AOS PROTOCOL AND PROCEDURE: SURFACE WATER CHEMISTRY SAMPLING IN WADEABLE STREAMS

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See configuration management system for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/31/2012	ECO-00680	Initial draft release
B_DRAFT	12/10/2012	ECO-00742	Updated draft protocol
C	04/23/2014	ECO-01121	Initial production release
D	08/29/2014	ECO-02233	Minor updates based on feedback from the field
E	11/07/2014	ECO-02445	Migration to new protocol template
F	03/26/2015	ECO-02646	Minor updates to shipping and labeling, and added information on ashing filters

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1 OVERVIEW

1.1 Background

This document describes the required protocols for field sampling of surface water chemistry in small, wadeable streams. Stream chemistry is determined by many factors, including rainwater chemistry, geology, volcanic activity, in-stream processes, and pollution. Thus, stream chemistry varies spatially and temporally, depending on the surrounding conditions and flowpaths of water on the land surface and subsurface. In streams, physical reactions, such as evaporation and adsorption, and biological reactions (immobilization and mineralization) occur which further influences stream chemistry.

Stream chemical parameters include concentration, load and yield. Concentration is the amount of a constituent in a volume of water (e.g. mg/L). Load is the total amount of a constituent transported per unit time:

$L = CQ$ where,

L = Load (mg/s)
C = Concentration (mg/L)
Q = Discharge (L/s)

Loads are typically calculated on an annual basis (e.g., Kg/year). Constituent yield is the transported load per unit of drainage area (e.g., Kg/Ha/year), and is useful in comparing loads from watersheds of differing sizes.

Stream chemistry provides scientists, managers and decision makers with valuable information to assess water quality responses to natural and anthropogenic changes, such as nutrient loading and point and non-point pollution sources. This includes insight about how changes in the surrounding landscape influence stream function and structure. For example, how is erosion at the top of a watershed influencing stream environment and biogeochemical dynamics? Stream biota are tolerant of small changes in chemistry; however, large shifts in chemistry can have dramatic effect on the biotic community structure and function through processes such as nutrient uptake and retention.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This protocol is derived from the United States Geological Survey, National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water Resources Investigations, Book 9, Chapter A4, Version 2.0, 9/2006 and Chapter A6.6, Version 4.0, 9/2012.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.014051	Field Audit Plan
AD[07]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002383	Datasheets for AOS Protocol and Procedure: Surface Water Chemistry Sampling in Wadeable Streams
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

2.3 Acronyms

Acronym	Definition
A/R	Acid-rinsed
ALK	alkalinity
ANC	Acid Neutralizing Capacity
ASR	Analytical Services Request
C/B	Cleaned and burned
^{o}C	Degrees Celsius
FIL	Filtered Chilled
GFF	Glass Fiber Filter
ha	Hectare
HDPE	High-density polyethylene
L	Liter
lb/in	Pounds per inch
m	Meter
M	Molar
m^3	Cubic meter
mg	Milligram
mg/L	Milligrams per liter
meq/L	Multiequivalents per liter
mL	Milliliter
N	Normal
P&P	Procedure and Protocol
PCN	Total Particulate Carbon and Nitrogen
PPE	Personal Protective Equipment
RAW	Raw Untreated
s	Second
µS/cm	Microsiemens per centimeter

2.4 Definitions

Alkalinity: The buffering capacity of a water body, or the ability of solution to neutralize acids to maintain a fairly stable pH, which is important for agriculture, wastewater, contamination determination, ecosystem health etc. Good water buffers include compounds such as bicarbonates, carbonates and hydroxides, which combine with H+ ions in the water and increase the pH to prevent acids from building up in a solution.

Acid Neutralizing Capacity (ANC): Measure of the overall (total) buffering capacity of water, or the ability to neutral acid and maintain a constant pH. Acid Neutralizing capacity is similar to Alkalinity, but is measured on an unfiltered water sample, rather than a filtered one.

Conductivity: A measurement of the electrical conductance per unit distance in an aqueous solution.

Headspace: A gaseous space above a closed liquid sample

Hydrograph: A diagram depicting the change in discharge (m^3) over a given time (s).

pH: A measure of the acidity or basicity of an aqueous solution.

Thalweg: The deepest part of a stream channel.

3 METHOD

The field protocol used by NEON for collecting surface water chemistry samples in small, wadeable streams follows the general requirements set forth by the USGS National Water-Quality Assessment (NAWQA) Program (U.S. Geological Survey, 2006). This protocol describes the collection, field processing, and shipping of total, dissolved, and particulate nutrients samples, as well as anions, cations and general chemistry (i.e., conductivity and pH). Additionally, samples will be collected for alkalinity and acid neutralizing capacity (ANC) and will be measured at the Domain support facility to reduce the error associated with changes in the chemical composition of a sample due to chemical dissolution or precipitation as well as the loss of CO_2.

The majority of the NEON stream sites are shallow and narrow, rendering the use of isokinetic (i.e., sampling at same velocity as the main stream) samplers for depth-integrative sampling impractical. Thus, the following protocol outlines the use of a dip sampling method in the main section of streamflow (i.e., thalweg). This method assumes the stream channel is completely mixed.

The water chemistry sampling location should be located, when possible, within 5 meters of the main stream sensor set so that the sensor measurements can be validated with stream water chemistry samples. The sampling location should be typical of the entire reach. The sampling location should be located away from, or upstream of, any major local disturbances and other areas where NEON sampling activities commonly occur. In streams with a shallow water column, field personnel must be cautious not to disturb the benthic sediments when sampling. Disruption of the sediments by walking or by sampling too close to the stream bottom can contaminate samples. Thus, always sample upstream from wading activity and minimize suspension of sediments when sampling. If sediments are disrupted, wait until the area has cleared before sampling.

Annually, NEON will ship known alkalinity and/or acid neutralizing capacity samples to be analyzed to ensure proper functioning of the digital titrator and sulfuric acid standards.

Verify the reproducibility of samples by completing a sample analysis on a replicate sample or a reference sample, at a minimum of every 10 samples. Reproducibility should be $\pm$5%. For low conductivity (<100 µS/cm), reproducibility should be within 10%.

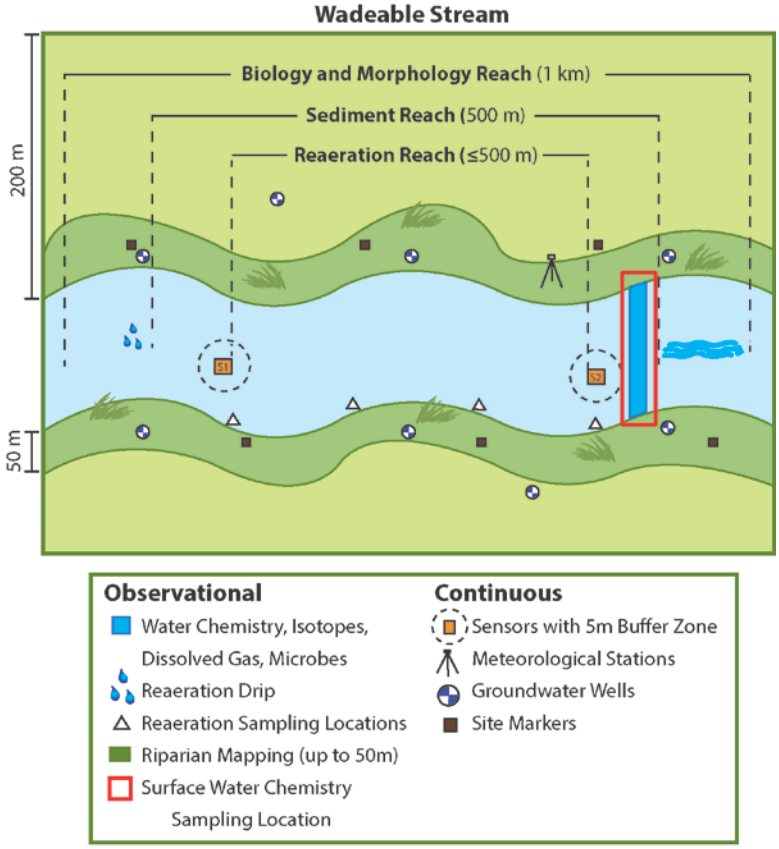


Figure 1. A generic wadeable stream site layout with water chemistry sampling locations

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[06]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[07]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Wadeable stream water chemistry sampling occurs up to 26 times per year (approximately every other week) at each NEON location. When applicable, chemistry samples should be collected on Tuesday to coincide with NEON atmospheric wet chemistry sampling, as well as the National chemistry sampling efforts. It is advised not to collect samples on Friday. A range of dates for each site will be determined *a priori*, based on historical stream discharge data. These criteria will be detailed in the NEON Aquatic Sample Strategy Document (RD[07]).

4.2 Criteria for Determining Onset and Cessation of Sampling

The timing of sampling allows researchers to assess aquatic biogeochemistry cycles, and therefore timing depends on the dominant driver(s) of nutrient flux within each system. Timing of sampling is site-specific and determined by rules developed using historical discharge and environmental data. For example, streams with little or no flow during the summer dry-season or frozen streams during the winter are sampled more intensively during wet periods. Systems that have a snowmelt-dominated or storm-dominated flow regime are sampled more intensively during time periods when the majority of the nutrients are moving through the system and sampled sporadically during times of baseflow. Stream systems that are heavily influenced by autumn leaf fall and winter rains are more heavily sampled in autumn and winter.

