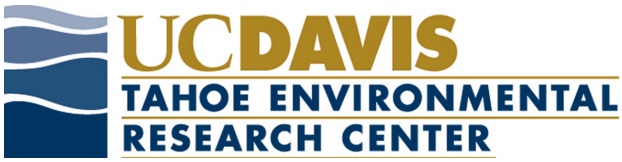
Laboratory Procedure, Field Protocol and Quality Assurance Manual



Tahoe Environmental Research Center, UC Davis
291 Country Club Drive
Incline Village, NV 89451
775-881-7560/530-754-8372

Tahoe City Field Station
2400 Lake Forest Road
Tahoe City, CA 96145
530-583-3279

UC Davis Limnology Lab
Dept of Environmental Science & Policy
One Shields Ave, UC Davis
Davis, CA
530-752-2913

Dr. Geoffrey Schladow, Director

Patricia Maloney, Associate Director

Tina Hammell, QA/QC officer-TERC, Lead chemist-UCD

Anne Liston, Lab manager, TERC

Steven Sesma, Lead chemist-TERC

Updated November 22, 2014

This document should be referenced as follows:

Liston, A., T. Hammell, V. Edirveerasingam, B. Allen, S. Hackley, J. Reuter and G. Schladow. 2013 Updated June 24, 2014). *Laboratory Procedure, Field Protocol and Quality Assurance Manual*, Tahoe Environmental Research Center, University of California, Davis. 158 p.

TABLE OF CONTENTS

PROJECT ORIENTATION

Project Description

This handbook describes the quality assurance procedures used by the Lake Tahoe Interagency Monitoring Program. The manual describes the procedures for the handling, sample preparation, analytical methods, use of instrumentation, written documentation and both internal and external quality control checks to be followed by the laboratory.

The purpose of the quality program is to provide an effective means of 1) monitoring the reliability of analytical results generated by analysts within the limnology project, 2) providing a measure of the accuracy and precision of the analytical methods, and 3) promoting continuity in long-term projects and a permanent record to assure integrity of sample results. Funding agencies that support this work have various quality assurance requirements. This handbook will address these requirements and outline our goals for the quality of data that is generated.

All programs managed through the limnology lab incorporate nutrient analyses of freshwater samples and sediment from highly variable ecosystems. Concentrations of most chemical constituents are very low (<100µg/l) with some analytes commonly less than 10µg/l. Our analytical methods are therefore specifically adapted for determination of low nutrient concentrations.

Project Organization and Responsibility

The Lake Tahoe Interagency Monitoring Program (LTIMP) is a consortium of the University of California and state and federal agencies. The program annually disseminates essential water quality information to support regulatory, planning and research activities in the Tahoe basin. The advisory committee of LTIMP is responsible for designating the type of data collection, the allocation of funding and the distribution of the reports. The University of California, as a member of LTIMP, is represented by the Tahoe Environmental Research Center (formerly known as the Tahoe Research Group and the Davis Limnology Group).

The limnology program at the University of California, Davis is a combined program called the Tahoe Environmental Research Center and stems from two home departments in Davis, CA: JMIE and the Department of Environmental Science and Policy. We operate three facilities. One lab is housed in 3117 Wickson Hall on the UC Davis campus. The second facility is located in Tahoe City, CA and serves as the field station. The overall managing facility is located in Incline Village, NV and is in close proximity to sample collections. Both the Incline Village labs and the Davis lab have USGS certification.

The director and the associate director manage all three facilities. Each project has a principal investigator who is responsible for contractual agreements, personnel supervision and reporting to the funding agencies. Sample collection and analysis is under the supervision of the senior staff members. The Davis lab and the Incline Village labs have a QA/QC officer to manage the overall QA/QC program. Each laboratory has a supervising chemist who is responsible for QA implementation and production.

Project Objectives

Lake Tahoe Interagency Monitoring Program

The goal of the Lake Tahoe Interagency Monitoring Program (LTIMP) is to disseminate, in a coordinated, cost-effective manner, water quality information needed to support regulatory, management, planning and research activities in the Lake Tahoe Basin.

The monitoring program objectives are:

1. To establish water quality baseline conditions and to identify trends in water quality in Lake Tahoe and its tributaries.

2. To measure and evaluate major pollutant inflows (mass loading) to Lake Tahoe, including nutrients and sediments.

3. To provide continuity of program direction and funding and to minimize overlap and duplication in water quality monitoring activities.

4. To assist management, planning, research and regulatory agencies in the implementation and evaluation of their programs.

5. To gain an understanding of the complex Lake Tahoe aquatic ecosystem.

6. To make environmental monitoring information available to agencies and individuals in a timely and understandable manner.

QUALITY ASSURACE PROCEDURES

Quality Assurance Objectives

Environmental analytical work requires qualitative and quantitative analysis. Qualitative analysis is the ability to correctly identify an analyte, whereas quantitative analysis is the ability to measure the **level** of analyte, once it is identified. Laboratories need operating guidelines that help the analyst and the client evaluate the validity of the data within a defined level of certainty. Quality Assurance (QA), therefore, is the set of operating principles that, if strictly followed during sample collection and analysis, will produce data of known and defensible quality.

The objective of the QA program is to present a definite QA plan that follows EPA recommendations. The plan will include both internal and external quality control measures as well as complete descriptions of the standard operating procedures. Our goal is to produce analytical results with a high level of confidence.

Laboratory Safety

In accordance with California, Nevada and federal safety laws, the laboratory has a Chemical Hygiene Manual. This manual outlines safe laboratory practices and provides procedural guidelines for emergencies. The manual also addresses the handling and storage of hazardous chemicals and the disposal procedures for hazardous waste. Every person working in the limnology laboratory **must** read this manual, read the IIPP and be trained to work in a UCD lab. Any questions or clarification should be directed to the laboratory supervisor. No one will be permitted to work in the laboratory without training and without signing the required UCD paperwork.

EMERGENCY MEDICAL CARE
CALL 911 IMMEDIATELY

FIRE EXTINGUISHERS ARE
LOCATED NEXT TO THE
LABORATORY EXITS

All work-related accidents, illness or injuries, no matter how small, must be reported to the laboratory supervisor **immediately**.

Safety Information

Be familiar with the substances you are working with in the lab. Each reagent has a Material Safety Data Sheet (MSDS) which is located in binders in room 302 in Incline Village, on the shelves in the lab in Tahoe City, CA and on the east shelves in Davis, CA. Refer also to the safety nets online for information about common hazards. All UCD lab workers must be familiar with safety Net #13 about spill clean up. All workers should be aware of the hazards related to their particular projects. If in doubt, consult the laboratory supervisor.

Safety Equipment

Everyone working in the chemistry laboratory should wear personal protective equipment (PPE). This includes long pants, closed-toed shoes, lab coat, gloves and goggles. Use the glove chart to find the appropriate gloves for the chemical you are using.

Concentrated acid should always be added to water and not vice-versa.

Safety showers are located in the halls in Incline Village, in the big room in Tahoe City, CA and in the hallway in Wickson Hall in the Davis lab.

Eyewash stations are located with each shower and also in most sinks in both Incline Village, NV and Tahoe City, CA.

Spill clean-up kits are located in each lab.

Hazard Management Practices

Monitor fume hood air-draw by looking at the digital readout and listening for the fume hood alarms. Never use a fume hood if it is not functioning properly. The fume hood should be cleaned regularly, be lined with absorbent paper for spill control. Store as few chemicals as possible in the hood.

Volatile and/or flammable substances should **ONLY** be used in a fume hood.

Portable gas cylinders must be firmly secured at all times.

All chemicals should be properly labeled and stored by segregated storage areas (flammables, toxic, oxidizers, acids, bases, etc.)

To avoid ingestion of toxic material, eating, drinking and chewing gum are prohibited in all laboratory areas. Smoking is never allowed.

Avoid floor clutter so that escape routes and fire extinguishers are not blocked

Broken glassware should **never** be put in the trash unless it is rinsed out and enclosed inside a cardboard box and labeled.

Waste Disposal

Solid and liquid chemicals must never be placed in the trash or poured into a drain for disposal unless the procedure has been approved by UC Davis Environmental Health and Safety (EH & S) in advance. Refer to Safety Net # 43 "*Identification and Segregation of Chemical Waste*" and Safety Net #6 "*Can this go Down the Drain?*" Identify and quantify the concentration of the hazardous substance on the label affixed to the disposal container. Do not needlessly create new waste containers for the hazardous substance and do not overfill the waste containers. When the container is ¾ full and/or at a 90-day storage period, call for a waste pick up. The information for waste pick-up is on the EHS website for UCD and for Tahoe City, CA. For Incline Village, NV, contact EHS for a contracted vendor with UCD to pick up your waste. Empty chemical and reagent containers may be placed in the trash provided that the container has been rinsed 3 times, the label has been defaced and caps left off. Update chemical inventory each time a chemical is added or removed from the lab.

Radiation hazards (This applies to the UCD lab ONLY)

All people working with radioactivity MUST be certified by the university and have their name listed on the radioactive license. A staff member has been appointed as custodian of radioactive materials for our lab. Please consult with this custodian in advance of performing any radioactive procedures. Also, consult with the lab supervisor to schedule the work.

Radioactivity should be confined to a specific place in the laboratory. The laboratory countertop must be covered with absorbent paper. Follow all recommendations for PPE for the handling of radioactivity and wear double gloves. Radioactivity can easily be spread throughout the entire lab by careless habits. Please use disposable gloves liberally and guard against spreading contamination. Contact our radioactive custodian and/or EHS on the UCD campus (530-752-1493) should an emergency occur. Our lab in monitored on a regular schedule for radioactive contamination. Refer to the **Radiation Safety Manual** on the bookshelf for more information.

Radioactive waste containers are well marked within the specified usage area. Dispose of solid waste in two-cubic-feet dry waste boxes with 4-mm clean plastic liners. Clearly identify the isotopes contained in each box. Disposes of liquid waste in a 5-gallon black jug with "Radioactive Waste" stenciled on the side. The jug should be sitting inside a secondary container to guard against accidental spills. Please do not overfill the just or

EH & S will not pick up the waste. When the jug is full, notify the Radioactive waste custodian that a pick-up is required. Refer to Safety Net #9 for more information.

All safety violations should be reported to the lab supervisor and the departmental safety officer.

Sampling Procedures

Sample Collection

It is essential to ensure sample integrity from collection to data reporting. The following guidelines for sample collection will facilitate consistent collection of representative environmental samples and provide further assurance of accurate and precise sub-sampling.

Sample collection is performed by trained personnel from the TERC or by personnel from contracting agencies. Samples are processed in the field, when possible, or soon after collection in the lab. Sample processing prepares the samples for storage so that the analysis can be cone at a future date. Water samples are collected in acid-washed polyethylene bottles with screw-top caps. The appropriate sample volume should be collected to ensure that detection limits can be met, that there is enough sample to perform the analysis and that there is extra sample in case the sample needs to be run again. Quality assurance of sampling operations is the responsibility of the project leader.

Lake Samples

All sampling will be accomplished following standard limnological methods (Wetzel and Likens 1979). Open-water samples are colleted with non-metallic Van Dorn sampler suspended from the vessel's hydrowire. Discrete depth raw samples are dispensed directly from the Van Dorn bottle into a clean, labeled polyethylene bottle. Unique field numbers are assigned to each sample. Affix labels to bottles before or at the time of sampling. Fill out the labels with waterproof ink.

Samples needing filtration are filtered through Whatman™ GF/C filters (we may be changing to GF/F filters). These samples are filtered on the boat or as soon as possible in the lab after collection. All filtration equipment and collection vessels are rinsed with Milli-Q water between samples to protect against cross-contamination.

Composite samples are created by taking water from discrete depth sample bottles within the composite depth range. The volume of water from each depth is proportionally

combined to represent an integrate water column sample. The composite samples are prepared in the field and distributed to the labeled sample bottles.

Raw and filtered water samples are kept cool at 4 ˚ C in a cooler until arrival at the lab.

Particulate material for carbon, nitrogen and phosphorus analysis is collected from water filtration of discrete depth (or composite) samples. The water is filtered through pre-combusted (500 ˚ C) Whatman™ GF/C filters (we may be changing to GF/F filters). Filtration pressure should not exceed 15 p.s.i. Care should be taken to keep clean filters from being contaminated from the sampler's hands. Handle the filters only with clean tweezers. The filters should be placed in clean, labeled containers and frozen until analysis. The volume of sample water filtered should be recorded for each sample.

Carbon and nitrogen can be analyzed from the same filter. Particulate phosphorus, however, must be filtered separately because the filter is preserved with 2 ml of 0.17M Na_2SO_4. Glassware used for the particulate phosphorus filtrations are dedicated only for this filtration because of possible contamination with the Na_2SO_4 in other chemistry assays.

After all the filtration has been completed, the manifold, side-arm flasks and graduated cylinders should be rinsed with deionized water 3-4 times and covered with aluminum foil caps.

Stream Samples

Stream sampling is problematic by the very nature of the system. Stream flow volumes and flow rates may be consistent or my drastically fluctuate in short time intervals. The type of collection devices and effective strategies for collecting representative stream samples are outlined in Appendix B. The discussion here will begin with the processing of the samples from the churn splitter.

Often stream water contains particulates that rapidly settle. It is important to keep these particulates suspended during sub-sampling so that reproducible sub-samples can be collected. The churn splitter is a device that facilitates that collection of reproducible sub-samples by churning the water as the sample is taken. Mix the water in the churn splitter 8-9 churning cycles using a consistent churn rate of approximately 7-9 inches/second. Take care not to aerate the sample by breaking the surface of the water in the churn. While continuously churning the sample, rinse each sample bottle and cap once with the raw water, then fill each bottle.

The filtered samples should be collected next. Rinse the filtration manifold thoroughly with deionized water. Place a cellulose nitrate filter (0.45µm) between the two filter screens. Be sure to keep the upper screen on the input or upper side of the manifold throughout the filtrations for all streams to prevent cross contamination with particulates.

Install the Tygon™ tubing in the GEP PUMP. Keep the tubing end that will be placed in churn sample in a clean bag when not in use. Before starting the pump, rinse this tubing end with a small amount of the raw sample water and place it in the churn splitter. Start the pump drawing sample water through the filter. To distribute the sample evenly across the filter, vent trapped air from the vent in the top of the manifold. Pre-draw approximately one liter of sample through the filter and discard. This removes any excess nitrate from the filter. Rinse each sample bottle and cp once with filtrate then fill.

Iron samples must be dispensed into their own sample bottle since 1.0ml of concentrated nitric acid (HNO_3) is added as a preservative. The nitric acid is sealed in a glass ampule. Before opening, rinse the outside of the ampule with deionized water and wipe dry with a Kimwipe™. Tap all acid down into the main part of the ampule. Carefully break open the ampule at the neck and pour the nitric acid into the sample.

After filling all bottles, discard the remaining churn sample and rinse the churn splitter 4-5 times with Milli-Q water. Remove the used filter from the manifold, rinse the manifold and Tygon™ tubing with deionized water and cover the manifold with a clean plastic bag.

