2024 QUALITY ASSURANCE PROCEDURE

BIOGEOCHEMISTRY LABORATORY

Air, Water & Aquatic Environments Program
Rocky Mountain Research Station
U. S. Forest Service
U.S. Department of Agriculture

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Acronyms / Abbreviations

AD	analytical duplicate
ASTM	American Society for Testing and Materials
BB	bottle blank
CFR	Code of Federal
cm	centimeter
DIC	Dissolved Inorganic Carbon
DIW	deionized water
DL	detection limit
DOC	Dissolved Organic Carbon
DSOL	Dissolved Solids
EPA	Environmental Protection Agency
FD	field duplicate
FEF	Fraser Experimental Forest
HDPE	High Density Polyethylene
IC	Ion Chromatograph
L	Liter
MDL	minimum detection limit
µeq	microequivalent
µg	microgram
µm	micrometer
µS	microsiemen
mg	milligram

mL	milliliter
MDL	Method Detection Limit
ML	Minimum Level of Quantification
ng	nanogram
NIST	National Institute of Standards and Technology
NPS	National Park Service
PPE	Personal Protective Equipment
ppb	parts per billion
ppm	parts per million
psi	pounds per square inch
QA	Quality Assurance
QAP	Quality Assurance Plan
QC	Quality Control
QCCS	Quality Control Check Sample
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SOP	Standard Operating Procedure
SRS	Standard Reference Sample
SSCS	Second Source Check Standard
SSED	Suspended Sediment
TDN	Total Dissolved Nitrogen
TDS	Total Dissolved Solids
TN	Total Nitrogen
TOC	Total Organic Carbon

TV (tv)	Transition Value
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
v/v	volume to volume ratio
w/w	weight to weight ratio
W&W	Water and Watersheds Program

Water Analysis Laboratory
Quality Assurance Procedure

1.0 Introduction

The Rocky Mountain Research Station W&W Biogeochemistry Laboratory was established in the spring of 1988 to provide baseline data characterizing the water and snow chemistry at the Fraser Experimental Forest. The Biogeochemistry Lab specializes in analyzing research samples originating from streams, standing water, precipitation, and sub-surface water. Services conducted by the laboratory include sample analysis, filtration, preservation, and extraction. The Biogeochemistry Laboratory uses standard analytical procedures and practices that in specific cases have been altered to meet the analytical needs of multidisciplinary research.

The W&W Biogeochemistry Laboratory Quality Assurance Procedure (QAP) describes protocols and procedures used in the laboratory. This living document will be updated and revised as new methods and procedures are implemented. Methods, detection limits, and acceptance parameters are tabulated for all analytical procedures. Standard Operating Procedures (SOPs) are listed in the appendix and are available as separate documents.

2.0 Project Organization and Personnel

The current staff consists of one full-time chemist, one full-time technician, and three student interns. Personnel and their primary responsibilities include:

> Timothy Fegel, Biogeochemist and Lab Manager: Integrion IC, Alkalinity, pH, conductivity, Lachat QuikChem AutoAnalyzer, Shimadzu, Turner Trilogy Fluorometer, FreeZone Lyophilizer, Perkins Elmer NexION ICP-MS, documentation, database management, staffing, quality assurance and reports.
>
> Technician and Student Interns: sample log-in, sample preparation and filtration, extractions, reagent preparation, sample storage and organization, cleaning of glassware and laboratory, general chemist support.

3.0 Quality Assurance Objectives

Quality Assurance (QA) Objectives for the W&W Biogeochemistry Laboratory are outlined in Table 3.1. Project specific QA Objectives supersede those listed.