4.3 Timing for Laboratory Processing and Analysis

Following sample collection, alkalinity and ANC samples should be kept on ice or refrigerated at 4°C +/- 2 °C. Laboratory analysis should be completed as soon as possible after returning from the field. Alkalinity and ANC samples should be processed within 24 hours. Samples analyzed after the 24 hours window will be flagged. The maximum allowable time period between sample collection and analysis is 72 hours.

Samples should be shipped to the water chemistry lab within 24 hours, when possible, to ensure sample integrity. Samples must be kept cold (~4°C) to reduce nutrient transformation. Water jugs must be shaken before filtration to re-suspend particulates and homogenize water. For further storage and shipping information see SOP E.

It is advised to not collect field samples on Friday.

4.4 Sampling Timing Contingencies

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

Samples should be processed (filtered and chilled) as soon as possible. If necessary, stream water may be collected in a large container (may require up to 2 4-L jugs in clear streams), kept on ice or ice packs, and filtered within 3 hours at a base camp or Domain Lab (i.e., if weather dictates the need to get out of the field immediately and stream discharge is increasing). Always make note of any weather or stream conditions that could influence chemistry, including but not limited to wind, activities in the surrounding watershed, prior flood or rain events, ice, and changes in sampling locations (RD[05]). Sample collection time, processing station and processing time must be recorded on the Water Chemistry Data Sheet (RD[05]).

Chemistry samples should be collected upstream of any solute injection work or any fieldwork disrupting the stream bottom (i.e., morphology mapping, invertebrate collection, macrophyte collection, etc.).

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If sampling stirred up sediments or added chemical constituents to the stream/lake (i.e., gas additions) within the past hour, allow the water to clear and disturbance to pass, sample upstream/upwind of the disturbance	No adverse outcome.
	Should time become limited during chemistry sampling, collect water samples in up to two 4-L jugs and return the samples on ice to the designated sample processing location to filter.	No adverse outcome.
	If water samples cannot be processed in situ, the filtration must be completed within 3 hours of sample collection.	No adverse outcome.
Days – Months	If low discharge renders some habitat dry or the flow is so low that the stream appears to be a series of pools not connected by surface water, continue sampling in the water chemistry sampling location provided the sample bottle can be filled without disturbing sediments. Be sure to notate this as a "non-flowing sample" (RD[05]) during data collection.	No adverse outcome.
	If the water chemistry sampling location is too shallow to obtain a sample, sample in a nearby pool where water is deep enough to obtain a clean, sediment free, sample, and notate this change during data collection.	No adverse outcome.
	If the stream is entirely dry or frozen, notate data.	No adverse outcome.
	If stream is ice-covered but still flowing, the ice should be broken in order to sample the stream. Be sure to bring a shovel or other long-handled tool if the surface ice is expected to be hard to break.	No adverse outcome.
	If temperatures are below freezing and filtration equipment is not functional in situ, collect sample and filter in a sheltered area, such as the field vehicle or return to the Domain Support Facility for filtration.	No adverse outcome.

4.5 Sampling Specific Concerns

Samples must be kept cold (~4°C) to reduce nutrient transformation. Water jugs must be gently shaken before filtration to re-suspend particulates and homogenize water. If you are using this water to subsample for dissolved gases (RD[10] do not shake rigorously as this will cause the dissolved gases to be released into the headspace and highly impact the sample. Always make note of any weather or other conditions that could influence chemistry, including but not limited to wind, activities in the surrounding watershed, prior flood or rain events, ice, and changes in sampling locations.

Chemistry samples should be collected up-wind or upstream of any fieldwork disrupting the stream bottom (i.e., morphology mapping, invertebrate collection, macrophyte collection, etc.).

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 10 ft^2/s (0.93 m^2/s).

Acid must be stored in acid-safe containment cabinets in compliance with the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]). Wear nitrile gloves and eye protection when dispensing acid. Ensure Safety Data Sheet (SDS) is readily available during use of acid and reviewed prior to exposure to acid.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 2. Equipment list – Field equipment and supplies

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
GB07270000	R	Pump Assembly - Easy-Load Peristaltic pump head (e.g., Masterflex® L/S® Easy-Load® pump head) with Peristaltic pump tubing (e.g., L/S® 15 or L/S® 24) - 18-V Drill Pump (Power source for pump head) - Tubing connectors	Pumping stream water into sample containers	1 1 1 2	N
	R	Pieces of C-Flex® tubing, 1/4 inch I.D. and 3/8 inch outer O.D., Site Specific Length (~4 feet and 2 feet in length)	Pumping stream water into sample containers	2	N
	R	18-V Drill battery charger	Pumping stream water into sample containers	1	N
	S	Velcro strap or c-clamp	Keeping the drill in the “on” position to pump stream water continuously	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	4-L jug	Collecting stream water	2	N
	S	Non-porous flat surface	Filtering and processing stream water	As needed	N
MX100514	R	Hand-held conductivity meter	Measuring conductivity	1	N
	R	Squirt bottle (125mL)	Rinsing tubing before placing in 4 L jug	1	N
Consumable items					
MX108215	R	Pall Supor capsule filter (0.45 µm)	Collecting stream water for filtered samples	1	N
	R	Sample labels (2*4 inch waterproof)	Labeling samples	3	N
	R	Permanent marker	Labeling samples	2	N
	S	1 L jug of DI	Rinsing tubing before placing in 4 L jug	As needed	N
	S	Conductivity calibration solutions	Calibrating hand-held conductivity meter	As needed	N

R/S=Required/Suggested

Table 3. Equipment list – Field water chemistry bottles for Raw and Filtered samples and totals (see also Figure 2 Stream Chemistry Bottle Types)

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	250 mL HDPE [a] - Alkalinity - Acid Neutralizing Capacity (ANC)	ALK and ANC sample containers, per site	2	N
Consumable items					
	R	1 L amber bottle – A/R and C/B	Filtered water sample (FIL) container	1	N
	R	250 mL amber bottle – A/R and C/B	Unfiltered water sample (RAW) container	1	N
	R	25 mm ashed GF/F filter	Filtering stream water for particulate samples (PCN)	1	N

R/S=Required/Suggested

[a] indicates sample bottles that will remain at NEON Domain Support Facilities to be analyzed. These bottles are labeled with different labels (Figure 4b) and bottles can be re-used. 125 mL bottles may be used if less volume is needed – site specific.

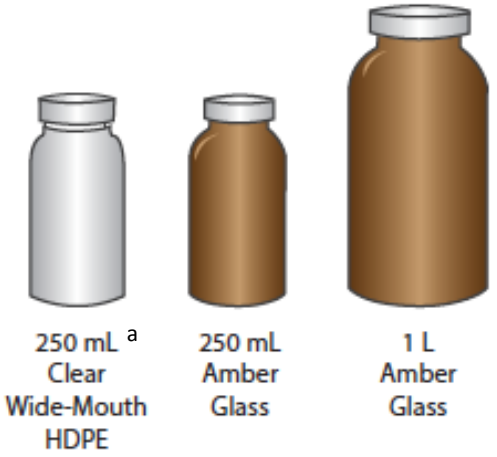


Figure 2. Water chemistry bottle types. [a] indicates sample bottles that will remain at NEON Domain Support Facilities to be analyzed. These bottles are labeled with different labels (Figure 4b) and bottles can be re-used. 125 mL bottles may be used if less volume is needed – site specific.

Table 4. Equipment list – Total Particulate Carbon and Nitrogen (PCN)

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	4 L jug	Collecting samples	1	N
	R	Vacuum hand pump and vacuum tubing	Filtering samples	1	N
	R	Filter Unit and Funnel	Filtering samples	1	N
	R	Graduated Cylinder, plastic, 250 mL	Measuring and adding the volume of sample into the filter funnel	1	N
	R	#8 rubber stopper for filter manifold	Filtering samples	1	N
	R	1 L PolyPropylene vacuum flask	Filtering samples	1	N
	R	Filter Forceps – forceps with flat ends to not poke holes in filter	Handling filter	2	N
	R	Squirt bottle (125mL)	Rinsing the sides of the filter funnel	1	N
Consumable items					
	R	25 mm pre-ashed GFF (0.7 µm) filters	Filters for particulate sample (PCN)	1*	N
	R	Aluminum foil, pieces (~4X4 inches)	Wrapping GF/F filters	1	N
	R	DI water	Rinsing the sides of the filter funnel	As needed	N

R/S=Required/Suggested

*Take extras in field

Table 5. Equipment list – Sample field storage and shipping

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Shipping cooler	Shipping samples	1	N
Consumable items					
	R	Packing material	Filling up extra space and adding absorbent material	As needed	N
	R	Resealable plastic bags (gallon and quart size)	Separately enclosing the shipping labels, ice packs and samples	As needed	N
	R	Ice or ice packs	Keeping the samples cool	As needed	N
	R	Clear packing tape, roll	Labeling shipment	1	N
	R	FedEx shipping labels	Labeling shipment and cooler return	2	N