Raw and filtered water samples are kept cool at 4 ˚ C in a cooler until arrival at the lab.

Sediment Samples

Sediment samples from the lake bottom or from the water column can be collected with a variety of devices. Typically lake bottom sediments are collected with a core and water column particulates are collected in sediment traps. Most sediment samples require some kind of physical preparation prior to chemical analysis. Samples require preparation to (1) reduce the sample to a size that is more conveniently transported; (2) increase the sample surface area to enhance the efficiency of subsequent chemical analysis; (3) homogenize the sample to ensure that a subsample is representative of the entire sample and (4) separate the sample into components based on characterizations such as mineralogy, grain size, location in the core, etc. Sample preparation is, arguable, the most important step in the process. Without careful preparation and attention to intersample contamination, the worth of the subsequent analyses is significantly diminished.

Wet sediment samples are fried in a forced-air drying oven at 30 degrees C. If the samples contain aggregates of material following drying they should be disaggregated. Remove pebbles and larger fragments from the sample by hand. Place the aggregates in a mortar, using the pestle to pulverize the sample. All sediment particles should be ground to approximately 180µm.

Field Documentation

Field Datasheet

Observations and physical measurements pertaining to the site location at the time of sampling are recorded on this datasheet. The number of samples collected, the field processing of these samples (filtration or preservation), the water column temperatures, light irradiance readings and Secchi depth are recorded. General observations such as the surface water conditions and recent weather conditions are also reported. All problems, equipments changes, procedural changes or modifications are noted. The original datasheet should be duplicated and the original sent to the data manager.

Field Notebook

Observations and physical measurements pertaining to the site location at the time of sampling are recorded in this hardbound notebook. Basic information such as: the site name, time of collection, sampler's initials, type of sediments sample, number of sediment samples, manometer or float tape readings from gauges in gauging house, tape measure reading from the outside staff, air temperature and water temperature are recorded in the notebook. General observations such as stream sediment load, unusual stream conditions, observations on the stream current and recent weather conditions are also recorded. All problems, equipments changes, procedural changes or modifications are noted. This notebook is kept at the TERC but should be duplicated on a bi-annual basis and a copy sent to the data manager.

Summary Notebook

All sampling events are recorded with location, date, time, sampler and sample identification number. The notebook is continually amended to provide a summary of results from each sample. This notebook is kept at the field station for use by the staff.

Sample Identification Labels

Each sample bottle must be labeled with location, date, time, sampler, assay(s) to be performed and field processing information.

Field Quality Control

Field Blanks

Field blanks are aliquots of Milli-Q water that have been treated as a sample, including contact with any field apparatus, collection vessel, preservative or storage technique. One field blank is collected quarterly per sampler. This

generates approximately 20 field blanks per year. Field blanks will help detect contamination arising during sampling (dust, vapors, poor quality preservation chemicals, or bad containers). Field blanks are also an attempt by samplers to see if the lab is capable of running low level samples without generating false positives. The criteria for acceptable field blanks are values < 5% difference from average field blank concentrations. The information should be recorded on the Field Blank Record Sheet.

Field Duplicates

Field duplicates are samples taken by duplicate operation of the field collection apparatus. For lake samples on field duplicate is collected per sampling event. For stream samples one field duplicate per stream per year is collected. For stream field duplicates, in particular, the samples should be collected one immediately following the other during stable stream flow conditions. This generates approximately 45 field duplicates per year from the lake sampling and approximately 25 field duplicates per year from the stream sampling. These duplicates can be used to evaluate the precision of the field collection methods and the homogeneity of the environment. The criteria for acceptable field duplicates are relative percent differences (RPD) < 15%. The information is recorded on the Field Duplicate Record Sheet.

Sample Preservation and Shipping

Sample Preservation

All water samples are processed in the field (filtration and/or preservation). Both filtered and raw samples are brought back to the laboratory and stored at 4°C. The samples are assayed within the specified time limits listed for each analysis. Iron samples are preserved with 1.0 M of concentrated nitric acid (HN03) per 250ml sample and stored at 4°C.

Precipitation samples are first analyzed for pH. The water is then filtered in preparation for cation analyses. These filtered water samples should be stored at 4°C for later analysis

The use of chemical preservatives, other than acidification of iron samples, is discouraged because of the potential for interference at the ~g/1 concentration range. Mercuric chloride (HgCI2) is a commonly used preservative in water sampling. However, we have documented matrix contamination from the HgCI2. IN addition mercury is a hazardous substance that requires special waste disposal. There is also the possibility of mercury contamination in a laboratory where low level mercury analyses are performed.

Shipping

Chemistry analyses are done at the Incline Village laboratory , the UC Davis limnology lab and the engineering lab at UC Davis. Samples to be assayed for ammonium, nitrate, orthophosphorus, total hydrolyzable phosphorus, particulate phosphorus, turbidity, DIC and total phosphorus are performed at the Incline Village, NV laboratory. Samples needing particle size analysis are performed at the UC Davis Engineering lab. Samples to be assayed for iron, Kjeldahl nitrogen, particulate carbon, particulate nitrogen, cations and total suspended solids are done at the UC Davis limnology lab.

Mail samples as soon as possible after collection. Samples should be packed in coolers with ample ice or ice packs to maintain the temperature near 4°C for up to three days. Enclose a completed chain if custody form, seal the cooler with tape and ship either via UPS or USPS to the appropriate lab.

UC Davis Limnology laboratory
c/o Tina Hammell
1 Shields Avenue
3117 Wickson Hall
Environmental Science and Policy
UC Davis
Davis, CA 95616
Phone: (530) 752-2913

UC Davis Engineering laboratory
c/o Geoff Schladow
1 Shields Avenue
Dept. Civil and Environmental
Engineering, room 2001
UC Davis
Davis, CA 95616
Phone: (530) 754-8372

Sample Custody

Chain of custody

The physical movement of each sample from one location to another is tracked with the use of a custody form. The sample name, identification number, sample time, collector, sample pretreatment, and assays to be performed must be noted on this form. The form should be addressed to the receiving lab. The sender's signature relinquishes the custody of those samples. The forms should be duplicated. The original form should be sent with the samples and copy should be filed at the field station. An example of this form can be found in Appendix C, page 82.

Laboratory Procedures

Sample Custody and Log-In

Chain of Custody Form

The laboratory receiving water samples should unpack the coolers upon arrival. A chain of custody (COC) form should accompany the samples and each separate bottle should be listed on the COC. Check the bottle labels against the custody form entry to verify for correctness. Note the condition of the samples in terms of bottle integrity on the COC. Take the temperature of the cooler upon
arrival and record the temperature in the temperature log-in book. If any temperatures are >4C, notify the client of the problem. Also, note the assigned storage space in the lab where the samples will be kept until assayed.

If all entries on the custody form match the enclosed bottles, the person who unpacked the cooler should sign the form. This signature acknowledges receipt of the listed samples. If there is a discrepancy between the enclosed bottles and the custody form, contact the sender for an explanation. Withhold signature from the form until notations have been made and discrepancies cleared. The signed form should be filed at the receiving lab as evidence of sample receipt.

Sample Log-In

The supervising chemist should log-in the samples into the Labtrack II database. The information entered is the date the samples are received, the project title, the client, the sample type, the sample site, the sample bottle number, the date and time of sample collection, the sample collector, any sample pre-treatment, and the assays to be performed.

Analytical Procedures

Glassware Management

Each chemist is responsible for their own glassware cleaning. All glassware must be cleaned and put away on a daily basis.

1. All stored glassware is rinsed with Milli-Q (deionized) water 3-5 times after use and partially filled with 0.1 M HCI. The containers are covered with Parafilm ^{TM} and placed on the appropriate storage shelf.

2. The glassware is filled with dilute acid and autoclaved for 15 minutes then rinsed 6 times with deionized water before use in an analysis.

3. The tubes are air-dried upside-down on clean Kimwipes before use and after cleaning in hot acid; for ammonium analysis, the tubes need to be rinsed the day of the analysis. For all other analyses, the tubes can be stored upside-down for a few days before analysis

4. Disposable plasticware can be reused but they should be thoroughly cleaned (same directions as above). All plastic pipette tips (except 1-10 ml Rainin™) should be discarded.

5. Tubes are grabbed randomly and filled with either standard, blank, SRM or sample. Immediately after filling, cover tube with Parafilm™.

Glassware Specifications

All glassware calibrated to contain (TC) or to deliver (TD) must meet the NBS specification for Class-A volumetric pipets. The volume of solution and the internal volume of the glass container itself changes with temperature. The temperature (20°C) at which the volumetric glassware was calibrated is indicated on the glassware. Solutions should be ±5°C (19 to 21 ° C) of the calibration temperature for accurate volume measurements. It is generally not necessary to re-calibrate Class-A glassware. However, be alert for unusual results when using any new equipment. Older glassware may be cracked or chipped. Make sure these imperfections do not interfere with accuracy.

Allow a glass pipet marked TD to drain freely then hold the tip against the inner wall of the vessel into which it is draining (do not touch the liquid already in the container). Some liquid always should remain in the tip of the pipet.

Standards Management

Stock Standard Solutions

For all wet chemistry assays, a liquid stock standard is prepared as directed in the assay procedures. In most cases the stock is then stored refrigerated at 4°C. For most of the assays it is necessary to make an intermediate stock solution that has a concentration value somewhere between the stock standard and the working standards. The **entire set** of working standards must be prepared at the same time. Use Class A glass volumetric flasks and pipettes for the dilutions. Do not use the automatic micropipettes.

These working standards are stored in the same manner as the stock standard or remade fresh daily. The intermediate stock solution is made only to logistically aid the analyst in pipetting the working standards. It should be remade each time a set of working standards are prepared. **The intermediate stock solution should not be stored and used at a later date because of potential inaccuracies in the analyte concentration over time.**

Calibration Verification of Standards

To ensure consistency and accuracy between old and new stock solutions it is essential that the old stock is compared to the new stock solution. Duplicate sets of working standards (both from the old and new stock) are prepared and run during the first assay where the new stock is being tested. The two resulting standard curves should be plotted on Excel. If each new working standard concentration differs by less than 5% from the old standard concentration and the new standard curve slope differs by less than 5% from the old standard curve slope, the new stock can be used from that time forward. File the comparative standard curve graph in the Quality Assurance notebook. Record the standard information in the Standards Logbook. If any of the new standard concentrations differ by more than 5% from the old standard concentrations the entire set of working standards must be remade from the intermediate stock solution.

Independent stock solutions used for calibration verification (spike addition stock and/or standard reference materials) are prepared as instructed for each method. Independent working standards can be made from these stock solutions and used routinely to verify the accuracy of the standard curve.

The **Stock Standard Logbook** is used to record the sources and quality of all standards. This logbook includes information such as the supplier, lot number and date of receipt. In addition, information about working standard preparation is recorded, such as the date of preparation and the analyst's name.

Reagent Preparation and Storage

Unless otherwise noted in a specific method, all chemicals used during analyses are reagent grade. When chemicals are received by the laboratory they must be entered into the chemical inventory. Storage location of chemicals will be segregated according to the type of chemical in the laboratory (acid, base, Oxidizer, Poison, Flammable, Corrosive, compressed gasses). Analytical reagents have a finite shelf life. Therefore, all chemicals received by the laboratory should be **dated when received** from the manufacturer and **dated when opened**. Unless otherwise specified by the manufacturer, inorganic chemicals have shelf life of 5 years at room temperature. Notify the laboratory supervisor for the immediate disposal of reagents that have undergone a change in color or consistency. Never stack reagent containers on top of one another. Only original manufacturer containers can be used for the storage of reagents. Ensure that all corrosive reagents are stored inside secondary containers in case of spills.

Reagent mixtures, specific to each assay, should also be safely stored. Liquid reagent mixtures can be stored in clean/new Nalgene™ plastic bottles or borosilicate glass bottles with ground glass stoppers. Note, however, that strong basic solutions should never be stored in the glass bottles because the stoppers are

impossible to remove. Do not use volumetric flasks for reagent storage. Each reagent mixture must be labeled with the **reagent name, data of preparation** and the **chemist's initials.** In addition, each reagent mixture should have special notation if the reagent requires refrigeration. The **Reagent Record** is used to record the inventory number of the reagents used in the Reagent mixture as well as the preparation date and the chemist's initials. This record helps tract impurities and the shelf life of the reagents. The Reagent record can be found in the Quality Assurance Notebook.

Deionized water is available in the Incline Village laboratory and in the Tahoe City Laboratory. These systems are maintained by the lab manager on a regular basis.

Distilled water enters the UC Davis Limnology lab through specially marked water faucets at each sink. The water used in preparing reagents is distilled but also deionized. This water is available at two sinks in the lab. The deionization process is achieved by passing distilled water through three resin columns that specifically remove inorganic contaminants. The resin columns are behind the wall and changed by UC Davis facilities. Let them know if these cartridges need maintenance.

Instrument Calibration and Verification

Instruments must be operated according to methods specified by the manufacturer or as outlined in the operational procedures found next to the instrument. Please read operation manuals for any instrument you intend to use. Do not operate the instrument without receiving personal instruction on its use and maintenance from the head chemist.

Equipment must remain in the laboratory at all times. Portable equipment can be checked out from the lab for a limited time. Contact the laboratory supervisor for assistance. Many of these instruments must operate within certain parameters. For those methods that require critical operation of the instrumentation we have a program of calibration and verification. Records are kept in **a Logbook,** located next to each of these instruments. The analyst doing the calibration should record the date, analyst's name, and calibration results.

If the calibration results do not meet the established criteria for acceptable performance, the analyst must not use the instrument. Report the malfunction to the supervisor as soon as possible. It is the supervisor's responsibility to resolve the problem by doing repairs, recalibration or actually discarding the instrument. Record all changes in the instrument logbook. If the instrument must be repaired by a professional, note the name of the contact person, date of service, and the nature of the repair problem. The QA officer is responsible for providing valid standard reference material (SRM) and for setting instrument performance criteria. The list of instrumentation (appendix D) provides an inventory of available equipment (make and model), common analytical uses, calibration procedures, performance criteria, calibration frequency and suggested maintenance schedule.

Sample Management

Sample management incorporates many laboratory procedural details that will help determine the outcome of the assay. Careful planning and preparation will make the assay procedures easier to follow. In addition, close attention to procedural protocol will aid in the assessment of the assay results.

Laboratory Scheduling

Arrange for use of the laboratory in advance. Sign up with the lab manager and make an appointment to get safety training if you haven't already done so. Staff projects always have priority over students. Nevertheless, everyone tends to be accommodating and usually there aren't problems.

Sample Preparation

Remove samples from storage and follow standard operating procedures for sample preparation. Samples that need to be pipetted should be at room temperature.

Workspace Preparation

Since countertop space is a valuable commodity, workers are responsible for clearing their workspace each day. When the countertop is cleared it should be wiped down with a wet paper towel. The laboratory supervisor must approve any project which requires countertop space for an extended period of time.