Table 3.1 Methods and Detection Limits

Analyte	Reference Method	Method Description	Minimum Detection Limit	Practical Quantitation Limit
Carbon, Total	EPA 415.1	Shimadzu TOC-L Combustion Analyzer	0.04 mg/L	0.1 mg/L
Carbon, Inorganic	EPA 415.1		0.04 mg/L	0.1 mg/L
Carbon, Dissolved Organic	EPA 415.1		0.04 mg/L	0.1 mg/L
Nitrogen, Total	ASTM D5176		0.01 mg/L	0.05 mg/L
Nitrogen, Total Dissolved	ASTM D5176		0.01 mg/L	0.05 mg/L
pH	EPA 150.1	Mettler Toledo InMotion Pro	NA	0-14 pH units
Conductivity	EPA 120.1		0.20 µS/cm	0.80 µS/cm
Acid Neutralizing Capacity	EPA 310.1		0.20 µeq/L	1.00 µeq/L
Anions - F, Cl, Br, NO2, NO3, PO4, SO4	EPA 300.0	Thermo Fisher IntegrIon Ion Chromatograph	0.01 mg/L	0.05 mg/L
Cations - Na, NH4, K, Mg, Ca	ASTM D6919-03		0.01 mg/L	0.05 mg/L
Soil Ammonium - N	Lachat 12-107-06-2-A	Lachat QuikChem AutoAnalyzer FIA+ 8000 Series	0.05 mg/L	0.1 mg/L
Soil Nitrate - N	Lachat 12-107-04-1-B		0.05 mg/L	0.1 mg/L
Metals, Total	USGS 2015-1010	Perkins Elmer NexION ICP-MS	0.005 mg/L	0.01 mg/L
Phosphorus, Total			0.001 mg/L	0.005 mg/L

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4.0 Sample Containers and Glassware Preparation

This section details the protocols for washing sample aliquot bottles, general laboratory glassware and analysis of specific vials/tubes. All laboratory preparation, filtering, and cleaning are done while wearing nitrile gloves and appropriate safety gear.

4.1 Sample Bottles

Remove tape labels and rinse bottles six times with DIW. Caps are rinsed along with bottles and placed on drying rack shelves. Soak bottles for 12-48 hours in a 1.0M HCl bath. Rinse bottles six times with DIW and place them upside down on

drying shelves. Fill with DIW until there is no headspace for storage. New bottles received the same wash treatment before use.

Bottle blanks are used bi-annually to verify the bottle-washing procedure. Bottles are filled with DI and remain at sealed at room temperature for a period no less than 10 days. Analytical results of the bottle blank samples should be lower than one standard deviation over the detection limit.

4.2 Glassware

Empty, remove tape and markings, and rinse five times with DIW. If residue is present, scrub with a brush and repeat rinsing. Fill and soak overnight. Rinse glassware one more time before drying. Invert glassware and place it on drying shelves with freshly lined absorbent matting. When dry, glassware is sealed with parafilm and stored in the proper cabinet for future use.

4.3 Filter Equipment

Thoroughly rinse each part individually with DIW before and after use, as well as between samples. Cover individual parts with Kimwipe before storage in the laboratory cabinet.

4.4 Carbon Analyzer Glassware

Rinse tubes and caps four times with DIW and soak in DIW overnight. Caps can then be covered and dried on drying shelves. Bake tubes in a muffle furnace at 500 ºC for no less than three hours. Allow tubes to cool overnight in the furnace. Tubes and lids are reassembled and stored in a laboratory cabinet under a sealed layer of aluminum foil.

4.5 Ion Chromatography Vials and Glassware

All glassware and plastic containers used for ion chromatography eluent, reagent, and dilution purposes are rinsed with DIW five times and allowed to soak overnight before a final DIW rinse. Items are to be dried upside down on drying racks. Sample vials for the Integrion IC arrive sterile from Thermo Fisher Scientific and are not reused.

4.6 Plastic Falcon Tubes For pH, EC, and Alkalinity

Plastic 50 ml falcon tubes are rinsed with DIW and soaked overnight. Falcon tubes are then rinsed three times with DIW and placed upside down on drying racks.

4.7 Miscellaneous Glassware, Carboys, Plastic Containers, Etc.

In general, all vessels or containers should be rinsed 6 times with DIW, soaked overnight, rinsed again, and placed upside down on drying shelves. All vessels or containers should be stored in enclosed areas protected from contaminants.

4.8 Laboratory Maintenance

Trace-level analysis requires exceptional laboratory hygiene. See the Appendix for regular laboratory cleaning and maintenance tasks.