R/S=Required/Suggested

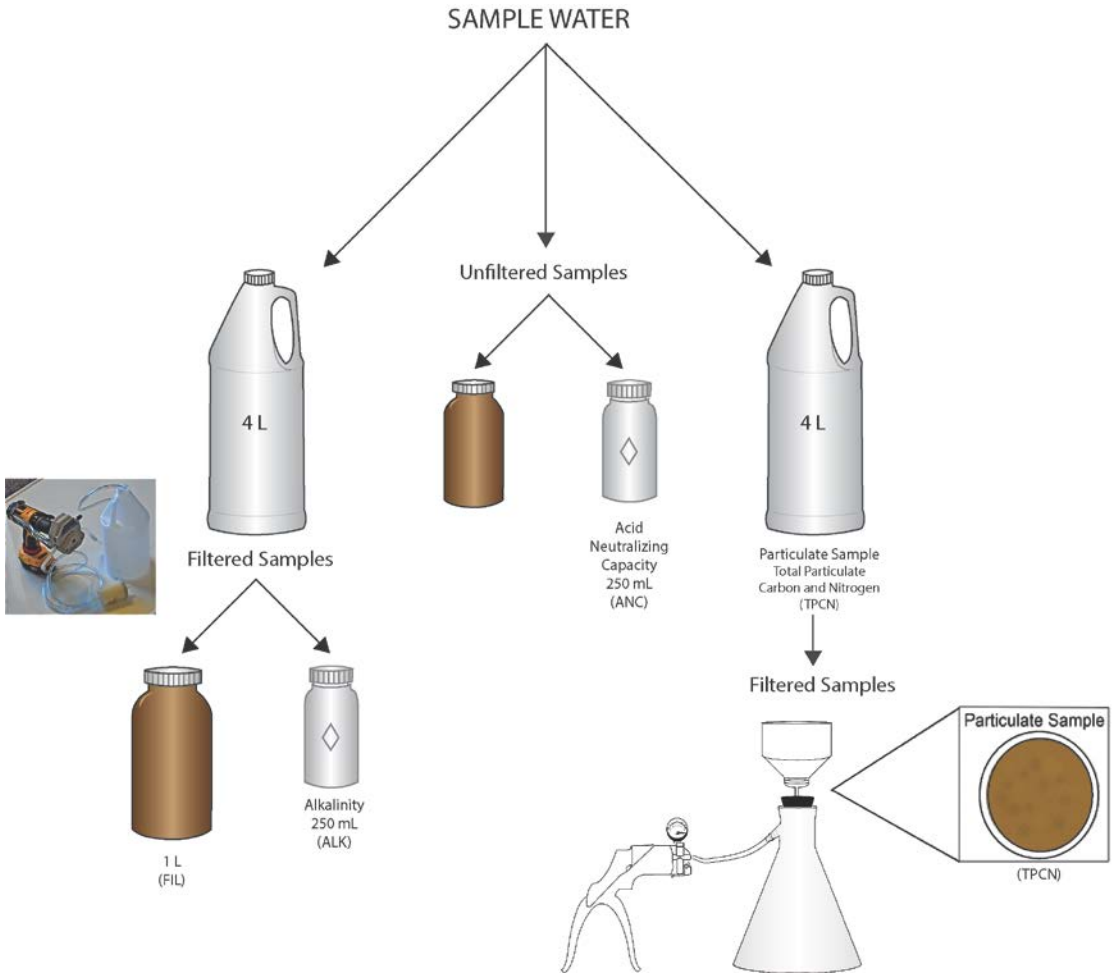


Figure 3. Flowchart of Water Chemistry Sample Collection and Filtration. ◊ Indicates 250 mL, wide-mouth sample bottles that remain at the Domain Support Facility for analysis. Letters in parenthesis indicate the codes that correspond to the chemistry labels (see Figure 4 a and b).

Table 6. Equipment list – Laboratory equipment: Materials and supplies required for the Alkalinity and ANC Laboratory Measurement Procedure

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX100556	R	pH meter, with automatic temperature compensator - pH electrode, calibrated Thermometer, calibrated	Reading pH of the samples	1	N
	R	Magnetic stirrer	Mixing the sample with the titrant solution	1	N
	R	Stir bars, Teflon® coated, smallest size	Mixing the sample with the titrant solution	2	N
	R	Volumetric pipets, Class A "TD" [a] – 25 mL – 50 mL – 100 mL	Measuring volume and transferring sample to glass beaker	1 1 1	N
	R	Graduated cylinders [b] – 25 mL – 50 mL – 100 mL	Measuring volume and transferring sample to glass beaker	1 1 1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Pipette squeeze bulb	Used with volumetric pipet	1	N
	R	Glass beakers – 50 mL – 100 mL – 150 mL	Sample container for pH readings	1	N
	R	Squeeze bottle with DI water	Rinsing pH probe	1	N
MX100384	R	Digital titrator and mounting assembly	Adding titrant solution to sample	1	N
	R	Delivery tubes, 90° angle, transparent	Adding titrant solution to sample, 1 per titrant solution	2	N
	R	Safety – gloves, glasses, acid spill kit, lab coat	Safety	1	N
	R	Acid waste container		1	N
Consumable items					
	R	Deionized (DI) water (max conductivity of 1 µs/cm)	Rinsing pH probe		N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Titrant solution - Sulfuric acid (H_2SO_4) 0.16*N* - Sulfuric acid (H_2SO_4) 1.6*N*	Added to samples in order to measure ANC and ALK		Y
	R	Sodium Bicarbonate, 1lb	Acid disposal		N

6.2 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

6.3 Specialized Skills

N/A

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

We estimate sampling requires 1-2 technicians for 2 hours each sampling day plus travel to and from the site and 2 hours for laboratory work at the Domain Support Facility per site.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. Check the water chemistry field sampling kit to make sure all supplies are packed and ensure batteries for the peristaltic pump are charged.
2. Check the hand-held conductivity calibration and recalibrate if necessary. See Conductivity User's Manual.
3. Prepare the appropriate bottles and collection devices based on type of water samples being collected (Figure 2 and Figure 3) *Note: prepare 2 sets of bottles; the second set will be used as a back up.
4. Pre-ash GF/F filters:
   a. Place layers of 25 mm GF/F filters on aluminum foil. Use multiple layers of foil if needed, filters can be touching and placed on top of one another but should not be stacked more than 3 filters deep.
   b. Place in muffle furnace (500 ^{o}C) for 6 hours.
   c. After 6 hours, remove from furnace, stack filters using filter forceps, and place in original box.
   d. Label box with permanent marker to read "ASHED, Your Name, Date".
   e. Place box in in sealed zip-top bag.
   f. Ashed filter may be stored indefinitely, as long as they remain in the box and stay dry.
5. Attach pre-printed labels (Figure 4 a and b) to bottles (Figure 3).
6. Use a permanent marker to fill out bottle labels (Figure 4 a and b) before going into the field. Note that there are two different labels depending on whether the samples will be shipped to the external analytical chemistry laboratory (Figure 4a) or will be analyzed at the Domain (Figure 4b). **Labels are waterproof but should be filled out before getting wet to ensure ink is dry. SampleID should be siteID.stationID.date.sampleType.** StationID is the 2-digit station code where sample was taken (i.e. Station ID for streams ='ss', non-wadeable streams/rivers = 'rs', in Lakes 'in', 'ot', 'c0', if center is stratified: 'c1', 'c2', 'c3' with one being the top layer. 'w1'-'w8' for groundwater wells) **or use basketID when STREON baskets are sampled. SampleType is FIL or RAW, with FIL indicating a filtered sample and RAW indicating an unfiltered sample, or domain Lab SampleType is ALK (filtered sample) and ANC (unfiltered sample).** Circle the correct bottle type code (Figure 3 and Figure 4) for each bottle.
7. Organize bottles as appropriate for your study sites.

a)

Sample ID: _____
(siteID.stationID.YYYYMMDD.sampleType)
Sample Type: **FIL** (Filtered) **RAW** (Unfiltered)
PCN (volume, mL units): _____ neon

b)

Sample ID: _____
(siteID.stationID.YYYYMMDD.sampleType)
Sample Type: **ALK** (filtered) **ANC** (unfiltered)
TO BE ANALYZED IN DOMAIN SUPPORT FACILITY
neon

Figure 4. Blank NEON Chemistry Labels for a) the External Analytical Laboratory and b) Internal NEON Domain Support Facility Measurements.

SOP B Field Sampling

In the field, fill out the General AQU Field Metadata Sheet (RD[06]) and the Wadeable Stream Water Chemistry Field Sampling Datasheet (RD[05]) before collecting samples.

B.1 Field Sampling

NOTE: ALWAYS sample in the THALWEG (the deepest location in the stream cross-section) with the bottle opening pointed upstream and into the main flow of water (Figure 5) and several centimeters below the surface (to avoid sampling floating material or surface film). You may step into the stream, but disturb the stream bottom as little as possible as you walk. Take samples UPSTREAM from where you are standing.