Datasheet Preparation

Raw data from each analysis is recorded on the water chemistry Labtrack II run sheet. Each run sheet is numbered sequentially in Labtrack II with a different run sheet for each assay being simultaneously analyzed. Information pertaining to the assay such as the date of the analysis, the project, the analyst, the sample site, etc. is recorded in the run sheet. The quality control information includes the standard curve calibration statistics (r coefficient, slope, y-intercept, etc.), the spike recoveries, the duplicate RPDs, the calibration verification standard and any analyst comments. The analyst is responsible for the completion of the run sheets and for typing in their raw data into Labtrack II. All chemistry laboratory personnel in Davis and in Tahoe have access data in Labtrack II via the web but must first obtain a log in and password from the Labtrack II manager.

Assay Set-Up and Design

Sample number

Consider the number of samples to be tested and the time it takes to perform the assay. With increasing technology, the number of samples that can be run in a

day has also increased. Nevertheless, each assay has limitation that dictate the optimum maximum number of samples that can be run.

Sample type
The unknowns may consist of several different environmental types (i.e. lake, stream, groundwater). If you decide to assay a conglomeration of sample types you should remember that you are likely to span a wide range of standard concentrations. It is important also to remember that each sample type is recorded on a different data sheet.

Paired aliquots
Some samples may be "paired", where one aliquot of the sample is raw and the other aliquot is filtered. Be sure to assay the "paired" samples in the same run.

Standards
Ideally, the range of standards should bracket the concentration range of the unknown samples. For most of the nutrient assays we run at least seven duplicate standard concentrations, including the blanks. It is acceptable to use more than seven standards if you need more resolution. Standards are always assayed in duplicate. If more than one rack of samples (40) are to be assayed it is a good idea to split up the duplicate standards so that one set of standards are in one rack and another set of standards are in the second rack.

Duplicates
Ten percent of the total assay samples have duplicates performed. The duplicates should not be placed next to each other in the rack. Many chemists run the duplicate and spike samples at the end of the run. If possible, when selecting which samples to duplicate, try to select one duplicate from each sample type. For analyses with high variability, a higher percentage of samples should be run in duplicate.

Spike Recoveries
Ten percent of the assay samples have spike additions. Spike additions relative to the sample concentration are added to the spike sample. The results of the spike addition samples are compared to the results of the same samples without spikes. The chemist can then evaluate the recovery of the spike addition. If possible, when selecting which samples to spike, try to select at least one spike from each sample type. Try not to spike diluted samples since dilutions not only decrease the analyte concentration but also the matrix. The matrix must not be perturbed for spiked samples.

Calibration Verification (CV)
Calibration verification can be done using an independent standard stock or by running standard reference material (SRM). These verification materials have

known or target values for the analyte being assayed. When they are analyzed they provide a very real measure of accuracy of the methods. When there is good agreement with the known value, there is more confidence in the run. At least two duplicate samples per run should be for calibration verification.

Reagent Preparation

Refer to the Standard Operating Procedures for the instructions on preparing mixed reagent chemicals.

Instrument Operation and Calibration

Refer to the instrument operation manual for instructions. After the instrument has been warmed-up, zero the instrument response. Analyze the analyte standards and measure the instrument response (absorbance). Construct a calibration curve by running a linear regression of the standard concentration versus the instrument response.

Sequence of Analysis - Quality Control Checks

(Typical Run of 40 Samples)
Blank
Blank spike
Test Samples 1-20
Reference Material #1
Reference material #2
Calibration Standard at Midrange
Test Samples 21-40
Reference Material #1
Reference Material #2
Duplicates
Spikes

This method of order of samples is not necessary except with the TKN analysis since the digester has variable heating in each hole. We use test tubes randomly after cleaning.

Concentration Calculation

Analyze the unknown samples and measure the instrument response. Relate this response to the standards by using the calibration curve. Be sure to make corrections for any diluted samples in the run. Using the linear regression
equation, a known absorbance will determine the concentration. The regression statistics will be used to evaluate the success of the run on Labtrack II.

Data Reduction and Validation

Quality Control Charts and Forms

Each assay is continually monitored for quality control. Records pertaining to each assay are kept in the laboratory quality assurance notebook, found at both the Incline Village and the UC Davis limnology labs. Examples of these QC forms can be found in Appendix C. The chemist and/or laboratory supervisor is responsible for maintaining the quality control updates. A QC **Checklist** (Appendix A) assists the analyst to meet all of the quality control limits.

1. Quality control charts for each assay contain warning (±1.5 standard deviations) and control limits (±3 standard deviations) for the standard curve slope. After each analysis the standard curve slope is plotted on the appropriate chart.

2. Once every six months the standard curve slope statistics for each assay are printed from the laboratory information management software (LIMS) Labtrack II. The output includes the LIMS assay run date and number, the limits are automatically calculated from the software and used for the new charts.

3. The relative percent difference for the duplicates is calculated in the LIMS when the data is entered. A separate quality control sheet is produced from the LIMS for each run.

4. The reagent blank values for each assay are entered into the LIMS as part of the calibration curve.

5. The spike concentrations and percent recoveries are calculated in the LIMS when the data is entered. A separate quality control sheet is produced from the LIMS for each run.

6. Quality control charts for each assay are maintained for the percent recovery of the spikes. After each analysis the percent recovery is plotted on the appropriate chart. The mean and standard deviation of the recoveries for each assay is updated to include data from analyses of the previous six months. At least 10 new data points must be plotted in the six-month period to initiate the update. The routine control limits for the analyses are 85-115% except for the nitrate and TKN assay. These two assays spike limits are calculated from the RPD warning limits of±1.5 standard deviations and control limits of±3 standard deviations.

7. Calibration Verifications using either an independent source stock solution or a standard reference material is entered into the LIMS with the run data. The percent difference between the actual value and the assay value is calculated. A RPD control limit for the routine analysis is 0-5%. For TKN, total phosphorus and particulate phosphorus an RPD control limit of 0-10% is used.

Assay Evaluation and Corrective Actions

A quality control **Flow Chart** allows the analyst to systematically interpret the quality control data and outlines the steps for corrective action. See Appendix A Pages A-5 and A-6.

Internal Quality Control Checks

Detection Limits

Instrument Detection Limit (IDL)

The IDL is a minimum value below which the signal cannot be distinguished from instrument noise. The IDL is a concentration of constituent that produces a signal five times the signal-noise ratio of the instrument. It is stated as 1.6s of blank analyses.

Method Detection Limit (MDL)

The method detection limit (MDL) is the minimum signal level required to qualitatively identify an analyte. There is a 99% probability that the true value is greater than O.

The method detection limit is calculated and recorded once a year for each analysis. For a given analyte, this is the point above which measurements are distinguishable from reagent blanks. Reagent blanks consist of a Milli-Q water matrix with reagent addition and treatment identical to the samples.

The matrix used for blanks and working standards is dependent on the specific ecosystem of interest. The distilled water matrix closely approximates the dilute conditions (low conductivity) found in waters of the Tahoe basin. The chemical methods outlined in this manual were to be applied to another system a different matrix might have to be employed in order to obtain valid results.
The method detection limit is calculated as:

MDL == Student t (s)

The MDL's are performed 3 times per year using 7 replicates of a standard 1-5 times the MDL. 21 points are used to calculate the final MDL using the one-sided student t value of 2.53 (21-1 == 20 degrees of freedom at the 99% confidence level). This procedure encompasses variability throughout the entire year.

Limit of Quantitation (LOQ)
A minimum value below which any measurement of data becomes unreliable. This limit has a 99% confidence level that the true concentration is ±30% of the measured values.

Reporting Limit
The production level detection limit (reporting limit) is the lowest concentration that can be reliably quantified within the specified limits under normal operations.

Accuracy

Accuracy is a measure of the difference between the mean of the measured value and the true value. We have both internal and external quality control checks on the accuracy of each procedure.

Since the measured concentration is determined from a standard curve (absolute concentration versus measured absorbance), the accuracy of our measured concentration is affected by the goodness of fit between the standard solutions and their respective absorbance readings. As a result, a great deal of effort goes into running the standard curve on each run. The analyst is required to run 10-14 standards (duplicates of 5-7 concentrations including blanks) for each assay. Specific standard concentrations are selected to span the range of ambient concentrations. The standard curves are generated by plotting the Absorbance readings from the working standards against the working standard concentrations. The statistics associated with that standard curve regression equation can be used as an indicator of accuracy. The correlation coefficient (r) must be 0.995 or greater to ensure the linearity of the standard curve. If samples fall outside the linear portion of the standard curve the sample is reanalyzed at a dilution.

The standard curve slope is also routinely plotted on quality control charts for each assay. These control charts monitor the drift in the standard curves. Each chart contains a mean value for the standard curve slope plus warning (±1.5 standard deviations) and control limits (±3 standard deviations). Both the analyst and the instrument are required to produce results within these standard deviations determined from previous runs. If the slope exceeds the statistically established control limit the assay is redone.

The accuracy of a method (relative to a particular natural water system) can also be

assessed from the recovery of spike solutions. The analyst is required to add spikes of known concentration to 5%-10% of the total number of field samples in each run. More than 5%-10% of the total sample number should be run as spikes if the data will be used in litigation. In that case, it is best to do the maximum quality control effort to validate the results.

Spiking concentrations are dependent upon the background levels in the original sample. If the background concentration is greater than the mid-point of the standard curve, the concentration of the spike should be approximately one-half of the original concentration. If the concentration in the sample is below the detection limit, the concentration of the spike should be 5 to 15 times the detection limit. Finally, if the concentration lies between these extremes, the concentration of the spike should be equal to the original sample concentration. Spike solutions are generally added on top of the total sample volume. The volume of spike solution added should change the total volume by less than 1%.

The recovery of the spikes is then calculated as follows:

$$\frac{[\text{Concentration of spiked sample} - \text{Concentration of unspiked sample}]}{\text{Concentration of spike}} \times 100$$

The percent recovery is plotted on quality control charts for each assay. Each chart contains an average percent recovery of 85-115% or for the nitrate and TKN assays, warning limits of (±1.5 standard deviations) and control limits of (±3 standard deviations), determined from previous runs. The percent recovery must fall within the control limits to be acceptable.

Calibration Verification

Calibration verification is assaying a specified constituent using a sample that has a known concentration. It is a routine procedure for all assays. When there is good agreement with the known value, you can have some confidence in your ability to perform that analytical procedure. It is particularly helpful to have known values when trying a new procedure or training a new analyst. There are two types of samples that can be used for calibration verification.

Standard Reference Material

Standard Reference Materials (SRM) are chemicals that are mixed in the lab or purchased form an outside vendor. They are then analyzed in each run alongside the samples to verify the Absorbance readings from the working standards. The reported values of the constituents are certified. The SRM's are therefore "target" or known values. When these samples are analyzed a very real measure of accuracy of the methods is applied. The

value must be within the published acceptable range for that SRM, usually within 5-10%.

Independent Stock

An independent Stock is a solution that is made from a constituent reagent other than that which was used for the Calibration Stock. Known concentrations of the constituent can be made from the Independent Stock as a calibration verification. These differ from SRM's in that they are not made in an environmental sample matrix and they do not have certified constituent values. Nevertheless, SRM's are not available for all constituents and often are not in the concentrations that mimic the samples being assayed. The value must be within 5% of the predicted constituent concentration for all of the assays but TKN, TP and PP, which have a 10% RPD limit.

Precision

Method Precision

Each analytical procedure has a measure of precision to be expected for that procedure. This precision refers to the reproducibility of a method when it is repeated on a homogeneous. sample under controlled conditions. Precision is expressed by the standard deviation of at least 10 replicates. This precision fore ach analytical procedure is performed when the method is first being certified.

Duplicate Precision

In addition to this traditional measure of precision we also monitor the reproducibility of the method from one run to the next. The analyst is required to duplicate 5% of the samples in each assay. These laboratory duplicates (i.e. 2 aliquots from the same sample bottle) are not true replicates in a statistical sense, however, they provide an indication of the precision of the method. The duplicate data is used to calculate the relative percent difference (RPD) using the equation shown below:

$$RPD = \frac{(x - y) \times 100}{(x + y)/2}$$

where x and yare the two duplicate concentrations. The RPD is a non-parametric evaluation of duplicates to determine whether the difference between the two numbers is meaningful. The significance of the RPD is dependent upon the laboratory method detection limit (MDL). The criterion applied for significance is stated as a multiple of the method detection limit. If the measured concentration is greater than a multiple of five times the MDL, a calculated RPD of 10 - 15% is allowed. If the measured concentration lies between the MDL and five times the MDL, a sliding scale is utilized as listed below. (Adopted from Environmental Protection Agency's contract language for Inorganic Analyses, 1984).

If a concentration is within x times the method detection limit the allowable difference is y (expressed as a percentage of the mean value of the two measurements) as shown below:

X	(Lab duplicate) y	(Interlab comparisons and field duplicates) y
2	90 – 95%	100%
3	70 – 75%	80%
4	50 – 55%	60%
5	30 – 35%	40%
>5	10 – 15%	20%

The criteria recognize the decreasing accuracy and increasing uncertainty of the analyses at measured concentrations near the detection limit. The criteria therefore recognize the difference between detectable concentrations and quantifiable concentrations.

For each analytical procedure the collection of calculated RPD data are formally evaluated on a bi-annual basis. However, the laboratory duplicates are also evaluated along with each run. Significant RPD values are not allowed to occur more than 20% of the time during the proceeding 6-month period. If so, general method procedures (e.g. temperature of water bath, reaction times, etc.) are checked. If the 15% RPD criterion (15% or higher; based on distance of measurement from the method detection limit is exceeded in more than two individual runs, checks are made for contamination of reaction flasks, pipetting errors, etc. Precision tends to vary within the system being analyzed. Lake samples have very few particulates but stream samples are variable. Samples with more particulates are usually less precise than samples without particulates.

Blanks

Method blanks are samples of Milli-Q water or DDW (distilled deionized water) that are subjected to the same preparation procedures as the routine samples being analyzed. Method blanks are also commonly referred to as reagent blanks. The analyst is required to run two reagent blanks per assay. These blanks are a check for random contamination which may have occurred in the laboratory preparation or analysis (contaminated glassware or reagents). The blank values for each procedure are recorded over time. The analyst should expect blank values to remain consistently low from one assay to the next within a procedure. If significant contamination is found, it probably invalidates any positive results found in the samples. These laboratory blank values are also used as "zero concentrations" in the standard curve construction.

Reporting and Document Storage

Data Entry

The original Labtrack II run sheets are used for data entry verification by the data management staff. The standard curve and absorbance values entered by the lab chemist are compared with the computer output. If there are any discrepancies the analyst responsible for the assay is contacted in an effort to clarify or correct the problem. The data management staff is responsible for the reporting format and issuing of the data to outside agencies.

Archival Storage

There are extensive collections of records for the LTIMP projects and all other projects. All data is kept, even after publication of the information. Record storage is managed by the data entry personnel and the supervising lab chemist. Annual log-in records, chain of custody forms, and the QA notebook are stored in each laboratory. Instrument calibration and maintenance records are kept for the life of the instrument and are also stored. in the respective laboratories.