5.0 Sample Custody, Preparation and Preservation

The accuracy of analytical data as a representation of true sample composition is dependent upon the collection and treatment of samples before they arrive at the laboratory. Sampling techniques and procedures must be such that the sample does not deteriorate or become contaminated before it reaches the lab. Recommended protocols for field collection are located in the appendix.

5.1 Sample Custody

A sample log, and labeled sample containers, should be delivered to the lab as soon as possible following sample collection. Requested sample analyses should be discussed and agreed upon before sample delivery. Once at the laboratory, samples are entered into the tracking system. Sample condition, number of samples, and date of receipt are recorded (see Sample Receipt and Tracking Form in the appendix). Before and after analysis, all samples will be stored below 4 ºC, or frozen if necessary.

Before analysis, samples are logged into the electronic database, a project code is assigned (see Table 5.1) and samples are numbered consecutively within the code for each project.

5.2 Sample Storage

Samples are stored in the walk-in cold room (4ºC), or one of the laboratory freezers (-8 ºC) / refrigerators (4ºC). Storage temperatures are monitored daily through the use of thermometers or HOBO temperature sensors. Analyzed samples are held for one month after submission of the final database or longer based on a preexisting agreement.

5.3 Sample Processing and Preservation

Samples requiring filtered aliquots should be filtered as soon as possible after collection to minimize biological and algal activity. Membrane filters (pore size 0.45 um) and glass-fiber filters (0.7 um) are most commonly used. The W&W Biogeochemistry Lab uses hydrophilic Millipore Durapore PVDF membrane filters (FisherSci #HVLP-047-00) for ion chromatography analysis and Millipore borosilicate glass fiber filters (FisherSci #APFF-047-00) for carbon analysis. See the Appendix for the RMRS lab filtering protocol. After filtering, both filtered and unfiltered samples should be stored in the dark at 4°C until delivery at the lab.

In general, the most reliable analytical results are obtained when samples are analyzed immediately after collection. This is rarely possible. The most commonly used sample preservation methods consist of the addition of chemical preservatives. For intermediate and long-term storage purposes we recommend freezing a filtered sample aliquot for most analyses; other aliquots should be kept cold and in the dark. See Table 5.2 for various analysis- specific preservation and hold time procedures used by the W&W Biogeochemistry Lab.

Regardless of the preservation method, complete stability for every constituent is unattainable. Strict rules for preservation of water samples do not exist and effectiveness of most preservation methods are questionable for various analytes. Extensive studies have been published supporting preservation of water samples by freezing and acidification for many analytes. Whatever methods are used, they should be consistent across the life of the project and procedures should be well documented. The lab should be notified in advance of the preservation method used.

Table 5.2 Sample Hold Times

Analysis	Storage Temperature		Hold Time*
	Filtered	Unfiltered	
Acid Neutralizing Capacity	-----	4ºC	7 days
Ammonium	-20ºC or 4ºC	-----	48 hrs unless frozen
Bromide	-20ºC or 4ºC	-----	28 days unless frozen
Calcium	-20ºC or 4ºC	4ºC	30 days unless frozen
Carbon, dissolved or total organic	-20ºC or 4ºC	4ºC	14 days or acidify
Carbon, inorganic	4ºC	4ºC	72 hrs
Chloride	-20ºC or 4ºC	-----	28 days unless frozen
Magnesium	-20ºC or 4ºC	4ºC	30 days unless frozen
Metals	-20ºC or 4ºC	-----	Acidify immediately with metals grade HNO_3 to 2% w/w
Nitrate	-20ºC or 4ºC	-----	48 hrs unless frozen
Nitrogen, total dissolved or total	-20ºC or 4ºC	4ºC	28 days or acidify
Phosphate, ortho	-20ºC or 4ºC	4ºC	48 hrs unless frozen
Phosphorous, total dissolved or total	-20ºC or 4ºC	4ºC	28 days until digestion unless frozen
pH	-----	4ºC	7 days
Potassium	-20ºC or 4ºC	4ºC	30 days unless frozen
Silica	4ºC	-----	28 days
Sodium	-20ºC or 4ºC	4ºC	30 days unless frozen
Conductivity	-----	4ºC	7 days
Sulfate	-20ºC or 4ºC	-----	28 days unless frozen

5.4 Sample Tracking

Requested analyses are entered into the database at the time of sample arrival. Sample analysis progress is tracked through data entry.

