SOP#2

c. Confidentiality SOP#4

d. Cellular Lysis and Preparation of Water Samples SOP #30

e. Microcystin, Cylindrospermopsin and Anatoxin-a extraction of algal material intended for human consumption (SOP #10)

f. Microcystin, Cylindrospermopsin and Anatoxin-a extraction of lyophilized material (SOP #11)

g. Microcystin, Cylindrospermopsin and Anatoxin-a extraction of tissues (SOP #12)

8. Responsibilities

a. The assay is to be performed by a trained analyst

b. A second qualified analyst will verify results

c. Final approval of laboratory results is the responsibility of the QA manager and/or Lab director

9. Materials and Equipment

a. US EPA National Wetland Condition Assessment 2021 specific equipment (not covered below)
   i. Glass vials (4 mL and 20 mL)
   ii. Norm-ject syringes (or equivalent)
   iii. Whatman Glass Fiber Syringe Filters (25 mm, 0.45 micron; Product #6894-2504 or equivalent)
   iv. Microcystins-ADDA Seawater Sample Clean-Up Kit (PN 529912)

b. Method 546 Sample Containers
   i. Amber glass bottles fitted with polytetrafluoroethylene (PTFE)-lined screw caps.
   ii. 15-40 mL Vials Borosilicate glass, clear or amber, with PTFE-lined closures. Vials with capacity in this range are recommended for the lysing procedure.
   iii. 4 mL Borosilicate glass, with PTFE-lined closures. Four milliliter vials are recommended for receiving and storing sample filtrate after lysing.

c. Sodium thiosulfate (CASRN 7772-98-7) to reduce residual chlorine in drinking water samples at the time of collection

d. Hach 2745050 Free & Total Chlorine Test Strips

e. Glass Luer-lock syringes (3 mL) or Plastic (Catalog Number S7515-3 or 03-377-27)
   i. 0.45 µm PVDF syringe filters
   ii. GFF for Method 546 Samples
      1. 25 mm glass fiber, 1.2 µm pore size, polypropylene housing, Environmental Express (Charleston, South Carolina) Catalog Number SF012G

f. National research Council of Canada certified reference material of MC-LR at 100ppb (µg/L) (NRC CRM-MCLR)

g. Secondary source of MC-LR (100ppb) for lab fortified matrix spike (LFSMs) and fortified blanks (LFBs)

h. Deionized water (18 MΩ-cm) or HPLC Grade water

i. 1.5 mL HPLC sample vials

j. 0.01 M Phosphate buffer solution at pH 7

k. Calibrated pipettes & tips
   i. Multichannel pipette (25-200 µL) & tips
   ii. 1-10 µL pipette & tips

iii. 50-200 µL pipette & tips
iv. 100-1000 µL pipette & tips

l. Reagent reservoirs
m. Parafilm
n. SpectraMax 340 PC
    i. Operating with Softmax Pro Software and Windows based computer
o. Abraxis Microcystins/Nodularin Adda ELISA kit (PN 52001)
p. Titer plate shaker
q. Timer
r. Lab safety protection
    i. Gloves
    ii. Eye protection

10. Sample Preservation, Shipment, Sample Collection, Receipt, and Storage

a. Sample Collection vials sent to clients for Drinking Water (Method 546 and other clients collecting from chlorinated water sources)
    i. Add sodium thiosulfate to each sample bottle for a final concentration of 100 mg/L and cap the vial
        1. 40 mL vial = 4 mg thiosulfate
    ii. Prohibition Regarding Ascorbic Acid
        1. Do not use ascorbic acid to reduce chlorine in drinking water samples. During studies to evaluate analyte stability during transport and storage, the authors discovered that microcystins degrade in the presence of ascorbic acid.
b. Sampling Instructions for client:
    i. Tap/Chlorinated Water
        1. Open the tap and allow the system to flush for approximately 5 minutes.
        2. Fill each bottle, taking care not to flush out the sodium thiosulfate, and invert several times to mix the sample with the reducing agent.
    ii. Ambient Water
        1. Method 546

a. The addition of sodium thiosulfate is not required for ambient water samples, but may be added if the laboratory chooses to prepare only one type of sample container.

2. Intake collections
   a. If the client is employing permanganate treatment at intakes to prevent mussel growth, use the same bottles and quenching agent as for drinking water.
3. General Instructions (Ambient water, blooms, etc.)
   a. 250 mL plastic is preferred, with a pre-rinse of sample bottle with site water (3x) prior to final collection recommended (to address potential MC adsorption concerns). See sampling Instructions Doc# Samp200511 for more information.

c. Sample Shipment:
   i. Method 546:
      1. Samples must be chilled during shipment and must not exceed 10 °C during the first 48 hours after collection.
   ii. US EPA National Wetland Condition Assessment 2021
      1. Samples will be frozen (first freeze) at the batching facility and shipped to GreenWater Frozen
   iii. General
      1. Overnight shipping on blue to ice to the lab. Refer to Sampling and Shipping Instructions Doc# Samp200511.

d. Sample Receipt Specifics by Method (Refer to Sample Receipt, Log, Handling and Disposal SOP#2 for general instructions)
   i. Method 546
      1. Temperature Samples must be confirmed to be ≤10 °C upon receipt
      2. A temperature of greater than 10° C is acceptable if transit time is short and the samples do not have sufficient time to chill. In this case, examine the ice packs in the cooler. If they remain frozen, the samples are valid.
      3. Residual Chlorine For drinking water
      4. Analyze one “Finished” sample from each cooler using the Hach Free & Total Chlorine Test strips using instructions on the box
      5. The total chlorine concentration should be less than 10 mg/L

a. Notate the Chlorine Level (pass/fail) on the Chain of Custody to be reported to the client
b. If the chlorine level is >10 mg/L, test the remainder of the samples
c. Notify the Lab Manager client that the sample failed the Chlorine test so the Lab Manager can notify the client

6. Considerations for Frozen Storage
   a. Use clear, or amber, borosilicate glass bottles with PTFE-lined septa.
   b. Select bottle capacity and sample volume to prevent breakage of bottles during freezing.
   c. Plan ahead to retain enough volume for preparing QC samples

ii. US EPA National Wetland Condition Assessment 2021

1. Report receipt of samples in the NARS IM sample tracking system (within 24 clock hours).
   a. Inspect each sample THE SAME DAY THEY ARE RECEIVED:
   b. Verify that the sample IDs in the shipment match those recorded on the:
   c. Chain of custody forms when the batching laboratory sends the samples to the microcystins laboratory; or
   d. Sample tracking form if the field crew sends the shipment directly to the State laboratory.
   e. Record the information in Table 7-1 into NARS IM, including the Condition Code for each sample:
      i. OK: Sample is in good condition
      ii. C: Sample container was cracked
      iii. L: Sample container is leaking
      iv. ML: Sample label is missing
      v. NF: Sample not frozen
2. If any sample is damaged or missing, contact the EPA HQ Laboratory Review Coordinator to discuss whether the sample can be analyzed. (See contact information in Table 2-1).
3. Store samples in the freezer until sample preparation begins.

4. Maintain the chain of custody or sample tracking forms with the samples.

e. Sample Holding Time
   i. Method 546 Samples
      1. Freeze samples upon arrival
      2. Samples must be analyzed within 14 days of collection
   ii. Water samples
      1. Analyze within 1 week (7 days) of collection
      2. Holding time increased to 6 months if frozen
   iii. Extracts
      1. To be analyzed within 1 week (7 days) from extraction
   iv. US EPA National Wetland Condition Assessment 2021
      1. Sample holding time (frozen) 90 days

11. Sample Prep and Lysing Procedure

a. **Method 546** (Follow procedure for cell lysing (freeze thaw) in SOP#30)
   i. Samples for QC to be prepped prior to cell lysis
      1. LFSM & LFSMD
         a. Fortify at least one paired LFSM with LFSMD, and 2 or more for >20 samples at (1 ng/mL – ppb)
            i. Add 10 µL MC-LR from 100 ppb PDS MC-LR to 990 µL reagent water
            ii. Freeze Thaw 3x (SOP#30)
      2. LFB
         a. Prepare a minimum of 2 separate Lab fortified blanks near the EC50 (1 ng/mL – ppb)
            i. Add 10 µL MC-LR from 100 ppb PDS MC-LR to 990 µL reagent water
            ii. Freeze Thaw 3x (SOP#30)
      3. QCS
         a. Assay 1 QCS for each new lot of calibration standards. Prepare the QCS near the EC50 with MC-LR from a source independent of the calibration standards (1 ng/mL – ppb)

i. If LFBs & LFSMs are prepared using GWL standard, use NRC standard for the QCS, or vice versa)

ii. Add 10 µL MC-LR from 100 ppb PDS MC-LR to 990 µL reagent water

iii. Freeze Thaw 3x (SOP#30)

4. LRB

a. Prepare at least one (1mL) LRB using freeze/thaw of reagent water (sodium thiosulfate 100 mg/L).

ii. Filter 1-2 mL of lysed sample into a 10x13 mm glass vial for analysis using a glass or plastic syringe and 1.2 µm GFF filter

1. Rinse glass syringe thoroughly with deionized water between samples
2. A pre-rinse of the syringe using sample water is also recommended, for both glass and plastic barrels

b. US EPA National Wetland Condition Assessment 2021

i. First freeze-thaw and filter procedure

1. Thaw received samples (in 125 mL bottle) to approximately 25 °C and verify there is no ice.
2. Shake well and transfer 10 mL labeled new 20 mL glass vial
   a. Archive remaining sample in 125 mL bottle in storage freezer
3. Freeze the 20 mL vial, thaw to 25 °C , refreeze and rethaw
4. Norm-ject syringe filter (25mm, GF 0.45 µm filter) the vial contents into a new, labeled 20 mL glass scintillation vial.
   a. One new syringe and filter should be used per sample.

ii. Additional Sample Preparation for Samples with Salinity>3.5 parts per thousand, which is equivalent to 6500 µS per EPA 3/23/2021

1. Place a small amount of glass wool into the top of a 5 ¾" glass Pasteur pipette. Using a 9" glass Pasteur pipette, push the glass wool into to the bottom of the 5 ¾" pipette to form the base of the column. The depth of the glass wool should be approximately 5 mm. Place the column into a 12x75 mm test tube.
2. Each column will require approximately 1.5 g of Seawater Sample Clean-Up Resin. Calculate and add the appropriate amount of Microcystins-ADDA Seawater Sample Clean-Up Resin to a 20 mL glass vial.

3. Add distilled or deionized water at an approximately 2:1 ratio to the Microcystins- ADDA Seawater Sample Clean-Up Resin (for example, 10 mL of deionized or distilled water per 5 g of Resin). Shake or vortex.
4. Pipette the Resin in water solution into the column using the 9" Pasteur pipette. Avoid the formation of air bubbles in the column bed by keeping the tip of the pipette at the surface of the bed being created. Fill the column to the indentation approximately 2 cm from the top of the pipette. This will create an approximately 8 cm column.
5. Allow the deionized or distilled water to drain from the column.4 1 Lift the tip of the column at least 1 cm above the surface of the water in the tube. Place the pipette bulb against theof the column. Avoid allowing the tip of the column to come into contact with the water in the tube to prevent aspiration of water back into the column.
6. Place the column into an appropriately labeled 4 mL glass vial.
7. Add 1 mL of the sample to a clean, appropriately labeled 4 mL glass vial. Add 50 µL of Microcystins-ADDA Seawater Sample Treatment Solution. Vortex.
8. Add 375 µL of the treated sample to the top of the column. Allow the sample to drain through the column and collect in the vial.
9. Add a second 375 µL aliquot of the treated sample to the column. Allow to drain through the column.
10. Lift the tip of the column at least 1 cm above the surface of the sample in the vial. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining sample out of the column. Avoid allowing the tip of the column to come into contact with the sample in the vial to prevent aspiration of the sample back into the column.
11. Lower the column back into the vial. Add 500 µL of distilled or deionized water to the top of the column. Allow the rinse to drain through the column and collect with the sample.
12. Lift the tip of the column at least 1 cm above the surface of the sample/rinse in the vial. Place the pipette bulb against the top of the column (do not attach the bulb to the column) and push the remaining rinse out of the column. Avoid allowing the tip of the column to come into contact with the sample in the vial to prevent aspiration of the sample back into the column.
13. Remove the column and discard (columns are single use only). Cap vial and vortex. The sample can then be analyzed using the Eurofins

Technologies (formerly Abraxis) Microcystins-ADDA ELISA Kit beginning with the next section (7.6.3).

12. Initial Demonstration of Capability (Method 546)

a. Precision & Accuracy Demonstration (9.1.1 Method 546)

i. Prepare 7 replicates at 0.5 ppb (ng/mL) in reagent water (sodium thiosulfate at 100 mg/L)

ii. Analyze the 7 replicates against the kit provided curve including

1. A Low-CV (0.15 ppb from kit)
2. Prepared LRB(s)
3. Calculate %RSD (must be ≤15%)

$$\%RSD = \frac{Standard\ Deviation\ of\ Measured\ Concentrations}{Mean\ Concentration} \times 100$$

4. Calculate the mean recovery (70-130%)

$$Average\ \%\ Recovery = \frac{Average\ Measured\ Concentration}{Fortified\ Concentration} \times 100$$

b. Minimum Reporting Level (MRL) Confirmation (9.1.3 Method 546)

i. Fortify seven replicate LFBs with MC-LR at 0.15 ng/mL (ppb) in reagent water (sodium thiosulfate 100mg/L)

1. Freeze-Thaw and filter the aliquots as in SOP #30 for Method 546 samples and filtered (syringe) using 1.2 µm GFF
2. The analysis batch must include two LRBs and Low CV
3. Calculate the MRL

a. Determine the Half Range for the Prediction Interval of Results (HRPIR) using the following equation:

$$HR_{PIR} = 3.963s$$

Where s = standard deviation

b. Calculate the Upper and Lower Limits for the Prediction Interval of Results (PIR = Mean ± HRPIR) as shown below:

$$Upper\ PIR\ Limit = \frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100\%$$

$$Lower\ PIR\ Limit = \frac{Mean - HR_{PIR}}{Fortified\ Concentration} \times 100\%$$

c. The MRL is confirmed if the Upper PIR Limit is less than, or equal to, 150% and the Lower PIR Limit is greater than, or equal to, 50%. If these criteria are not met, the MRL may be set too low and the confirmation should either be repeated, or the MRL established and confirmed at a higher concentration.

c. Quality Control Sample (QCS)

i. Verify an external standard (NRC-certified reference standard of MC-LR) spiked near EC50 (1 ppb) is recovered 70-130%

Figure 1: Example Plates for set-up by Method (e.g. Method 546)

GreenWater and Method 546 General Plate Set-up Example (minimum QC)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ppb	1.0 ppb	BLANK	Sample 1 LFSM	Sample 5	Extract 1 LFSM	Sample 7 10x DF	Sample 9	Sample 12	Sample 15 LFSM	Sample 19	BLANK
B	0 ppb	1.0 ppb	BLANK	Sample 1 LFSM	Sample 5	Extract 1 LFSM	Sample 7 10x DF	Sample 9	Sample 12	Sample 15 LFSM	Sample 19	BLANK
C	0.15 ppb	2.5 ppb	Low CCC (0.15 ppb)	Sample 2	Sample 5 LFSM	Extract 1 LFSMD	Sample 7 100x DF	Sample 9 LFSM	Sample 13	Sample 16	Sample 20	Low CCC (0.15 ppb)
D	0.15 ppb	2.5 ppb	Low CCC (0.15 ppb)	Sample 2	Sample 5 LFSM	Extract 1 LFSMD	Sample 7 100x DF	Sample 9 LFSM	Sample 13	Sample 16	Sample 20	Low CCC (0.15 ppb)
E	0.25 ppb	4.0 ppb	LFB (1ppb)	Sample 3	Sample 6	Extract 2	Sample 7 1000x DF	Sample 10	Sample 14	Sample 17	Sample 20 LFSM	LFB (1ppb)
F	0.25 ppb	4.0 ppb	LFB (1ppb)	Sample 3	Sample 6	Extract 2	Sample 7 1000x DF	Sample 10	Sample 14	Sample 17	Sample 20 LFSM	LFB (1ppb)
G	0.5 ppb	5.0 ppb	Sample 1	Sample 4	Extract 1	Extarct 2 LD	Sample 8	Sample 11	Sample 15	Sample 18	Sample 21	Sample 21
H	0.5 ppb	5.0 ppb	Sample 1	Sample 4	Extract 1	Extract 2 LD	Sample 8	Smple 11	Sample 15	Sample 18	Sample 21	Sample 21

Standard Curve and Low CCC is certified reference standard of MC-LR
LFB and LFSMs are an external source of reference material
Blank is deionized water (or diluent used to dilute samples); LRB is reagent water
At least one LFSM per client and per 10 samples.