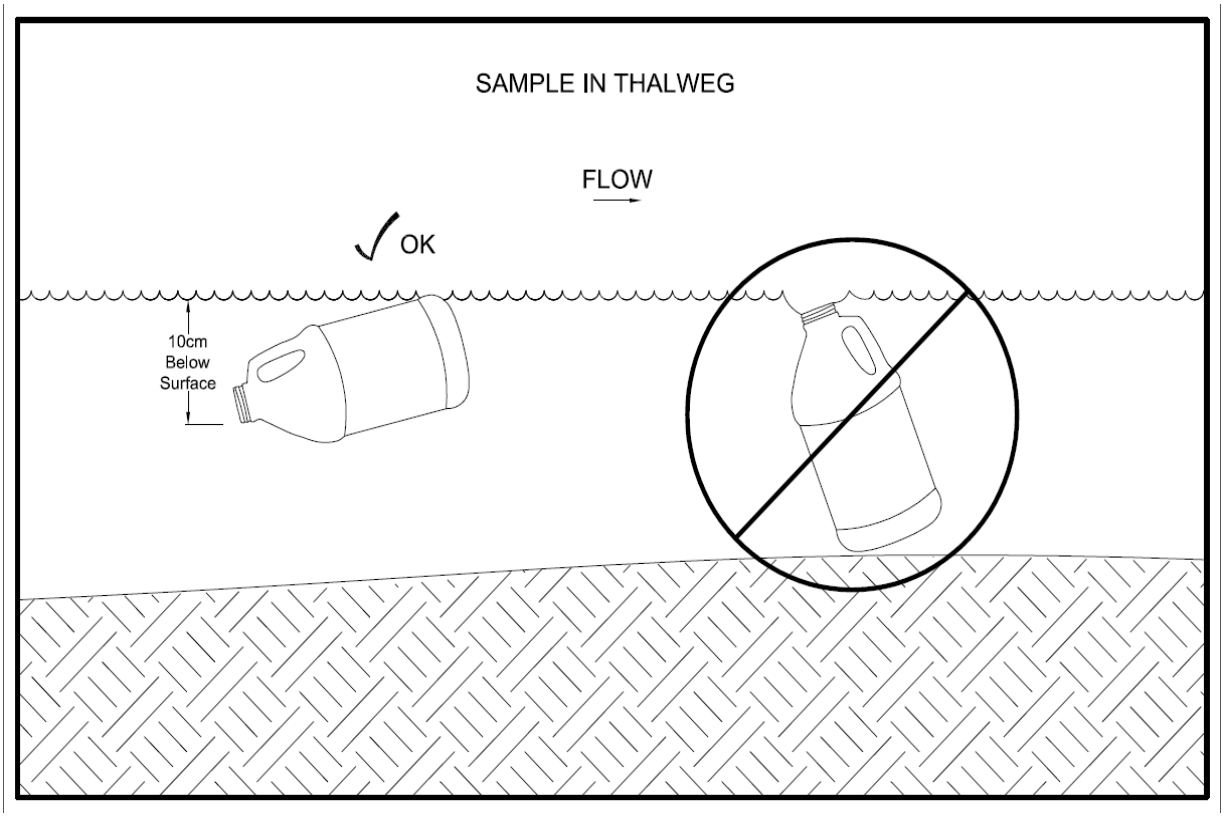


Figure 5. Diagram of proper and poor placement of a water sampling bottle

8. Record the Date (YYYYMMDD) and the time of day (use local, military time; ex. 13:46) that samples were collected from the stream Water Chemistry Field Sampling Datasheet (RD[05]).
   a. **NOTE:** Use the same time for all bottles filled at the same sampling point during each sampling event (i.e., the time the water was withdrawn from the stream).
9. Measure and record temperature-corrected conductivity values on the water chemistry field sampling Datasheet (RD[05]) and the sample shipping inventory remarks section of the 'per sample' tab. Conductivity should be measured as temperature-corrected conductivity at 25°C, whenever possible. Ensure conductivity measurements are on the appropriate temperature-corrected and unit setting (i.e., setting SPC, uS/cm).
10. Rinse the collection bottles and caps with the appropriate sample water (i.e., use filtered water to rinse filtered samples):
    a. Bottles to be rinsed with stream water:
       1) 4 L jug (can be used for filtered samples and/or PCN, see below)
       2) 250 mL burned amber glass bottle for external lab
       3) ANC - 250 mL wide-mouth, HDPE – ***to be analyzed at the Domain Support Facility**.
    b. To rinse: Hold the cap in your hand (setting the cap down increases risk of contamination). Lower the collection bottle under the water surface (approximately 10 cm below the surface) so that the opening of the bottle faces upstream. Allow stream water to fill approximately $^{1}/_{5}$ of the collection bottle. Remove bottle from stream, cap and shake. Discard water downstream. Repeat 2 more times. **Be cautious when sampling. Items can easily fall into stream while bending to sample.**

B.2 Collect Unfiltered Water Samples (RAW)

Fill the collection bottle by placing the bottle 10 cm below the water surface with the opening pointed upstream (Figure 5).

1. If you are not filtering directly out of the stream, collect water in a 4 L jug to be filtered into appropriate containers (Figure 3). Cap the jug, set aside for Section B.3 (Filtered Water). Keep jug cool and in the dark.
2. Collect water for the samples that do not require filtering (Figure 3). Fill glass bottle to the bottom of bottle neck, and fill ANC completely to reduce any changes in CO_2 concentrations due to headspace:
   a. 250 mL burned glass amber bottle – Filled to neck (Code RAW)
   b. Acid Neutralizing Capacity: 250 mL wide mouth HDPE – **FILLED** (Code ANC) ***to be analyzed at the Domain Support Facility.**
3. Collect an additional 4 L jug to be filtered for Total Particulate Carbon and Nitrogen (code PCN) analysis (NOTE: the filter will be analyzed for PCN)
4. IMMEDIATELY, chill samples (4°C +/- 2 °C). DO NOT FREEZE.

B.3 Filtered Water (FIL)

The water sample can be filtered directly from stream by placing inlet end of tubing into the stream. Be sure the tubing end is below the water surface and is not on stream bottom. Alternatively, you may use a 4 L jug to collect stream water and filter directly out of the jug into the appropriate bottles (Figure 3).

1. Set-up of Peristaltic Pump Apparatus (Figure 6):
   a. The peristaltic pump should be fitted with peristaltic tubing connected to ¼ inch Inner Diameter (I.D.) C-Flex tubing on either end (**a**).
   b. Place a **CLEAN** end of the tubing into the stream or into the 4 L collection jug (**b**). Rinse tubing with DI before placing in jug if necessary.
   c. Attach the other end of the tubing to a $^{3}/_{8}$ – ¼ inch tubing connector, which is then attached to the peristaltic tubing and pump (**c**).
   d. The other end of the pump should connect to a $^{3}/_{8}$ – ¼ inch tubing connector.
   e. Attach one end of ¼ inch C-flex tubing (2 feet long) to the tubing adaptor (**d**).
   f. Using peristaltic drill pump, rinse tubing with approximately 100 mL of sample water. The direction of the drill pump can be changed, if necessary.
   g. When tubing has been rinsed and is mostly filled with water (i.e., no large air pockets), attach the end of the outflow tubing to an unused filter capsule fitted with a tubing connector (**e**). **NOTE:** make sure to attach filter so that the direction of flow follows the flow arrow on the capsule filter.

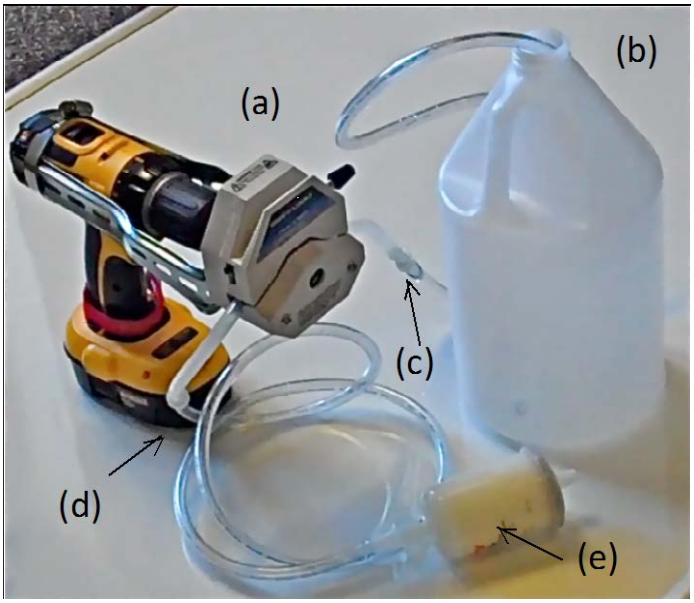


Figure 6. Pump and filter setup. Includes (a) a peristaltic sampling pump (modified from Woessner 2007), (b) a 4 L sample bottle, (c and d) tubing connectors to connect peristaltic and C-flex tubing, and (e) a capsule filter.