6.0 Field Sampling Quality Assurance and Control

An additional 10% of the total number of samples collected in the field should be collected as either field blanks or field duplicates. Field blanks should use the same bottle type as the regular sample. Blanks should be filled completely (no headspace) with previously tested deionized water before leaving the laboratory. The blank should travel into the field in the same manner as a regular sample. Field duplicates should be collected in an independent bottle of the same type as the regular sample.

7.0 Calibration and Analytical Procedures

Standard Operating Procedures (SOPs) are available as individual documents for each analysis used by the W&W Biogeochemistry Laboratory. A complete list of methods is found in Table 3.1 and general laboratory procedures are documented here. Additional methods may be developed upon request, and as new instrumentation is obtained.

Run logs are maintained for each instrument. They contain information such as analysis run details, samples analyzed, instrument maintenance, problematic symptoms, troubleshooting, and response.

Descriptions of analytical procedures including instrument calibration are detailed in each analyte-specific SOP. General laboratory procedures are outlined below.

7.1 Balance and Pipette Calibration

All laboratory balances are calibrated monthly. Pipette calibration is checked daily by weight to within 0.1% of the theoretical weight of the aliquot volume.

7.2 Calibration Standard Preparation

Standards are prepared by serial dilution of stock solutions purchased from vendors that provide traceability to National Institute of Standards and Technology (NIST) standards. Preparation of stock and working standards is recorded on worksheets (see example in appendix) and documented by the weight of standard added to a given flask before dilution to volume with DIW. The weight of the standard dispensed must be within 1% of the expected value. All records of certification and standard preparation are kept on file.

7.3 General Calibration and Analysis Procedures

Analytical instrumentation is calibrated at the beginning of each analysis set with

three to seven working standards. Thermo Fisher IntegrIons are calibrated with 7-point standard curves using Inorganic Ventures stock solutions IC-FAS-1A for anions and IC-SCS1 for cations. Shimadzu TOC-V is calibration using independent 5-point calibration curves for DOC and TDN using Aqua Solutions DC843-155. The Mettler Toledo InMotion Pro is calibrated for pH using a 3-point curve with Fisher Scientific pH standards 4, 7, and 10 and is calibrated for EC using RICCA Scientific 84µS/cm standard. A second source check standard (SSCS) is analyzed after the calibration and after every 5-15 samples. For most analyses, the SSCS is followed by a blank. The SSCS is prepared from a source or lot different than that used for the calibration standards. Check standard recovery must be within 5% of theoretical value, or within normal observed limits of variability, to accept the sample data preceding it. In addition to the SSCS, a detection limit standard and/or a bulk quality control check standard (QCCS) may be analyzed once at the beginning and end of each run. Approximately 5% of the samples analyzed are duplicated; duplicate values must be within 5% of the original value.

7.4 Minimum Detection Limits

Minimum Detection Limit Testing is performed annually in February and March to determine the lowest possible reporting values for each of the instruments. Updated values are recorded in this Lab Annual Quality Assurance Procedure and the laboratory website (Table 3.1). The minimum (aka method) detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte (U.S. EPA, 40CFR136, App. B). The MDL is determined by repeated analysis of a standard solution approximately five times the concentration of the estimated detection limit. The standard sample used in the determination of the MDL should complete all normal sample processing steps used in the analytical method. At least ten measurements are recommended for determining the MDL. The MDL is calculated as follows:

$$MDL = s \times t_{(n-1,1-\propto=0.99)} \quad (1)$$

Where

n = number of replicate spike determinations at 1 to 5 times the estimated MDL.

s = standard deviation of measured concentrations of *n* spike determinations

t = Student's *t* at *n-1* degrees of freedom and 1-α (99%) confidence level.