Method 546 General Plate Set-up

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ppb	1.0 ppb	BLANK	Sample 1 LD	Sample 3	Sample 7	Sample 10 LFSM	Sample 13	Sample 17	Sample 21	Sample 25	LRB (Blank)
B	0 ppb	1.0 ppb	BLANK	Sample 1 LD	Sample 3	Sample 7	Sample 10 LFSM	Sample 13	Sample 17	Sample 21	Sample 25	LRB (Blank)
C	0.15 ppb	2.5 ppb	Low CCC (0.15 ppb)	Sample 2	Sample 4	Sample 8	Sample 10 LFSMD	Sample 14	Sample 18	Sample 22	Sample 26	Low CCC (0.15 ppb)
D	0.15 ppb	2.5 ppb	Low CCC (0.15 ppb)	Sample 2	Sample 4	Sample 8	Sample 10 LFSMD	Sample 14	Sample 18	Sample 22	Sample 26	Low CCC (0.15 ppb)
E	0.25 ppb	4.0 ppb	LFB (1ppb)	Sample 2 LFSM	Sample 5	Sample 9	Sample 11	Sample 15	Sample 19	Sample 23	Sample 27	LFB (1ppb)
F	0.25 ppb	4.0 ppb	LFB (1ppb)	Sample 2 LFSM	Sample 5	Sample 9	Smple 11	Sample 15	Sample 19	Sample 23	Sample 27	LFB (1ppb)
G	0.5 ppb	5.0 ppb	Sample 1	Sample 2 LFSMD	Sample 6	Sample 10	Sample 12	Sample 16	Sample 20	Sample 24	Sample 28	Sample 29
H	0.5 ppb	5.0 ppb	Sample 1	Sample 2 LFSMD	Sample 6	Sample 10	Sample 12	Sample 16	Sample 20	Sample 24	Sample 28	Sample 29

Standard Curve is certified reference standard of MC-LR (Kit curve can be used if desired)
Low CCC is the 0.15 ppb standard from kit
LFB and LFSMs are an external source of reference material
Blank is deionized water (or diluent used to dilute samples); LRB is reagent water
At least 1 LD or LFSMD per 20 samples (Batch)

US EPA General Plate Set-up (NWCA 2021)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 (S1)	2.0 (S1)	Sample 1 (P)	Sample 5 (P)	Sample 9 (P)	Sample 13 (P)	Sample 17 (P)	Sample 21 (P)	Sample 25 (P)	Sample 29 (P)	Sample 33 (P)	Sample 37 (P)
B	0 (S1)	2.0 (S1)	Sample 1 (D)	Sample 5 (P)	Sample 9 (D)	Sample 13 (D)	Sample 17 (D)	Sample 21 (D)	Sample 25 (D)	Sample 29 (D)	Sample 33 (D)	Sample 37 (P)
C	0.15 (S1)	5.0 (S1)	Sample 2 (P)	Sample 6 (P)	Sample 10 (P)	Sample 14 (P)	Sample 18 (P)	Sample 22 (P)	Sample 26 (P)	Sample 30 (P)	Sample 34 (P)	Sample 38 (D)
D	0.15 (S1)	5.0 (S1)	Sample 2 (P)	Sample 6 (P)	Sample 10 (D)	Sample 14 (D)	Sample 18 (D)	Sample 22 (D)	Sample 26 (P)	Sample 30 (D)	Sample 34 (D)	Sample 38 (D)
E	0.4 (S1)	KC (0.75)	Sample 3 (P)	Sample 7 (P)	Sample 11 (P)	Sample 15 (P)	Sample 19 (P)	Sample 23 (P)	Sample 27 (D)	Sample 31 (P)	Sample 35 (P)	Sample 39 (P)
F	0.4 (S1)	KC (0.75)	Sample 3 (P)	Sample 7 (P)	Sample 11 (D)	Sample 15 (D)	Sample 19 (D)	Sample 23 (D)	Sample 27 (P)	Sample 31 (D)	Sample 35 (P)	Sample 39 (D)
G	1.0 (S1)	NC (Blank)	Sample 4 (P)	Sample 8 (P)	Sample 12 (P)	Sample 16 (P)	Sample 20 (P)	Sample 24 (P)	Sample 28 (P)	Sample 32 (P)	Sample 36 (D)	Sample 40 (P)
H	1.0 (S1)	NC (Blank)	Sample 4 (P)	Sample 8 (P)	Sample 12 (D)	Sample 16 (D)	Sample 20 (D)	Sample 24 (D)	Sample 28 (D)	Sample 32 (D)	Sample 36 (D)	Sample 40 (D)

Key: S0-S5 = Standards; KC = Control supplied with Kit (i.e., Kit Control);
NC = Negative Control (Laboratory Reagent Blank);
P = Primary aliquot for each unknown sample collected by field crew;
D= "DUPLICATE" aliquot for each matching unknown Primary sample.

13. Analysis Procedure

a. Remove the ELISA kit from fridge and allow it to come to ambient temperature before use

b. Wear appropriate protective safety equipment while preparing and conducting the Microcystins/Nodularins ELISA assay

c. Samples previously prepared (e.g. SOP #10) are put in 10 x 130mm glass vials

d. Standard Curve Preparation (to be analyzed alongside kit curve for additional QC for each new Lot of kits or in lieu of kit curve. – **Use the Kit Provided Curve Only for US EPA per National Wetland Condition Assessment 2021 Laboratory Operations Manual**

   i. An MC-LR certified reference standard is used to prepare calibration curve solutions

   ii. Phosphate buffer at pH 7 (0.01 M) is used as the diluent

   iii. Solutions are prepared in 1 mL aliquots using 1.5 mL HPLC vials at the following concentrations

      1. 0.00, 0.15, 0.25, 0.50, 1.00, 2.5, 4.0, 5.0 ppb

   iv. Solutions are stored in the refrigerator between uses

e. Lab Fortified Sample Matrix (LFSM) & LFSMD (do not conduct for US EPA National Wetland Condition Assessment 2021)

   i. Water samples

      1. Duplicate samples are spiked at a concentration of 1 ppb (µg/L)
      2. 990 µL sample + 10 µL of spiking solution MC-LR
         a. Spiking solution is 100 ppb MC-LR (PDS – secondary source)
      3. At least 20% samples in an assay are spiked
         a. At least one LFSM & LFSMD are prepared per 20 samples for Method 546
            i. Spike both raw and finished sets separately
            ii. Prepare an LFSMD for each LFSM
            iii. Use lyse and filter method outlined in section 11.a & SOP #30

   ii. Other matrices (*e.g.* soil, lyophilized algae)

      1. Spikes are prepared pre-extraction and analyzed at the same dilutions as samples

f. Lab fortified Blanks (LFBs) (do not conduct for US EPA National Wetland Condition Assessment 2021, instead use kit supplied 0.75 ppb control)
    i. Two or more LFBs are prepared per test
    ii. 990 µL deionized water or 0.01 M Phosphate buffer at pH 7 + 10 µL of spiking solution MC-LR
        1. Spiking solution is 100 ppb MC-LR (PDS – secondary source)
    iii. Method 546
        1. 990 µL REAGENT WATER + 10 µL of spiking solution MC-LR
            a. Spiking solution is 100 ppb MC-LR (PDS – secondary source)
            b. Follow the lyse and filter protocol outlined in section 11.a and SOP#30
    iv. Space LFBs in both ends of plate
g. Lab Reagent Blanks (LRB)
    i. One or more blanks are prepared and run in duplicate
    ii. For all other sources (non-Method 546 samples) the solvent source is the same solution used for the LFB and dilution preparations, either deionized water or 0.01 M Phosphate buffer at pH 7
    iii. Method 546
        1. Two blanks are prepared for Method 546
        2. The LRB is reagent water (sodium thiosulfate added for finished water) or deionized water, which is also lysed (SOP #30 – freeze thaw) and filtered prior to analysis
            a. Follow the lyse and filter protocol outlined in section 11.a and SOP#30
        3. The 2 Blanks must be analyzed at two ends of the curve
        4. If finished water is analyzed in the run, at least one LRB must be in sodium thiosulfate (reagent water)
h. Low Calibration Verification (Low-CV)
    i. The 0.15 ppb kit provided standard to be loaded outside curve for additional verification
    ii. Method 546
        1. Do not add sodium thiosulfate, do not lyse, and do not filter the Low-CV.
    iii. Do not conduct for US EPA National Wetland Condition Assessment 2021

i. Quality Control Sample (QCS)
    i. Prepare one additional 1ppb LRB using an alternate source of MC-LR
        1. If NRC used for LFMs, LFBs, use GWL sourced MC-LR
        2. If GWL sources MC-LR used for LFMs/LFBs, use NRC MC-LR
    ii. Do not conduct for US EPA National Wetland Condition Assessment 2021

j. Dilution
    i. Dilutions are conducted, when required, to put sample absorbance (ABS) values within the standard curve range
        1. Dilute samples using 0.01 M phosphate buffer at a pH of 7, deionized water or kit diluent
        2. For Method 546 samples, dilute in kit provided diluent
        3. For US EPA National Wetland Condition Assessment 2021, dilute in deionized water (DI) in 10-fold increments
            a. e.g. 900 µL DI + 100 µL sample

k. All glassware used for holding samples and QC (LFSMs, Blanks, Dups, LFBs) is single use only and not reused
    i. Glassware is to be discarded in Glass disposal container

l. The Adda ELISA (Abraxis PN 520011) is conducted utilizing manufacturer's instructions with modifications
    i. All 50 µL calibration standards, samples or extracts, the blanks (LRBs), LFBs, LFSMs, Low-CV & QCS are loaded in wells with duplicates
        1. Load Kit standards only for US EPA National Wetland Condition Assessment 2021
        2. For each new lots of Kit, run both the kit curve and certified reference standard (CRM) curve (prepared in 13.d.) for comparison
        3. For Method 546 and general GreenWater samples, use CRM curve
    ii. Add 50 µL of the antibody solution to the individual wells successively using a multichannel pipette
    iii. Cover the wells with parafilm and incubate for 90 minutes at room temperature on titer plate shaker
    iv. After incubation, remove the covering and vigorously shake the contents of the wells into a sink.
    v. Wash the strips three times using the 1X washing buffer solution and pat dry on paper towel.

vi. Add 100 µL of enzyme conjugate solution to the individual wells successively using a multichannel pipette.

vii. Cover the wells with parafilm or tape and incubate the strips for 30 min at room temperature on titer plate shaker

viii. After incubation, remove the covering and vigorously shake the contents of the wells into a sink.

ix. Wash the strips three times using the 1X washing buffer solution and pat dry on paper towel.

x. Add 100 µL of color solution to the wells using a multi- channel pipette and incubate 10-20 minutes in the dark.

1. **For Ohio EPA, US EPA Method 546 samples, and US EPA National Wetlands Assessment, incubate for 20 minutes

xi. Add 50 µL of stop solution to the wells in the same sequence as for the color solution using a multi- channel pipette and read within 15 min

m. Data Acquisition

i. When the assay is completed, turn on the SpectraMax 340 PC or M2

1. The lamp does not require a warm-up time

ii. Open the SOFTmax pro software on computer (7.0)

iii. Connect the computer to the SpectraMax using USB port

iv. Open SoftMax Pro Software (Version 7.X)

v. Open the previously constructed MC template with sample names and import or transcribe to SoftMax software

vi. Load the ELISA tray in microplate drawer

vii. Click "Read" button

1. Settings are automatic and the tray will mix (5s) and be read

2. 450 nm wavelength

viii. Immediately save the .sda file on computer and process data

14. Data Processing

a. Fit the calibration curve to a 4-parameter curve

b. Export the raw data to excel

i. Click  , click Export (

), check the box for Ept1 for plate1, standards and unknowns and check 'Raw' and 'Plate (.txt .xls)' options. Hit OK, change the file type option to 'Excel files .xls' and save the file under the 'ELISA' file on the computer

ii. Open the newly made .xls file and click OK when the warning comes up about 'file format and extension…' and copy pertinent data over to the 'MC Spreadsheet template' found on the server under the 'ELISA_'current_year'' file.

iii. Update client, sample, and collection date information.

iv. Update name of analyst, % CV of standards, R2 value and date of analysis

v. Copy **curve** image from SpectraMax to the bottom of the sheet

vi. Calculate final [Conc] based on dilution factors (if used)

vii. Calculate the QC returns (low CCC, LRB, LFB, %RPD etc.) based on section 15 equations

viii. Flag (highlight) any outlier data (failed CV, failed curve data, failed QC samples, elevated spike returns)

c. Print the ELISA data and put in ELISA book

15. Quality Control

a. Curve Interpretations

i. Non-linear coefficient of determination (R^2)

1. The ELISA plate data is not accepted and all samples must be rerun if the standard curve exhibits <0.99 R-square value

a. Specific for Method 546 ≤0.98 R-squared value

ii. US EPA National Wetland Condition Assessment 2021

1. S0 – 0ppb kit supplied standard must have an average absorbance of ≥ 0.8 (i.e., $\bar{A}0 \geq 0.8$) or plate needs to be rerun

b. Blank (LRB – Lab reagent Blank)

i. The Blank control must be below the 0.15 ppb calibrator

1. If the blank exceeds 0.15 ppb, the assay is compromised and all samples must be re-run

ii. Method 546

1. LRB (lab reagent blank) must return < ½ the MRL or reanalysis required

iii. US EPA National Wetland Condition Assessment 2021

1. When two or more negative control samples have detectable concentrations of microcystins (i.e., values > 0.1 µg/L), plates need to be rerun

c. Kit Control (0.75 ppb)

i. Only used for US EPA National Wetland Condition Assessment 2021

1. When control samples are outside the acceptable range of 0.75 +/- 0.185 ppb. (< 0.565 or > 0.93), the plate needs to be rerun

d. Lab fortified blank (LFB) (when conducted)

i. Calculate the percent (%) recovery of the LFB

1. % Recovery = (average LFB assay value – average blank assay value)*100

ii. The acceptable recovery for the lab fortified blank is 70-130% (60-140% for Method 546)

1. If the LFB is outside this range, all samples must be re-analyzed

a. If outside this range and reanalysis is not possible, the results must be appropriately qualified (J) and noted in the final report.

2. For Ohio EPA data, the LFB return must be 75-125% percent

a. If outside this range and reanalysis is not possible, the results must be appropriately qualified (J) and noted in the final report.

e. Lab fortified matrix (LFSM & LFSMD)

i. Calculate the percent (%) recovery of the LFSM

1. % Recovery = (average LFSM assay value – average sample assay value)*100

ii. The acceptable recovery for the lab fortified matrix (LFSM) is 70-130%If the LFSM is outside of this range, data may be reported and a note must be made to the client

1. If the LFSM is <70%, sample matrix may have caused interference or destroyed the microcystin

2. If the LFSM >130%, salts or other unknown compounds may have resulted in non-specific binding

iii. Method 546

1. Calculate the Mean % recovery of the LFSM & LFSMD

$$\%R = \frac{(A - B)}{C} \times 100\%$$

Where,

A = mean measured concentration of the LFSM and LFSMD,

B = measured concentration in the unfortified sample, and

C = fortification concentration.

a. The acceptable % recovery ±40% (60-140%)

b. If the percent recovery falls outside this range, and the laboratory performance is in control for the LFBs within the same Analysis Batch, the recovery may be matrix biased. Qualify the result for the sample from which the LFSM was prepared as "suspect–matrix"

2. Calculate the RPD for the LFSM and LFSMD

$$RPD = \frac{|LFSMD - LFSM|}{(LFSMD + LFSM)/2} \times 100\%$$

a. The RPD for each LFSM and LFSMD set should be less than, or equal to, 40%. If the RPD falls outside this range, and the laboratory performance is in control for the LFBs within the same Analysis Batch, the precision may be matrix biased. Qualify the result for the sample from which the LFSMD was prepared as "suspect–matrix".

f. QCS – Method 546

i. The percent recovery of MC-LR in the QCS must be greater than, or equal to, 70% and less than, or equal to, 130%.

g. Reportable Data

i. GreenWater Specific Requirements for general reporting

1. Only values above 0.15 ppb are considered above the detection limit, with 0.30 ppb used as the current method reporting limit (MRL)

a. Matrix effects are common below this level

b. If the level of MCs was above 0.15 ppb, and the LFSM was >130%, a note is made to the client that ELISA data may be over-estimating the true MC levels

2. Levels above 4 ppb (but below 5 ppb) may be flagged as estimates

3. All samples over 5 ppb must be re-analyzed with dilution until they are within range of the calibration curve (0.15 – 5.0 ppb)

ii. Method 546 Reporting

1. The MRL is 0.30 ppb

2. Dilute and re-analyze samples that exceed the calibration range (> 5 ppb)

iii. US EPA National Wetland Condition Assessment 2021

a. The MDL is 0.1 ppb and the MRL is 0.15 ppb
    i. Data above the MDL, but below the MRL is reported and qualified.
b. If the values are above 5 ppb, the leave the conc column of EDD blank and flag as 'HI'. Dilute the sample for the reanalysis per Section 7.6.5 of the LOM (10-fold DF using DI).

h. Assay Precision
    i. Coefficient of Variation (% CV)
        1. The % CV average must be <10% for calibration standards (does not apply to US EPA Wetlands 2021 assessment)
            a. If all calibrators are <10% except 1, then the 1 must be <15%
            b. For Ohio EPA methods, this refers to the QC and standards used for the curve. Per Ohio EPA methods
        2. The accepted % CV for replicate assay values is 15% (includes LFBs, LFSMs, ambient water)
            a. Although the coefficient of variation can be used to measure the precision of the ELISA test, values achieved on the low end of the assay may be outside the typical accepted %CV, but still reportable. A note is made to the client to qualify this. For Ohio EPA requirements, the qualifier used is "J"
            b. US EPA National Wetland Condition Assessment 2021
                i. If the %CV > 15% between the duplicate absorbance values for a sample, the sample needs to be re-analyzed.
            c. Method 546
                i. If the %CV exceeds 15% for a field sample or QC sample (Low-CV, LRB, LFB, LFSM, and LFSMD), then the sample is invalid

i. Calibrator Control Check (Method 546 = Low-CV or Low CCC) (does not apply to US EPA Wetlands 2021 assessment)
    i. The 0.15 ppb low control should quantify between 0.1-0.2 ppb to verify the low end of the curve
        1. Method 546 ±50%
    ii. If calibrator control check fails, the assay must be rerun
        1. Ohio EPA data is still reportable if the low calibrator is within 40% of true value

j. For Clients with a Laboratory Duplicate (LD)

i. Ohio EPA protocol

1. LDs must be analyzed on 5% (1 of every 20) of samples in any analytical batch and both results must be reported to the Ohio EPA.

The Relative Percent Difference (RPD) must be calculated and records must be maintained.

Calculate RPD as follows:

$$RPD = [(D2 - D1)/DAvg] * 100$$

Where: D1 = Laboratory Duplicate 1

D2 = Laboratory Duplicate 2

DAvg = Average of Laboratory Duplicates 1and 2

k. Qualifiers

i. Ohio EPA Qualifiers for clients requiring Ohio EPA SOP methodology

B Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.

CL Analytical result is estimated due to ineffective quenching.

J Analyte was positively identified; the associated numerical value is estimated.

PT The reported result is estimated because the sample was not analyzed within required holding time.

UJ The analyte was not detected above the sample quantification limit (QL). However, the QL is estimated.

To be used when any of the three conditions apply

1) sample was collected in an improper sample container
2) received warm (>4°C)
3) low LCRC recovery

l. US EPA National Wetland Condition Assessment 2021

i. DATA FLAGS have codes for the following special cases:

1. ND if the sample was non-detected;
2. J if the value is detected but at a level below the reporting limit of 0.15 µg/L (for undiluted samples);
3. HI if the concentration value registers as HIGH (exceeds the calibration range).

ii. QUALITY FLAGS have codes for the following special cases:

1. QCF if there is a QC failure per step 23 above. The QCF code must be used for all failures to facilitate data analysis.
2. Q for any other quality issue (describe in COMMENTS)

m. US EPA Method 546 Qualifiers:

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated. Qualifier may be used when LFSM data exceeds acceptable criteria.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values Achieved were outside calibration range.
N	Spiked sample control was outside limits
ND	The analyte was analyzed for, but was not detected above the reported method detection limit

16. Reporting Limit (RL) Verification for Ohio EPA

a. To be calculated annually by each analyst, when a new analyst begins work or whenever a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate the reporting limit must be recalculated. In order to maintain a reporting limit of 0.15 µg/L, each analyst must demonstrate the capability to achieve a reporting limit of 0.15 µg/L.