Original data sheets are organized chronologically in project binders after data entry. These binders are stored in room 3117 Wickson Hall and in the Incline Village facility. These rooms are kept locked when unoccupied by the staff, even during normal work hours. The computer records are backed up daily. Access to Labtrack II and UTD are secured with the use of a password.

Supervisor and/or QA Officer Review

The analyst often makes notations on the data sheet about that particular assay reagent dates, spike concentrations, quality control evaluations, corrective actions, and/or assay problems. These notations are reviewed by the supervising chemist in Davis through the Labtrack II information system.

If the supervisor spots potential problems with any aspect of the assay, the analyst of the assay is contacted for discussion. The supervisor and/or QA Officer will then note any clarifications on the data sheet or determine if corrective action is necessary. The QA Officer will maintain an Observation Log. Records of problems and implementation of corrective action will serve as evidence of actual implementation.

Performance and System Audits

External quality control checks for accuracy provide information for laboratory evaluation.

Biannual reference samples are received from the U.S. Geological Survey, Western Region. This Round Robin analysis of reference samples for ammonium, nitrate, orthophosphate, total phosphorus, Kjeldahl nitrogen and iron ensures accurate data.

Occasionally we participate in inter-laboratory comparisons of replicate samples from collaborative studies. While the analyte concentration is unknown to both laboratories, the comparison of results still yields a quasi-quantitative check on laboratory accuracy (i.e. we are able to calculate relative percent difference but not an absolute percent difference). These comparisons have been initiated both by our laboratory and also at the request of the other laboratories.

Analytical Method Validation

Format for Writing Analytical Methods

Each analytical method must be written in the specified format to conform to the established methods in the standard operating procedures.

Introduction
This section gives the reader information about (1) the usefulness of the method, (2) a short summary of the procedures, mentioning required instrumentation, (3) discussion of the method ruggedness, (4) potential interferences, (5) applicable analyte concentration range, (6) method detection limit and method precision.

Pre-treatment of Water Samples
Mention special processing of the samples that occurs prior to analysis. This includes filtration, storage requirements, and preservation. Holding times of the samples should be specified.

Reagents
This section gives a complete listing of reagents required to perform the assay. Instructions are included for making the various reagent solutions, reagent storage requirements, reagent shelf life, and any special instructions or warning that will assist the analyst.

Procedure
Methodically detail the procedures such that the assay can be performed by another person without special training. Each procedural step should set one goal and not be compound tasks.

Notes
This section is perhaps the most useful to the analyst because it gives helpful information about the assay that will insure the success of the run.

Calculations
Provide instructions to assist the analyst in calculating the final concentration of the specified analyte.

QA/QC Procedures
List any quality control procedures that are used during the set-up and final evaluation of the run.

References
Make a detailed list of references that help validate the method and assist the analyst in finding helpful information.

Method Validation and Modification

There may be occasions when standard methods cannot be used or when no standard method exists for a particular constituent. Therefore, method development may be required. Whether an entirely new method is developed by accepted research procedures or an existing method is modified to meet special requirements, validation by a three-step process is required:

1. **Determine method detection limit**

   Analyze at least 7 but preferably 21 portions of a standard at a concentration that is 1-5 times the anticipated MDL. This must be done for each matrix that may be used.

   Determine the concentration range of the method and the relationship between the method and the concentration of the constituent. This relationship may be linear or curvilinear. Use 7-10 replicates at each standard concentration. Be sure to measure several concentrations of standards in several different matrices. Use one relatively high standard so that the range of concentrations can be specified.
   Calculate the precision and bias at each concentration and in each matrix.

2. **Validate method accuracy**

   Assay a minimum of 10 replicates for a variety of matrix reference materials. The matrix reference materials should cover the lower, midpoint, and upper end of the calibration range. The replicates should be run on at least three non-consecutive days. Calculate the arithmetic mean, μ, and the standard deviation, σ. Accuracy is generally considered adequate if:

$$\mu - V < 3 \text{ standard deviations}$$

where V is the reported value of the SRM. (The validation test is based upon one proposed by Sutarno and Steger, 1985, p445).

3. Determine method precision

Assay a minimum of 15 replicates from one "real" sample. It would be most effective if these replicates could be measured on non-consecutive days by the same analyst. However, if there is a problem with prolonged holding time, the replicates should be run on the same day. Calculate the arithmetic mean, μ, the standard deviation, σ, and the Relative Standard Deviation (RSD):

$$RSD == (\sigma / \mu) * 100$$

Depending upon the analytical objective; for major elements (composition grater than 1%) a RSD less than 1-2% may be considered adequate, for minor elements (0.1-1%) a RSD less than 5%, and for trace elements (less than 0.1%) less than 15%.

The key with QA is to report analytical information on the accuracy and precision of a method as estimates of typical performance capability.

After this work has been completed the method must be formally presented and approved by the supervising chemist, the QA officer, and the project leader. The format of the presentation should be:

Analytical method in proper written format, include some discussion of method ruggedness

Research report that includes the QA information above and the results of the new method when run concurrently with an approved standard method (if one exists)

Summary statement of cost estimate (equipment and supplies)

Personnel Training and Qualification Records

Personnel Training Record

Personnel training records are tracked in the **Personnel Training Binder at each location.** Laboratory training includes evidence that staff personnel have taken an internal lab safety class, read the IIPP, the QA Manual and the Chemical Hygiene Manual. It also includes campus based training classes for EH&S classes in radiation and chem. lab safety, fire department classes in fire safety, or Staff Development or University Extension classes.

Proficiency skills are also listed on the **Personnel Training Binder.** Defined skills, when completed successfully are signed and dated by the responsible trainer.

QA Report to Management

Biannual laboratory reports are prepared by the QA officer. The reports summarize the quality control parameters obtained for each assay at each laboratory during the previous 6 month period. A detailed discussion of each parameter is reported.

Tables of on-going slope values from 1995 to the present are presented in the appendix of the report as well as other statistical analysis.

References

Literature

Standard Methods for Examination of Water and Wastewater, L.S. Clesari, A.E. Greenberg, R.R. Trussel, 17th Edition, 1989.

Standard Methods for Examination of Water and Wastewater, Andrew Eaton, Lenore Clesceri, Arnold Greenberg, 19th Edition, 1995.

Environmental Sampling and Analysis, a Practical Guide, Lawrence H. Keith, Lewis Publishers, Chelsea, Michigan. 1991

Detection in Analytical Chemistry - Importance, Theory, and Practice. L.A. Currie, Ed. ACS Symposium Series 361. 1986

Report on Minimum Criteria to Assure Data Quality, *EPA/530-SW-90-021* , 1989., p62

Quality Assurance Plan Requirements for Nuclear Facilities, NQA-1-1989, 1989.

Interim Guidelines and Specifications for Preparing Quality Assurance Project Plans, *QAMS-005/90,* EP*A-600/4-* 83-004, 1883

Test Methods for Evaluating Solid Waste, SW-846, Third Edition, Chapter One. 1986

Code of Federal Regulations, 40CFR Part 792, Good Laboratory Practice.

Guidance on Preparation of Laboratory Quality Assurance Plans, State of Alaska, 1992

Sutarno, R. and Steger, H.F., 1985. The Use of Certified Reference Materials in the Verification of Analytical Data and Methods. *Talanta* v.32, no. 6, Oxford, England, Pergamon Press.

USGS QA Manual for the Branch of Geochemistry. Open File Report 90-668. B.F. Arbogast (ed.)

USGS *QAIQC* Manual, Open File Report 95-443. J.W. Pritt, J.W. Raese (ed.)

Standard Reference Material Sources

National Institute of Standards and Technology (NIST)
Standard Reference Material Program
Bldg. 202, Room 204
Gaithersburg, MD. 20899
Phone: (301) 975-6776
Fax: (301) 948-3730

Analytical Quality Control Services (AQCS)
International Atomic Energy Agency
Agency's Laboratories Seibersdorf
P.O. Box 100
A-1400 Vienna (Austria)
Phone: 43 2254/3361/226
Facsimile: 43 2254/2951222

Community Bureau of Reference (BCR)
Commission of the European Communities
Rue de la Loi, 200
B-1049 Brussels (Belgium)
Phone: 32 2 235 31 15
Telefax: 32 2 235 80 72

United States Geological Survey
Branch of Quality Systems
Denver Federal Center, Bldg. 53
Denver, CO 80225
(303) 236-1875

Environmental Resource Associates
5540 Marshall St.
Arvada, CO 8002
1-800-372-0122

High Purity Standards
P.O. Box 41727
Charleston, SC 29423
(843) 767-7900

APPENDIX A

Quality Control Summary

Sequence of Analysis

The sequence in which the test samples, standards, and quality control samples are read will have an effect on the assay outcome. The following sequence of analysis will assist the chemist to utilize the quality control samples in the best possible manner.

1. Allow adequate time for instrument warm-up and calibration.
2. Read one set of calibration standards.
3. Re-zero the instrument, if necessary.
4. Read samples #1-20.
5. Read the Calibration Verification sample. Save this sample.
6. Read the duplicate set of calibration standards.
7. Read Samples #21-40.
8. Read the Calibration Verification sample.
9. Read sample duplicates and spikes.

Quality Control Checklist

Quality Control Checklist - Analyst

Data entry of assay results into LabTrack by the analyst who performed to assay:

- Enter the absorbency values for the standards in LabTrack II Select the Results menu item. The first option, **Set Result Status** must be highlighted. This will change the status of the assay from design complete to run complete. Go back into Results and select the second option, **Standards**. Assays that use one or more spectrophotometer cell lengths must define these lengths in the data entry. The program stores the standard concentration data with each cell length that was used for that particular assay. All standard absorbency values should be entered. However, the analyst can choose to accept or reject any of the individual values for each concentration. Next to each absorbency value, select either **T** for acceptance or **F** for rejection. The standard curve statistics will be calculated only from the accepted values. Re-check numbers entered for verification before exiting the standards worksheet.

  For some projects, (defined in the Projects module) the standard curve will also be corrected so the y-intercept passes through zero. This correction is historically significant for the long-term data set from the Tahoe project. It was used in the past to compensate for background absorbency contributed by the assay reagents. The program will automatically correct the standard curve if this option was chosen for that particular project.

- Enter the absorbency values for the samples in LabTrack II, under the Results menu item. Select the fourth option, **Read Data**. The program will prompt the user to enter the most common cell length used during the assay. It will also ask the user to enter the most common dilution factor. These common values will automatically be entered into the database. The user must therefore make specific corrections to the worksheet to enter any deviation from the most common values. The user can enter notation for each individual sample, if needed. Re-check numbers entered for verification.

- When data entry is complete, the user must prompt LabTrack II by highlighting **Input Complete**. The program will then calculate sample concentration values.

Quality Control Checklist – Quality Assurance Officer

- LabTrack II automatically calculates the standard curve statistics, the Relative Percent Difference (RPD) of sample duplicates, the Percent Spike Recovery, and the reference material performance. These values are reported on the QA report from each run. The QA officer must evaluate the overall assay performance by either accepting or rejecting these criteria. Each cell length used during the assay will have its own standard curve statistics and will have to be evaluated individually.

- Standard curve slope:
  The standard curve slope values are plotted on the QC charts. If the slope falls within the 1.5 standard deviation units from the average, it is acceptable. If the slope falls beyond the warning limits but still within the control limits (1.5σ-3σ), it is generally acceptable but a notation must be made. Corrective measures include re-evaluation of the standard absorbencies and dropping outlying values. Other possible corrective measures include evaluation of reagents, procedural changes and equipment performance. When the slope falls beyond the control limits ($>3\sigma$) the assay is automatically rejected and all samples have to be rerun.

* Standard curve y-intercept:
  The standard curve y-intercept should be qualitatively evaluated. The value should not vary much from one assay run to the next. If the value is high or low, notation should be made and corrective measures begun. Corrective measure may include evaluation of reagents, procedural changes and equipment performance. The QA officer can reject the Y-intercept value but still accept the assay.

- Standard curve regression:
  The standard curve regression coefficient (r^2) should be greater than 0.999 for all assays except TKN. If the statistics do not meet these criteria, corrective measures should be taken. At the discretion of the QA officer, the run may have to be repeated.

- Laboratory duplicate performance:
  The Relative Percent Difference (RPD) between duplicates must be less than 15% for acceptance. One exception to this rule is if the sample concentrations are within 5% of the method detection limit. The rules for acceptance under these conditions are within the QA text. The QA officer can reject the duplicate performance but still accept the assay run. Corrective measures include rerunning only that field sample which was rejected or rerunning 5% of the samples as a validation check.

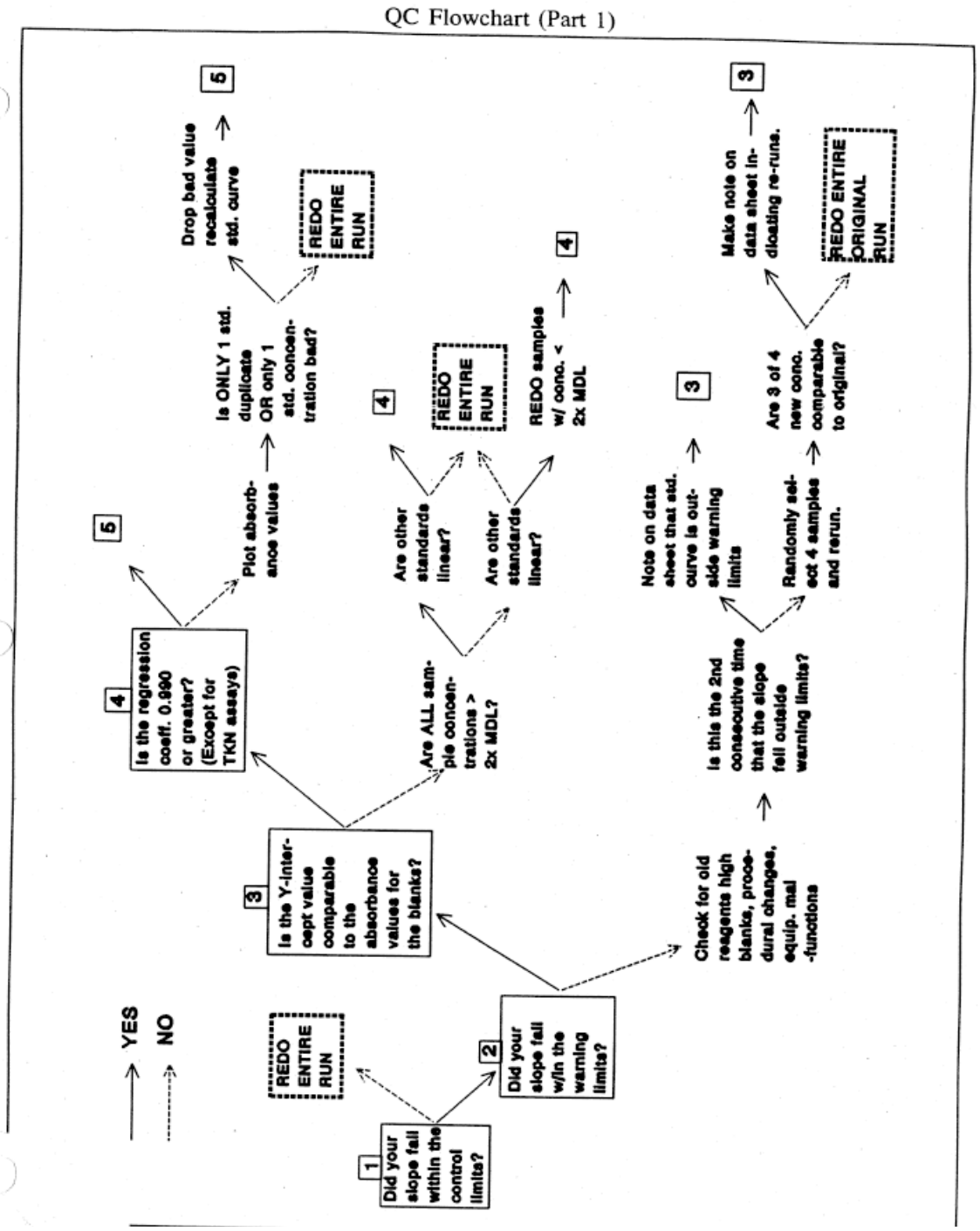
- Laboratory spike recovery performance:
  When the percent spike recovery falls between 85-115%, it is acceptable. These criteria apply to all assays except TKN and nitrate. For these two assays the spike recovery percentage must be plotted on the QC charts and fall within the acceptable range. The QA officer can reject the spike recovery performance but still accept the assay run. Corrective measures include rerunning only that field sample which was rejected or rerunning 5% of the samples as a validation check. At the discretion of the QA officer, the run may have to be repeated if all the spiked samples are rejected.