8.0 Internal Laboratory Quality Control Checks

Analytical instrumentation is calibrated using standard solutions of the analyte of interest as described in section 7.0. The calibration correlation should be greater than 0.999. Data for each sample is visually analyzed within the instrument software for preliminary identification of problems including rogue peaks, unusual peak shapes and tailings, baseline drift, and noise. Drift is monitored with check standards and blanks throughout the analysis run. Check standards are from a source or lot other than that of the calibration standards. If drift outside 5% recovery is observed, the run is stopped and the instrument recalibrated, or the analysis is repeated. Samples beyond the last acceptable check standard are reanalyzed.

For most analyses, a bulk, surface water Quality Control Check Standard (QCCS) is analyzed twice during each analysis run. Action is required for results outside three standard deviations of expected values, and may include recalibration and reanalysis, instrument maintenance, and/or repair. Some analyte concentrations may change over time and this must be taken into account when determining appropriate response.

Sample duplicates are used to estimate precision. When sample volume allows, 5% of the samples are duplicated in the laboratory for every analysis. Values for both lab and field duplicate samples should be within 5% of the regular sample value. Blanks are run throughout the analysis to monitor carry-over, detection limit standards, and filter and/or bottle blanks. Blank values should be within 5% of the baseline for every analyte.

9.0 Performance and System Audits

The W&W Biogeochemistry Laboratory participates in the USGS inter-laboratory comparison study for laboratory quality assurance testing semiannually. The program provides the laboratory with a Standard Reference Sample for precipitation. The accuracy of analytical results is ascertained based on performance in the program.

10.0 Calculation of Data Quality Indicators

At lower concentrations, precision objectives are equivalent to the MDL, and based upon the standard deviation (*sd*) of a set of repeated measurements:

$$sd = \sqrt{\sum {(x - \bar{x})^2} \Big/ (n - 1)}$$

Where x is an individual measurement and $\bar{x}$ is the mean of the measurement set.

For higher concentrations, the precision objectives are based on the percent relative

standard deviation (*%RSD*).

$$\%RSD = \frac{sd}{x} * 100$$

This reduces the problems of unreasonable objectives for low or high analyte concentrations. Concentration ranges are specified to determine the concentration at which absolute or relative terms apply. The division between the ranges, the Transition Value (*tv*), is estimated by:

$$tv = \frac{\sqrt{\frac{sd}{2} * sd}}{RSD} - \frac{sd}{2}$$

where *RSD* = *%RSD*/100.

To use difference instead of the standard deviation to evaluate precision, the difference between two measurements is used for the absolute term and the relative percent difference (*RPD*) is used for the relative term:

$$RPD = \frac{|x_1 - x_2|}{\overline{x}} * 100$$

For any given sample run, the RPD is calculated for the check standards within the run by subtracting the value from the actual concentration divided the annual average for the check standard. This data is available upon request.

11.0 Data Analysis and Validation

Raw data are exported from instruments in various formats. These data are then concatenated into single .xlsx and .csv files for examination and validation. Samples that do not meet the following validations are flagged and highlighted for reanalysis. Validation of analytical results may include the following calculations:

- For projects requesting a complete analytical suite of anions, cations, pH and alkalinity, an ion balance may be performed to check for completeness and identify any outlying values. The balance may be skewed if there is an abundance of an ion not analyzed (including heavy and transitional metals), but the balance check works well for most water samples.

$$Ion\ Balance = \frac{\sum anions}{\sum cations}$$

Where:

$\sum anions = HCO_3 + SO_4 + Cl + NO_3 + PO_4$

$\sum cations = H + Ca + Mg + K + Na + NH_4$

(All ion concentrations are in units of ueq/L)

- Total nitrogen concentration should be equal to or greater than the sum of ammonium and nitrate.
- Total phosphorus concentration should be greater than orthophosphorus.
- Total (unfiltered) results should be greater than dissolved (filtered) results.
- Comparison of field and laboratory duplicates.
- A mostly linear trend between Ca concentration and acid neutralizing capacity should exist.

12.0 QA/QC Reports for Long-Term Datasets

Sampling sites with long term data collection are subject to further quality assurance and control procedures. Sample values for each analyte are compared to historical values within the database. Samples are statistically analyzed on a site basis using a Tukey Honest Significant Difference test with the parameters set to flag samples two standard deviations away from the mean of the collection record for any analyte. Annual and monthly box and whisker plots using the Tukey HSD parameters are created for every analyte at each site within the dataset. Flagged samples are highlighted for reanalysis. QA/QC reports for any site from the long term database are available on request.