To calculate the reporting limit (RL) value, take seven (or more) replicate aliquots of the low calibrator standard (0.15 ppb) and process them through the SOP. Once the results for the replicates have been obtained, calculate the RL as follows:

$$RL = (t)*(SDR)$$

Where: t = Student's t value for a 99% confidence interval and a standard deviation estimate with n-1 degrees of freedom

t = 3.143 for the seven replicates (6 df)

t=2.650 for 14 replicates (13 df))

SDR = Standard deviation of the replicate aliquot analyses

The RL study will be valid if the resulting value of the RL is no more than ten times lower than the replicate standard concentration level and does not exceed the replicate standard concentration level. Save and print a copy of each analyst RL verification in the ELISA lab notebook.

17. Safety Precautions – Hazard Communication

a. Microcystins/Nodularins

DANGER! Acute Toxicity Category 1. Fatal if swallowed, fatal if inhaled.

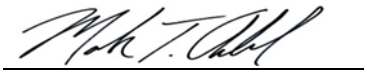
Warning! Irritant. May cause allergic skin reaction, may cause serious eye irritation.

b. Tetramethylbenzidine

i. The Microcystins/Nodularins Adda ELISA Kit contains a STOP solution made of. If skin comes in contact with the ELISA kit solutions, wash with water

18. Revision History

Revision	Description of Changes	Date	Requested by
0.1	Incorporation of NRC standards	5/1/2013	Foss
0.2	Additional calibrators added to curve	4/1/2015	Foss
0.3	Format change	6/29/2015	Foss
0.4	Changes per Ohio EPA verification requirements	8/22/2015	Foss
0.5	Addition of Method 546 QC	12/9/16	Foss
0.6	SOP# change to reflect Master List	1/27/17	Foss
0.7	Addition of 546 IDC data	4/20/17	Foss
0.8	Addition of Ohio EPA qualifier	5/15/18	Foss
0.9	Addition of US EPA (National Wetland Condition Assessment 2021 Laboratory Operations Manual) requirements	3/18/2021	Foss

Approved by: 

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SOP No.: 321.4500PE

Title: Phosphorus, All Forms

Scope and Application: This method covers the determination of all forms of phosphorus, including o-phosphate, organic phosphate, and total phosphorus. This method is applicable to all drinking water, surface water, ground water, industrial and domestic waste, and soil samples and their leachates. The range for this procedure is 0.010-1.0 mg/L. Samples above 1.0 mg/L can be analyzed by sample dilution prior to digestion. The limit of detection is 0.010 mg/L.

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates, and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms. These forms of phosphate arise from a variety of sources. Small amounts of certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.
Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macro-organisms in nuisance quantities.

I. Summary of Method

A. Ammonium molybdate and potassium antimony tartrate react in an acid medium to form a heteropoly acid (phosphomolybdic acid) which is reduced to an intensely blue-colored complex by ascorbic acid. The intensity of the blue color is directly proportional to the concentration of orthophosphate in the sample.

B. Since the above reaction only occurs with the o-phosphate form of phosphorus, pretreatment of condensed and organically bound is required for their

determination. Condensed phosphates must undergo acid hydrolysis at boiling-water temperature. Organically bound phosphates must undergo a digestion using acid, heat, and an oxidizing agent. These treatments transform the bound phosphates to o-phosphate, where they can be quantified.

C. For dissolved phosphate, filter the sample through a 0.45um filter before analysis. If only particulate phosphate is to be determined, analyze for dissolved phosphate and for total phosphate, and subtract dissolved from total.

D. Total Phosphorus (P)- all of the phosphorus present in the sample, regardless of form, as measured by the persulfate digestion procedure.

1. Total Orthophosphate (P, ortho) or Total Reactive Phosphorus- inorganic phosphorus [$(PO_4)^{-3}$] in the sample as measured by the direct colorimetric analysis procedure.
2. Total Hydrolyzable Phosphorus (P, hydro) – phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus pre-determined orthophosphates. This hydrolyzable phosphorus includes polyphosphorus. [$(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$, etc.] plus some organic phosphorus.
3. Total Organic Phosphorus (P, org) – phosphorus (inorganic plus oxidizable organic) in the sample measured by the persulfate digestion procedure, and minus hydrolyzable phosphorus and orthophosphate.

E . Dissolved Phosphorus (P-D)- all of the phosphorus present in the filtrate of a Sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure.

1. Dissolved Orthophosphate (P-D, ortho) or Dissolved Reactive Phosphorus- as measured by the direct colorimetric analysis procedure.
2. Dissolved Hydrolyzable Phosphorus (P-D, hydro)- as measured by the sulfuric acid hydrolysis procedure and minus pre-determined dissolved orthophosphates.
3. Dissolved Organic Phosphorus (P-D, org)-as measured by the persulfate digestion procedure, and minus dissolved hydrolyzable phosphorus and orthophosphate.

F. The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration, may be calculated:

1. Insoluble Phosphorus (P-I) = (P)-(P-D).
2. Insoluble orthophosphate (P-I, ortho)=(P,ortho)-(P-D,ortho).
3. Insoluble Hydrolyzable Phosphorus (P-I,hydro)=(P,hydro)-(P-D,hydro).
4. Insoluble Organic Phosphorus (P-I, org)=(P,org) – (P-D,org).

G. See Appendix B for flow chart.

II. Interferences

A. Glassware must be scrupulously cleaned, as carry over from phosphates is common. Refer to Appendix A in this SOP for the Phosphorus Glassware Cleaning Instructions.

B. Arsenate present in the sample to be tested is a positive interference, as it reacts with the molybdate reagent to produce a blue color similar to the blue formed by o-phosphate. Concentrations as low as 0.1 mg/L are enough to cause interference. By adding sodium bisulfite to the sample, arsenate is reduced to arsenious acid.

C. High concentrations of iron in a sample will give erroneously low results, as it will take away some of the reducing agent. Sodium bisulfite treatment will eliminate this interference.

D. Hexavalent chromium and nitrite interfere to give low results. Concentrations of these ions need to be at least 10.0 mg/L to interfere signifcantly.

E. Particulates may interfere with spectrophotometric analysis by absorbing light. Samples may be filtered after hydrolysis or digestion but not once the color reagent is added.

F. In the determination of orthophosphate, color can interfere. A color blank may be performed.

III. Sample Collection, Preservation and Storage

A. Samples can be collected in plastic or glass. They must be stored at 4°C until time of analysis.

B. Orthophosphate samples must be unpreserved and analyzed within 48 hours from time of collection.

C. Total Phosphorus samples must be H_2SO_4 preserved to pH<2 and analyzed within 28 days of collection.

IV. Equipment and Supplies

A. Spectophotometer, 880nm.

B. Environmental Express 54-position Hot Block with custom glass digestion vials.

C. Pro Clean Environmental Containers glass 60mL vials, P/N GV60/PCLB-72.

D. Graduated cylinder, 50mL.

E. Volumetric flasks, 50mL.

F. Class "A" volumetric pipets; assorted.

G. Hengar granules, boiling stones

V. Reagents and Standards

A. Sulfuric Acid, 5N. Dilute 139mL conc H_2SO_4 to 1000mL with DI water.

B. Potassium antimony tartrate solution. Dissolve 1.3715 g $K(SbO)C_4H_4O_6$ 1/2 H_2O in 400 mL DI water in a 500ml volumetric flask and dilute to volume. Store in a plastic, stoppered bottle.

C. Ammonium molybdate solution. Dissolve 20 g $(NH_4)_6$ Mo_7O_{24}*$4H_2O$ in 500ml DI water. Store in plastic, stoppered bottle.

D. Ascorbic acid solution, 0.01M. Dissolve 3.52g ascorbic acid, reagent grade, in 200mL DI water. Make fresh daily.

E. Combined color reagent. Mix 100mL 5N H_2SO_4, 10mL potassium antimony tartrate, 30mL ammonium molybdate, and 60mL ascorbic acid solution in a clean 200mL erlynmeyer flask. If more reagent is needed, increase volumes of each solution proportionally. **Add reagents in the order given, and mix after the addition of each reagent.** Combined reagent is only stable for 4 hours.

F. Sulfuric acid, 11N. Slowly add 300mL conc. H_2SO_4 to 600mL DI water. Cool in a water bath, and dilute to 1 liter. Alternatively, make from 18N (1:1) H_2SO_4 by diluting 600mL to 1 liter of DI water.

G. Ammonium persulfate $(NH_4)_2\ S_2O_8$ solid, reagent grade. Potassium persulfate may be used as an oxidizing reagent.

H. Sodium bisulfite solution. Dissolve 5.2 g of $NaHSO_3$ in l00mL of l N H_2SO_4.

I. Sodium hydroxide solution, 2N. Dissolve 80 g NaOH into 1 liter DI water.

J. Phenolphthalein indicator solution, alcohol-based. Dissolve 5g of phenolphthalein in approximately 500mL 95% ethyl or isopropyl alcohol. Dilute to 1 L with DI water.

K. Stock phosphorus solution. Dissolve 0.4393g of oven-dried and desiccated KH_2PO_4 in 1L DI water. 1.0mL = 100ug P. Record preparation of standards in standards logbook.

L. Standard phosphorus solution. Dilute 100mL of stock phosphorus solution to 1L with DI water. 1.0mL= 10ug P. Record preparation of standards in standards logbook.

VI. Definitions

A. Laboratory Control Sample (LCS)- A purchased standard of a known concentration that is from a different source than the calibration standards. The LCS is used to determine laboratory performance.

B. Preparation Blank (Prep Blank)- An aliquot of reagent water that is treated exactly as a sample. The prep blank is used to determine if method analytes are present in the lab environment, reagents or glassware.

C. Calibration standard- A solution prepared from the dilution of stock standard solutions. The calibration solutions are used to calibrate instrument response with respect to analyte concentration.

D. Matrix Spike (MS)- An aliquot of environmental sample to which a known quantity of the method analytes are added in the laboratory. The MS is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results.

VII. Procedure

A. Determination of total phosphorus (TP-WM), all forms of phosphate.

1. Check pH of the sample and record in Phos batching program.

2. Into a digestion vessel, measure 50mL sample or an aliquot diluted to 50mL, hengar granules, and add a drop of phenolphthalein solution. If it turns pink, add 11N H_2SO_4 drop-wise until color disappears.
3. Add 0.4g solid ammonium or potassium persulfate.
4. Add lmL 11N H_2SO_4. Use disposable pipets to reduce chances of contamination.
5. Set the Hot Block to approximately 140°C, enough to get the samples to 100°C. Boil gently under a ventilation hood until a final volume of less than 10mL is achieved. Do not allow sample to go to dryness.
6. For samples suspected to contain iron interference, add 5mL of sodium bisulfite and mix. continue heating for 20 min. Do not allow sample to go to dryness.
7. Cool, dilute to 30mL in the digestion vessels (filter sample, if needed), add 1 drop phenolphthalein solution, and neutralize to a faint pink color using 2N NaOH solution. *Do not filter while sample is neutral, as phosphate-containing precipitates form that will dissolve under acid conditions.*
8. Bring sample up to 50 mL volume with DI water in digestion vessel and mix well. Transfer a 25 mL aliquot to 60 mL glass vessel. If a larger dilution is necessary, transfer a small volume to glass vial and bring to 25 mL final volume with DI water.
9. Determine phosphorus as outlined in the determination of orthophosphate, C.2. and C.3. below.

B. Determination of total phosphates (code "TPFATE") hydrolyzable and condensed phosphates.

1. Into a digestion vessel, measure 50mL sample or an aliquot diluted to 50mL and add a drop of phenolphthalein solution. If it turns pink, add 11N H_2SO_4 drop-wise until color disappears. Record pH of original sample in Phos Batching sheet.
2. Add 1 mL of H_2SO_4 to sample. Use a disposable pipet to reduce risk of contamination.
3. Set the Hot Block to approxiamately 140°C, enough to get the samples to 100°C. Boil gently with hengar granules under a ventilation hood until a final volume of less than 10mL is achieved. Do not allow sample to go to dryness.
4. Treat samples containing interferences, as above.
5. Cool samples, filter if needed, then add 1 drop phenolphthalein solution, and neutralize to a faint pink color using 6N NaOH solution. *Do not filter while sample is neutral, as phosphate-containing precipitates from that will dissolve under acid conditions.*
6. Bring sample up to 50 mL volume with DI water in digestion vessel and mix well. Transfer a 25 mL aliquot to 60 mL glass vessel. If a larger dilution is necessary, transfer a small volume to glass vial and bring to 25 mL final volume with DI water.

7. Determine phosphorus as outlined in the determination of orthophosphate, C.2. and C.3. below.

C. Determination of orthophosphate (OP-WM).

1. Decant 25mL of unpreserved sample or an aliquot diluted to 25mL into a glass vial, add 1 drop of phenolphthalein solution. If a pink color appears, add 5N H_2SO_4 drop-wise until color disappears.
2. Add 4 mL combined color reagent and mix thoroughly.
3. After 10 (but before 30) minutes, measure the absorbance of each sample at 880nm, using DI water as the reference solution. The absorbance is correlated to a point on a linear regression which correlates to a concentration of phosphorus.

D. For other forms of Phosphorus, see Section 1.C – 1.F.

VIII. Calculations

A. Refer to the Standards Logbook for the most current calibration curve. To generate a new calibration curve, use phosphorus standard solution. Make the following dilutions: blank (DI water), 0.01mg/L, 0.025 mg/L, 0.05 mg/L, 0.10 mg/L, 0.20 mg/L, 0.50 mg/L and 1.0 mg/L. Add phenolphthalein and 5N H_2SO_4 to each, as you would a sample. Add 8 mL combined color reagent, and read at 880 nm after 15 minutes. Record absorbances, and plot them on the x-axis against the corresponding concentrations. Follow with a linear regression, and use the following calculation:

$Y = (MX + B) * DF$, where: Y = concentration (mg/L)
M = slope of the linear regression
X = absorbance at 620nm
B = y-intercept of linear regression
DF = dilution factor, if any

Note that M and B values will differ with each calibration.

B. Calculating RPD:

The RPD is the relative percent difference, and is a measure of the consistency of the sample replicate for the sample set. First, calculate the results for both the sample and the sample replicate. Then apply the two results to the following calculation:

$$RPD, \% = \frac{(R1 - R2)}{(R1+R2)/2} \times 100$$

where: R1 = value achieved for sample, mg/L
R2 = value achieved for sample replicate, mg/L

C. Calculating the sample spike recovery:

The sample spike is a test if the sample matrix is suitable for accurate testing. To calculate the % recovery, first calculate both the sample and the spiked sample. Then apply the two results to the following calculation:

$$\% \text{ Recovery, } \% = \frac{(SR - R)}{STV} \times 100$$

where: SR = value of sample with added spike, mg/L
R = value of sample, mg/L
STV = true value of spike added to sample, mg/L

D. Calculating the LCS:
The LCS is the laboratory control standard and is a test of the accuracy of the calibration and the test overall. To calculate the % recovery of the LCS, calculate the LCS sample, and then apply it to the following calculation:

$$\% \text{ Recovery, } \% = \frac{(RL)}{(LTV)} \times 100$$

where: RL = achieved value for LCS sample, mg/L
LTV = true value of LCS, mg/L

E. Calculating poly-phosphate, as P is done during data transfer, automatically, when needed.

IX. Quality Control

A. All reagents and standards are to be labelled with the following information: date prepared, date expires, preparer's initials, analyte, contents, and concentration of contents. Record preparation of all reagents in the reagent prep logbook.

B. A sample prep blank of dilution water must be analyzed with each batch of twenty or fewer samples. Prep blanks must be determined to be BRL (below reportable level).

C. Two purchased, second source, laboratory control standards (LCS) are run with each batch of twenty or fewer samples. One is a "complex" solution, which goes through the digestion process, while the other is a "simple" solution, which is not digested. The acceptable recovery of a LCS is 85-115%.

D. An in-house standard (CC) made from the standard solution used to calculate the curve is run after every ten samples. The true value of the CC is 0.25mg/L. The acceptable recovery of an in-house standard is 90-110%.

E. A DI water blank, (CB), is analyzed after the CC every ten samples. The CB must be BRL (below reportable level).

F. A sample spike and a sample duplicate are run with every batch of twenty or fewer samples. The acceptable recovery for a sample spike is 75-125%. The acceptable relative percent difference (RPD) for a sample duplicate is less than or equal to 20%.

G. A new calibration curve must be analyzed annually or whenever a new stock solution is made. The correlation coefficient of the curve must be >0.9975.

H. Samples labeled as trip blanks, field blanks, or equipment blanks are ineligible samples for spiking and duplicating.

X. Safety

A. The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material data handling sheets are available to all personnel involved in the chemical analysis.

B. Always wear safety glasses for eye protection as well as lab coats.

C. When diluting H_2SO_4, always add acid to water.

D. Refer to Phoenix SOP #805: Hazardous Chemical and Laboratory Safety Procedures

XI. Pollution Prevention

A. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation.

B. Reagents and chemicals should be purchased and/or prepared in volumes consistant with laboratory use to minimize the volume of disposal.

XII. Waste Management

A. It is the laboratories responsibility to comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

XIII. Method Performance

A. This method was validated through internal QA/QC monitoring, including annual method detection limit studies, precision and accuracy studies, initial and continuing calibration verifications, blank analysis, laboratory control samples and matrix spikes and duplicates.

B. See section IX Quality Control in this SOP for acceptable limits.

XIV. Corrective Action for Out-of-Control or Unacceptable Data

A. Should the calibration curve have a correlation coefficient of <0.9975, remake and reanalyze curve before processing samples.

B. Should the preparation blank, LCS or inhouse standard fail acceptance criteria, redigest and reanalyze batch.

C. Should the matrix spike or sample duplicate analysis fail acceptance criteria, a non-conformance report must be generated or the sample QC must be reanalyzed.

XV. Batching Program for Phosphorus

A. Double click on Phoenix MDB (2K MDE) to get to main menu.

B. Click on QA/QC Batching and double click on Prep Batching.

C. Go to the Batch square and type in "Phos-T".

D. Go to New Batch and click, this brings up the last batch made.

E. Click again and this brings up a new list of samples to pick for each batch to be made.

F. Click on numbers under Batches to build for the number needed.

G. Click on Build Batch.

H. Finish making the batch by filling in QC sample, weight/volume, initials and finish date.

I. All samples MUST be batched. They cannot be added to the Sequence Program if they are not in a batch.

J. All batches must have Initial sample weight/volume entered by the analyst before the batch can be added to the Sequence Program.