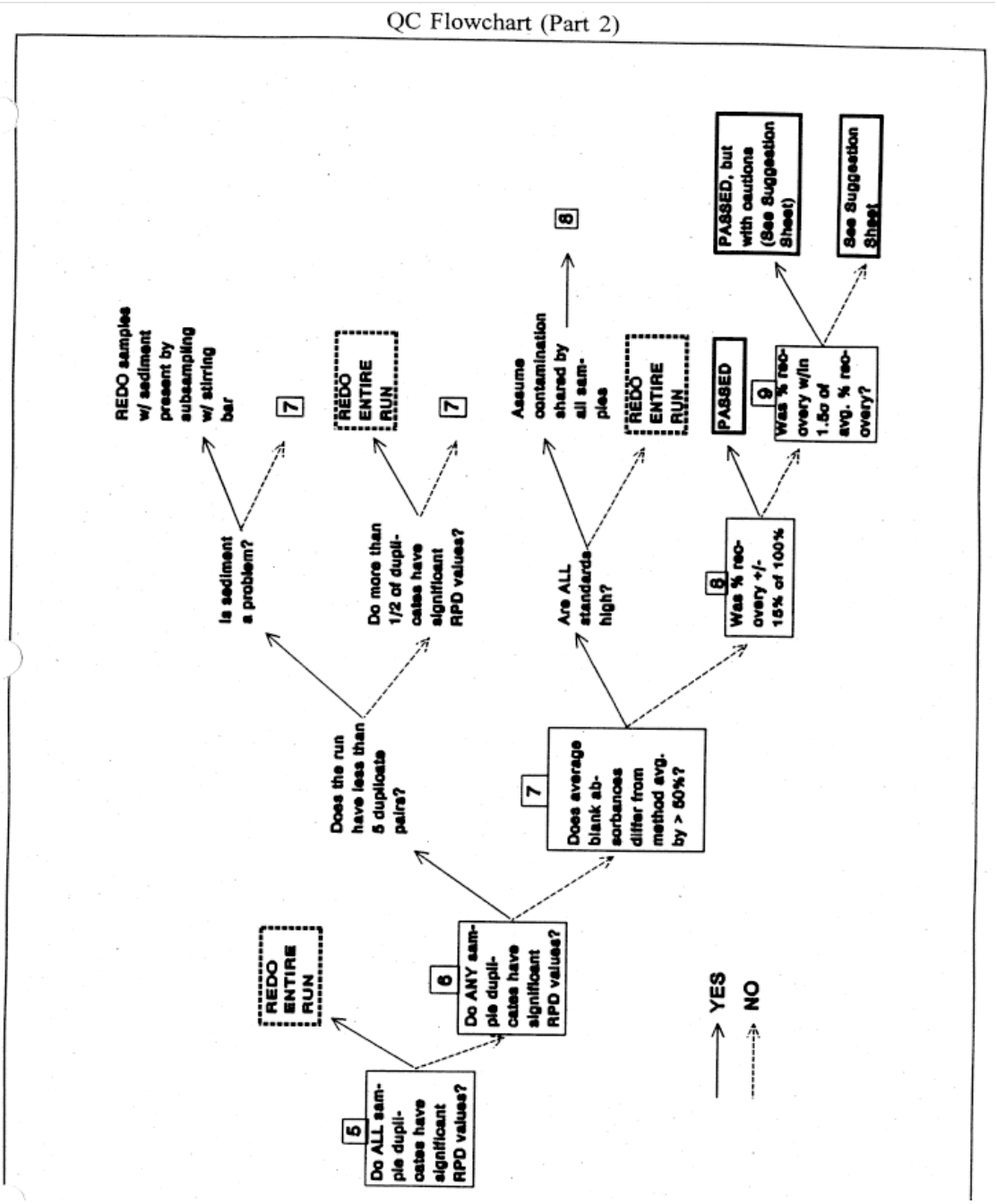
- Reference material performance:
  The concentration of the reference material should agree with the known concentration within 5%. At the discretion of the QA officer, the run may have to be repeated if this condition has not been met.

- Individual sample performance:
  If there is reason to doubt the sample concentration the QA officer can reject individual samples and request reruns. Most commonly the analyst has entered notes about particular samples that invalidate the reported concentration. Another evaluation procedure, although somewhat subjective, is to view the sample concentration as part of an ecological system, comparing it to "like" samples. Samples from the same station, but separated by depth, will behave in predictable patterns of concentration. Seasonal and inter-annual patterns are sometimes known in advance and can be used to evaluate sample performance.

- Assay acceptance or rejection:
  The QA officer has to accept or reject the assay run based on the performance of the quality control. If an assay is accepted, the status of individual samples from that run changes from complete to accepted. When a project report is requested, sample concentration values will be reported. If an assay is rejected, all the samples return to the Scheduled status to be rerun. When a project report is requested, sample concentration values will not be reported. Also, the quality control for the rejected run is not included in the six-month statistical summary for the bi-annual QC report.

QA Flowchart




QC Flowchart (Part 2)
5
Do ALL sample duplicates have significant RPD values?
REDO ENTIRE RUN
6
Do ANY sample duplicates have significant RPD values?
Does the run have less than 5 duplicate pairs?
7
Does average blank absorbances differ from method avg. by > 50%?
Is sediment a problem?
REDO samples w/ sediment present by subsampling w/ stirring bar
7
Do more than 1/2 of duplicates have significant RPD values?
REDO ENTIRE RUN
7
Are ALL standards high?
Assume contamination shared by all samples
REDO ENTIRE RUN
8
8
Was % recovery +/- 15% of 100%
PASSED
9
Was % recovery w/in 1.5σ of avg. % recovery?
PASSED, but with cautions (See Suggestion Sheet)
See Suggestion Sheet
YES
NO

Troubleshooting Suggestion Sheet

Method Failure Review

1. Use the *QA/QC* data to troubleshoot the assay.
2. Review the chemical/biological principles of the test.
3. Think about each part of the assay (equipment, reagents, standards, etc.).
4. What does the failed QC tell you?
5. Change one condition and see what happens.
6. Repeat the last step of the procedure changing one thing at a time until you see some impact on the problem.
7. Take and document the corrective action.

Potential Problems/Suggestions

Matrix Problems

The sample matrix will limit the analytical certainty. Matrix problems include chemical or physical interferences (particulates). Typically it is best to eliminate the interference from the matrix, if possible. Particulates, for example, might be removed through filtration or lessened through dilution of the original matrix. Chemical interferences might be removed through precipitation of the interference, pH changes, or lessened through dilution of the original matrix.

One indication of a matrix problem is poor spike recoveries and/or poor precision between duplicates. To confirm a suspected matrix problem you can perform a standard addition where standards are made up in the matrix rather than DDW water. When the sample concentrations are calculated from the matrix-standard curve, the spike recoveries should be close to 100%.

Precision Problems

When numerous duplicate samples have poor replication this could be attributed to one or more potential problems. First, as mentioned above, check the sample homogeneity. Does the sample have particulates that limit the effectiveness of sub sampling duplicates? If so, try to remove the particulates or lessen the impact by diluting the original sample. Mix the sample continuously while pipe ting to ensure homogeneity.

Second, poor replication may be diagnostic of changes that are occurring in your samples over time. If the duplicates are correctly set-up in the assay, they should not be run one next to the other. Thus, if poor replication can be attributed to nothing other than time, take a careful look at the method instructions. Have you

allowed the color to develop in the samples to full intensity before reading them? Have you completely mixed the reagents in the sample to enhance homogeneous color development?

Third, poor replication may also be an indication of spot contamination. The assay procedures may be prone to cross-contamination (such as TKN) where it is very difficult to control the source of contamination or the analyst may be causing contamination by careless habits.

Accuracy Problems

There are two QC tests for accuracy within a run that can indicate problems: spike recoveries and calibration verification (SRM). Poor spike recoveries can indicate matrix problems, incorrect or low spike additions relative to the sample concentration, inaccurate pipette calibrations, or method procedural inadequacies. If the calibration verification sample has poor agreement with the known value, this indicates method failure. Review the chemical principles of the method and try to diagnose what portion of the assay is suspect. One suggestion for a diagnostic tool is to spike sample replicates at different times in the assay to determine where the analyte is lost. This is an effective tool to monitor the procedural methods. Never alter the matrix (i.e. by dilution) when you are performing this test.

Contamination Problems

Laboratory contaminants, other than spot contamination, are introduced in a variety of ways. The contamination can affect all the samples and standards or only the method blanks. The most common suspect of contamination to all the samples **and** the standards is a reagent contamination. If all the standards have high absorbencies you can assume that all the samples have similarly been contaminated. Try to identify the suspect reagent and **replace only this reagent** in the next run. If the problem is not solved, select another reagent, replace, and rerun the assay. It is important to replace only one reagent at a time in order to diagnose the original source of contamination.

If only the method blanks have high absorbencies, this indicates possible DDW contamination. However, the standards are also prepared from the same DDW so all of the standard curve absorbances would be higher than usual.

Rerun Procedures

1. Save all the sample bottles and insure that they are stored appropriately.
2. It may be helpful to do a smaller run if your initial run had greater than 40 samples. Sometimes greater care is given to fewer samples and you will get better results.

3. Carefully consider the type(s) of problem you had with the initial run. If you can pin-point the source of the error it will save lots of time and effort.
4. Fill out new data sheets, making a notation that this is a rerun. If possible, list any changes that you've made to hopefully improve the performance of the next run.
5. To monitor the performance of the run I would suggest any or all of the following changes: increase the number of duplicates, increase the number of spikes and increase the number of standards.
6. Be sure to check the results from this rerun carefully. Monitor the quality control samples. Check to make sure that the paired samples have appropriate values, i.e. totals> soluble.

Corrective Action Request

UC DAVIS LIMNOLOGY LAB-ENVIRONMENTAL SCIENCE AND POLICY
DATA QUALITY ASSURANCE
QUALITY CONTROL CORRECTIVE ACTION REQUEST

Date: _____

Analysis: _____

Labtrack Run #: _____

Analyst: _____

QC Parameter out of Control: _____

- ❑ Duplicate Precision: _____

_____

_____

- ❑ Spike Accuracy: _____

_____

_____

- ❑ Standard Curve Slope: _____

_____

_____

- ❑ Sample: _____

_____

Comments
_____
_____
_____

APPENDIX B

Field Sampling Procedures

Tahoe Stream Sampling and Processing Procedures

Equipment

Churn splitter
Sediment bottle carrier and glass sediment bottles
DH 81 hand held sampler for collecting chemistry samples
DH 81 quart size sampling bottles
DH 48 hand held sampler for collecting sediment samples
DH 59
USGS "GEO PUMP" peristaltic pump
Battery (12 volt)
Tygon™ tubing for pump
USGS filtration manifold
Box of cellulose nitrate filters (0.45 μm, 14.2 cm diameter)
Tweezers
Box of nitric ampoules
Disposable gloves
Amber plastic bottles and black caps (250 ml)
White plastic bottles and red caps (nitric acid washed, 250 ml)
Deionized water
Thermometer
Dissolved oxygen meter and probe
Barometer
pH meter
Conductivity meter
Measuring tape (50 ft.)
Waders
Field notebook
Bottle labels
Ice chest
Ice Bottles
Kim Wipes™
Waste container for empty nitric acid ampoules

Stream Sampling Methods

1. Upon arrival at the stream, check the hydrograph and gauge readings in the gauging house to see what the stream gauge has been doing recently. Decide whether conditions are safe for wading the stream; if not, sample from a bridge or the stream bank.

2. Measure the air temperature in the shade, using the dry thermometer.

3. Set up a "tag line" (measuring tape) across the regular sampling site. If sampling from a bridge, use the tag line or some other regularly spaced markings on the bridge.

4. Take the suspended sediment sample. This sample is generally a depth-integrated sample taken near the center of flow in the channel. Determine the sampling point and rinse the sampling device (DH 48 or DH 59) several times in flowing stream water, then attach a clean suspended sediment bottle. Take the sample by lowering and raising the bottle at a constant rate through the water column (this rate should be about equal to the rate which will fill one sediment bottle to the shoulder at the deepest/fastest moving part of the stream with one lowering/raising sequence). If the sample bottle is overfilled, discard the sample and take a new sample in a clean bottle. Take two replicate samples per stream.

5. Take the chemistry sample. This sample is generally a equal-width-increment (EWI) sample taken using either the DH81, a Teflon coated DH48, or Teflon coated DH 59 "Bomb".

- From the tag line note the total width of the stream cross section, divide this width by the number of equal width intervals you want in a cross section, usually 8-15. For instance, if the stream width is 20 ft., 20 ft. divided by 10 intervals = 2 ft. per interval.
- Rinse the churn splitter, sampling device (DH 81/DH 48/DH 59), and sampling bottle 3-4 times with flowing stream water.
- Determine the sampling rate to use. This should equal the rate which will fill 1 bottle approximately to the shoulder at the deepest/fastest moving part of the stream with one lowering/raising sequence. Use this rate for each vertical. Begin sampling at a distance of 2 a full interval from the bank, i.e. 1 ft. from the bank for the above example. Then proceed to take verticals at the predetermined intervals across the channel i.e. 1 ft, 3 ft, 5 ft, 7 ft. etc. When a sampling bottle is filled to the shoulder, note the stopping point on the tag line, pour the sample into the churn splitter, and continue on. For normal chemistry sampling, we need to fill the churn splitter about $^{2}/_{3}$ full. Once you start taking samples across the stream cross section, you must continue to sample all verticals to the opposite bank. Even if you have enough water after sampling part way across the stream you need to continue until you reach the other bank.