2. To obtain filtered water samples (Figure 3) use a peristaltic drill pump and filter capsule (Figure 6):
   a. Using the drill pump, begin pumping water through the filter. Make sure the tube was filled with stream water to reduce large amounts of air being forced through the filter and potentially blowing a hole in the filter. Do not pump too fast, or you could damage the filter. Do not fully engage trigger.
   b. Filter approximately 100 mL of sample water to rinse filter, and discard this water.
   c. Rinse sample bottles and caps with filtered water. You may wish to secure the drill trigger at desired speed, thus freeing one hand while filtering.
      1) Filter approximately 25 mL into the bottles. Cap and shake to rinse, and discard the rinse water.
      2) Repeat rinsing 2 more times.
   d. Ensure the tubing stays completely submerged in the water at all times.
   e. Fill filtered sample bottle (FIL) (Figure 3) to the neck. **FILL ALK bottles completely (NO HEADSPACE).**
   f. Place samples in cooler with ice or ice packs to keep cool (4°C +/- 2°C) until returned to lab. Group ALK and ANC samples together and ensure they will not be accidentally shipped to the water chemistry analytical laboratory.
   g. IMMEDIATELY, chill samples (4°C +/- 2°C) DO NOT FREEZE.
3. Dispose of the capsule filter after all samples have been filtered. These are one time-use filters.

B.4 Total Particulate Carbon and Nitrogen Sample Collection

Filter with hand pump and filter funnel to obtain a particulate sample on filter (Figure 7)

1. Rinse filter unit, filter screen and filter funnel (Figure 7) with DI water, making sure no particulates remain on the filter screen or funnel.
2. Insert the stem of the filter unit into the hole in the middle of the rubber stopper and insert the stopper into the filter flask.
3. Remove the filter funnel from the base, leaving the filter unit and screen resting on the manifold stem.
4. Use filter forceps to remove a 25-mm pre-ashed GFF filter from the box and place the filter on the screen of the filter unit.
5. Replace the filter funnel, rinsing with DI if necessary before replacing. Make sure that the filter is in the center of the filter unit. Ensure there are no gaps between the side of the filter and the filter unit, and that there are no holes in the filter itself.
6. Replace the filter box in the Ziploc bag to keep the filters from getting wet or blowing away.
7. Attach the vacuum pump tubing to the filter flask (Figure 7).
8. Rinse the filter with DI water. Use the hand pump to create suction in the flask and draw the DI water through the filter.

9. Shake the 4 L jug of water you collected at the stream in order to resuspend and uniformly mix the particles (approximately 15 seconds).
10. RINSE the clean plastic 250 mL graduated cylinder with 25 mLs of sample water. Turn cylinder on its side and rotate to rinse all sides of cylinder. Discard the rinse water.
11. Resuspend particulates (shake for 15 seconds) and immediately fill the 250 mL cylinder to the 200 mL marks. Immediately pour the water into the filter funnel. Be careful: the funnel only holds 200 mL of sample.
12. Pump the hand vacuum to pull water through the filter (do not exceed more than 15 lb/in of pressure). **IMPORTANT: BE CAUTIOUS OF THE VOLUME OF WATER IN THE FILTER FLASK SO YOU DO NOT SUCK WATER INTO THE VACUUM PUMP. DISCARD FILTERED WATER WHEN WATER IN FLASK REACHES THE FLASK NECK.**
13. Repeat steps 11-12 until water starts to move more slowly through the filter and particulates are visible on the filter. In streams with low particulate concentrations (i.e., clearwater systems), filter a minimum of 2 L of sample.
14. **IMPORTANT: Keep track of the amount of water you filter**. The external lab will need the total volume filtered for the PARTICULATE calculation.
15. Use a DI squirt bottle to rinse down the particulates on the sides of the filter funnel.
16. Continue to pump until all the water is drawn through the filter.
17. Release the vacuum and record the TOTAL volume of water filtered for the PCN sample on the chemistry lab sheet in the “Comments” section. Be sure to include the appropriate units (mL).

    NOTE: All the water in the tower must be filtered once poured because particles will start to settle. DO NOT add more water than you can filter.
18. Using clean filter forceps, remove filter funnel from the filter unit; **fold filter into quarters**. Folding filter helps reduce loss of particulate sample. DO NOT touch filter with your hands to reduce risk of sample contamination.
19. **Be Careful.** If filter tears or rips, begin filtration over with a new filter and sample water.
20. Place filter on 4 X 4-inch piece of aluminum foil, fold foil around filter and add label. Circle lab code PCN.
21. **Record total volume of water filtered** on label, Wadeable Stream Water Chemistry Field Datasheet (RD[05]) and AOS shipping inventory).
22. Double bag foiled filter in resealable plastic bag and place in ice in cooler (4°C +/- 2 °C).
23. Return to the Domain Support Facility for sample storage and shipping. Samples should be filtered as soon as possible and must be filtered within 3 hours of collection. Store the samples to be analyzed at the Domain Support Facility in the refrigerator (4°C +/- 2 °C). See Section 11 for sample processing details.

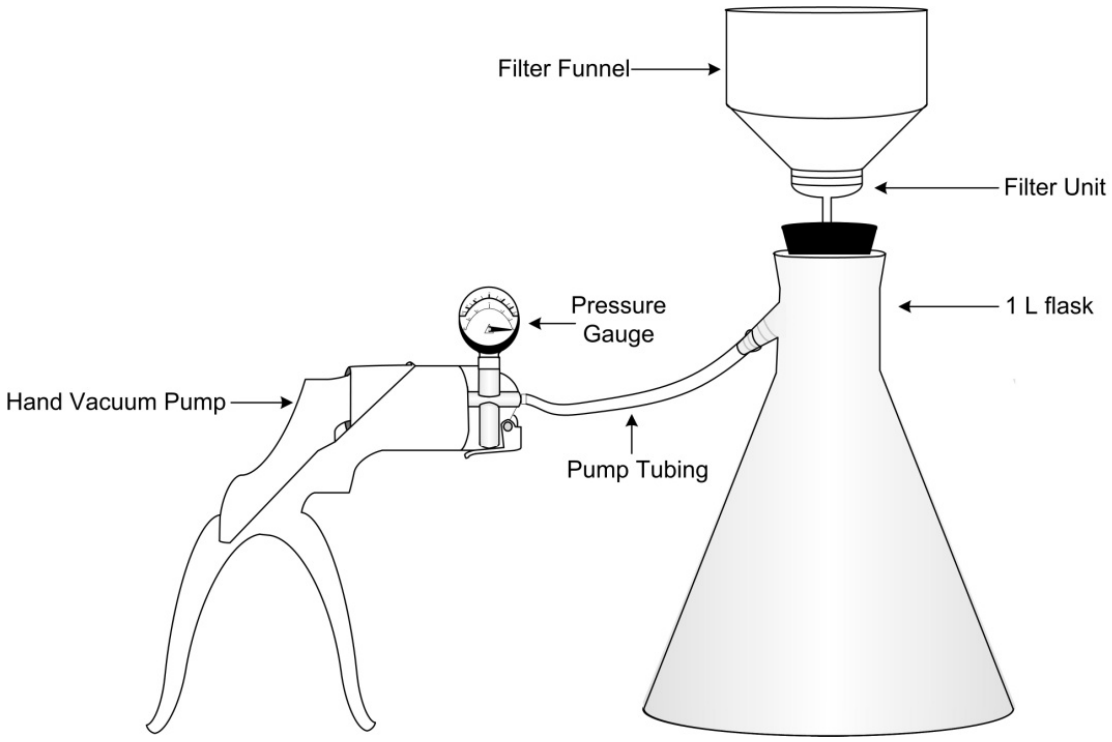


Figure 7. Filter apparatus setup for particulates. Includes the hand vacuum pump, attached to the filter flask and filter funnel/manifold.

B.5 Ending the Sampling Day

1. Refreshing the sampling kit
   a. Restock the sampling kit (shipping cooler) with new water chemistry sampling bottles with new labels attached, (alkalinity and ANC bottles can be rinsed with DI water and reused), nitrile gloves, filters, resealable plastic bags, foil, etc. Refer to Section 6.1.
2. Equipment maintenance, cleaning and storage
   a. Run clean water through the peristaltic pump to rinse tubing. Make sure to pump all water out of tubing before storage.
   b. Charge drill pump batteries.
   c. Triple rinse Alkalinity and ANC sample bottles with DI to be re-used.
   d. Using 1 L DI water jug (or more, as necessary), rinse the filter set-up (filter unit, funnel and flask) and equipment (forceps, graduated cylinder).

SOP C Laboratory Analysis

Alkalinity and Acid Neutralizing Capacity (ANC) are measures of the water's ability to buffer systems from changes in pH by neutralizing strong acids for filtered and non-filtered (i.e., whole-water samples), respectively. Thus, alkalinity and ANC are identical in systems without titratable particulates. Alkaline compounds include bicarbonate, carbonate and hydroxides, each of which removes H^+ ions from the water, ultimately increasing the system pH. Streams without these alkaline compounds are unable to buffer against changes in acidity, and therefore any acid added to the system, such as from acid rain or wastewater effluent, will result in an immediate decrease in stream pH. Thus, alkalinity and ANC are important measures to understand and predict how a system will respond to acidic inputs.

To determine alkalinity and ANC concentrations, a known strength of acid is added until the three main forms (bicarbonate, carbonate and hydroxide) are converted to carbonic acid. At pH 10, ~8.1, and ~5, hydroxide, if present, carbonate, and bicarbonates are converted to carbonic acid, respectively. By a pH 4.5, all bicarbonate and carbonate species should be converted to carbonic acid. The pH at which the species are converted is the equivalence point. The amount of acid needed to convert the species to carbonic acid is correlated with the amount of alkalinity and ANC in the sample.