K. Samples cannot be transferred if % Solid is still pending.

L. Samples cannot be transferred if the batch LCS fails.

M. Changing the QC Sample:

1. A batch cannot be loaded in the Sequence Program until it has been assigned a QC sample. However, a QC sample can be changed.
2. To change a QC sample, select the batch in Prep Batching and Clear the finish date.
3. Change the QC sample to the desired QC sample. The LCS and Blank will automatically change in the Sequence Program.
4. The original spike and dup will remain. Do not select them for transfer and the results will go nowhere.
5. The spike and dup for the new QC sample will have to be added manually to the end of the sequence.

N. Updating the Calibration:

1. When recalibrating the instrument, change the dates in the set-up to reflect the effective ending date of prior calibration.

2. Enter a new record. This can be done by copying an existing record and pasting it on a new line.
3. Update the appropriate date, Slope, y-intercept, LCS true value and source, spike true value, etc.

O. Selecting or Deselecting Samples for Transfer:

1. Before transferring samples, the Batch Blank and Batch LCS must be selected. This is necessary so when a sample is selected, the program knows which QC is associated with it.
2. If a Blank or LCS is deselected, all samples in the batch will be deselected.

P. Rerunning QC Samples:

1. QC can be re-analyzed by re-adding the samples and appropriate QC record to the end of the sequence.
2. Deselect the original run(s) and select the new one, and the corresponding samples.

XVI. Data Transfer Program for Phosphorus

A. Click on Phoenix MDB (2K MDE) to go to the main menu.
B. Click on Data Transfer.
C. Double Click on Phosphate Sequence and Data Transfer.
D. Select "P" on Run Type drop down box.
E. Click on New Sequence or Add Batch for however many to be done.
F. After batches are completed, add analysts initials to bottom and click on Transfer to Labworks.
G. Click on Present Log to print a copy and exit page.

XVII. References

A. Standard Methods for the Examination of Wastewater, 22nd ed., 2011.
Methods used: 4500-P B2 for hydrolysis; 4500P B5 for digestion; 4500-P E for orthophosphate and color formation.
B. Phosphorus, All Forms, Environmental Express Hot Block Standard Procedure.
Method used: Colorimetric, Ascorbic Acid, Single Reagent.
C. USEPA Method 365.3, Phosphorus, All Forms, 1978.

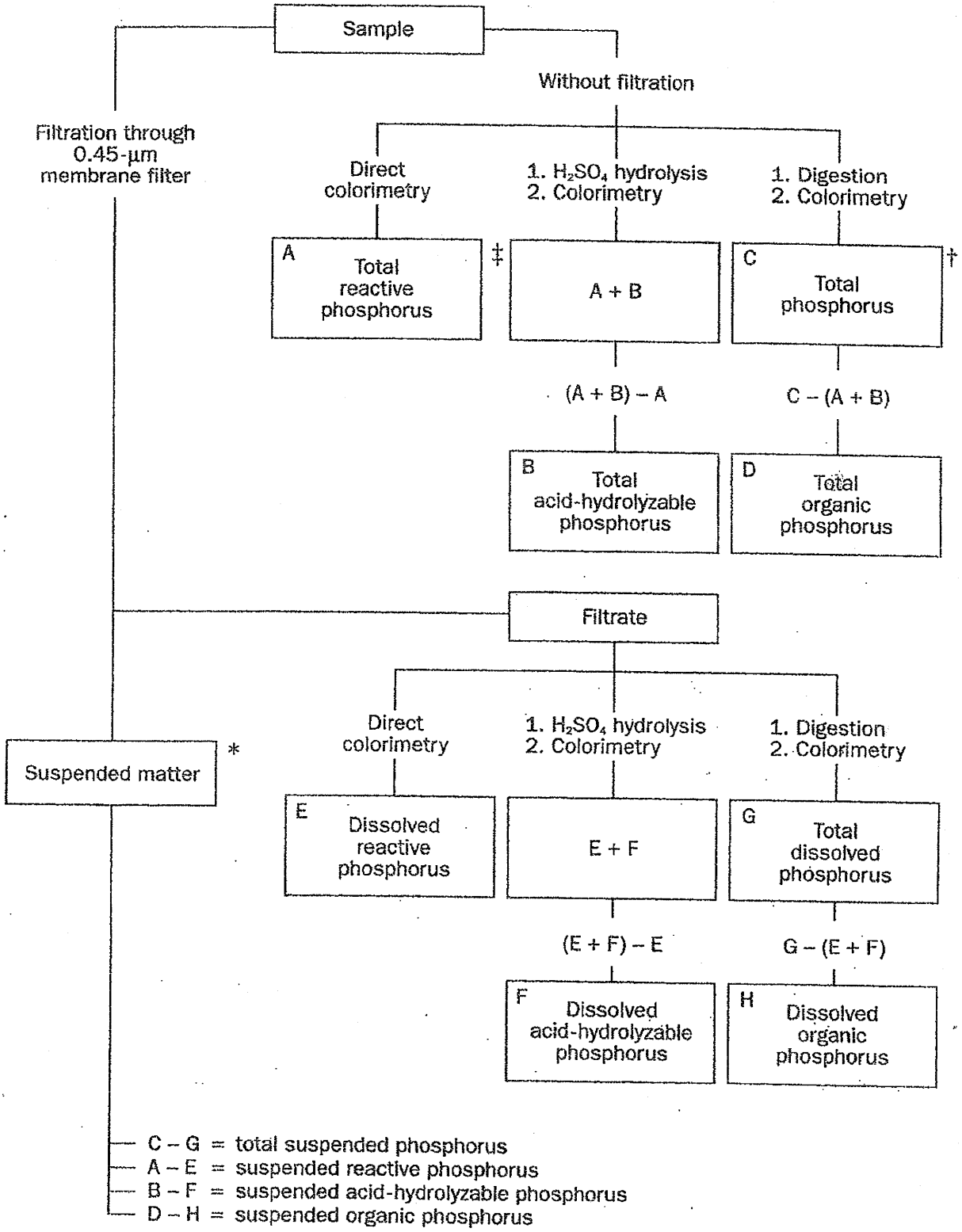
Appendix A- Phosphorus Glassware Cleaning Instructions

1) Make new 10% HCl solution at least once a week.
   a) Dump old solution in acid waste.
   b) Rinse bottles and caps thoroughly with deionized water.
      i) Bottles are usually in hood #8.
   c) Fill bottles to 900 mL line with deionized water.
   d) In a hood, fill bottles to 1000 mL line (the bump near the top), with concentrated HCl.
   e) Cap bottles and CAREFULLY invert several times to mix.

2) PUT 10% HCl bottles on stir/heat block in Hood #8 to heat.
   a) Turn on power switch for block.
   b) Turn heater switch to high. (Downward is low, upward is high, middle is off.)
   c) Set the heat knob to 150°C. Bottles should heat up within the hour.
   d) Monitor bottles to make sure they don't boil.

3) Handwash the 60 mL vessels (NOTE: Some vessels have a 50mL volumetric line for digestions, while others have a 25mL volumetric line for color developement. They are washed the same way!)
   a) Pour out the water that the vessel has been soaking in, while in the white floats.
   b) Fill with hot water, add a squirt of Phosphate free detergent and scrub with brush.
      i) Brush is the clean, white bristled brush hanging to the right of the sink.
      ii) The Phosphate free detergent is in a squeeze Nalgene bottle near brush.
      iii) Remove any sharpie marks on the outside of the vessel while scrubbing.
   c) Rinse washed vessels numerous times with DI water.
   d) Place the scrubbed / rinsed vessels upright in the plastic storage trays.
      i) Trays can usually be found piled up in the phosphorus area.
         (1) Blank trays are for "25 mL" vessels
         (2) Numbered trays are for "50 mL" vessels.
   e) In a hood, carefully fill each vessel to the brim with HOT 10% HCl solution, which was heated in Step #2.
      i) Use a hotglove borrowed from the ammonia setup if necessary; the acid solution should be hot enough to be slightly uncomfortable, but not boiling hot.
   f) Let the vessels soak in HCl solution for a few minutes
      i) The time necessary to pour another tray should be sufficient.
   g) Using the funnel that was stored with the bottles, pour the hot HCl solution back into its bottle. Cap it and store in hood #8 when finished.
   h) Take the tray of acid washed vessels to the sink for rinsing.
      i) Rinse three times with deionized water and pour out.
      ii) Fill vessels to the brim with deionized water
   i) Store clean deionized water filled tray of vessels either on the phosphorus countertop or in the shelves in the rear storage room.
      i) Cover vessels with clean paper towel to protect them.
   j) Dump DI water in vessels and rinse once more with DI before use.

Appendix B


Sample
Without filtration
Filtration through 0.45-µm membrane filter
Direct colorimetry
1. H_2SO_4 hydrolysis 2. Colorimetry
1. Digestion 2. Colorimetry
A Total reactive phosphorus
‡
A + B
C Total phosphorus
†
(A + B) – A
C – (A + B)
B Total acid-hydrolyzable phosphorus
D Total organic phosphorus
Filtrate
Direct colorimetry
1. H_2SO_4 hydrolysis 2. Colorimetry
1. Digestion 2. Colorimetry
Suspended matter
*
E Dissolved reactive phosphorus
E + F
G Total dissolved phosphorus
(E + F) – E
G – (E + F)
F Dissolved acid-hydrolyzable phosphorus
H Dissolved organic phosphorus
C – G = total suspended phosphorus
A – E = suspended reactive phosphorus
B – F = suspended acid-hydrolyzable phosphorus
D – H = suspended organic phosphorus

Revision History Log for SOP #321 TP

Date:	Revision #:	Summary of Changes:	Submitted By:	Approved By:	Effective Date:
1/6/14	5	Simple & Complex LCS added, recording pH of sample, updated vessel cleaning procedure	Kathy Cressia	Eric Geyer	1/6/14
10/04/16	5.1	Section 1.0- added D, E, F, G (forms of phos), Section VII- added D, added EPA method to references, just referring to forms of Phos, added Appendix B- flow chart	Kathy Cressia	Eric Geyer	10/04/16
3/15/18	5.2	Doubled combined reagent recipe, updated method reference year for MUR	Kathy Cressia	Eric Geyer	3/15/18

Expiration Date

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SOP No: 360.PCT

Title: PC Titrator for pH, Alkalinity, Conductivity & Turbidity

Scope and Applications:

pH: This method covers the electrometric determination of the pH of samples. Corrosivity is then determined as a function of the pH of a sample. The range of this method is from 0 to 14 pH units. A sample is deemed "corrosive" if its pH is less than 2.00 or greater than 12.50 pH units. Ideally, the pH is determined in the field by the sampler because the holding time is 'immediate'. Samples received in the lab should be analyzed as soon as possible, before the sample is submitted to the sample storage refrigerators by the sample receipt department. All pH samples analyzed in the laboratory are flagged as past holding time.

Turbidity: Turbidity is a measure of water's clarity, and is important in producing products destined for human consumption. This method covers the determination of a sample's turbidity in the range of 0 to 40 NTU. Samples that have a turbidity of greater than 40 NTU require sample dilution prior to analysis. This method is suitable for any water that is free of debris and rapidly settling coarse sediment. Samples should be stored at 4°C prior to analysis, and analyzed within 48 hours from the time of sampling.

Conductivity: This method is applicable to the measurement of conductivity in drinking, surface, and saline waters, domestic and industrial wastes. This method is suitable for samples with conductivity values of 5.0 to 10000*u*mhos/cm. Samples with conductivity values of greater than 10000*u*mhos/cm may be diluted prior to analysis. Resistance may be determined from the conductivity result, as follows: 1/C = R; where C = conductivity (*u*mhos/cm), and R = resistivity (Mohm/cm).

Alkalinity: This method covers the determination of alkalinity in drinking, surface, and saline waters, domestic and industrial wastes. This method is suitable for samples with alkalinity values between 5.0 mg/L to 2000 mg/L. Samples with an alkalinity greater than 2000 mg $CaCO_3$/L need to be diluted prior to titration.

I. Summary of Methods:

A. This method covers the operation and maintenance of the PC Titrator auto-analyzer which measures for 4 analytes: pH (electrode), Alkalinity (titration), Conductivity (electrode) and Turbidity (nephelometric).

pH: The pH of the sample is determined electrometrically using a combination electrode. The pH meter is calibrated before each use using a series of standard solutions of known pH.

Turbidity: A turbidimeter is used to quantify the amount of light that is scattered as it is passed through a sample.

Conductivity: Conductivity is the numerical expression of the ability of an aqueous solution to carry an electric current. The conductivity is measured by use of a conductivity meter (wheatstone bridge).

Alkalinity: The Alkalinity of a sample represents the acid-neutralizing capacity of a sample. Strong acid is added to the sample until it reaches a designated pH. The amount of acid added is a function of the alkalinity of the sample.

II. Interferences:

pH:

A. Coatings of oily material or particulate matter can impair electrode response. These coatings can usually be removed by gently wiping or detergent washing, followed by rinsing with DI water. An additional treatment with 1:10 HCl may be necessary to remove any remaining film.

B. Temperature effects the electrometric determination of pH. It is important to analyze standards and samples at the same temperature or use an automatic temperature compensation (ATC) probe if the temperatures are different.

Turbidity:

A. Dirty glassware and the presence of air bubbles are positive interferences, both of which can be avoided. Be sure all glassware is

free of scratches, smears from fingerprints, and stray dust. Discard any scratched or otherwise permanently marred piece of glassware.

B. True color, i.e., water color due to dissolved substances that absorb light, causes measured turbidities to be low. This effect is usually not significant in treated water.

Conductivity:

A. Because conductivity is a function of temperature, samples are analyzed at a standard 25°C. Remove samples from refrigerator and allow warming to room temperature before analysis.

Alkalinity:

A. Dissolved gases may be lost or gained during sample storage, and can add to or reduce the alkalinity of a sample. Minimize this interference by titrating promptly, by avoiding vigorous shaking, and by keeping the sample at a constant temperature.

III. Sample Collection, Preservation and Holding Times:

pH:

A. pH is a field parameter and should be analzyed immediately after sampling. Samples analyzed for pH in the laboratory must be flagged as analyzed past holding time.

B. Collect sample in a clean plastic or glass bottle with no preservative.

Turbidity:

A. Collect sample in a clean plastic or glass bottle with no preservative and stored at 4°C until time of analysis.

B. The holding time for Turbidity is 48 hours from time of collection.

Conductivity:

A. Collect sample in a clean plastic or glass bottle with no preservative and stored at 4°C until time of analysis.

B. The holding time for Conductivity is 28 days from time of collection.

Alkalinity:

A. Samples should be collected in a separate plastic 100mL container. They must be unpreserved and stored at 4°C until time of analysis.
B. Samples must be analyzed within 14 days of collection.

IV. Equipment and Supplies:

A. Man-Tech PC-Titration Analyzer with Autosampler; Cat. No. PC-1000-102.
B. 50mL Conical Vials; Environmental Express Cat. No. SC461 (or equivalent).

V. Reagents and Standards:

A. Primary Standard Buffer, 4.00 pH units; RICCA # IBF-040-L (or equivalent).
B. Primary Standard Buffer, 7.00 pH units; RICCA # IBF-070-L (or equivalent).
C. Primary Standard Buffer, 10.00 pH units; RICCA # IBF100-L (or equivalent).
D. 1412 µmhos/cm Potassium Chloride Conductivity Standard, Ricca Cat. No. 5890-1 (or equivalent).
E. NIST Traceable Conductivity Standard, 10.0*u*mhos/cm at 25°C. RICCA P/N 2236-16 (or equivalent).
F. NIST Traceable Conductivity Standard, 100*u*mhos/cm at 25°C. RICCA P/N 2237-16 (or equivalent).
G. NIST Traceable Conductivity Standard, 1000*u*mhos/cm at 25°C. RICCA P/N 2243-16 (or equivalent).
H. NIST Traceable Conductivity Standard, 10000*u*mhos/cm at 25°C. VWR Cat.#23226-625 (or equivalent).
I. NIST Traceable Conductivity LCS, 100*u*mhos/cm at 25°C. VWR Cat.#23226-589 (or equivalent second source).
J. 100 NTU Turbidity Standard; Man-Tech Cat. No. PC-1000-164 (or equivalent).
K. 0.02N H_2SO_4 Alkalinity Titrant: In a 2-Liter volumetric flask containing approximately 1500mL DI water, add 1.12mL concentrated H_2SO_4 and bring up to volume with DI water. **Carefully** invert to mix.
L. 0.02N NaOH: In a 1-Liter volumetric flask containing approximately 500mL DI water, add 2.0mL 10N NaOH (VWR Cat. No. JT5674-2 or

equivalent) and bring up to volume with DI water. **Carefully** invert to mix.

M. LCS Solution (pH, Conductivity and Alkalinity)- Ultra Minerals # QCI-710 or equivalent.

N. LCS Stock Solution (Turbidity)- Amco Clear Turbidity Standard 40 NTU #8017 or equivalent.

O. LCS Working Solution: 100mL LCS Stock Solution into 1L DI Water.

VI. Definitions

A. Laboratory Control Sample (LCS)- A purchased standard of a known concentration that is from a different source than the calibration standards. The LCS is used to determine laboratory performance.

B. Preparation Blank (Prep Blank)- An aliquot of reagent water that is treated exactly as a sample. The prep blank is used to determine if method analytes are present in the lab environment, reagents or glassware.

C. Calibration standard- A solution prepared from the dilution of stock standard solutions. The calibration solutions are used to calibrate instrument response with respect to analyte concentration.

D. Sample Replicates (Rep)- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of the sample and duplicate indicate precision associated with laboratory procedures.

VII. Procedure:

A. Daily Maintenance

1. Prior to running calibrations and samples the instrument must go through a daily maintenance check to ensure accurate results.
2. The pH probe must be rinsed with deionized water, wiped with a kimwipe, and filled with pH fill solution.
3. The conductivity probe must be taken out of the holder, rinsed with deionized water, and gently wiped with a kimwipe on the outside only. NOTE: **DO NOT** wipe the black sensors on the inside of the probe as this will adversely affect the probes performance.
4. Rinse and wipe the metal turbidity sample tube. Check the plastic tubing that goes from the metal turbidity sample tube to the turbidimeter for anything that might affect the result.

This tubing should be changed on a monthly basis or earlier if needed.

5. Fill the DI water reservoir at the back of the instrument to the "fill" line and also empty the two waste containers (check the pH of the waste, if it is neutral then it can be poured down the sink. If the waste is either acidic or alkaline then dispose of the waste in the appropriate satellite container.)
6. Fill the Alkalinity titrant bottle with 0.02N H_2SO_4 titrant.