6. Water temperature is taken by submerging the thermometer completely below the surface of the water in the flow, out of direct sunlight. Allow the thermometer to equilibrate for 30-60 seconds, then read while still submerged to the nearest 0.1˚C.

7. Dissolved oxygen is measured during expanded sampling according to USGS standard procedures.

8. Before leaving the stream, note the stream height measured on the outside staff measuring tape and note the general stream conditions (ice cover, flooding etc.) and the clarity of the stream (clear, light sediment, heavy sediment).

9. Go back to the stream gauging house, make a "tic" mark on the hydrograph corresponding to the time sampled and adjacent to it record: date and time sampled, your initials, type of sediment sample (single vertical, EWI, or DIP), number of sediment samples, manometer or float tape readings from gages in gauging house, outside staff measuring tape reading, air temperature, water temperature, and general observations on stream sediment load, any unusual conditions in the steam, and observations on current and recent weather. Record similar information in the Field Notebook.

Sample Processing Procedures

Field Processing

Often stream water contains particulates that rapidly settle. It is important to keep these particulates suspended during sub-sampling so reproducible sub-samples can be collected. The churn splitter facilitates the collection of reproducible sub-samples by churning the water as the sample is taken. Mix the water in the churn splitter 8-9 churning cycles using a consistent churn rate of approximately 7-9 inches/second. Take care not to aerate the sample by breaking the surface of the water in the churn. While continuously churning the sample, rinse each sample bottle and cap once with the raw water then fill each bottle.

The filtered samples should be collected next. Rinse the filtration manifold thoroughly with Milli-Q water. Place a cellulose nitrate filter (0.45 μm) between the two filter screens. Be sure to keep the upper screen on the input or upper side of the manifold throughout the filtration, for all streams, to prevent cross contamination with particulates. Install the Tygon™ tubing in the GEO PUMP. Keep the tubing end that will be placed in churn sample in a clean bag when not in use. Before starting the pump, rinse this tubing end with a small amount of the raw sample water and place it in the churn splitter. Start the pump drawing sample water through the filter. To distribute the sample evenly across the filter, vent trapped air from the vent in the top of the manifold. Pre-draw approximately one liter of sample through the filter and discard. This removes any excess nitrate from the filter. Rinse each sample bottle and corresponding cap once with filtrate, then fill.

Iron samples must be dispensed into their own sample bottle since 2.0 ml of concentrated nitric acid (HNO_3) is added as a preservative. The nitric acid is sealed in a glass ampule. Before opening, rinse the outside of the ampule with Milli-Q water and wipe dry with a Kim Wipe™. Tap all acid down into the main part of the ampule. Carefully break open the ampule at the neck and pour the nitric acid into the sample.

After filling all bottles, discard the remaining churn sample and rinse the churn splitter 4-5 times with deionized water. Remove the used filter from the manifold, rinse the manifold and Tygon™ tubing with deionized water, and cover the manifold with a clean plastic bag.

Raw and filtered water samples are kept cool at 4˚C in a cooler until arrival at the field station.

Lab Processing (within 2 hours of collection)

Measure conductivity of the raw sample water in the lab following standard operating procedures.

Measure pH of raw expanded samples using standard operating procedures.

Lake Sampling Procedures

The sampling program for Lake Tahoe began in 1969. It was called the Lake Tahoe Project (LTP). The sampling site was called the "Index" station, located several hundred yards offshore the Fleur de Lac estate on the west side of the lake in 175 m of water. This continues to be the sampling site. This station is sampled approximately 30 times each year, generally every ten days. Each sampling event has been numbered sequentially since 1968 (i.e. LTP 25, LTP 26 etc.). Water collection and the primary productivity experiments are conducted on water collected from 13 depths (0, 2, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105 m).

The second site for routine sampling was selected in 1980, at the middle of the lake, with 475 m of depth. This project was titled the Mid-Lake Tahoe Project (MLTP). This station has been sampled continuously since 1980, at least once a month. The sampling events have also been numbered sequentially (MLTP 100, MLTP 101..etc.). Water is collected from eleven depths (0, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 m).

SAMPLE COLLECTION SCHEDULE

This is largely representative of the recent sample collection schedule. Exact frequency and list of parameters may be modified with contract renewal every three years starting in the period 2013-2016.

Sample Frequency	Station	Sample Type
once/month	Mid-lake	Particulate CHN
once/month	Mid-lake	Particulate Phosphorus
once/month	Mid-lake, Index	Raw water samples for nutrient chemistry
once/month	Mid-lake, Index	Filtered water samples for nutrient chemistry
once/month	Mid-lake	Dissolved oxygen
3x/month	Index	Dissolved inorganic carbon
once/month 3x/month	Mid-lake Index	Chlorophyll a
once/month 3x/month	Mid-lake Index	Phytoplankton
3x/month	Index	Primary Productivity
3x/month	Index	Fluorescence
once/month 3x/month	Mid-lake Index	Zooplankton
once/month 3x/month	Mid-lake Index	Temperature
once/month 3x/month	Mid-lake Index	Secchi Transparency
3x/month	Index	Underwater Light Irradiance

Samples are collected from the R/V John Le Conte, a 37' diesel-powered, aluminum boat. The boat is equipped with 6 hydraulic winches and has a hydraulic-electrical generator. There is also a refrigerator/freezer onboard.

Field Equipment

6 or 8L Van Dorn Samplers with brass wire messengers
4L Nalgene™ polyethylene bottles
500ml Nalgene™ polyethylene bottles, acid-washed and labeled
Ice chest for cool storage and transport of samples
8 Ea. - 125ml glass bottles, with ~1ml Lugol's solution
1 Ea. - 125ml glass bottle, with 25ml Sucrose/Formalin solution
26 Ea. - 125ml glass bottles with glass stoppers, fitted with removable light shields
13 Ea. - 125ml glass bottles with glass stoppers, permanently darkened
Storage holding box for primary productivity bottles (darkened)
Martek™ thermistor
Kahlsico™ reversing thermometers
Thermometer, non-mercury (NBS Standardized)
Belfort™ pyreheliometer - Land-based station
Li-Cor™ P.A.R (Photosynthetically Active Radiation) Irradiance meter
Secchi disk, 25cm in diameter
YSI™ Dissolved oxygen/temperature meter
Zooplankton net, 80µm mesh, 4m X 0.75m, fitted with a Kahlsico™ flow meter at the mouth of the net
2 Ea. - Filtration manifolds, six filter holders per manifold
2 Ea. - 1L glass filtration towers, fitted to filter frit holders (25mm) with aluminum clamps
2 Ea. - 4L side-arm filtration flasks
30ml glass filtration tower, fitted to filter frit holder (25mm) with aluminum clamp
Vacuum pump, electric, with PSI gage, Tygon™ tubing, overflow device, and plastic quick- connectors
Hand-held Nalgene™ vacuum pump with psi meter
Filters, Whatman™ GF/C 24mm, pre-combusted at 500˚C
Filters, Whatman™ GF/C 24mm, non-combusted
Filters, Millipore™ 0.45µm, HA cellulose
Pre-filtration funnel, fitted with 80µm mesh
C^{14} glass ampoules, 10µCi/ml, ~ 20 ml volume
Syringe pipette, 0.5ml for delivery of C^{14}
13 Ea. - Horizontal PPR bottle hangers with 3 to 6 dog snaps
Millipore™ filter forceps
C^{14} Radioactive waste container - 5 gal.
Seltzer water
Disposable gloves, plastic
Labeling tape, ½"or 1" wide, multi-colors
Aluminum weighing pans
Aluminum foil
0.17M Na_2SO_4 with a 2ml syringe pipette for delivery
Distilled water
Graduated cylinders, plastic, various sizes (50 - 1000 ml)
Paper goods, Kimwipes™ and paper towels
Data sheets
Ink marker pens, permanent

LTP Index Station Procedures

Water Collection

Water is collected from 13 depths (0, 2, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105 m) using the 8 liter Van Dorn bottle attached to the boat's hydrowire. The water is distributed to collection bottles immediately.

- For each depth fill three 125ml glass-stoppered bottles (2 clear glass in light shield holders and 1 darkened bottle) for the primary productivity (PPr) experiments. Place the PPr bottles in the darkened storage box.
- At each depth fill one 500 ml Nalgene™ bottle for THP analysis and another 500 ml Nalgene™ bottle for shipboard filtration (nitrate and chlorophyll), DIC and fluorescence assays. Place the chemistry bottles in the ice chest.
- At the 5m, 20m, 40m, 60m, 75m, and 90m depths - fill a 125 ml glass bottle (containing Lugol's solution) for phytoplankton enumeration. The phytoplankton samples can remain at ambient temperature, shielded from sunshine. Tighten caps securely before storage to exclude air and prevent water loss.

A water chemistry and chlorophyll field duplicate should be collected from a second hydrocast to the 10 m depth. Fill one 500 ml Nalgene™ bottle for THP analysis and another 500 ml Nalgene™ bottle for shipboard filtration for nitrate and chlorophyll.

Generally three LTP index samplings occur monthly. One of the three is called a "full" LTP. The word "full" alludes to the expanded sampling for chemistry and chlorophyll assays. Nitrate and Total Hydrolyzable Phosphorus (THP) chemistry profiles and discrete depth chlorophyll collections are performed on the water chemistry bottles. The other two monthly LTP's are scheduled 10 days apart. They do not require water collection for nitrate, THP, or discrete depth chlorophyll. Nevertheless, water from each depth is still required for DIC, fluorescence, and chlorophyll composites.

Primary Productivity Experiment

When water collection is complete, each of the three PPr bottles from every depth must be injected with 0.5 ml of 10μCi radioactive C^{14}. The person doing the injection of the radioactive C^{14} should be certified by the University to work with radioactive substances. Two pairs of disposable gloves should be worn for this procedure. Keep the PPr bottles inside the storage box but remove the glass stoppers. The glass ampule of C^{14} can be broken at the neck and the radioactive bicarbonate is ready for pipetting into the PPr bottles. After the C^{14} has been added, re-stopper the glass PPr bottles. Make sure the stopper label and bottle label correspond. Invert the bottles several times to mix the contents. Keep the bottles in the storage box, away from light, until they can be placed *in situ.* When the PPr *in situ* line is ready, place the three bottles from each depth on a

horizontal hanger. Remove the light shields from the clear bottles, and place the hanger on the *in situ* line at the appropriate depth marker. The *in situ* line, when fully loaded with sample bottles, has a bottom anchor on one end and a surface float on the other. The experiment time begins when all samples are in the lake. This time should be recorded on the data sheet. The bottles incubate at ambient light and temperature levels for approximately 4 hours.

During the incubation, the filters needed for the end of the experiment should be prepared. The filters are HA cellulose Millipore™ (0.45 μm, 24mm). They should be pre-labeled using a ballpoint ink pen. Along the outside circumference of the filter write the LTP number, depth, and light (lt) or dark bottle (dk) bottle designation.

The *in situ* line is retrieved 4 hrs. after the beginning of the experiment. When the bottles are removed from the horizontal hangers they should immediately be placed in the darkened storage box. This officially ends the experiment. The time should be recorded on the data sheet. After all the bottles are retrieved, the filtration of the samples should begin as soon as possible. The Millipore™ filters should be placed on the manifold frits. The clear ("light") bottles from each depth should be filtered first. With 12 manifold filter places, stagger the filtration by doing 4 bottles at a time to keep a smooth operation going and prevent filters from becoming dry all at once.

Keep the filtration pressure less than 10 psi so that the cells are not overly stressed and do not lyse. Rinse the filter towers with lake-water from a squirt bottle before the last drop of sample water has been filtered. Remove the filters when filtration is complete and place on a paper towel in a filing folder. Round metal rings are placed on top of the filters so that they dry flat. After all the clear PPr bottles have been filtered, begin filtering the dark bottles. Follow the same procedures. Check the 4-liter waste flask so it does not overflow into the vacuum pump. The entire filtering operation creates almost 5 liters of waste. Dispose of the radioactive filtrate waste in a labeled waste container.

The filters dry over-night. They should then be handled with filter tweezers and placed in a specially labeled plastic vial container. The filters are stacked on top of each other, separated by the blue filter dividers. The filter container should be sealed with radioactive tape and placed inside a Ziploc™ bag that has been labeled and dated. The bag can be stored at ambient temperature. The filters are sent, by courier, to the UC Davis lab where they will be counted in the Geiger counter.

Zooplankton Collection

Vertical zooplankton tows from 150 m to the surface are taken at each LTP sampling. The zooplankton net is 4 m in length with a mouth diameter of 0.75 m. The netting has 80 μm mesh size. A flow meter is secured in the middle of the circular opening to the net. There is a fly wheel attached to the flow meter that spins when it meets water resistance. The number of revolutions of the fly wheel and the distance towed will

determine the volume of water filtered. When the net and attached collection bucket are ready for sampling, the number on the flow meter should be recorded on the field data sheet.

The net is attached to the hydrowire at the mouth opening. The hydrowire with the attached weighted net is lowered slowly into the water. The line meter wheel is zeroed when the mouth of the net is resting on the surface of the water. The net descent is continued until the line-out reads 150 m. The net descent is stopped. The net ascent is begun, taking care to keep a constant speed, approximately 1 meter per second. This speed is maintained until the mouth of the net breaks the surface of the water. The entire 4 meters of net is brought out of the water and lowered again until the mouth of the net rests on the lake surface. The net is again raised completely out of the water for the second time, the process is repeated once more for a total of three rinses. This "rinse-down" technique forces the zooplankton into the cod-end. The net is then lowered to a level where the researcher can read the flow meter. The number is recorded on the field data sheet next to the initial reading. The numerical difference between the two readings is the number of fly wheel revolutions for that zooplankton tow. Record the number of revolutions on the data sheet and on the sample bottle cap label.

The cod-end bucket is hauled on-board. A squirt bottle filled with lake-water is used to rinse down the lower half meter of netting, above the bucket. When rinsing is complete, carefully remove the bucket filled with water and zooplankton. Make sure water in the net is drained below the connection between net and bucket or part of the sample will be lost. To avoid this, swirl the bucket contents, to drain off excess water through the netted drain holes in the bucket. When the contents of the bucket are reduced to only 1/4 full, add approximately 20 ml of seltzer water. This addition of CO_2 will anesthetize the zooplankton. Continue to drain off the liquid contents of the bucket through the netting until about 75 ml remain. Pour the contents of the bucket into the 125 ml glass bottles containing sucrose-formalin. Rinse the bucket to capture remaining zooplankton, and add them to the sample bottle. Cap the glass bottle and invert it several times to mix the contents. This sample can remain at ambient temperature but the color of the zooplankters are preserved better if the sample is kept in cold (4°C) storage.