NEON will largely follow the USGS procedures for the analysis of alkalinity and ANC using a digital titrator (Rounds 2012). Measurement will be determined at the Domain Support Facility following the Inflection Point Titration (IPT) Method for most of the NEON Aquatic sites. The IPT method is a titration method in which the sampler titrates on both sides of the expected equivalence points. The point at which the slope of the titration curve is the steepest is the inflection point. However, when alkalinity or ANC is extremely low (<0.4 meq/L or 20 mg/L) or conductivity is low (<100 µs/cm), the Gran function plot (Gran) method will be followed. This protocol focuses on the use of the IPT method, and briefly mentions the Gran method. For additional details on the IPT method or the Gran method, see the USGS protocol (Rounds 2012).

C.1 Sample Processing Timing

Following sample collection, alkalinity and ANC samples should be kept on ice or refrigerated at 4°C +/- 2 °C. Laboratory analysis should be completed as soon as possible after returning from the field. Alkalinity and ANC samples should be processed within 24 hours. Samples analyzed after the 24 hours window will be flagged. The maximum allowable time period between sample collection and analysis is 72 hours. It is advised to not collect field samples on Friday, unless alkalinity and ANC can be measured in the Domain Support Facility on Friday afternoon.

C.2 Preparation

1. Turn on pH meter well in advance of sample analysis.
2. Allow pH buffers 4.0 and 7.0 to come to room temperature before calibration by allowing the sample bottle to sit on a lab bench until the temperature has equilibrated.
   a. Make sure buffer solution has not expired and is not reused.
   b. Ensure the bottle has been capped during storage to reduce contamination.
3. Check the pH meter calibration at pH 4 and 7 and record the meter readings on the Water Chemistry Datasheet (RD[05]).
4. If the pH meter is off by ≥0.1 pH units, calibrate pH meter following pH meter manual.
5. Ensure sulfuric acid titrant solutions have not expired.
6. Allow samples to come to room temperature by letting the sample bottle(s) sit on the lab bench until the temperature has equilibrated. You can pour out the volume of sample you will use in the titration in a labeled glass beaker, covered with plastic, to help sample come to room temperature more quickly.

C.3 Sample Processing in the Lab

1. Determine the method (Inflection Point Titration (IPT) or the Gran method) of measurement you will use by evaluating known stream conductivity or alkalinity measurements. Most waters will use the IPT method. However, when alkalinity or ANC is <0.4 meq/L or 20 mg/L or conductivity <100 µS/cm the Gran method should be followed. Record method type on the Water Chemistry Datasheet (RD[05]]). For additional details on the IPT or the Gran method, see the USGS protocol (Rounds 2012).

   Note for Gran Method Users: This protocol details the IPT Method, although the information in the steps is still useful to the Gran Method users. See USGS Gran method (Rounds 2012) for detailed instructions on using the Gran Method to calculate alkalinity. Contributing carbonate species will not be determined). In short, titrate to **pH 5.5** (DO NOT GO PAST 5.5 TOO FAST), and then add acid in small increments (to change pH 0.2 -0.3 pH units). Titrate to pH of 3.5. Do NOT use a stir bar, but swirl solution gently (do not create a vortex) between additions. Wait 15 - 30 seconds before recording data and adding more acid.
2. Determine the sample volume and acid normality you will use (Table 7). The majority of measurements will require a 50 mL volume with 0.16 *N* titrant. Thus, if you do not know the expected alkalinity or ANC values, start with a sample volume of 50 mL and 0.16 *N* titrant, and adjust as necessary. 1.6 *N* will only be used when alkalinity or ANC is greater than 4.0 meq/L, although it may not be necessary. Note: Table 7 provides suggested sample volume and titrant normality, but should be adjusted as necessary per site. Record sample titration normality on the Water Chemistry Datasheet (RD[05]]).

Table 7. Suggested sample volume and titrant normality for alkalinity and ANC measurements based on approximate concentration ranges. ANC is acid neutralizing capacity. Table modified from Rounds 2012.

Alkalinity or ANC (meq/L)	Alkalinity or ANC (mg/L as $CaCO_3$)	Sample Volume (mL)	Titrant Normality (*N*)	Minimum Beaker Size (mL)
0-1.0	0-50	100	0.1600	150
1.0-4.0*	50-200	50	0.1600	100
4.0-20	200-1000	100	1.600	150
>20	>1000	25	1.600	50

* indicates the suggested volume and titrant solution if alkalinity or ANC is unknown.

3. Attach the titrant cartridge to the digital titrator body. Chemical resistant gloves and safety glasses are needed when handling the cartridge and setting up the titrator.
   a. Depress the plunger-release button and retract the plunger.
   b. Insert cartridge into the end slot of the titrator (Figure 8) and **rotate cartridge** one-quarter turn to lock into place.

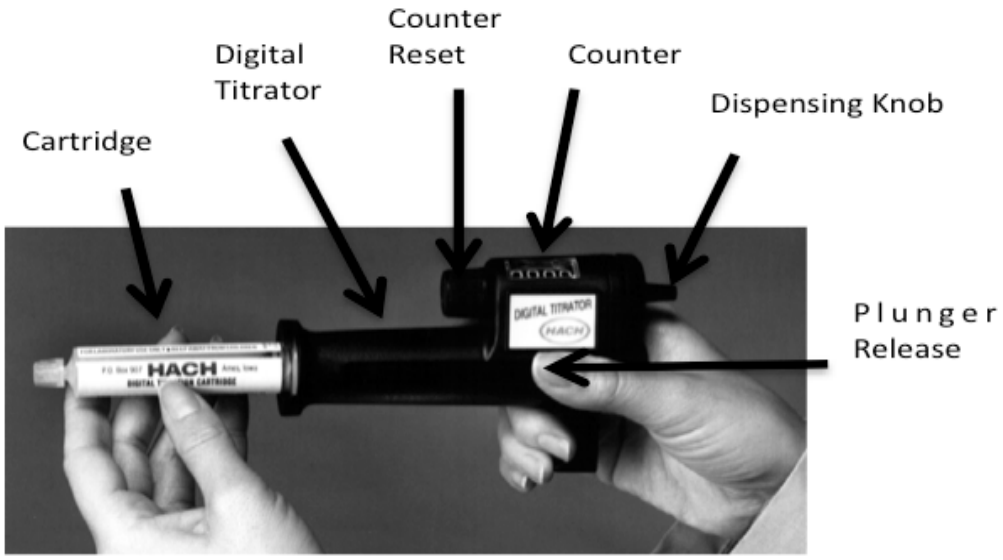


Figure 8. Inserting titrant cartridge into digital titrator. Photo from the Hach digital titrator manual.

   c. Depress plunger-release button and push plunger forward until it is touching inside of cartridge. If plunger will not engage with the cartridge, ensure that the cartridge has been rotated one-quarter turn and is locked into place.
   d. Attach titrator set up to titrator bracket on the mounting bracket.
4. While wearing gloves, remove cap on titration cartridge and insert a clean titration tube into the cartridge tip (Figure 9). If tube is new, label tube with correct normality (Figure 10). You may need to turn the titrator upright so the bubble comes to the tip. Store the cap in alkalinity test kit, so that you do not lose the cap. You will need to recap the cartridge when finished.

Figure 9. Digital titrator with titrant cartridge and titration tube attached. Photo from the Hach digital titrator manual.

5. Turn the dispensing knob to expel a few drops of titrant into a discard/acid waste container (Figure 10). This should remove air bubbles from the tube.

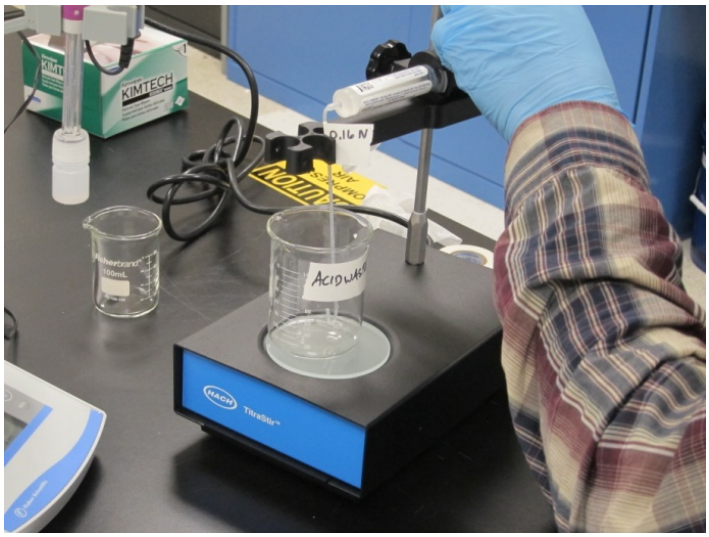


Figure 10. Expelling acid from digital titrator set-up into a temporary acid waste container

6. **Reset the counter to zero using the counter reset button** Figure 8) and wipe the tip of the tube with a soft, lint-free tissue, such as a Kimwipe®. Once set to zero, do not turn the delivery knob.
7. Place a clean, small, magnetic stirrer into the appropriate sized beaker (Table 7). Do not use a stir bar if conductivity is less than 100 µs/cm. Using a stir bar in low conductivity water will increase the diffusion of gases into the sample and alter the pH. If you have low conductivity water, after each titration, swirl the sample lightly by moving the beaker slowly in one circular motion. Do not swirl so fast that you create bubbles or a vortex in the sample. Allow the sample to stabilize before recording the data and continuing titrations.
8. Shake sample bottle for 30 seconds to homogenize.