B. Calibration

pH Calibration

1. The pH calibration is done daily. First, click the PC Titrator icon  on the desktop to open the software. Next, select "Run Titration" from the drop-down menu under the Titration tab (see Figure 4).
2. When the "Run Titration" screen opens, click on the "Add x Rows" button and type in the number of rows needed. For the pH calibration, normally 4 rows are added.
3. Double click on the "Schedule" box for Row1.
4. When the "Schedule Search Form" window opens select **pH only** from the "Available Selections" list and click "OK".
5. Repeat the last step for Rows 2, 4, & 5. Row 3 is similar except **pH calibration** is selected from the "Available Selections" list.
6. Next, click on the "Auto-Generate Order Number" button. The software will automatically assign an Order Number to the run, which is put in the "Order Number" box for row 1.
7. Click on & drag the order number until the entire column is full.
8. Type in a description of each sample in the "Sample Name" column. The first two rows are Blanks, the third row is the "pH Calibration", the fourth row is a LCS, and the last row is a "pH 7.00" calibration check.
9. Under the Vial column, type in "1" for row 1, "2" for row 2, "3" for row 3, "**6**" for row 4 (the pH cal for line 3 needs 3 vials), "7" for the last row. The pH timetable should mimic that in Figure 7.
10. Next, pour 45mL of deionized water into a conical 50mL Vial for each of the two BLANKS. Place the 2 vials into the tray holes marked 1 & 2.
11. Pour 45mL of a pH 4.00 buffer into a vial and place vial into hole #3.

12. Pour 45mL of a pH 7.00 buffer into a vial and place vial into hole #4.
13. Pour 45mL of a pH 10.00 buffer into a vial and place vial into hole #5.
14. Pour 45mL of LCS into a vial and place vial into hole #6.
15. Pour 45mL of a pH 7.00 buffer into a vial and place vial into hole #7.
16. Once the vials are all in the tray click the green "Start" button (Figure 7.) to start the calibration.
17. Once the calibration is complete, the system will verbally notify you whether the calibration passed or failed.
18. Print out the passing calibration and include a copy with the data report.

Turbidity Calibration

The Turbidity calibration is done for any of the following: new standards, new tubing, new turbidity lamp, or calibration standards fail to meet QA/QC requirements.

1. First, click the PC Titrator icon  on the desktop to open the software. Next, select "Run Titration" from the drop-down menu under the Titration tab (see Figure 4).
2. When the "Run Titration" screen opens, click on the "Add x Rows" button and type in the number of rows needed. For the turbidity calibration, normally 5 rows are added.
3. Double click on the "Schedule" box for Row1.
4. When the "Schedule Search Form" window opens select TURBIDITY from the "Available Selections" list and click "OK".
5. Repeat the last step for Rows 2, 4-6. Row 3 is similar except Turbidity Calibration is selected from the "Available Selections" list.
6. Next, click on the "Auto-Generate Order Number" button. The software will automatically assign an Order Number to the run, which is put in the "Order Number" box for row 1.
7. Click on & drag the order number until the entire column is full.
8. Type in a description of each sample in the "Sample Name" column. The first two rows are Blanks, the third row is the "Turb Cal", the fourth row is a LCS, the fifth row is a "2.0 NTU Std" and the last row is a "Blank".
9. Under the Vial column, type in "1" for row 1, "2" for row 2, "3" for row 3, "**11**" for row 4 (the Turbidity cal for line 3 needs 8

vials), "12" for the fifth row & "13" for the last row. The turidity timetable should mimic that in Figure 8.

10. Next, pour 45mL of deionized water into a conical 50mL Vial for each of the two BLANKS. Place the 2 vials into the tray holes marked 1 & 2.
11. Pour 45mL of deionized water (0.02 NTU Std) into a vial and place into hole #3.
12. Pour 45mL of 0.20 NTU Standard into a vial and place vial into hole #4.
13. Pour 45mL of 0.50 NTU Standard into a vial and place vial into hole #5.
14. Pour 45mL of 1.00 NTU Standard into a vial and place vial into hole #6.
15. Pour 45mL of 2.00 NTU Standard into a vial and place vial into hole #7.
16. Pour 45mL of 5.00 NTU Standard into a vial and place vial into hole #8.
17. Pour 45mL of 10.0 NTU Standard into a vial and place vial into hole #9.
18. Pour 45mL of 50 NTU Standard into a vial and place vial into hole #10.
19. Pour 45mL of LCS into a vial and place vial into hole #11.
20. Pour 45mL of 2.0 NTU Std into a vial and place vial into hole #12.
21. Pour 45mL of deionized water (Blank) into a vial and place into hole #13.
22. Once the vials are all in the tray click the green "Start" button (Figure 8) to start the calibration.
23. The calibration is a point-to-point calibration and has no pass/fail criteria. However, should any of the calibration equations have a negative slope then the calibration must be rerun.
24. Print out the calibration and include a copy with every relevant data report.

Conductivity Calibration

The Conductivity calibration is done when QA/QC samples fail to meet acceptable criteria or when a new standard is opened.

1. First, pour a fresh aliquot of 1412 µmhos/cm conductivity standard into a 50mL conical vial.

2. Immerse the conductivity probe into the vial containing the 1412 standard until a stable reading is achieved.
3. Press the "CAL/CLR" button twice on the Mantech 4510 Conductivity Meter.
4. Check at least one other conductivity standard to ensure the calibration is valid.
5. If the calibration is not valid repeat steps 1-4.

C. Building a Sequence

1. To build a sequence for the PC Titrator open up the Phoenix LIMS program, select "QA/QC Batching" from the Main Menu, and "PCT Sequence File" from the right hand list (see Figure 1).
2. The LIMS program will automatically list all pending non-soil samples from the WET3 & WET6 backlogs that require pH, turbidity, alkalinity, & conductivity (see Figure 2). The analyst has the choice of analyzing *just* the samples from WET3, *just* the samples from WET6, or the samples from *both* backlogs.
3. At this point any sample that is deemed "too dirty" to run on the PC Titrator can be unselected from the list and the necessary analysis can be completed using other approved methods.
4. To select a sample replicate simply click on a line that is designated "Rep-19" (19 is just an example as shown in Figure 2. The number will change to whatever vial number it is) and select a sample from the drop-down list. A replicate sample will be listed every tenth sample.
5. At this point click the "Build Sequence File" button at the bottom of the window. The file will be saved as the date (ie. 03-03-09A.txt) in the folder **P:/PCT**. When the new window opens and asks "Generate PCT Sequence File?" click "YES" and then "OK".
6. Before proceeding, ensure that the correct LCS lot numbers and true values are entered in the "LCS TV" tab (see Figure 3). These values are used to automatically calculate the percent recoveries during the data entry procedure.

D. Importing the Sequence

1. First, click the PC Titrator icon on the desktop to open the software. Next, select "Run Titration" from the drop-down menu under the Titration tab (see Figure 4).
2. Click on the "Load from Text File" button (see Figure 5), select the file you wish to import (ie. 03-03-09A.txt) and click "Open".
3. If a dilution is needed you must click on the Phoenix sample ID (ie. AR33245) and place a "space", dilution factor, & "x" after the sample ID (ie. AR33245 10x) so that the automatic data transfer program will calculate the correct result.
4. Once the sequence is ready to run, click the "Print Existing Timetable" button (see Figure 5). When the new window opens up (Figure 6) click the "Printer" option in the "Destination" box and then click the "Print" button. Press "Done" after the timetable prints out.

E. Loading the Sample Trays

1. Using the sequence timetable printout as a guide, pour the samples, blanks & QC samples into 50mL conical vials (up to the 45mL mark) and place the filled vials into the corresponding holes of the 122-position sample tray.

NOTE: Vial positions 121 & 122 are always reserved for turbidity cleaning solution & pH 4 buffer (to store probe) respectively.

2. If more than 120 vials are needed then the sequence will repeat starting again at vial position 1. The analyst must wait for the first set of duplicated vial numbers to finish being analyzed before replacing them with the second set.
3. Once the samples have been poured the instrument can be started simply by clicking the green "Start' button (see Figure 5).

F. Data Entry

Upon completing the samples the instrument will automatically print out the data (Water Quality Report) at the defaulted printer. Check the printout to ensure all QC parameters are met as defined in the QA/QC section of this SOP. If all the data passes the QA/QC requirements it can be entered using the Phoenix LIMS program:

1. First, double-click the "Copy PCT Data to Server" icon on the desktop of the Titrator computer. This will send the data to the server so it can be reviewed and entered.

2. From the LIMS Main Menu click the "Data Transfer" → "PCT Data Transfer" option (see Figure 9) to open up the data entry screen.
3. Select the file you are transferring. Generally, it is the very last file in the folder.
4. When the data file opens up the file will display the results with a checkmark in the "S" column for all pending analysis (unchecked boxes mean the sample is either already entered in Labworks or it is an unreportable QC sample). If a result is not needed, for any reason, uncheck the box.
5. Once all the data has been closely looked over & all the appropriate boxes have been checked, the analyst enters their initials in the space provided at the bottom of the screen and clicks the "Transfer Results to Labworks" button.
6. Click the "Print" button at the bottom left of the screen to print the results.
7. The Final Report will consist of the Run List, Sequence List, Calibrations, Raw Data (Water Analysis Report), & the Data Transfer Log. The completed report will then be put on the Wetlab Supervisor's desk for review.

VIII. Calculations

A. Multiply sample result by dilution factor where applicable.

B. Calculating RPD:
The RPD is the relative percent difference, and is a measure of the consistency of the sample replicate for the sample set. First, calculate the results for both the sample and the sample replicate. Then apply the two results to the following calculation:

$$\text{RPD, \%} = \frac{(R1 - R2)}{(R1+R2)/2} \times 100$$

where: R1 = value achieved for sample, mg/L
R2 = value achieved for sample replicate, mg/L

C. Calculating the LCS:

The LCS is the laboratory control standard and is a test of the accuracy of the calibration and the test overall. To calculate the %

recovery of the LCS, calculate the LCS sample, and then apply it to the following calculation:

$$\% \text{ Recovery}, \% = \frac{(RL)}{(LTV)} \times 100$$

where: RL = achieved value for LCS sample, mg/L
LTV = true value of LCS, mg/L

IV. Quality Control

A. All reagents and standards are to be labeled with the following information: date prepared, date expires, preparer's initials, analyte, contents, and concentration of contents. Record preparation of all reagents in the reagent prep logbook.

B. The primary pH buffers and calibration standards are poured fresh at each calibration.

C. A sample prep blank of dilution water must be analyzed with each batch of twenty or fewer samples, as well as at the end of every run. Prep blanks must be determined to be BRL (below reporting level).

D. A bought standard (LCS) is run with each batch of twenty or fewer samples. The acceptable recovery of a LCS is 85-115%.

E. Sample replicates are analyzed every 10 of fewer samples. The RPD must be $\leq$20%, else repeat sample and duplicate analysis to prove matrix effect.

V. Safety

A. The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material data handling sheets are available to all personnel involved in the chemical analysis.

B. Always wear safety glasses for eye protection as well as lab coats.

C. Refer to Phoenix SOP #805: Hazardous Chemical and Laboratory Safety Procedures

VI. Pollution Prevention

A. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation.
B. Standards should be purchased in volumes consistent with laboratory use to minimize the volume of expired standards to be disposed.

VII. Waste Management

A. It is the laboratories responsibility to comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

VIII. Method Performance

A. This method was validated through internal QA/QC monitoring, including annual method detection limit studies, precision and accuracy studies, initial and continuing calibration verifications, blank analysis, laboratory control samples and matrix spikes and duplicates.
B. See section IX Quality Control in this SOP for acceptable limits.

IX. Corrective Action for Out-of-Control or Unacceptable Data

A. Should the preparation blank, LCS or in-house standard fail acceptance criteria, reanalyze batch.
B. Should the sample duplicate analysis fail acceptance criteria, a non-conformance report must be generated or the sample QC must be reanalyzed.

X. References

A. Standard Methods for the Examination of Water and Wastewater, 22nd edition, Turbidity Method 2130B, Revised 2011.

B. Standard Methods for the Examination of Waste and Wastewater, 22nd edition, pH Method No. 4500-H^+ B, Revised 2011.

C. Standard Methods for the Examination of Waste and Wastewater, 22nd edition, Alkalinity Method No. 2320B, Revised 2011.

D. Standard Methods for the Examination of Water and Wastewater, 22nd edition, Conductivity Method 2510B, Revised 2011.

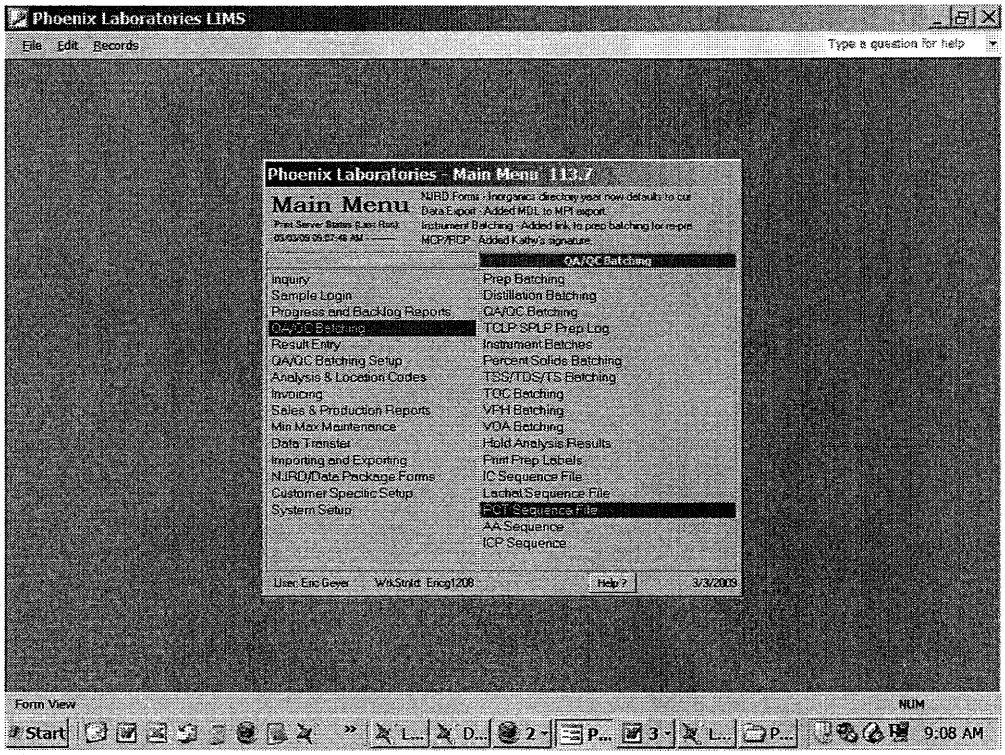

Figure 1.