Phytoplankton Collection

Presently, six discrete samples (as mentioned earlier under "Water Collection") and two composite phytoplankton samples are taken. The discrete depth samples (5m, 20m, 40m, 60m, & 75m) are collected directly from the Van Dorn bottle into 125 ml glass bottles with the Lugol's preservative. The phytoplankton composite samples are comprised of water from discrete depth raw water samples bottles. Refer to the composite sample guide. Proportional volumes from each depth are listed for phytoplankton composites. The first composite is compiled from water from all thirteen depths (0-105 m). The second composite is compiled from water above the Secchi depth. This composite can be compiled only after the Secchi depth measurement has been taken. This composite

includes water from 0 m to the nearest depth value on the composite sample guide. For example, if the Secchi depth is 19.5 m, the composite would include the 20 m depth sample. If it was 25.5 m, include the 30 m depth sample. Pour each of the composite samples into a 125 ml glass bottle with the Lugol's preservative.

Temperature

Temperature measurements of the water column, down to 98 m, are taken at each LTP sampling. The thermistor has a temperature probe attached to the end of a 98 m wire. Initially the probe is calibrated at the surface, using a non-mercury thermometer for comparison. The thermistor probe needs to be calibrated on three range scales, using the small screwdriver for all 6 potentiometers. After calibration, the probe is lowered from the surface to 2 m. A reading is taken at each of the LTP sampling depths, except 105 m. Extra temperature readings are taken when the probe encounters the thermocline and/or micro-stratifications. The last reading is taken at 98m. All temperature data is recorded on the data sheet along with the time the readings were taken.

Underwater Light Irradiance

Light irradiance measurements in the water column, down to 145 m, are taken at each LTP sampling. The Li-Cor™ P.A.R. Irradiance meter has a deck cell and a sea cell. The deck cell measures surface irradiance and should be positioned in a place on the boat where artificial shading is avoided and the cell is kept level with the horizon. The deck cell will monitor changes in the surface irradiance over time. The sea cell can take readings on five range scales (100x, 100x, 10x, 1x, & 0.1x). The sea cell is attached to the end of a 150 m wire. The boat should be positioned such that the hydrowire is lowered on the sunny side. The sea cell is lowered along side the boat on the hydrowire as the connecting meter cable is paid out. Usually on a sunny day both deck and sea cell readings will start out on the 100x scale.

A reading is taken just above the surface of the water with the sea cell switch on the "air" position. Lower the sea cell just under the surface of the water and move the switch to "in water" position. Take another reading on the sea cell irradiance. Record both of these readings on the data sheet. Take a reading for the deck cell. Continue lowering the sea cell and take readings at all the LTP sampling depths plus 125 m and 145 m. Record all the irradiance readings on the data sheet.
When the irradiance sea cell reading is below 2.00 on any particular range scale, take two readings. One reading will be on the higher scale and the other will be on the lower scale. The two readings should nearly correspond; separated by an order of magnitude. Record the deck cell reading at this time as well. However, on a sunny day it is not necessary to read the deck cell irradiance at every sample depth. If the surface irradiance

is constantly changing (scattered clouds passing in front of the sun), then more readings will be necessary. The observer should also describe on the data sheet the surface water conditions, time, and the cloud cover situation during the readings. Also record any significant wire angle caused by boat drift.

Water Transparency

Measurements of water transparency are taken at each LTP sampling using the Secchi disk. The weighted disk is attached to a hydrowire. The boat should be positioned so that the hydrowire is lowered on the shady side. The line meter wheel is zeroed when the disk is at the lake surface. The disk is lowered in the water until it disappears from the observer's sight. This depth reading is noted and later recorded on the data sheet. The disk is then raised in the water column until it is visible again. This depth reading is also recorded. Transparency depth is the mean of these two depths. One would think that the readings would be somewhat subjective. However, when a second observer also takes the Secchi depth readings, they are generally quite similar to the first recorded readings. Both observers should record sky, surface water, wind conditions and the time. Make note of any unusual events (i.e. forest fire smoke etc.). The water surface may be "shaded" with a clipboard or hat to darken it, increasing contrast and reducing sky reflection. Polaroid (or other types of) sunglasses are not allowed.

Sample Pre-Treatment

Chemistry and Chlorophyll

The LTP raw water chemistry bottles will be used for water chemistry and chlorophyll samples. The samples should be handled carefully at all times to prevent contamination. One bottle from each depth will be assayed in the laboratory for THP analysis. The other bottle from each depth will be used for nitrate and chlorophyll filtration on the ship, fluorescence, and DIC analysis. Keep the raw water chemistry bottles cool.

During the "full" LTP, the raw water collected for nitrate and chlorophyll filtration need to be processed. The filtration should be done on-station if possible.

Chlorophyll filtration is done first. Chlorophyll is an unstable compound and will break down quite easily. We take precautions to lessen filtration stress and photo-degradation. To filter chlorophyll samples, a filtration apparatus should be assembled using a clean side-arm flask and filtration funnel. The apparatus should be rinsed with distilled water and positioned in an area shaded from direct sunlight. A Whatman™ GF/C filter (24mm , non-combusted) should be placed on the filter frit. Shake the raw water sample bottle and measure out 100 ml into a clean graduated cylinder. Turn on the vacuum pump to a pressure not greater than 5 psi. Filter the water, shutting off the vacuum pressure before

the filter goes completely dry. This will lessen the stress on the algal cells. Quickly remove the filter using filter forceps. Place the filter in a labeled aluminum weigh pan in the freezer. Keep it shielded from light.

Conveniently, the filtrate in the side arm flask can be used for the nitrate analysis. For each depth, pour the filtrate into labeled bottles. The bottles should be kept cool (4°C) until analysis. The filter funnel and side-arm flask must be rinsed between sample depths.

One additional chlorophyll sample needs to be filtered, the composite. Composite samples are comprised of water taken from the discrete depth raw water sample bottles. Use only the samples within the composite depth range, 0-105 m. The volume of water from each depth is proportionally combined to represent an integrated water column sample. Refer to the composite sample guide on the boat for instructions on volumes from each depth. Once the composite sample is made, pour 100 ml of the sample into the clean graduated cylinder. Filter the sample as instructed above. The filtrate does not need to be saved.

When all the filtration is complete, stack the aluminum weighing pans from the chlorophyll assay and bind them together with green labeling tape. Keep the filters frozen and in the dark.

During the other two monthly LTP samplings only one chlorophyll sample is taken, the composite. The composite is created in the same manner and the filtration of the sample is the same. As before, the filtrate does not need to be saved. The raw water chemistry bottles should be kept in the ice chest and taken back to the field lab for fluorescence readings and DIC analysis. There are not any other chemistry assays performed on these LTP samples.

MLTP Sampling Procedures

Water Collection

Water is collected from 11 depths (0, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 m) using a 9 liter Van Dorn bottle for each depth. Three hydrocasts are done. The first hydrocast collects water from 0 m and 10 m. The second hydrocast collects water again from 10m to be used as a field duplicate. The deep hydrocast collects water from the remaining depths. The Van Dorn bottles are attached in sequence on the hydrowire. Reversing thermometers are also placed on the hydrowire, interspersed between the Van Dorn samplers. The thermometers are placed at 50, 100, 200, 300, and 400 m. They are placed approximately 1 m below the Van Dorn bottle for that corresponding depth. A brass messenger is attached to each of the water samplers and thermometers on the hydrowire. When the deep hydrocast reaches the maximum depth the hydrowire is left

alone for 2-3 minutes while the reversing thermometers are allowed time to equilibrate at the correct depth. To "trip" the sampling sequence, a brass messenger is released down the hydrowire from the boat. The messenger should first strike the Van Dorn bottle at 50 m and trigger the bottle to close. The next sequential messenger should be released to strike the reversing thermometer. The thermometer should invert and the next messenger should be released. This sequence continues for 3-4 minutes until all the samplers have been released. On calm days you can place your hand on the hydrowire and feel all of them "trip".

When the Van Dorn bottles are brought onboard they are placed in a bottle holder on the boat's deck. Dissolved oxygen must be measured immediately by lowering the oxygen probe directly into the bottle. Follow the detailed instructions written in the next section. Record these results on the field data sheet. The water is then drained from the Van Dorn bottle into a labeled 4L Nalgene™ bottle. The Nalgene™ bottle should be rinsed with the sample water before being filled. The filled bottle should be kept cool and protected from the sun until the water can be processed.

For the deep hydrocast, the sampler bottles and thermometers are removed from the hydrowire one at a time, leaving the remaining samplers on the line, suspended in the water. Obviously this tactic is dependent upon surface wind conditions. The first Van Dorn bottle brought to the surface is the 50 m sampler. This bottle is removed from the hydrowire, followed by the removal of the reversing thermometer. The thermometer is placed in a cool, protected location until it can be read. The next sequential Van Dorn bottle is brought onboard and the above routine is repeated until all the samplers have been retrieved. Set the thermometer frames in a vertical position to maintain the temperature readings at depth. If they are laid flat on the deck, the temperatures may shift in the glass tubes and be lost.

Dissolved Oxygen

Dissolved oxygen is the first parameter that needs to be measured from the Van Dorn sample bottles. The YSI™ Dissolved Oxygen (DO) meter should be switched on when the boat leaves the mooring to travel to the sampling station. The instrument has to be calibrated before readings can be taken. When the boat is idle, on-station, the calibration can begin. Oxygen concentrations are dependent on temperature and barometric pressure. It is necessary to calibrate the instrument's thermometer to an independent thermometer.

For the surface samples, the "bucket" thermometer can be used for calibration. Suspend the dissolved oxygen probe from the port or stern of the boat into the surface water. Using the "bucket" non-mercury thermometer, take a reading of the surface water temperature. If possible, set the thermistor dial on the DO meter to match this temperature reading. Keep the DO probe wet at all times when it has been removed

from the protective sheath. Set the correct altitude setting to calibrate for barometric pressure.

For deeper samples, the reversing thermometer readings should be used for calibration of the thermistor dial on the DO meter. For those depths that do not have a reversing thermometer, the temperature must be estimated by interpolation. This may seem imprecise but the instrument's dial accuracy is only an estimate between 0.2-0.3°C.

The Van Dorn bottles are a microcosm of the depth being sampled. When the bottles are brought to the surface and onto the boat, the ambient conditions are quite different from the sampled depth. Warmer surface temperatures will affect the dissolved oxygen concentration. Dissolved oxygen readings should therefore be taken as soon as possible after the water has been sampled. The top suction cup of the Van Dorn bottle should be pulled out and secured on the side of the bottle. Drop the DO probe into the water and raise it up and down in the bottle for 20-30 seconds. Set the DO meter to the correct depth temperature. Record the DO concentration and enter that reading on the data sheet .

Remember to keep the oxygen probe wet at all times between samplings. One practical "hint" is to leave the probe inside the previously sampled Van Dorn bottle until it can be transferred to the next bottle.

Temperature

The subsurface temperature is measured using reversing thermometers. These instruments are very accurate because they consider the expansion and contraction properties of mercury in glass. Temperature and pressure changes at each sampling depth will affect these properties of mercury. The thermometers are specialized instruments with two side by side glass cases enclosing thermometer pairs. One pair of thermometers ("protected") is inside a sealed glass case. One thermometer of the "protected" pair is constructed with a coiled and constricted stem (pigtail) which has a glass knife inside. This thermometer will be used to measure the temperature at depth. The other smaller thermometer is used to measure ambient temperatures. The other large and small thermometer pair ("unprotected") is inside an un-sealed glass case. They are "free-flooding" and record temperature caused by pressure at depth. They will not give the true depth temperatures and are only used to make pressure corrections to the protected thermometers so that the true temperature can be determined.

It is important that the field sampler sets the thermometers correctly and checks both thermometers in the reversing frame after the instrument has been placed on the hydrowire and rotated 180°. The "depth" thermometer should have a continuous column of mercury and the mercury should be drained down toward the "pigtail". Place the messenger on the bottom of the reversing frame so it will travel down the wire to"trip" and release the bottle or thermometer set. The instrument is lowered on the hydrowire to depth and equilibrated to temperature. The release mechanism is triggered when the

brass messenger is sent down the hydrowire. The thermometer, once released, pivots vertically 180°. When the position of the thermometer is inverted, the mercury column in the larger thermometers separates at the point of ambient temperature. Since the once continuous column of mercury is broken, it will not move as the temperature changes. The thermometers are retrieved from the hydrowire and placed in a cool place. The thermometers should be read soon, but immediate attention is not imperative. Remember to keep the thermometers as vertical as possible as they come off the hydrowire.

A special "reading" device with a rubber cup and magnifying lens is used to read the temperatures from the thermometers. Record temperatures on the data sheet from each pair of thermometers. Also, record the serial number of each thermometer pair (usually a 4 or 5 digit number located inside each glass case). The temperature from the larger "depth" thermometers is the point at which the column of mercury has broken. The temperature from the smaller ambient thermometers is the height of the continuous mercury column. The ambient temperature is necessary to make corrections for thermal expansions of mercury in glass.

Water Transparency

Measurements of water transparency are taken at each MLTP sampling using the Secchi disk. The procedures are described in detail for the LTP sampling on page B-10.

Zooplankton Collection

Only one zooplankton tow is taken for each MLTP sampling. The vertical tow is taken from 150 m to the surface. The procedures are described in detail for the LTP sampling on page B-8.

Phytoplankton Collection

Two phytoplankton composites are collected from each MLTP sampling. One composite is comprised of water from 0-100 m; the second is 200-450 m. Most of the viable phytoplankton will, of course, be in the shallower composite. The second composite will indicate if viable cells remain at these deep depths. To create the composite samples, water from the discrete depth, 4-liter Nalgene™ bottles is used. The volume of water from each depth is proportionally combined to represent an integrated water column sample. Refer to the composite sample guide on the boat for instructions on volumes from each depth. Invert the 4 liter bottle several times to re-suspend particulates. Measure the required volume using a graduated cylinder. Combine all the aliquots of water from each depth in one container. When all the composite depths have been combined, fill the appropriately labeled phytoplankton sample jar (containing Lugol's solution). Fill the jar to the top to eliminate air space. The jars can be kept at ambient temperature.

Sample Pre-Treatment

The water collected from the MLTP depths, in the 4-liter Nalgene™ bottles, should be processed on-station. Water collected from the eleven depths plus the field duplicate is used for measurements of water chemistry and chlorophyll. The samples should be handled carefully at all times to prevent contamination.

Raw water collection

For each sample depth, invert the 4-liter Nalgene™ bottle several times to re-suspend settled particulates. Pour the mixed water into another clean, labeled 500ml sample bottle. Place the bottle in an ice chest to keep it cool.