13.0 Reanalysis of Flagged Samples

Samples identified as being potentially erroneous in sections 9.0 and 10.0 are reanalyzed within the specified holding time for analyte(s) in question. The QA/QC procedure is then repeated with the new value. If the new value meets the analysis criteria within sections 9.0 and 10.0, the new value is reported. If the new value does not meet the QA/QC criteria, but is within 5% of the original value, then the old value is reported but flagged as an outlier. Potential reasoning for irregular values (e.g contamination, long holding times, etc.) is noted in the comments section of the dataset.

14.0 Data Reporting

Analytical results, sample information and calibration summaries are sent electronically to the project PI in Excel and CSV formats. Unless other arrangements are made, investigators have three weeks to review the results and request reanalysis. QA/QC reports, including statistical code and figures and data validation calculations, are available upon request

15.0 References

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

14.2 Code of Federal Regulations. Protection of Environment. Section 40, Appendix B to Part 136. Definition and procedure for the determination of the method detection limit. Revision 1.11. Revised July 1, 1990. Office if the Federal Register, National Archives and Records.

14.3 ASTM. American Society for Testing and Materials. Standard Specifications for Reagent Water. D1193-77 (reapproved 1983). Annual Book of ASTM Standards, Vol. 11.01. ASTM: Philadelphia, PA, 1991.

14.4 Water Chemistry Laboratory Manual, Wadeable Streams Assessment. U.S. Environmental Protection Agency, Office of Water, Washington DC; EPA841-B-04-008, 2004.

14.5 Recommended Guidelines for Sampling and Analyses in the Chesapeake Bay Monitoring Program, U.S. Environmental Protection Agency; EPA 903-R-96-006, 1996.

14.6 D.T.E. Hunt and A.L. Wilson, "The Chemical Analysis of Water: General Principles and Techniques". Royal Society of Chemistry; Burlington House, London; 1986

14.7 Chaloud, D. and Peck, D.V. (Eds) 1994. Environmental Monitoring and Assessment Program: Integrated Quality Assurance Project Plan for the Surface Waters Resources Group, 1994 Activities. EPA 600/X-91/080, Rev. 2.00. U.S. Environmental Protection Agency, Las Vegas, Nevada.

14.8 Patton, C.J. and Gilroy, E.J. 1999. U.S. Geological Survey; Nutrient Preservation Experiment – Experimental Design, Statistical Analysis, and Interpretation of Analytical Results; Water-Resources Investigations Report 98-4118; U.S. Geological Survey. Denver, Colorado.

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

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

14.10 Environment Canada, Analytical Methods Manual; August 1979. Inland Waters Directorate; Water Quality Branch; Ottawa, Canada.

Appendix A – Water Sampling Protocol

- Streams should be sampled upstream from any man-made structure.. Collect from the same sampling site for each repeated measure. Avoid disturbing the bottom as additional particulates may affect analysis.
- If a temperature reading is required, leave the thermometer immersed for five minutes before reading. Avoid disturbing the bottom with the thermometer at the sample site.
- Label bottle with location (geographic area name and stream or lake name), date, and time of day, water temperature, and sampler's initials. Label the bottle before immersion using a black permanent marker.
- Use nitrile gloves when handling bottles during sampling. Bug repellents or sunscreen are particularly troublesome as contaminants. Once the gloves are on, be careful not to touch your skin, the ground, etc.
- Be sure to immerse the sample bottle completely, 10 cm (4 inches) deep, with the mouth of the bottle pointing upstream, so no water flows over your hand into the bottle. Remove the cap under water. Be sure the bottle does not get near the bottom of the stream where sediments can be disturbed. Fill bottle at least half full, replace cap loosely, remove from water, and shake. Pour out rinse water downstream of the sample point. Pour some rinse water over the inside of the cap. Do not touch the bottle mouth or the inside of the cap. Partially fill the bottle, cap, shake, and rinse three times.
- Collect the sample on the fourth immersion. Use the same procedure as before but fill the bottle completely. Be careful not to contaminate the sample with surface film, contact with human skin, breathing in/on the bottle or cap, etc. If necessary, squeeze the bottle slightly as the cap is tightened so no air remains in the bottle. If the stream is too shallow to immerse the bottle fully, collect as much as possible, being very careful not to touch the bottom. Note depth on field notes.
- Collect a "duplicate" sample if instructed. Generally, every 10th sample collected is duplicated. Sample sites chosen for duplicate sampling are selected at random among the streams sampled. If a duplicate is required for your site, repeat procedures as with normal stream samples. The duplicate is the second when