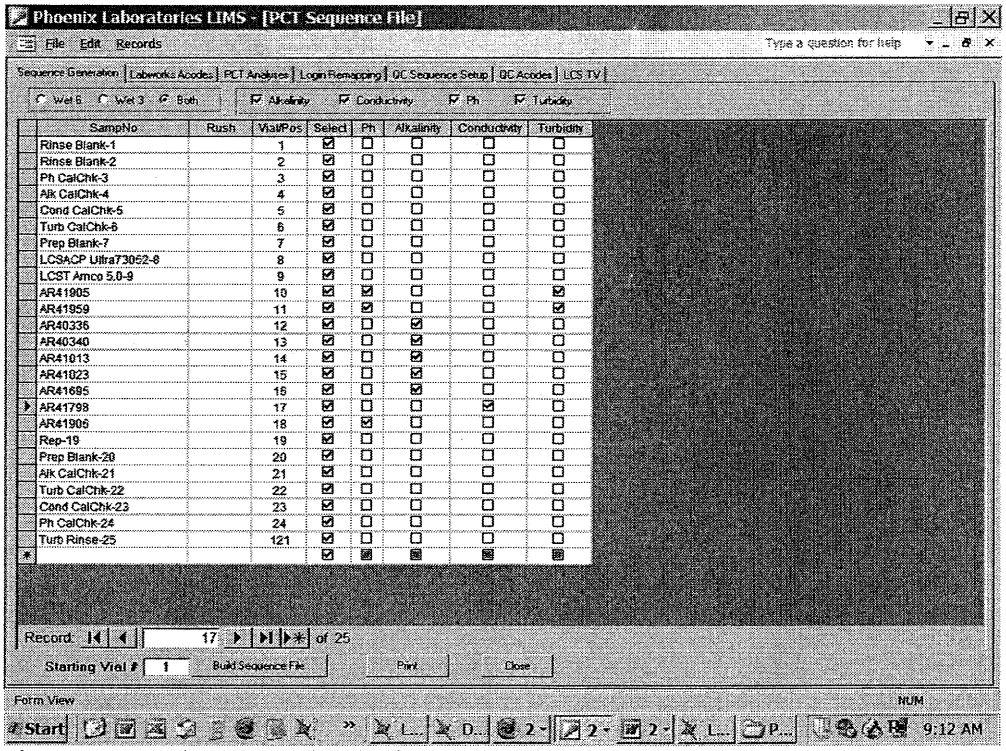

Figure 2. Sample & Analysis selection

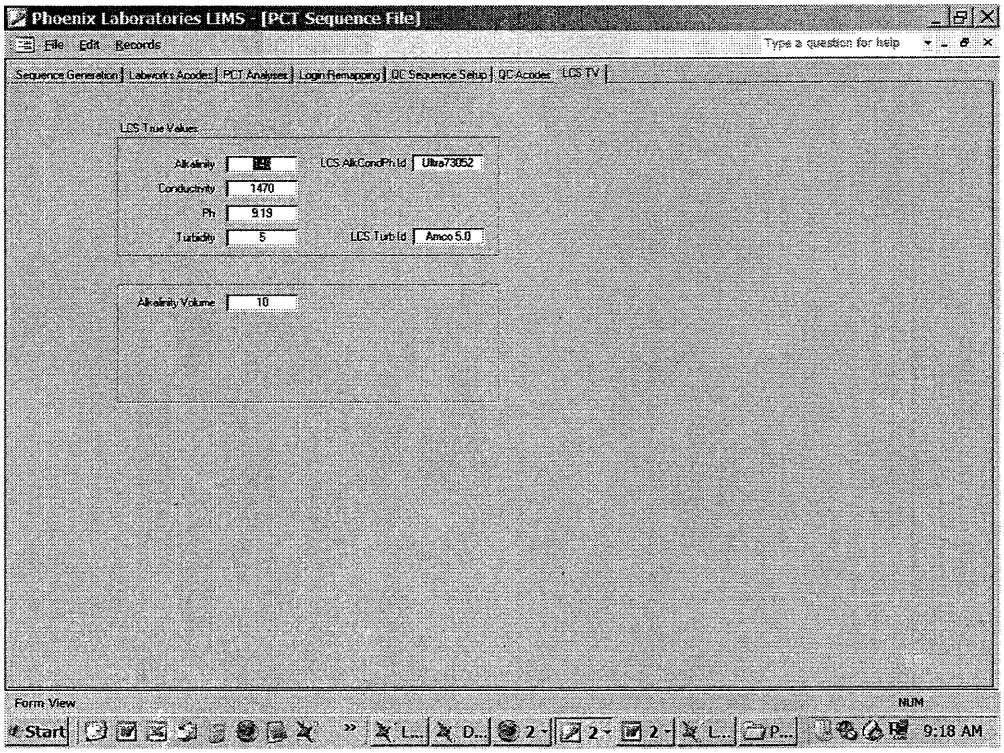

Figure 3. LCS Lot #'s and TV's

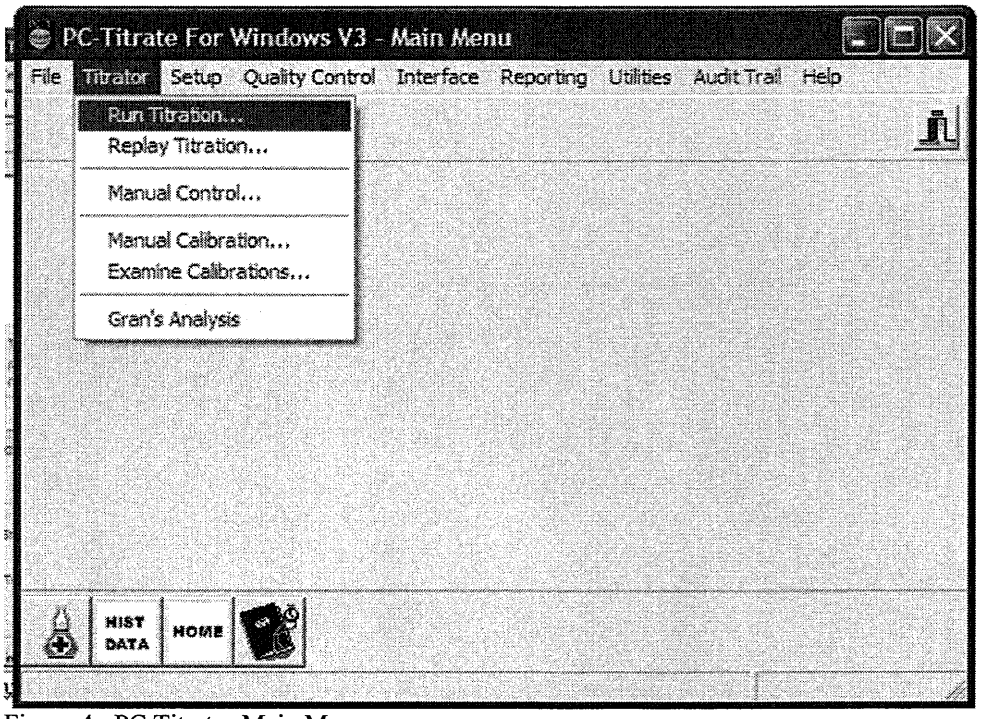

Figure 4. PC Titrator Main Menu

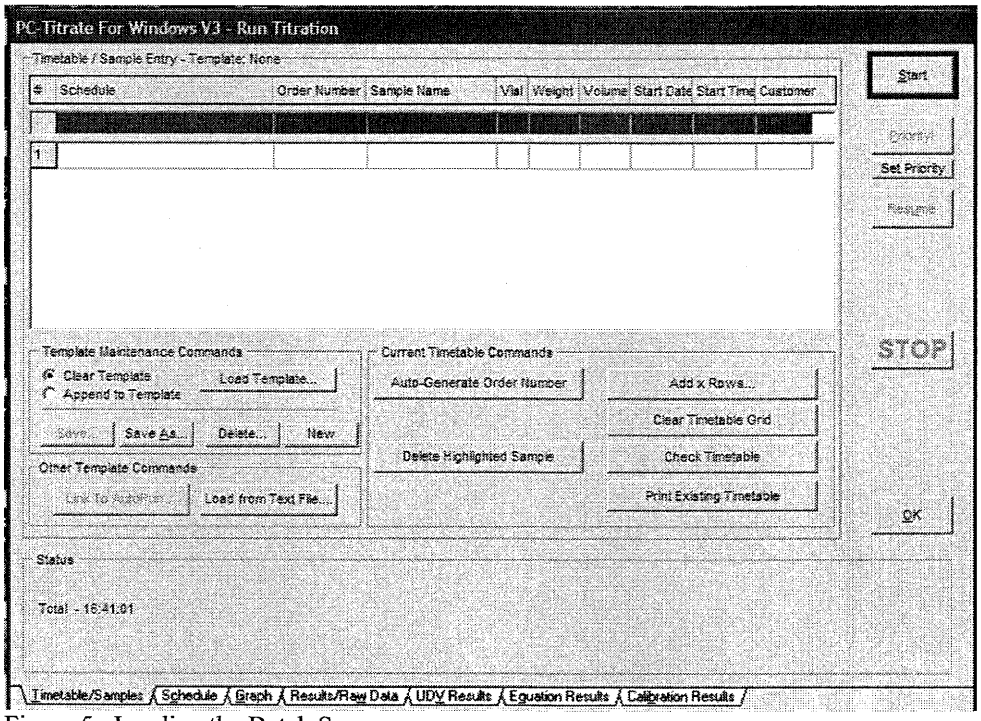

Figure 5. Loading the Batch Sequence

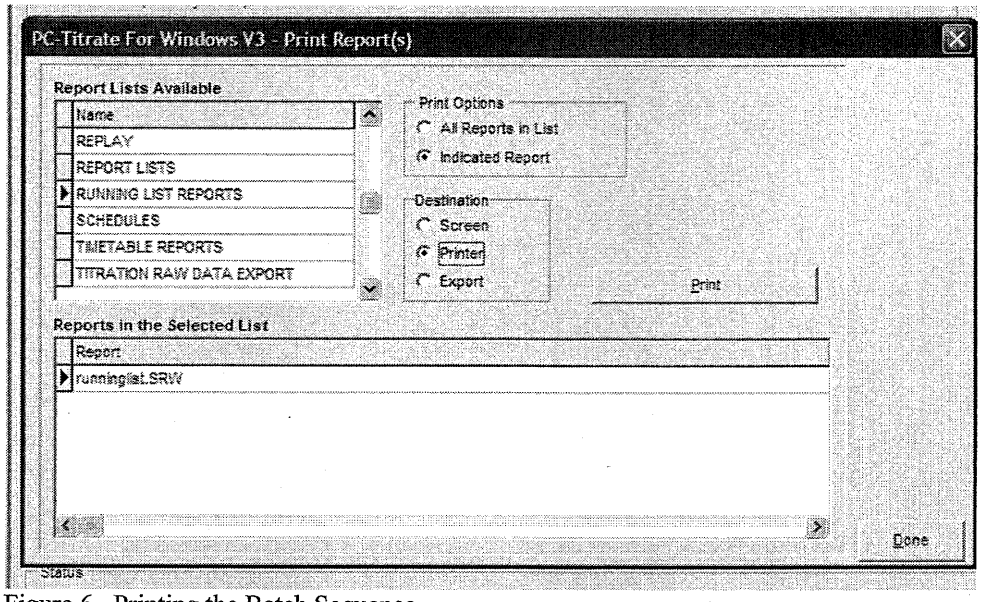

Figure 6. Printing the Batch Sequence

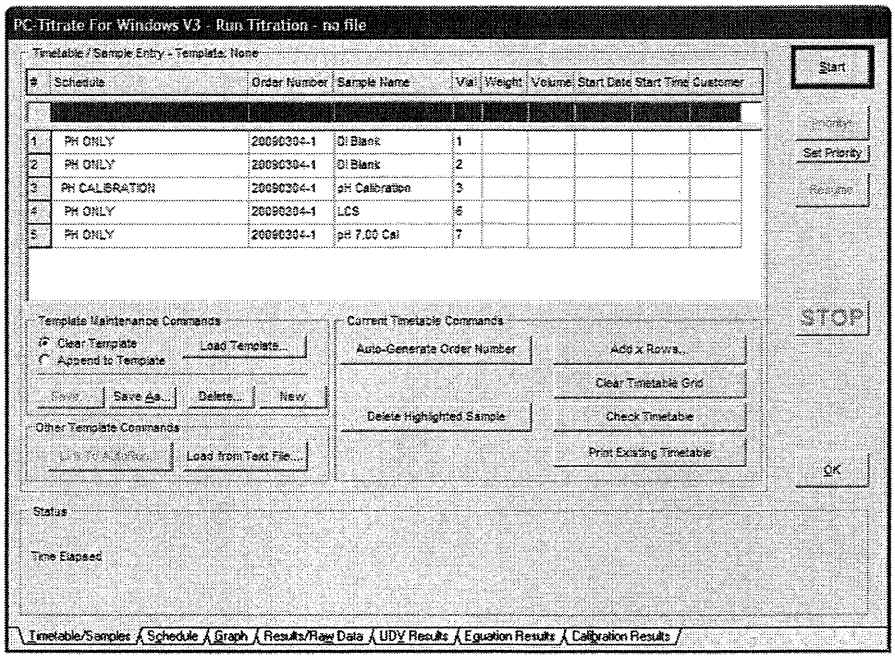

Figure 7. pH Calibration Timetable

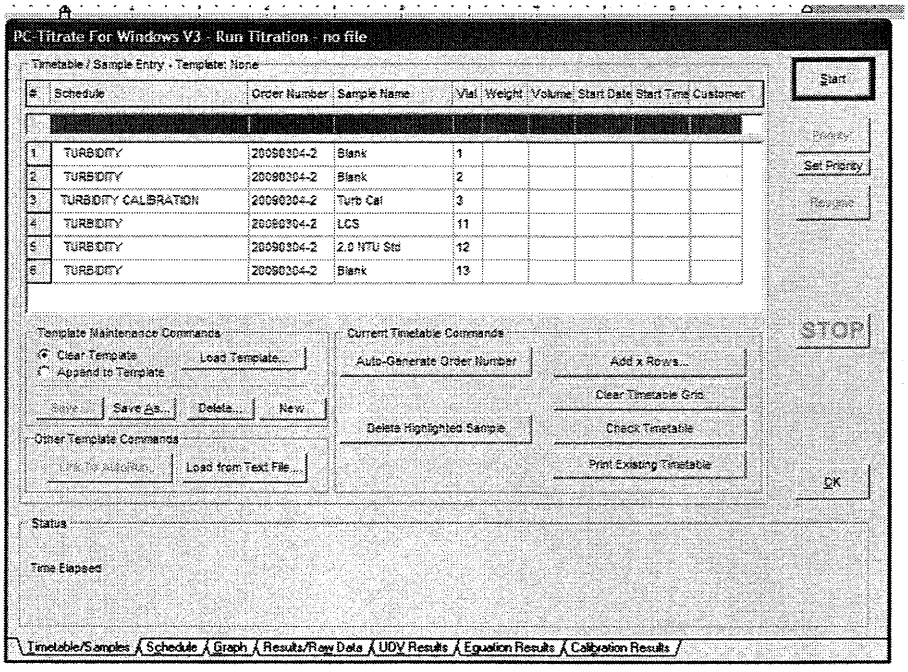

Figure 8. Turbidity Calibration Timetable

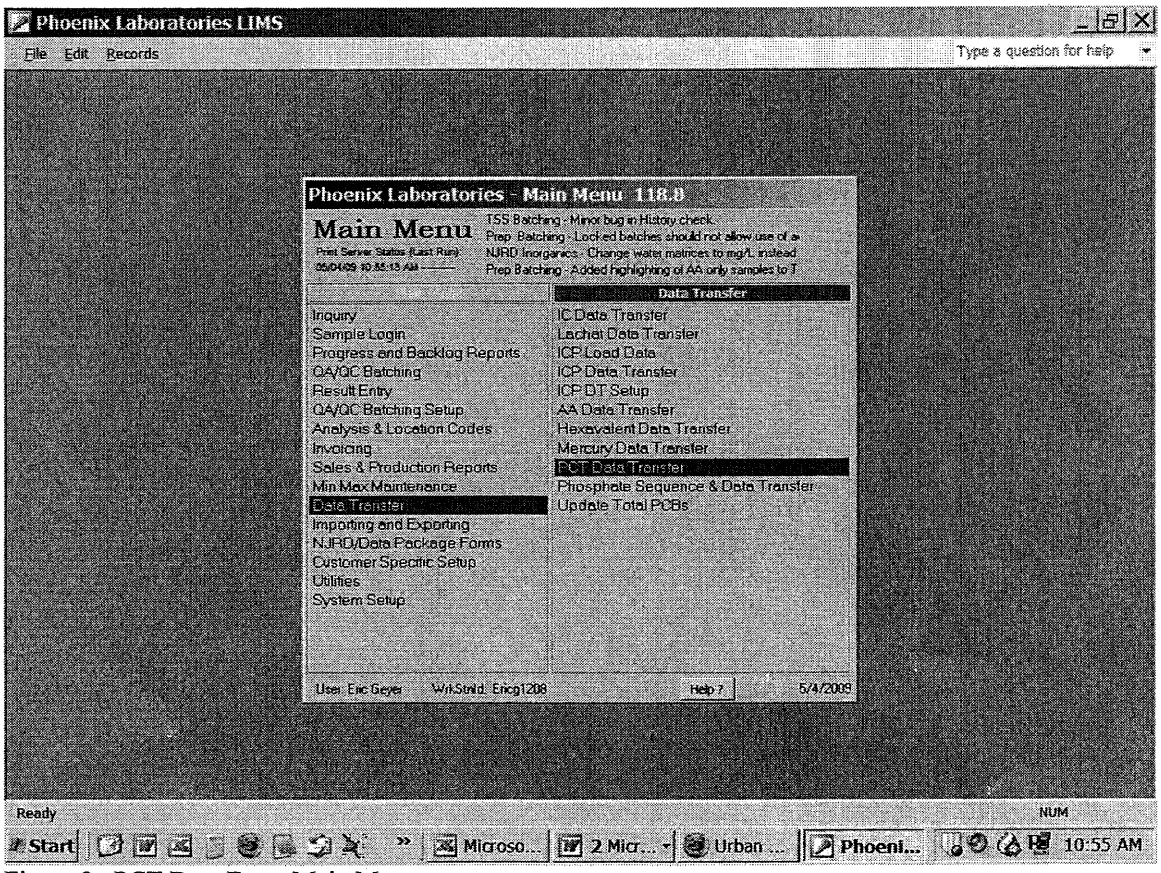

Figure 9. PCT Data Entry Main Menu

PCT Data Entry Instructions

Checking the Results Sheet

1) A (-1.00) on the results sheet indicates the sample was NOT analyzed for that analyte.
2) A pH of 5.80 – 6.10 MAY indicate a missing vial or is DI water (check rack to ensure a vial is present)
3) Brian will leave a note of which samples he did not run due to dirtiness or that he couldn't find.
4) Erratic Alkalinity results can be caused by a few different factors such as:
   - a leaking drain valve
   - a clogged sample line
   - no titrant
   - no fill solution in pH probe
   - sample valve is clogged
   - sample swaps

I don't expect you to fix these problems but it is important to be aware of them. Do not enter Alk results if the QC fails or if it appears any of these problems had occurred.

5) If a sample has a high turbidity result (over ~200) I usually do not enter the sample after it just in case there is contamination. If a sample is extremely high (~1000) do not enter any sample you feel has been contaminated (history should give you a good idea of what was contaminated).
6) Conductivity results are very reliable. The only problem we have sometimes is that the blanks come out slightly high (2.0 – 5.0) if they follow a sample with a high result. If this happens talk to Kathy or Greg L. I usually just raise the detection limit of the blank.

Entering results

1) In the Phoenix MDB click "Data Transfer → PCT Data Transfer"
2) Each analyte is entered separately (alk, cond, pH, & turb)
3) Click "Load Data" and select the two newest runs (2 highest numbers) & click "Load selected Data Files".

   The second highest run number is the beginning QC
   The highest run number is the subsequent samples and QC

The reason we split the beginning QC from the rest of the run is to ensure the system is running properly before we run it for 20 hours. Brian checks the beginning QC and will rerun anything that needs to in order to get the system 100% before starting the sample run.

4) Once the data loads you ill notice "check marks". These check marks indicate the sample has not been entered into labworks or that it is a non-reportable value (such as a calcheck). All pending results will default to be "checked".
5) Should a sample need a dilution, is contaminated, or should not be entered for any reason then it needs to be "unchecked".
6) Any sample that falls outside of past history will show the actual history in the right-hand column and will be in yellow. It is up to your discretion on whether these outliers should be entered or rerun.
7) Any failing QC will be hi-lighted in RED. Again it is your discretion on whether the failing QC warrants reruns.
8) Once all the appropriate data has been checked, enter the initials of the person who set the samples as well as your own in the provided field and click the "Transfer Data to Labworks" button.
9) Print out the Data using the print button at the bottom left corner.
10) Repeat steps 4 – 9 for each analyte.
11) Staple all sheets together. The report should include:
    a) Run list (make sure Brian wrote in the QC Lot #'s)
    b) Calibration curves for pH & turbidity
    c) Data sheets for the opening QC run & the sample run
    d) Data Transfer Logs for all analytes

LCS TV's for Ultra Lot# 77133 (must be 85% - 115% recovered):
pH = 9.10 (8.95 – 9.25)
conductivity = 510 umhos/cm (433 – 586)
Alkalinity = 226 mg/L (192 – 260)
Turbidity = 4.00 NTU (3.40 – 4.60)

CALCHK TV's (must be 90% - 110% recovered):
pH = 7.00 (6.85 – 7.15)
conductivity = 100 umhos/cm (90 – 110)
Alkalinity = 125 mg/L (112 – 138)
Turbidity = 5.00 NTU (4.50 – 5.50)

Highest reportable concentrations
(anything over these limits MUST be diluted) Leave Brian a note of dilutions that need to be run.
pH = no upper limit
conductivity = 10000 umhos/cm
Alkalinity = 2000 mg/L (sample must be run by hand)
Turbidity = 40 NTU

Revision History Log for SOP # 360

Date:	Revision #:	Summary of Changes:	Submitted By:	Approved By:	Effective Date:
3/7/18	2.1	Out of MDB, section V. A, B, C- new supplier and part numbers, V.O- new dilution for working solution, section VII.4- added metal to turbidity sample tube, section VII.B Calibration- Conductivity 1-5- new due to error in last SOP, section X- references updated per MUR	Kathy Cressia	Eric Geyer	3/7/18

Expiration Date

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Effective Date: 06/09/17
Version Number: 3.3
Initiated By:
Approved By:

SOP Number: 336.353.2

Title: Total Nitrite as Nitrogen / Total Nitrate as Nitrogen

Scope: This method is applicable for the determination of concentrations of nitrites and nitrates in drinking, surface, and ground water, domestic and industrial waste, and solid samples. This method is used for wastewater samples originating from states that do not approve the IC for wastewater samples. This method is applicable for nitrite levels of 0.01 - 0.75 ppm and nitrate levels of 0.02 - 2.5.0 ppm. Samples with nitrite levels in excess of 0.750 ppm and/or nitrate levels in excess of 2.50 ppm require sample dilution prior to analysis.