9. Using a pipette (for alkalinity, filtered sample) or a graduated cylinder (for ANC, unfiltered sample), measure out the appropriate volume of sample and transfer to glass beaker (Table 7). Note: a pipette is a more accurate measuring device, and should be used on filtered alkalinity samples. Since particulates may get caught in the pipette tip, use graduated cylinders when measuring for non-filtered ANC samples.
    a. A small amount of sample will remain in the pipette tip when dispensing the sample from the pipette to the beaker. Touch and hold the tip of the pipette to the beaker wall and allow pipette to drain. Once flow from pipette stops, hold tip against the beaker wall for 10 more seconds to remove the majority of sample. A small volume of sample will be retained in the pipette.
10. Place the beaker on the stir plate and turn the power on. Stir should be slow and steady to avoid creating a vortex in the beaker.
    a. If sample splashes on wall of beaker, spray it down with DI water. Adding DI will not influence the titration reactions.
    b. If sample splashes out of beaker, start over.
11. DI rinse pH meter and temperature sensor and carefully blot dry with lint-free cloth. Be cautious not to rinse probes over sample.
12. Insert **pH meter and temperature sensor** into sample water, making sure to not touch the stir bar or the sides and bottom of the beaker.
    a. Sample solution must cover the pH sensor bulb, sensor reference electrode and temperature sensor (Figure 11). Increase volume, using pipettes or graduated cylinders, as necessary, or change beaker size, being sure to transfer the entire sample by rinsing beaker with DI into the smaller beaker. Volume of rinse DI should not be included as part of the sample volume.
13. **Record:** Start time of titration, Initial pH and temperature (°C), sample volume, titrant normality (0.16 or 1.60 *N*), and initial titrator count (should be reset to zero) (RD[05]).
14. Insert the digital titrator tip into the sample in the beaker without touching the stir bar. Tip should be immersed in the sample (Figure 11).

Figure 11. Titration setup with digital titrator, stir bar, pH meter, and temperature probe

Ensure nothing is touching the sides and bottom of the beaker or the stir bar.

15. **Add Titrant** (Table 8). After each addition of titrant, allow the stirrer to homogenize the sample for 15 – 30 seconds. Record pH and counter reading on the Alkalinity/ANC laboratory data sheet (RD[05]). You do not need to fill out the grey-celled columns. They will be calculated in a spreadsheet. **Near equivalence points (pH ~10, 8.1 and 5), pH can change rapidly (Figure 12). If you add titrant too fast or in too great of increments, you will miss the inflection point completely! Therefore, you must add titrant in smaller increments around these points, being sure to provide ample mixing time before the readings. The readings should be stable for at least 30 seconds before recording and continuing the titration.**
    a. pH ≥8.1 – Carbonate equivalence point (~pH 8.1) (Figure 12). As you approach pH 8.1, cautiously and slowly add titrant in small (but not less than three counts on the digital titrator) increments until sample pH is less than 8.0, and you are sure you have passed the equivalence point (~pH 8.1). If sample carbonate concentrations are high, larger increments can be used. **After each addition of titrant, allow the stirrer to homogenize the sample for 15 – 30 seconds. Record pH and counter reading on the Alkalinity/ANC laboratory data sheet (RD[05]).**
    b. pH <8.1 and ≥5.0 - Titrate with larger increments to just above a pH 5.0. If using the Gran method, record measurements every 0.2 to 0.3 pH units. Do not add in increments that are so large that you skip this region completely. **After each addition of titrant, allow the stirrer**

to homogenize the sample for 15 – 30 seconds. Record pH and counter reading on the Alkalinity/ANC laboratory data sheet (RD[05]).

c. pH <5.0 - Bicarbonate equivalence point. Cautiously and slowly add titrant in small (but not less than three counts on the digital titrator) increments from pH 5.0 to ≤4.0. If using the Gran method, titrate to pH ≤3.5. Titrate to pH ≤3.0 for samples with high organic acids or if sample range is unknown. **After each addition of titrant, allow the stirrer to homogenize the sample for 15 – 30 seconds. Record pH and counter reading on the Alkalinity/ANC laboratory data sheet (RD[05]).**

Table 8. Guidelines for sulfuric acid titration for alkalinity and ANC sample analysis

pH	Titrant addition guidelines
≥8.1	Add in small increments, no less than 3 counts
<8.1 and ≥5.0	Add in larger increments, but do not skip region entirely
pH <5.0	Add in small increments, no less than 3 counts

16. When possible, enter data into computer spreadsheet and graph the titration curve (change in pH divided by change in titrant volume (y-axis) by volume of titrant added (X-axis) (Figure 12).
    a. If more than one inflection point occurs in proximity, the true inflection point has been missed, and a duplicate sample should be analyzed being sure to take precaution and add titrant in smaller increments around the inflection point.

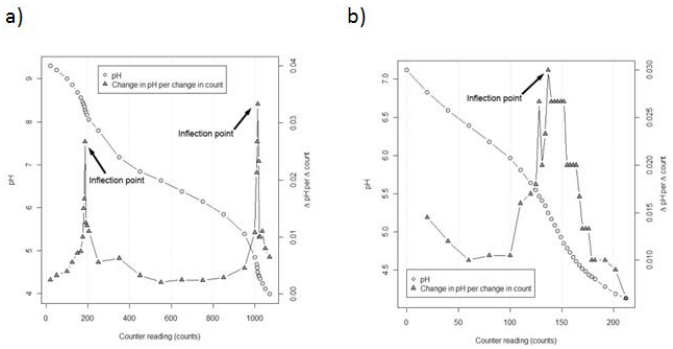


Figure 12. Example of inflection point titration using a digital titrator of a) a high alkalinity sample and B) a low alkalinity sample. Note difference in Y-axis scale. (Modified from USGS TWRI Book 9, Alkalinity, Version 3.0 7/2006).

17. When titration is finished, use soda ash or soda bicarbonate to return the sample pH to a pH 6 - 9. Use a pH meter to ensure the proper pH level.
18. Dispose of sample.
19. Repeat steps 1-18 for the second alkalinity/ANC sample.

20. Remove digital titrator from beaker. Depress plunger release and retract plunger to remove cartridge. Remove titrator tube. Cap cartridge tip.
21. Immediately double rinse titration tube and glassware with DI water and blot dry with lint-free soft paper tissue.
22. Place titration tubes in clean, sealable bag labeled with the titration normality (0.16 or 1.6 *N*).
23. Titration tubes can be reused if rinsed well, but should be only used for the same titrant normality. When tubes begin to show wear (e.g., stretching at the end that attaches to titrant cartridge), replace with a new one.
24. Store all glassware, titrator, titrator tubes and chemicals in the blue field case.
25. Rinse and re-use 250 mL alkalinity and ANC sample bottles.

C.4 Ending the Processing Day

1. Refreshing the laboratory supplies
   a. Check expiration date of sulfuric acid titrant and pH buffer solutions. Order more if expiration has passed or will be passed within the next month.
   b. Ensure you have enough equipment for the next sampling event. Refer to Section 6.1.
2. Equipment maintenance, cleaning and storage
   a. Double rinse glassware and titrator tubes with DI water immediately after use. Glassware, titrator, titrator tubes and chemicals should be clean and dry before storage. Titrator tubes should be stored in resealable plastic bags and labeled with the titrant normality for which they were used. Store alkalinity kit parts in the blue field case. Store cartridges in a resealable plastic bag in a cool, dark place or a frost-free refrigerator.
   b. Titrators do not require calibration. However, to ensure that the titrators have maintained calibration, annually perform a calibration check, using a known sample, of the accuracy and precision of the digital titrator.

SOP D Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

SOP E Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the CLA shipping document on CLA's NEON intranet site.

Shipments are to have a hardcopy of the "per Sample" tab of the shipping inventory (RD[08]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. ShipmentID must be included in the electronic version of the shipping inventory, but is not necessary for the hard copy. Also include the shipment tracking # in the email.

E.1 Handling Hazardous Material

N/A

E.2 Supplies/Containers

NOTE: Shipping vessels and materials vary with the number of sites and site type.

1. Pack glass bottles in packing material for protection from breaking.
2. Place samples into the cooler (12 qt for 1 site) and add ice packs (0˚ ice packs). **ALL** water chemistry samples should be surrounded by the ice packs, including the filter (wrapped in foil and placed in a resealable plastic bag). There should be at least an equal volume of ice and samples in the cooler. More ice will be required in the summer, since coolers may sit outside in the sun for several hours.
3. Surround bottles with absorbent packing material.
4. Fill remaining space with regular packing material.
5. Place 'per sample' tab of AOS shipping inventory (RD[08]) in a resealable plastic bag and tape to the inside top of cooler.
6. Tape the cooler shut and ship to appropriate address

E.3 Timelines

1. Ship samples to the External Water Chemistry Laboratory immediately following processing, when possible. Ship water chemistry samples **overnight** to the external laboratory within 24 hours from sample collection in order to minimize chemical speciation and sample degradation. Ship only samples that will be analyzed by an outside laboratory. Make sure ALK and ANC samples remain at the Domain Support Facility.
2. Ship samples "**Priority Overnight**."
   a. **DO NOT** send them "FedEX First Overnight."
   b. **DO NOT ship samples on Friday.**

E.4 Conditions

Keep samples at 0.5˚C – 6˚C. DO NOT FREEZE.