two samples are collected. Duplicates document the repeatability of individual sample collections and reproducibility of laboratory results.

- One "field blank" sample should be taken to the field and remain unopened each sample day. Write sample study area, stream name, date, time of day, sampler's initials, and 'FB' on the bottle. Field blanks are necessary to quantify chemicals from non-sample sources such as water bottles, DI water, filter paper, handling procedures, etc. The extra bottle for the FB can be used for the sample in an emergency if the sample bottle is lost or contaminated.

- Place the sample immediately in a Ziploc bag in a cooler after collection. Do not expose sample bottles to the sun. Fill out the field data sheet, noting any unusual conditions such as wind or rain. Measure air temperature (shaded) and record. Keep gloves clean for next use. Rinse clean, dry, and transport gloves in a clean zip lock bag.

- Samples are filtered in the lab. Keep samples cool while transporting. Zip lock bags (double bagged) filled with snow work well if frozen icepacks are unavailable for transport from the field. Store at 4 C but do not freeze. Ship to the lab in a picnic cooler with frozen “blue ice” packs via FedEx or UPS overnight. Do not ship so the sample arrives on a weekend. If necessary, keep samples refrigerated for arrival on weekdays. Hand delivery to the lab is preferred, or arrange for a contact to pick up the samples.

Ship or deliver samples to:

Timothy Fegel
240 W. Prospect
Fort Collins, CO 80526
(970) 498 – 1017
timothy.fegel@usda.gov

Appendix B – Ion Chromatography Calibration Standard Table

Anions (mg/L)

Level	Conc.	F	Cl	NO3	PO4	SO4
2	DX.25	0.050	0.075	0.250	0.375	0.375
3	DX.5	0.100	0.150	0.500	0.750	0.750
4	DX1	0.200	0.300	1.000	1.500	1.500
5	DX2	0.400	0.600	2.000	3.000	3.000
6	DX3	0.600	0.900	3.000	4.500	4.500
7	DX4	0.800	1.200	4.000	6.000	6.000
8	DX5	1.000	1.500	5.000	7.500	7.500

Cations (mg/L)

Level	Conc.	Na	NH4	K	Mg	Ca
2	CDX.025	0.050	0.100	0.050	0.050	0.250
3	CDX.05	0.100	0.200	0.100	0.100	0.500
4	CDX.1	0.200	0.400	0.200	0.200	1.000
5	CDX.2	0.400	0.800	0.400	0.400	2.000
6	CDX.3	0.600	1.200	0.600	0.600	3.000
7	CDX.5	1.000	2.000	1.000	1.000	5.000
8	CDX1	2.000	4.000	2.000	2.000	10.000

Appendix C – Ion Chromatography Eluent Procedures

Cation Eluent

1. Fill a 1000mL volumetric flask with DI and place it in a vacuum chamber.
2. Degas the eluent under pressure for 2 hours.
3. Pour degassed DI into eluent carboy and add 40mL of 1N methylsulfonic acid.
4. Repeat steps 1 and 2 and add to the eluent carboy, bringing the total volume of eluent to 2L.

Anion Eluent

1. Use the analytical balance to weigh out 1.145 g of Sodium Carbonate (Na_2CO_3) and 0.101 g of Sodium Bicarbonate ($NaHCO_{3)}$ and put both into a 4-liter carboy.
2. Add deionized water to the 4-liter mark on the carboy, tighten the lid (make sure to use a lid with a washer inside for a tight seal) and shake to dissolve the salts. The solution may need to sit for a while for the salts to completely dissolve.
3. Degas the eluent with helium gas for several minutes or let sit overnight.