I. Summary of Method

A. Nitrate in a sample is reduced to nitrite by passage through a cadmium column in an alkaline environment. The resulting nitrite is combined with a sulfanilamide color reagent, which produces a strong pink color. The intensity of the pink color is proportional to the amount of nitrite in the sample.

B. To determine the amount of Nitrate in the sample it is necessary to subtract concentration of ambient nitrite from the concentration of transformed nitrite.
NO_3 = (NO_2 after Cd) – (NO_2 before Cd)

C. All samples that require a nitrate result require analysis of nitrite as well, regardless of whether or not it is requested by the client.

II. Sample Collection, Preservation and Storage

A. Samples are collected in clean plastic or glass containers.

B. Samples are not preserved and need to be analyzed within 48 hours from time of collection. If the Nitrite analysis is completed within the 48-hr hold time, the sample may be preserved with sulfuric acid and stored for up to 28 days at 4°C prior to completion of nitrate analysis.

C. Samples that are preserved with sulfuric acid cannot be used to test for nitrite. Furthermore, if there is no nitrite result to be subtracted form the "NO_2 after Cd" result, then no determination can be made as to how much of the "NO_2 after Cd" result was present as NO_2 initially. The best the lab can do to accommodate these clients is to report the combined result of nitrate and nitrite in a sample; that is, to report the "NO_2 after Cd" result or the "NO_2NO_3" result.

III. Interferences

A. Oxidizing agents such as Residual Chlorine may interfere by oxidizing the cadmium column. Remove residual chlorine by adding Sodium Thiosulfate ($Na_2S_2O_3$) solution.

B. EDTA is added to the buffer to reduce the negative interference of high concentrations of iron, copper, and other metals.

C. Samples that contain high concentrations of oil and grease can coat the surface of the cadmium, reducing its reducing capabilities. Extracting oils from the sample using an organic solvent prior to analysis can eliminate this interference.

D. As with all colorimetric analysis, turbidity or suspended matter can be an interference. Remove by filtering sample through 0.45um pore diameter membrane filter prior to analysis.

IV. Equipment and Supplies

A. Lachat QuikChem 8500 with both Nitrite and Nitrate Manifolds.

B. Cadmium column, Lachat Part #50237.

C. Refer to Lachat SOP #302 for consummables.

V. Reagents and Standards

A. Sodium Hydroxide, 6N. Borrowed from ammonia or phosphate method. Since we do not use a lot of this reagent, it is not worth having a sequestered reagent solely for the preparation of Ammonium Chloride Buffer for Nitrate analysis. (NOTE: When preparing 6N NaOH, decant some from the liter bottle into a separate receptacle. This will reduce the risk cross-contamination between analyses).

B. Ammonium Chloride Buffer. Into a 2-Liter volumetric flask, dissolve 170g Ammonium Chloride and 2.0g EDTA in about 1 liter DI water. Bring up to volume with DI water. Adjust the pH to 8.50+/-0.05 w/ 6N NaOH using a pH meter.
When adjusting pH of the buffer, use a separate receptacle to hold the buffer as the pH is taken. To reduce risk of contamination of the buffer from the pH probe, do not immerse the probe directly into the 2 liters of buffer.

C. Sulfanilamide Color Reagent. (NOTE: This reagent is good for both NO_2/NO_3 and NO_2.) Into a 1-Liter volumetric flask, dissolve 40g Sulfanilamide and 1.0g NED (N-(1-naphthyl)ethylenediamine dihydrochloride) in around 500mL DI water containing 100mL Phosphoric acid. Bring up to volume with DI water. Store in a dark bottle at 4°C. Prepare fresh monthly.

D. Nitrite Stock Solution, 1000ppm. RICCA Catalog # 5445-16 or equivalent.

E. Nitrite Standard Solution, 10ppm. Dilute 10mL Nitrite Stock Solution to 1 liter with DI water. Prepare fresh as needed, up to 3 months.

F. Nitrate Stock Solution, 1000ppm. RICCA Catalog # 5307-16 or equivalent.

G. Nitrate Standard Solution, 10ppm. Dilute 10mL Nitrate Stock Solution to 1 liter with DI water. Prepare fresh as needed, up to 6 months.

H. Nitrite LCS Stock Solution, 1000ppm, Environmental Express Cat # IC-N-M.

I. Nitrite Working LCS, 0.25ppm, 0.205mL of Nitrite Stock LCS into 250mL DI.

J. Nitrate LCS Stock Solution, 1000ppm, Environmental Express Cat # IC-NO-M.

K. Nitrate Working LCS, 1.00ppm, 1.106mL of Nitrate Stock LCS into 250mL DI.

L. **NOTE:** The Nitrite and Nitrate Working Standards in this SOP can be combined together along with the Chloride Working Standards of SOP 346 and the ortho-Phosphate Working Standards of SOP 355 to form a set of "4-point" Working Standards.

Nitrite Working Standards A-F. Prepare fresh as needed, at least monthly.

A. Dilute 37.5mL Nitrite Standard Solution to 500mL with DI water; t.v.= 0.750ppm
B. Dilute 25mL Nitrite Standard Solution to 500mL with DI water; t.v.= 0.50ppm
C. Dilute 12.5mL Nitrite Standard Solution to 500mL with DI water; t.v.= 0.25ppm
D. Dilute 5.0mL Nitrite Standard Solution to 500mL with DI water; t.v.= 0.10ppm
E. Dilute 2.5mL Nitrite Standard Solution to 500mL with DI water; t.v.= 0.05ppm
F. Dilute 50mL of Nitrite Working Standard "D" to 500mL with DI water; t.v.= 0.01ppm

Nitrate Working Standards A-F. Prepare fresh as needed, at least monthly.

A. Dilute 87.5mL Nitrate Standard Solution to 500mL with DI water; t.v.= 1.75ppm
B. Dilute 50mL Nitrate Standard Solution to 500mL with DI water; t.v.= 1.00ppm
C. Dilute 25mL Nitrate Standard Solution to 500mL with DI water; t.v.= 0.50ppm
D. Dilute 5.0mL Nitrate Standard Solution to 500mL with DI water; t.v.= 0.10ppm
E. Dilute 2.5mL Nitrate Standard Solution to 500mL with DI water; t.v.= 0.05ppm
F. Dilute 50mL of Nitrate Working Standard "D" to 500mL with DI water; t.v.= 0.01ppm

M. Carrier is DI water.

VI. Procedure

A. Refer to SOP #302 on use of the Lachat QuickChem 8500 for general information on the operation of the instrument. This method includes information unique to the nitrite and nitrate chemistries.

B. Attach manifold for Nitrite chemistry.

C. Attach manifold for Nitrate chemistry. (NOTE: Be sure to keep the Cadmium column closed until buffer is running through the lines. Efficiency of the reducing capacity of the cadmium reduces as it is exposed to air. Once the reagents are flowing through the manifold to the waste line, it is safe to open the column by turning the dial next to the Cd column on the manifold).

D. At this time, make a visual assessment of the Cadmium column. Check for air bubbles, lines, gaps in the column or any change in the cadmium surface characteristics (Cd granules should appear dark gray). If air bubbles cannot be dislodged by ramping the pump speed and tapping on the column, then the column needs to be replaced with a new one. Keep at least two prepared columns on hand at any time. See the QC section for assessing the efficiency of the column.

E. Load background.

F. Create a worksheet for the samples to be analyzed, including all QC requirements. The two standards for the cadmium column efficiency check (see IV.E.) should be the first two samples on each run (The column efficiency needs to be >90% to pass). Check standards will be

included automatically after 10 samples. They must pass within 90-110% of true value, otherwise the system recognizes the error and stops automatically.

G. Start standards and samples.

H. If the correlation coefficient is at least 0.9975 and the RPD values are at 10% or less, the calibration passes. It may be necessary to remake standards.

I. After samples have been analyzed, any dilutions needed have been run, and all QC criteria have been met, print out calibration statistics, and staple them along with the chart records to the sample report. Highlight all reported results on the report sheet. Leave the run on the WetChem supervisor's desk for review.

J. Close the Cd column.

K. Run DI water through the manifolds for 10 minutes to wash out any chemicals that might dry, encrust the tubing and cause blockages the next time. Remove all the tubes from the DI water and dry out the tubes by allowing the pump to run for an additional 10 minutes.

VII. Definitions

A. Laboratory Control Sample (LCS)- A purchased standard of a known concentration that is from a different source than the calibration standards. The LCS is used to determine laboratory performance.

B. Preparation Blank (Prep Blank)- An aliquot of reagent water that is treated exactly as a sample. The prep blank is used to determine if method analytes are present in the lab environment, reagents or glassware.

C. Calibration standard- A solution prepared from the dilution of stock standard solutions. The calibration solutions are used to calibrate instrument response with respect to analyte concentration.

D. Matrix Spike (MS)- An aliquot of environmental sample to which a known quantity of the method analytes are added in the laboratory. The MS is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results.

VIII. Calculations

A. Calculating Nitrate:
It is necessary to subtract the result from Nitrite analysis (done separately) from the result off the Lachat (NO_2+NO_3) to get a value for Nitrate.

$NO_3 = (NO_2NO_3) - (NO_2)$

Where: NO_3 = final result for Nitrate as N, ppm
NO_2NO_3 = result from Lachat, ppm
NO_2 = result from Lachat, ppm

NOTE: Do not subtract negative NO2 values. Only positive NO2 values are subtracted.

B. Calculating RPD:
The RPD is the relative percent difference, and is a measure of the consistency of the sample replicate for the sample set. First, calculate the results for both the sample and the sample replicate. Then apply the two results to the following calculation:

$$\text{RPD, \%} = \frac{(R1 - R2)}{(R1+R2)/2} \times 100$$

where: R1 = value achieved for sample, mg/L
R2 = value achieved for sample replicate, mg/L

C. Calculating the sample spike recovery:
The sample spike is a test if the sample matrix is suitable for accurate testing. To calculate the % recovery, first calculate both the sample and the spiked sample. Then apply the two results to the following calculation:

$$\text{\% Recovery, \%} = \frac{(SR - R)}{STV} \times 100$$

where: SR = value of sample with added spike, mg/L
R = value of sample, mg/L
STV = true value of spike added to sample, mg/L

D. Calculating the LCS:
The LCS is the laboratory control standard and is a test of the accuracy of the calibration and the test overall. To calculate the % recovery of the LCS, calculate the value of the LCS sample, and then apply it to the following calculation:

$$\text{\% Recovery, \%} = \frac{(RL)}{(LTV)} \times 100$$

where: RL = value for LCS sample, mg/L
LTV = true value of LCS, mg/L

IX. Quality Control

A. Prep Blank must be analyzed every batch of 20 or fewer samples. This is a DI water aliquot treated like a sample, undergoing all treatments the samples undergo. The prep blank must yield levels of nitrite and nitrate below the reporting level. The minimum detectable limit is 0.01mg/L for both nitrite and nitrate.

B. Laboratory Control Standards must be analyzed every batch of 20 or fewer samples. This standard comes from RICCA (or equivalent second source) and has a certified value. It must be recovered 90-110%. A failing LCS for a batch of samples requires re-analysis of the batch.

C. Continuing calibration checks must be analyzed every 10 samples, as is written into the software of the Lachat QuickChem 8000. They must pass within 90-110%. The calibration check for both nitrite and nitrate is the C standard from the calibration curves.

D. A Cadmium column efficiency check must be analyzed at the beginning of the run. Run a second source, 0.5 ppm Nitrate only standard through the column. Run a second source, 0.5 ppm Nitrite only standard through the column. The column efficiency is determined by this calculation: $\frac{\text{Nitrate peak Area}}{\text{Nitrite peak Area}} \times 100 = \%\ \text{Efficiency}$

The acceptable limits for this standard is +/-10%. If the % Efficiency result is outside of this range, the column must be repacked or replaced.

E. Sample replicates must be done every batch of 20 or fewer samples. They must have a RPD of no more than 20%.

F. Sample spikes must be done every batch of 10 or fewer samples. A known amount of nitrate is added to a sample to test the sample matrix. The spike need be recovered 90-110%, however, if recoveries fall outside of this range, and the LCS and Blank spike are acceptable, it is judged to be sample related and not system related. Report % recovery.

G. MDL studies are performed annually or when a different analyst is performing the analysis. Seven replicates of a 0.05 mg/L standard are analyzed for both nitrite and nitrate.

H. A Limit of Detection (LOD) study must be analyzed annually. All sample processing steps of the analytical method shall be included in the determination of the LOD. The LOD study is achieved by analyzing a standard at no more than 2-3x the LOD. In many cases, a 1:1 dilution of the lowest calibration standard is prepared and analyzed. LOD recoveries must be 50 – 150%.

I. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by ± 10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

J. An initial demonstration of capability (IDOC) must be performed to prove the generation of acceptable data with regard to accuracy and precision. An initial demonstration must be performed by each analyst and consists of analyzing 4 LCS solutions, usually over a series of days.

K. Standards preparations are documented in the Standards Prep Logbook.

L. Reagent preparations are documented in the Nitrate/Nitrite Reagent Prep Logbook.

M. Lachat use and maintenance is documented in the Lachat Maintenance Logbook.

X. Safety

A. The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material data handling sheets are available to all personnel involved in the chemical analysis.

B. Always wear safety glasses for eye protection as well as lab coats.
C. Refer to Phoenix SOP #805: Hazardous Chemical and Laboratory Safety Procedures

XI. Pollution Prevention

A. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation.
B. Standards should be purchased in volumes consistant with laboratory use to minimize the volume of expired standards to be disposed.

XII. Waste Management

A. It is the laboratories responsibility to comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

XIII. Method Performance

A. This method was validated through internal QA/QC monitoring, including annual method detection limit studies, precision and accuracy studies, semiannual proficiency tests, initial and continuing calibration verifications, blank analysis, laboratory control samples and matrix spikes and duplicates.
B. See section IX Quality Control in this SOP for acceptable limits.

XIV. Corrective Action for Out-of-Control or Unacceptable Data

A. Should the calibration curve have a correlation coefficient of <0.9975, remake and reanalyze curve before processing samples.
B. Should the preparation blank, LCS or inhouse standard fail acceptance criteria, do not proceed. Find problem and reanalyze. All samples must be bracketed by passing Cal checks.
C. Should the matrix spike or sample duplicate analysis fail acceptance criteria, a non-conformance report must be generated or the sample QC must be reanalyzed.

XV. References

A. Nitrate by Automated Cadmium Reduction, Method 353.2, Methods for the Chemical Analysis of Water and Wastes, EPA 600 Series, Revision 2, 1993.
B. Nitrate Nitrogen by Automated Cadmium Reduction, Method 4500-NO3 F, Standard Methods for the Examination of Water and Wastewater, 22nd edition, 2000.
C. Nitrate/Nitrite Analysis, Method 10-107-04-1-C, Lachat Instruments QuikChem 8000, July 1995.
D. Nitrite Analysis, Method 10-107-05-1-O, Lachat Instruments QuikChem 8000, July 1995.

Revision History Log for SOP # 336

Date:	Revision #:	Summary of Changes:	Submitted By:	Approved By:	Effective Date:
2/12/15	3.1	Scope= new curve ranges, added new sections out of Summary, Sec. V.H adjusted A Std to reflect new range, added Sections VII, X – XIV, took Cd Column efficiency calc out of Sec. VIII, added D, H, I, J to Sec. IX, updated SM Reference in Sec. XV.	Kathy Cressia	Eric Geyer	2/12/15
5/10/17	3.2	Sec VIII.A- added "NOTE" to calculation, sec IX.D- updated Cd column efficiency check and added calculation, Sec IX.F- changed MS frequency to 10%, Sec XV.A- updated method revision and year	Kathy Cressia	Eric Geyer	5/10/17
6/9/17	3.3	Section IX.D- updated calculation based on area, section V.D, F, H-K- new sources of stock solution and second source	Eric Geyer	Kathy Cressia	6/9/17

Expiration Date

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Effective Date: 07/26/19
Version Number: 8.5
Initiated By: Kathy Russia
Approved By:

SOP Number: 304.NH3/TKN

Title: Ammonia/TKN Phenate Method

Scope: This method covers the determination of ammonia, organic nitrogen, and Kjeldahl nitrogen in drinking, surface and saline waters, domestic and industrial wastes, soils and leachates. The working range for water samples is 0.05-8.0 mg/L, and 2.0-400 mg/kg for solids. Higher concentrations may be determined by sample dilutions.

I. Summary of Method

A. The sample is buffered at a pH of 9.5 with borate buffer in order to decrease hydrolysis of cyanides and organic nitrogen compounds, and then distilled into a sulfuric acid solution. The ammonia ion in the sulfuric acid solution is then determined by colorimetric analysis. This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction produced is measured at 630nm, and is directly proportional to the original ammonia concentration.

B. The determination of organic nitrogen (ORGN) is based on the ammonia (NH3) and TKN results of a sample. ORGN conc. = TKN conc. – NH3 conc. Using this relationship, it is also possible to determine the concentration of TKN, when the ORGN and NH3 are known.

II. Interferences

A. When present, residual chlorine must be removed by pretreatment of the sample with sodium thiosulfate before distillation. During sample development calcium and magnesium ions may precipitate if present in sufficient concentrations. EDTA is added to the sample in-line in order to prevent this problem. Distillate turbidity may interfere and can be removed by manual filtration.