E.5 Grouping/Splitting Samples

N/A

E.6 Return of Materials or Containers

1. Include a return shipping label to your address with account information so the analyzing laboratory can return the cooler to you.
2. Place return shipping label and the AOS sample shipping inventory (RD[08]) in a resealable plastic bag and securely tape the bag to the inside cooler lid to help keep the forms dry.

E.7 Shipping Inventory

Fill out the AOS Sample Shipping Inventory (RD[08]). Each box sent should have a copy of the 'per sample' tab of the shipping inventory of its contents. The 'Shipment ID' does not need to be filled out on the hardcopy. The electronic shipping inventory that includes ShipmentIDs and IDs of all samples shipped should be emailed to the appropriate contact at the receiving analytical laboratory as well as the NEON CLA contact on the day that samples ship. Include shipping IDs and estimated arrival date(s)/time(s) in the email as well.

E.8 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

Rounds, S.A., 2012, Alkalinity and acid neutralizing capacity (ver. 4.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., sec. 6.6, September 2012, accessed December 2, 2012, from http://pubs.water.usgs.gov/twri9A6/.

U.S. Geological Survey, 2006, Collection of water samples (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A4, September 2006, accessed March 1, 2011, at http://pubs.water.usgs.gov/twri9A4/.

W.W. Woessner. 2007. Building a Compact, Low-Cost, and Portable Peristaltic Sampling Pump. Ground Water 45(6) 795-797.

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 9. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC. 002383	Datasheets for AOS Protocol and Procedure: Surface Water Chemistry Sampling in Wadeable Streams
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory

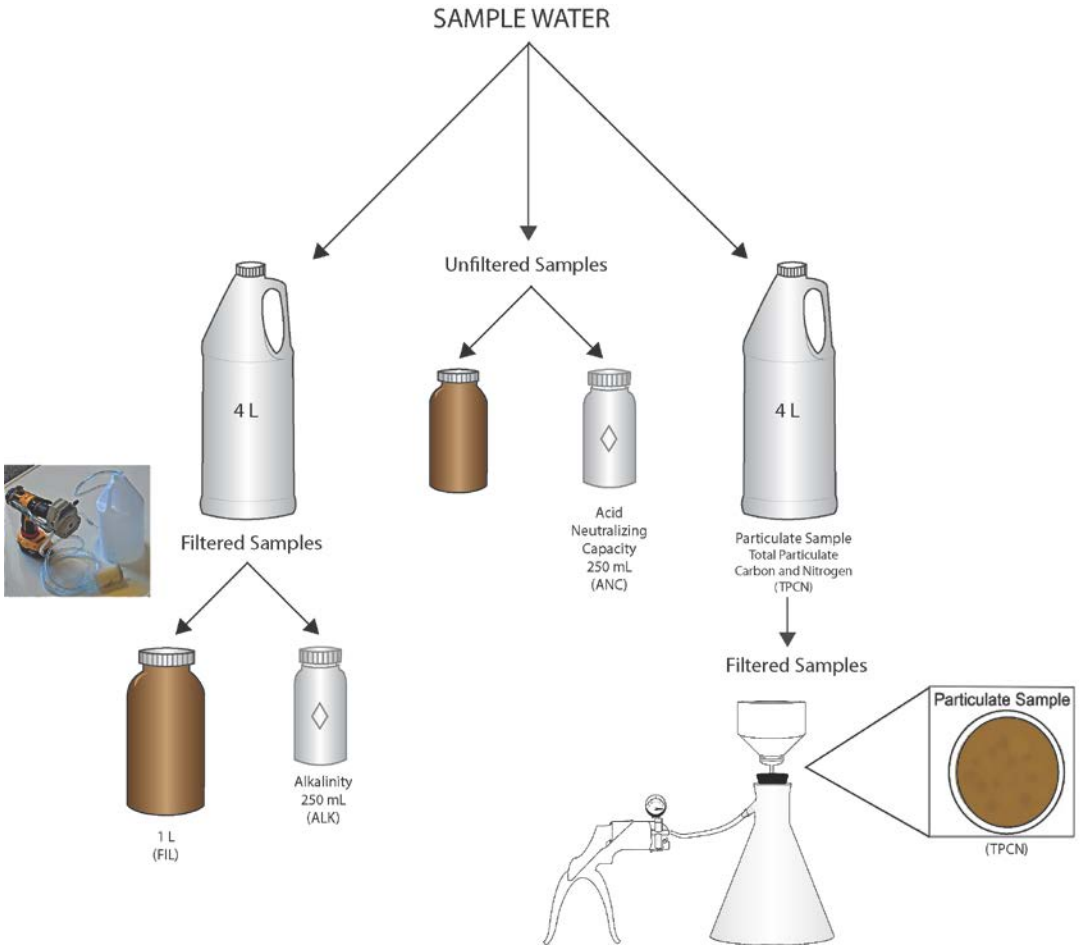
These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

B.1 Considerations for Implementation

Samples must be kept cool at all times (4°C +/- 2°C). Samples should be processed (filtered and within 3 hours. The sooner the samples are processed, the better the data quality. If there is a problem with sample filtration and particulates can still be seen in the filtered samples, samples can be re-filtered, ONLY if it is within the 3-hour time window from collection.

B.2 Flowchart of Sample Collection and Filtration



*◊ Indicates 250 mL, wide-mouth sample bottles that remain at the Domain Support Facility for analysis. Letters in parenthesis indicate the codes that correspond to the chemistry labels (see **Figure 4** a and b).

B.3 Steps for Water Chemistry Sampling

Step 1 – Check the water chemistry field sampling kit to make sure all supplies are packed.

Step 2 – Prepare labels (2" * 4").

a)

Sample ID: _____
(siteID.stationID.YYYYMMDD.sampleType)
Sample Type: **FIL** (Filtered) **RAW** (Unfiltered)
PCN (volume, mL units): _____

b)

Sample ID: _____
(siteID.stationID.YYYYMMDD.sampleType)
Sample Type: **ALK** (filtered) **ANC** (unfiltered)
TO BE ANALYZED IN DOMAIN SUPPORT FACILITY

Blank NEON Chemistry Labels for a) the External Analytical Laboratory and b) Internal NEON Domain Support Facility Measurements.

Step 3 – Ensure the General AQU Field Metadata Sheet (RD[06]) is completed per field site visit.

Step 4 – Navigate to the sampling location near sensor set 2.

Step 5 – Rinse the collection bottles and caps with the appropriate sample water (i.e., use filtered water to rinse filtered samples).

Step 6 – Collect samples

1. Filtered Samples: Fill glass bottle to the bottom of bottle neck, and fill ALK completely to reduce any changes in CO_2 concentrations due to headspace:
   a. 250 mL burned amber glass bottle for external lab (code FIL)
   b. ANC - 250 mL wide-mouth, HDPE – FILLED (code ALK) *to be analyzed at the Domain Support Facility.
2. Unfiltered Samples: Fill glass bottle to the bottom of bottle neck, and fill ANC completely to reduce any changes in CO_2 concentrations due to headspace:
   a. 250 mL burned glass amber bottle – Filled to neck (Code RAW)
   b. Acid Neutralizing Capacity: 250 mL wide mouth HDPE – RINSED – FILLED (Code ANC) *to be analyzed at the Domain Support Facility.
   c. 4 L jug (for PCN and/or Filtered Sample)

Step 7 – Filter for PCN particulate samples.

Step 8 – Ship samples overnight with ice pakcs within 24 hours of collection.

Step 9 – Complete ALK and ANC titrations in Domain Facility.

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment including labels.
- ☑ Pre-print labels on waterproof paper.
- ☑ Fill out the labels before they get wet.

Sample collection: Be sure to…

- ☑ Sample in the thalweg with the bottle pointed upstream, into the main flow of water, and several centimeters below the surface.
- ☑ Do not sample anywhere you or other field technicians have walked in the reach, or locations that appear recently disturbed. Wait for disturbance to pass.
- ☑ Use caution when sampling as items can easily fall into stream while bending to sample.
- ☑ Fill ALK and ANC bottles completely (no headspace), and fill all other sample bottles to the bottom of the neck.
- ☑ DO NOT FREEZE samples.

Sample filtering: Be sure to…

- ☑ Keep track of the volume of sample water filtered for PCN.
- ☑ Once poured, filter all of the water in the tower because particles will start to settle.
- ☑ DO NOT add more water into the filter tower than you can filter.

Sample titrations: Be sure to…

- ☑ Add titrant in smaller increments around equivalence points (pH~10, 8.1 and 5)
- ☑ After each addition of titrant, allow the stirrer to homogenize the sample for 15-30 seconds. In low conductivity samples, stir manually.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

APPENDIX E SITE-SPECIFIC INFORMATION

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.