Appendix D – Sampling Filtering Procedure

A. Introduction

Solutes present in surface waters are primarily derived from precipitation inputs and geochemical weathering. Because samples must be shipped out for chemical analysis, it is important that the original chemical composition remains unchanged before and during transport. Particulates in a sample can affect chemical results as well as clog lines of sensitive instrumentation. Biological activity in the sample can alter chemical composition. Therefore, it is important to filter samples as soon as

possible post sampling to remove particulate materials, spores, and bacteria in sample water that might affect chemical analysis.

B. Equipment and Supplies

Equipment and supplies are located in the Water Lab 201A.

Bottle(s) with sample

Absorbent Surface Liner Sheet (~20”x20”)

Millipore* Sterifil* 47mm Aseptic Vacuum Filter System (funnel, holder and receiver flask)

Gast* Pressure/Vacuum Pump or Nalgene* PVC Hand-Operated Vacuum Pump

Millipore* Durapore® Membrane Filters 0.45µm (IC Samples)

Millipore* Glass Fiber Prefilters 0.7µm (DOC Samples)

Clean forceps (blue) and tweezers

Deionized (DI) water for rinsing

Waste bucket

Clean 60-ml bottle(s) labeled with site name, date, temp., precip. Amt. or discharge, Sample ID#, etc. (copy all information from sample bottle)

C. Estimated Time to Complete Procedure

Allow approximately 5-10 minutes per sample, although filtering times may vary greatly depending on the amount of particulate matter in the sample.

D. Preparation

Washing

All pump apparati, except receiver flask, should be DI-washed after each use. This can be done in the sink in the Water Lab. To clean, triple rinse with DI water, soak in a bucket of DI for 24 hrs., triple rinse again, and air dry in a drying rack covered with a couple of Kimwipes. Conductivity of the rinse water should be < 2 µS cm-1.

Glass bottles must be triple rinsed in DI water and baked at 500°C for 5 hrs. The lids are washed as per above. These should be washed and baked in the RMRS Lab. They are stored empty, dry and capped in their original boxes and should be returned to the RMRS Lab in their original boxes when possible.

E. Procedures for filtering sample

Rinsing Pump

4. Rinse 250mL funnel and funnel holder with DI for ~3-5 sec. and place on clean surface (e.g. Kimwipe or on clean part of surface liner sheet)

Loading Filter

5. Using tweezers, remove blue papers over filter, throw away and carefully remove one filter (Millipore® 0.45µm or Glass Fiber 0.7µm) from box. Place filter paper on the holder. If filter is not centered, carefully adjust its position by pulling on edge with tweezers.

   Note:

   Millipore* Glass Fiber filters do not have any papers separating each filter. It is necessary to look carefully at each filter to make sure that you do not have more than one filter stuck together.

WARNING!

Be careful handling filters, as any contact of filter by forceps in any area except its extreme outer edge will result in contamination or damage to filter.

DO NOT breathe, sneeze, cough on or touch filter paper or any apparati that may come in contact with sample.

6. Screw filter holder onto funnel.

Filtering Sample

7. Place pre-labeled 60mL bottle in receiver flask using blue forceps. Make sure to place as little of the forceps tip into the bottle as possible to reduce the risk of contaminating the bottle or sample.

8. 2. Pour a few milliliters of sample into the funnel and pump through.

9. Remove 60mL bottle with filtrate using blue forceps. Replace lid, shake, remove lid and pour into waste bucket.

10. Repeat steps 1-3 three times before filling.

11. Pour 50mL into funnel for final sample.

    Note:

    If sample contains enough matter to clog the filter before the 60mL bottle is filled, it may be necessary to stop, change filter, and resume filtering. Remember to rinse the new filter with sample before beginning to fill bottle again.

12. Change filters between samples. Follow pump rinsing procedure as mentioned previously.

13. After all samples are filtered clean all equipment following the washing procedures described in section D above.

14. Store the 250mL samples and 60-mL filtered sample bottles in the refrigerator or in a cooler with a sufficient number of ice packs to keep the samples at ~40°F.