III. Sample Collection, Preservation and Storage

A. Samples can be collected in plastic or glass. They must be stored at 4°C until time of analysis.

B. Samples must be H_2SO_4 preserved to pH 1.5-2 and analyzed within 28 days of collection.

IV. Equipment and Supplies

A. Kjeldahl tubes.
B. Snap Cap, 70mL with 50mL lines.
C. Graduated cylinders, 50 mL.
D. Lachat QuickChem 8000 autoanalyzer with XYZ autosampler.
E. Digestion heating block with temperature control.
F. Reflux condenser distillation unit with temperature control.

V. Reagents and Standards

(NOTE: Use only reagent grade chemicals for standards and reagents)

A. Ammonia Stock Solution (1.0 ml=1000mg NH3-N): Dissolve 3.819g reagent grade ammonium chloride (NH4CL), that has been oven dried and desiccated, in DI water and bring to volume in a 1 liter volumetric flask.
B. Intermediate Ammonia Standard (1.0 ml=20 mg NH3-N): Dilute 20.0mL ammonia stock solution in 1-liter volumetric flask. Bring up to volume with DI water.
C. Ammonia Spiking Solution (1.0 ml=100 mg NH3-N): Dilute 100mL ammonia stock solution in 1-liter volumetric flask. Bring up to volume with DI water.
D. Ammonia Working Standards:

Std. ID	mL of Inter. Std.	Dilute to	Concentration
A	80	200mL	8.0 mg/L
B	50	250mL	4.00 mg/L
C*	40	500mL	1.60 mg/L
D	5	250mL	0.40 mg/L
E	1.0	200mL	0.10 mg/L
F	0.5	200mL	0.05 mg/L
G	0	250mL	0.00 mg/L

Note: All working standards are to be diluted and taken to volume with DI water. They are to be 1:1 H2SO4 preserved. *Working standard C is the calibration check standard.

E. Sodium hydroxide solution, 6 N: While stirring in a cold water bath, dissolve 240g Sodium Hydroxide (NaOH) in 1 liter of DI water.
F. Sodium hydroxide solution, 0.2 N: In a 1-liter volumetric, dissolve 8g of Sodium Hydroxide (NaOH) in 800mLs of DI water. Dilute to volume and mix.
G. Borate buffer: Add 44mls of 0.2 N NaOH solution to 500 ml of DI water. Add sodium tetraborate (2.5g anhydrous $Na_2B_4O_7$ or 9.50g $Na_2B_4O_7$ *$10H_20$) and dilute to 1 liter.
H. Sulfuric Acid Solution, 1.0N, to about 800mL DI water, add 28mL concentrated H_2SO_4,then bring to a final volume of 1L with DI. Invert 3 times.
I. Sulfuric acid solution, 0.02N: To about 800mL DI water, add 20mL 1.0N H_2SO_4 (see N above). Dilute to 1L with DI water.
J. Phenolphthalein Indicator Solution: Dissolve 5.0g phenolphthalein in 500mL 95% ethyl or isopropyl alcohol and dilute to 1L with DI water.
K. Sodium Phenolate: In a 1 liter volumetric flask, dissolve 88ml of 88% liquefied phenol or 83g crystalline phenol in approximately 600ml of DI water. While stirring, slowly

add 32g sodium hydroxide. Allow to cool, then dilute to volume and mix. **CAUTION: WEAR GLOVES! Phenol causes severe burns and is rapidly absorbed through skin!**

L. Sodium Hypochlorite: In a 500-ml volumetric flask, dilute 250ml "Regular Strength" bleach (5.25% NaOCl) to volume with DI water. (NOTE: DO NOT use "Industrial Strength" or "Ultra Strength" Bleach as they contain higher concentrations of NaOCl.)

M. Buffer Solution: In a 1-liter volumetric flask, dissolve 50.0g disodium ethylenediamine tetraacetate (Na_2EDTA) and 5.5g sodium hydroxide in about 900mls DI water. Dilute to volume and mix.

N. Sodium Nitroprusside: In a 1-liter volumetric flask, dissolve 3.50g sodium nitroprusside (sodium nitroferricyanide) and dilute to volume with DI water. **CAUTION: WEAR GLOVES! Health Hazard -- Affects the Central Nervous System.**

O. Carrier: DI water.

P. TKN Digestion Reagent: Dissolve 134g K_2SO_4 and 7.3g $CuSO_4$ in 500mL DI water. Slowly add 134mL concentrated H_2SO_4. Bring to a final volume of 1L with DI water.

Q. TKN Sodium Hydroxide-thiosulfate reagent: Slowly dissolve 500g NaOH and 25g $Na_2S_2O_3*5H_2O$ in water and dilute to 1 liter with DI water. **Caution: Prepare this reagent in a cooling waterbath, as it is exothermic.**

VI. Definitions

A. Laboratory Control Sample (LCS)- A purchased standard of a known concentration that is from a different source than the calibration standards. The LCS is used to determine laboratory performance.

B. Preparation Blank (Prep Blank)- An aliquot of reagent water that is treated exactly as a sample. The prep blank is used to determine if method analytes are present in the lab environment, reagents or glassware.

C. Calibration standard- A solution prepared from the dilution of stock standard solutions. The calibration solutions are used to calibrate instrument response with respect to analyte concentration.

D. Matrix Spike (MS)- An aliquot of environmental sample to which a known quantity of the method analytes are added in the laboratory. The MS is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results.

VII. Procedure

A. Samples are preserved with H_2SO_4 to pH of <2 at time of sampling, and stored at 4°C until time of analysis. Samples to be determined for ammonia or TKN have a holding time of 28 days from time of collection.

B. Sample screening:

1. Verify pH and record in Ammonia Prep Batching Program.
2. If residual chlorine is present, destroy with Sodium thiosulfate. Record check in Ammonia Prep Batching Program.

C. Ammonia distillation:

1. Rinse Kjeldahl tubes with DI water. Measure 50mL of aqueous sample (or 0.01g of soil diluted to 50mL DI water) into a Kjeldahl tube containing boiling stones. Add 5mL borate buffer, 2-3 drops of phenolphthalein, and enough 6N NaOH to turn the sample light pink. If too much 6N NaOH was added, add 0.02N H_2SO_4 drop-wise until sample is light pink. Phenolphthalein is light pink at a pH of 9.5; this is the pH at which the distillation should occur. Add 1.0mL of Ammonia Spiking Solution to all matrix and blank spike tubes (T.V.= 2.0mg/L). Label the tubes with sample ID number and sample volume using a permanent marker. Record information in the Ammonia - TKN batching program.
2. Place Kjeldahl tubes containing prepared sample onto the distillation heating block, and attach reflux unit. Turn on condenser water.
3. Label clean snap cap tubes with appropriate sample ID number and sample dilution information, and add 10mL 0.02N H_2SO_4 to each tube. Place the snap cap under the condenser, and raise it so that the tip of the condenser is below the surface of the sulfuric acid, but not touching the bottom.
4. Turn on the block and set upper temperature limit to 300°C.
5. Some sample matrices require the addition of an anti-foaming reagent. If a sample begins to bubble and climb up the sides of the tube, add a few drops of antifoam. If a sample is suspected or expected to foam, antifoam may be added as part of sample pretreatment.
6. After 40mL has been distilled, remove snap caps from under the condensers, and bring up to 50mL with DI water. Refrigerate at 4°C until time of color development on Lachat.
7. If sample requires TKN or organic nitrogen determination, save the remainder of sample in the Kjeldahl tube for digestion.

D. Organic Nitrogen/TKN digestion:

1. If samples require NH3 & TKN (or ORGN only), perform ammonia distillation procedure as described in III.B. above, discard distillate, and allow the remainder of the sample in the Kjeldahl tube to cool. If only TKN is requested, place 50mL sample in a clean Kjeldahl tube containing boiling stones.
2. Move to TKN digestion heating block, carefully add 10mL TKN digestion reagent to Kjeldahl tube. Add 1.0mL of Ammonia Spike Solution to all matrix and blank spike tubes (T.V. = 2.0mg/L). Do not attach reflux unit. Turn on block and set upper limit temperature to 400°C. Boil under a ventilation hood until copious white fumes are observed. Continue to digest for another 30 minutes after copious fumes are observed and after the temperature is at least 375°C.
3. Allow samples to cool up to 2 minutes, and dilute with 50mL DI water (NOTE: If samples are allowed to cool longer than 2 minutes, the residue has a tendancy of sticking to the bottom of the tube even with vortexing). Vortex to mix. Make sure that boiling chips are free from the dried slurry in the bottom of the tubes and that the acid residue is dissolved into the water!
4. Move to distillation heating block.

5. Tilt tube away from personnel, towards the back of the hood, and carefully add 10mL sodium hydroxide-thiosulfate reagent to form an alkaline layer at tube bottom. Connect tube to distillation apparatus and swirl tube to insure complete mixing. Do not swirl the tube until the reflux unit is attached. The pH of the solution should exceed 11.0.
6. Label clean snap cap tubes with appropriate sample ID number and sample dilution information, and add 10mL 0.02N H_2SO_4 to each tube. Place the snap cap under the condenser, and raise it so that the tip of the condenser is below the surface of the sulfuric acid, but not touching the bottom.
7. Turn on condenser water. Turn on the block. Make sure the sample ID numbers on the Kjeldahl tubes match the ID numbers on the other end of the condenser.
8. Some sample matrices require the addition of an anti-foaming reagent. If a sample begins to bubble and climb up the sides of the tube, add a few drops of antifoam. If a sample is suspected or expected to foam, antifoam may be added as part of sample pretreatment.
9. After 40mL has been distilled, remove snap caps from under the condensers, and bring up to 50mL with DI water. Refrigerate at 4°C until time of color development on Lachat.

E. Development Procedure on Lachat Autoanalyzer:

1. Refer to SOP #302, Lachat operation, for a more detailed description of instrument use.
2. Turn on power to all modules except pump. Turn on heating unit, set on 65°C, and allow to warm to temperature.
2. Attach ammonia manifold. Be sure to use the 650cm heater block. Refer to manifold diagram if necessary.
3. Load background and generate a sample tray. Be sure to include all sample dilutions and quality control samples (blank, prep blank, LCS, replicates, spikes, check standards, blank spikes) for each form of nitrogen (ammonia, TKN).
4. Use helium to degas all Lachat reagents except for sodium phenolate reagent and sodium nitroprusside.
5. Place feed lines into proper reagent containers. Check that the correct waste container is in place. The sample waste stream is acid waste.
6. Hook pump tubing onto pump tube cassettes, and push into place onto pump. Start pump. Wait while all reagents flow through lines to establish a steady baseline.
9. Place calibration standards in the tray marked S1-S8 in order of descending concentration.
10. Start calibration and samples. The calibration curve correlation coefficient must be 0.9975 or better. If the calibration fails, the standards may have to be remade.
11. Print out calibration statistics and attach it to the last page of the report. Start the run with a free ammonia LCS. Once the run is complete, staple a copy of the lachat run to all associated batch sheets and results sheets. Leave the completed report on the Wet Chemistry supervisor's desk for review.

12. For data transfer, see Appendix A.

VIII. Calculations

A. Calculating RPD:
The RPD is the relative percent difference, and is a measure of the consistency of the sample replicate for the sample set. First, calculate the results for both the sample and the sample replicate. Then apply the two results to the following calculation:

$$\text{RPD, \%} = \frac{(R1 - R2)}{(R1+R2)/2} \times 100$$

where: R1 = value achieved for sample, mg/L
R2 = value achieved for sample replicate, mg/L

B. Calculating the sample spike recovery:
The sample spike is a test if the sample matrix is suitable for accurate testing. To calculate the % recovery, first calculate both the sample and the spiked sample. Then apply the two results to the following calculation:

$$\text{\% Recovery, \%} = \frac{(SR - R)}{STV} \times 100$$

where: SR = value of sample with added spike, mg/L
R = value of sample, mg/L
STV = true value of spike added to sample, mg/L

C. Calculating the LCS:
The LCS is the laboratory control standard and is a test of the accuracy of the calibration and the test overall. To calculate the % recovery of the LCS, calculate the value of the LCS sample, and then apply it to the following calculation:

$$\text{\% Recovery, \%} = \frac{(RL)}{(LTV)} \times 100$$

where: RL = value for LCS sample, mg/L
LTV = true value of LCS, mg/L

D. The Phoenix LIMS automatically calculates all recoveries and RPDs.

IX. Quality Control

A. Prep Blank must be analyzed every batch of 20 or fewer samples. This is a DI water aliquot treated like a sample, undergoing all treatments the samples undergo. The prep blank must yield ammonia concentrations below the reporting limit.

B. Laboratory Control Standards must be analyzed every batch of 20 or fewer samples. It must be recovered 90-110%. A failing LCS for a batch of samples requires re-analysis of the batch.

C. Blank spike must be analyzed every batch of 20 or fewer samples. This is a DI water aliquot fortified by the lab with a known quantity of ammonia. The blank spike must be recovered 90-110% for ammonia and 85-115% for TKN.

D. Continuing calibration checks must be analyzed every 10 samples, as is written into the software of the Lachat QuickChem 8000. They must pass within 90-110%. The calibration check for ammonia is the 1.6mg/L standard (Std C) from the calibration curve.

E. Sample replicates must be done every batch of 20 or fewer samples. They must have a RPD of no more than 20%.

F. Sample matrix spikes must be done every batch of 20 or fewer samples. A known amount of ammonia is added to a sample to test the sample matrix. The spike recovery for ammonia 90-110% and 85-115% for TKN, however, if recoveries fall outside of this range, and the LCS and Blank spike are acceptable, it is judged to be sample related and not system related. Report % recovery.

G. MDL studies are performed annually. Seven replicates of a 0.10 mg/L standard are distilled and analyzed for ammonia. Seven replicates of a 0.50 mg/L standard are distilled and analyzed for TKN. Two MDL spikes are performed quarterly to complete a full MDL over the course of a year. An MDL study must also be performed on prep blanks.

H. A Limit of Detection (LOD) study must be analyzed annually. All sample processing steps of the analytical method shall be included in the determination of the LOD. The LOD study is achieved by analyzing a standard at no more than 2-3x the LOD. In many cases, a 1:1 dilution of the lowest calibration standard is prepared and analyzed. LOD recoveries must be 50 – 150%.

I. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by ± 10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

J. An initial demonstration of capability (IDOC) must be performed to prove the generation of acceptable data with regard to accuracy and precision. An initial demonstration must be performed by each analyst and consists of analyzing 4 LCS solutions, usually over a series of days.

K. Sample preparations are documented in the Standards Prep Logbook.

L. Reagent preparations are documented in the Reagent Prep Logbook.

M. Lachat use and maintenance is documented in the Lachat Maintenance Logbook.

X. Safety

A. The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. A reference file of material data handling sheets are available to all personnel involved in the chemical analysis.
B. Always wear safety glasses for eye protection as well as lab coats.
C. Refer to Phoenix SOP #805: Hazardous Chemical and Laboratory Safety Procedures

XI. Pollution Prevention

A. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation.
B. Standards should be purchased in volumes consistant with laboratory use to minimize the volume of expired standards to be disposed.

XII. Waste Management

A. It is the laboratories responsibility to comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

XIII. Method Performance

A. This method was validated through internal QA/QC monitoring, including annual method detection limit studies, precision and accuracy studies, initial and continuing calibration verifications, blank analysis, laboratory control samples and matrix spikes and duplicates.
B. See section IX Quality Control in this SOP for acceptable limits.

XIV. Corrective Action for Out-of-Control or Unacceptable Data

A. Should the calibration curve have a correlation coefficient of <0.9975, remake and reanalyze curve before processing samples.
B. Should the preparation blank, LCS or inhouse standard fail acceptance criteria, redistill/redigest and reanalyze batch.
C. Should the matrix spike or sample duplicate analysis fail acceptance criteria, a non-conformance report must be generated or the sample QC must be reanalyzed.

XV. References

A. Method 4500-NH3 B. and G., Standard Methods for the Examination of Water and Wastewater, edition, 1997.
B. Method 4500-Norg B., Standard Methods for the Examination of Water and Wastewater, edition, 1997.
C. USEPA Method 350.1, Determination of Ammonia Nitrogen by Semi-automated Colorimetry, Revision 2, August 1993.
D. USEPA Method 351.1, Determination of Total Kjeldahl Nitrogen by Semi-automated Colorimetry, 1978.
E. Methods for QuikChem Automated Ion Analyzer, Lachat Instruments, Method 10-107-06-1-C, August 1993.

XVI. Appendices and Diagrams

A. Appendix A- Data Transfer

Appendix A: Data Transfer to LIMS

From the Phoenix MDE Icon
Left: Data Transfer
Right: IC/Lachat Data Transfer
File Icon (Browse, Local Disk C Drive, Program Files, Lachat, Data, 2015, Data, All Files)
Select Excel File by Date and Time

Enter Initials
Load and Review Data

Edit Data:
Reject samples not needed by checking them (such as Organic Nitrogen)
Look to reject over-range samples that need a diluted sample reported
Make sure that the diluted sample is there (usually at the end of the batch)

Load Data Tab (if edit changes were made)
Load and Review Data (if edit changes were made)

Process Data
After verifying history issues, select sample by checking it under OK column
History issues include: Minimum, Adjusted Maximum, Number of Samples,
All Time Maximum
Results over the All Time Maximum may have to be prepped
(distilled, digested, etc.) again.
History verification issues can usually be ignored from other laboratories, as their location codes are not historically accurate.
All passing reps and spikes should already be selected with a check

Transfer To Labworks
Print Log

Revision History Log for SOP # 304

Date:	Revision #:	Summary of Changes:	Submitted By:	Approved By:	Effective Date:
2/6/15	8.1	Changed the range in scope to "0.05ppm", section V.D changed 10 ppm std to 6.0 ppm, and changed 0.04 ppm to 0.05ppm, added dechlorination to section VII.B, added sections H, I, & J to IX (LOD, LCR, IDOC), changed acceptance criteria of LCS, blank spike, & MS to 90-110%, added EPA method references to section XV.	Kathy Cressia	Eric Geyer	2/6/15
3/8/17	8.2	Changed "A" standard to 8.0mg/L, fixed typo in borate buffer reagent, added Appendix A on data transfer	William McKernan	Eric Geyer	3/8/17
4/20/17	8.3	Fixed typo in section IX Quality Control- CCC 90-110% recovery, it said 90-100%	Kathy Cressia	Eric Geyer	4/20/17
7/26/17	8.4	During the 8.2 revision, it was missed in the Scope that the range now goes to 8.0 mg/L.	Kathy Cressia	Eric Geyer	7/26/17
7/26/19	8.5	Section IX.C & F – Added spike recovery range for TKN. Section IX.G. – Added information on MDL studies.	Kathy Cressia	Eric Geyer	7/26/19

Expiration Date

Appendix C – Approved Sampling & Analysis Plan (SAP)