Chlorophyll

Discrete chlorophyll samples are taken from 0, 10, 10 dup., 50, 100 m. In addition, a composite chlorophyll sample is collected using water from all eleven depths (0-450m). Chlorophyll samples need to be filtered. Water for the filtration is taken from the 4-liter Nalgene™ bottles. A filtration apparatus should be assembled using a clean side-arm flask and filtration funnel. The apparatus should be rinsed with distilled water. A Whatman™ GF/C filter (24 mm , non-combusted) should be placed on the filter frit. For each discrete depth sample, invert the corresponding 4-liter bottle several times to re-suspend particulates. Measure out 100 ml into a clean graduated cylinder. Turn on the vacuum pump to a pressure not greater than 5 psi. Filter the water, shutting off the vacuum pressure before the filter goes completely dry. (This will lessen the stress on the algal cells. Chlorophyll is an unstable compound and will break down quite easily.) Quickly remove the filter using filter forceps. Place the filter in a labeled aluminum weigh pan in the freezer, shielded from light. Discard the filtrate.
The chlorophyll composite (0-450m) is also comprised of water from the 4-liter Nalgene™ bottles. Refer to the composite sample guide on the boat for instructions on the volume required from each depth. Combine all the composite water in one container. From this container, pour 100 ml into a clean graduated cylinder. Filter this water as instructed above. Discard the filtrate.

After each sample has been filtered, the filtration apparatus needs to be rinsed with DI water to prevent cross-contamination. When all the filtration is complete, stack the aluminum weigh pans from the chlorophyll assay and bind them together with green labeling tape. Keep the filters frozen and protected from light.

Particulate Phosphorus

Water for the particulate phosphorus samples comes from the individual 4-liter Nalgene™ bottles. The water needs to be filtered. The filter is the sample while

the filtrate water is thrown out. One liter of water is filtered for each depth plus the field duplicate. A filtration apparatus should be assembled using a clean side-arm flask and filtration funnel. The apparatus should be rinsed with distilled water. A Whatman™ GF/C filter (24mm , combusted) should be placed on the filter frit. The corresponding 4-liter bottle should be inverted several times to re-suspend particulates. Measure 1000 ml of the water into a clean graduated cylinder. Pour the water into the filtration funnel through an 80µm mesh for pre-filtration of zooplankton. Turn on the vacuum pump to a pressure less than 15 psi. Filter the water. When approximately 20 ml of sample remains in the filtration flask, add two rinses of 2 ml Na_2So_4 (0.17M). Shut off the vacuum pressure before the filter goes completely dry. Quickly remove the filter using filter tweezers. Do not touch the filter with your hands. Place the filter, filtered side up, in the center of a 2" x 2" square of aluminum foil. Fold the filter in half using the forceps. Fold the aluminum foil around the filter. Label the foil packet with the depth and place it in an aluminum pan in the freezer.

It is necessary to collect field blanks for particulate phosphorus. A field blank usually consists of DI water filtered through the filtration apparatus. In this case, most all the significant particulate phosphorus contamination would come from the added Na_2So_4 (0.17M). Filter blanks are collected using the combusted GF/C filters. Place the filters on the filtration frit with the filtration tower. Add two rinses of 2.0 ml Na_2So_4 (0.17M). Turn on the vacuum pump and filter the Na_2So_4. Remove the filter from the frit and follow the procedures as instructed above. Usually three filter blanks are collected every MLTP sampling.

After each sample has been filtered, the filtration tower needs to be rinsed with DI water to prevent cross contamination. When all the filtration is complete, stack the aluminum weighing pans from the particulate phosphorus assay and bind them together with blue labeling tape. Keep the filters frozen.

CHN and Filtered Water Collection

Water for the particulate CHN samples comes from the individual 4-liter Nalgene™ bottles. The water needs to be filtered. The filter is the sample. However, the filtrate water is saved for soluble chemistry assays. One liter of water is filtered for each depth plus the field duplicate. A filtration apparatus should be assembled using a clean side-arm flask and filtration funnel. The apparatus should be rinsed with distilled water. Do not use the same filtration apparatus as you used for the particulate phosphorus filtration. A Whatman™ GF/C filter (24 mm , combusted) should be placed on the filter frit. The corresponding 4-liter bottle should be inverted several times to re-suspend particulates. Measure 1000 ml of the water into a clean, graduated cylinder. Pour the water into the filtration funnel through an 80µm mesh for pre-filtration of zooplankton. Turn on the vacuum pump to a pressure less than 15 psi. Before the filter goes dry, shut off the vacuum

pressure. Quickly remove the filter from the frit and place it in a labeled aluminum weigh pan in the freezer.

The left-over filtrate water from each CHN filtration is collected and poured into a clean, labeled 500 ml sample bottle for dissolved chemistry analysis (NH_4, NO_3, SRP, DP). The sample bottle can be rinsed with the sample water and then filled. Discard the remainder of the filtrate. These bottles should be kept cool in the ice chest. They will be used for the soluble chemistry assays.

After each sample has been filtered and the filtrate sampled, the filtration apparatus needs to be rinsed with DI water to prevent cross-contamination.

It is necessary to collect field blanks and filter blanks for CHN. A field blank usually consists of DI water filtered through the filtration apparatus. One liter of DI water is filtered in the same manner as above. The filtrate is discarded. The field blanks are usually collected twice for every MLTP. One field blank is filtered before beginning the filtration of the depths. The other field blank is filtered after the 150m sample.

The filter blanks consist of 2 filters taken from the box of combusted filters that is being used. Place these filters in the labeled aluminum pans, just as the samples have been stored.

When all the CHN filtration is complete, stack the aluminum weighing pans from the CHN assay and bind them together with yellow labeling tape. Keep the filters frozen.

Precipitation Sampling Procedures

Field Equipment

Ward Bench Station

- Equipment on 20 ft. tower:
  - Sierra Misco™ Model 2500E electric rain and snow gage
  - Weathermeasure™ Event Recorder
  - Taylor Instruments™ Thermograph
  - Snow tube and cap
  - 12 volt gel cell battery
  - 120 volt AC power outlet
  - Alter Type windshield
- Equipment required when servicing:
  - Weathermeasure™ event recorder chart #M699191
  - Taylor Instruments™ thermograph chart #132

Thermometer
2 ea. - 2 liter polypropylene bottles, thoroughly cleaned
1 snow tube bag

Ward Lake Level Station

- Equipment on 6 ft. tower:
  Weathermeasure™ P511E electric rain and snow gage
  Sierra Misco™ Event Recorder
  12 volt gel call battery
  Aerochem Metrics™ Wet/Dry Deposition Sampler Model #301
  120 volt AC power outlet
- Equipment required when servicing:
  Sierra Misco™ event recorder chart #2515W
  1-HDPE 28.575 cm dia. 5 gallon bucket for "wet-side"
  1-HDPE 25.4 cm dia. modified 5 gallon bucket for "dry-side" (fitted with baffles and coarse mesh screen in summer: fitted with aquarium heater in winter)
  Carboy of deionized water
  1 liter graduated cylinder
  2 ea. - 1 quart jugs for collection of rain gage water

Mid-lake Buoy

- Equipment on PVC buoy anchored on lake bottom:
  Dry deposition bucket holder
  Snow tube and cap
  Rain gage
  Flashing Warning Light (darkness-activated), 2 lantern batteries.
- Equipment required when servicing:
  1- HDPE 25.4 cm dia. modified 5 gal. bucket (fitted with baffles)
  Carboy of deionized water
  1 liter graduated cylinder
  1 snow tube bag
  mineral oil for rain gauge
  2 lantern batteries

Laboratory Equipment

1 liter side-arm flask
Filtration manifold capable of accepting 2.4 cm filters
Filtration pump
2.4 cm GF/F filters
Tweezers
Aluminum weighing pans
Aluminum foil
Corning® 610A pH meter and calomel reference electrode

pH buffer solutions (pH 4 and 7)
0.17M Na_2SO_4
5 x 10^{-5} N HNO_3 Quality Control Standard
5 ml Oxford® adjustable pipette and tips
250 ml Nalgene® bottles
1 liter graduated cylinder
Drying oven
Freezer

Station Servicing Procedures

Ward Valley Bench Station

Non-storm Procedures:
Visit station every week during non-storm periods, change thermograph chart, rewind event recorder clock, and reset the chart back to the proper time on the first day on chart, note the reset date/time on the chart, refill the pen with ink if necessary. On a monthly basis, check the calibration of the thermograph with a thermometer placed in the shade at the top of the tower.

Check the calibration of the tipping mechanism in the rain and snow gage when the chart recorded precipitation amount and the actual volume collected differ significantly. The gage tipping mechanism can be adjusted by adjusting the height of the stops beneath each side of the tipping mechanism and by adjusting the weight height on the mechanism. The mechanism should tip with each addition of 8.2 ml of deionized water.

Record procedures in the field notebook and general notes on current weather conditions.

Storm Event Procedures:
As soon as possible after a storm event visit the station. Remove event recorder chart, note date and time removed. Rewind clock and attach a new chart, note date/time, reset pen to baseline of chart, refill the pen with ink if necessary.

Remove the 2 liter bottles containing precipitation from the rain/snow gage, seal with caps from clean 2 liter bottles, place clean 2 liter bottles in gage.

Remove snow tube containing precipitation and seal by tying off end, replace with clean snow tube, rinse snow tube cap with deionized water and replace.

Record procedures in the field notebook and general notes on current weather conditions.

Ward Valley Lake Level Station

Non-storm Procedures:

Visit station every week during non-storm periods, rewind event recorder clock, and reset the chart back to the proper time on the first day on chart, note the reset date/time on the chart, refill the pen with ink if necessary.

Remove the 25.4 cm dry deposition bucket (and aquarium heater if attached) and enclose in bag from clean deposition bucket. Add 4 liters of deionized water to clean dry deposition bucket and place in dry side of Aerochem Metrics9 Wet/Dry atmospheric deposition sampler (a support has been placed to adjust height of bucket to provide for proper seal by the Aerochem Metrics9 lid).

Record procedures in the field notebook and general notes on current weather conditions.

Storm Event Procedures:

As soon as possible after a storm event visit the station. Remove event recorder chart, note date and time removed. Rewind clock and attach a new chart, note date/time, reset pen to baseline of chart, refill the pen with ink if necessary.

Remove 28.5 cm diameter deposition bucket from "wet side" of Aerochem Metrics9 Wet/Dry atmospheric deposition sampler, cap with lid from clean 5 gallon deposition bucket and replace with clean bucket.

Replace the quart size bottles in the P511E tipping bucket with fresh bottles.

Mid-lake Buoy

Every 7-10 days visit buoy. Check the light operation by covering the light with blacken cloth. If light is dim or not functional, replace batteries.

Remove the dry deposition bucket and enclose in plastic bag from clean deposition bucket. Fill the clean deposition bucket with 4 liters of deionized water and place in bucket holder on buoy.

Remove the snow tube and replace with clean snow tube. Rinse the snow tube cap with deionized water and replace.

Note precipitation accumulated in rain gage. If necessary empty the gage and add new mineral oil.

Laboratory Processing

Wet and Dry Precipitation

If sample is frozen, thaw by partially immersing sealed sample collection container in warm water.

Determine the total volume of the sample by keeping track of volumes measured during processing. Record the volume in the field notebook.

Mix the sample thoroughly. Measure a known amount of sample (ideally 250-500 ml) and filter through a pre-combusted 2.4 cm GF/F filter. Transfer the filtrate to a labeled 250 ml Nalgene™ bottle and freeze for later analysis of nitrate, ammonium, orthophosphorus, total dissolved phosphorus, and sodium. Place the filter in a labeled aluminum weighing pan and dry overnight at approximately 60˚C. After drying, freeze the filter for later analysis of particulate nitrogen. Be sure to include the volume filtered on the sample label.

Mix the sample again then filter a known amount of sample (ideally 250-500 ml) through another pre-combusted GF/F filter. Before the filter goes dry, add two 2ml additions of 0.17M Na_2SO_4. (Note: if saving the filtrate for chemical analyses be sure to use filtrate prior to adding the Na_2SO_4 because it will contaminate the sample for the sodium assay) Fold the filter in half, with the filtered particulates on the inside. Wrap the filter in foil, label, and freeze for later determination of particulate phosphorus. Be sure to include the volume filtered on the sample label. For every 20 samples, include an unused GF/F pre-combusted filter to be used as a blank.

Mix the sample again them transfer at least 50 ml of "raw" sample to a labeled 250 ml Nalgene™ bottle and freeze for later TKN analysis.

Measure the remaining volume of precipitation and save approximately 50 ml for measurement of pH as described below. Note: Lake level station dry deposition pH is not usually measured, mid-lake buoy dry deposition pH may be measured when contamination with lake water splash is suspected. A pH greater than 5.6-6.0 may indicate lake water contamination since typically lake water pH is near 7.8.

APPENDIX C

Data Entry and Quality Control Forms

UCD Water Sample Log-In Record

SAMPLE	Date Collected	Time Collected	Sampler	Date Received	NOTES	TKN	BAFe	Other

TERC Water Sample Log-In Record

Page:_____

SAMPLE	Date Collected	Time Collected	Sampler	Date Received	Filt-ered	Raw	NH4	NO3	SRP	TP	THP	Turbi-dity	Other	NOTES

UCD Limnology Group

1/1/93

Relative Percent Difference (RPD) Worksheet

Assay:	Method Detection Limit (MDL): µg/L

SAMPLE	Assay Date	Duplicate		RPD	Significant		NOTES
		1	2		No	Yes	

Matrix Spike Recovery Worksheet

Assay:_____

Sample	Assay Date	Spike Conc.	Sample Concentrations			Percent Spike Recovery	Passes Control Limit Test	
			Initial	Final	Difference		Yes	No

Standard Curve Statistical Summary

Assay:________________

Assay Date	Working Standard Range	Slope	Intercept	Coefficient of Regression (r)	Passes QC Criteria?		Notation
					Yes	No	

Standard Reference Material List

Material: _____

Assay: _____

Reported Value: _____

Date	Replicates			Avg. of Repli-cates	% Differ From Reported	Within accept-ance range?		Notation
	1	2	3			Yes	No	

Method Blank Absorbance Summary

Assay:_____

Assay Date	Blank 1	Blank 2	Blank 3	Blank 4	Blank Average	Method Average	Percent Differ.	Notations

UCD Limnology Group

1/1/93

Personnel Training Record

Name: _____

Hire date: _____

Task/Class	Date of Completion	Trainer	Supervisor

Water Chemistry Data Sheet __

Project:______________________

Assay:_________________ Assay date:________ Sample size:______ Wavelength:____ Chemist:____

Tube #	QC Code	Station	Sample	Sample Id #	Collection Info			Absorb-ance	Dilution	Con-centra-tion	Std. Code
					Date	Time	Sampler				

Notes: Standards and QC information on reverse side. Indicate standard curve used (e.g. A, B, etc.) in "Std Code" column. Indicate QC spikes, duplicates and reference material in "QC Code" column. (Code S1, S2, etc. for spikes, code D1, D2, etc. for duplicates, and code R1, R2, etc. for reference material.)

Comments:

Water Chemistry QC Data

STANDARD A			Cell size:			
Conc.	Reps		Range used:			
Blank			150 ppb:			
			Corr. 150 ppb:			
				Value	Passes QC	
					Yes	No
			Slope:			
			y-int:			
			r:			

STANDARD B			Cell size:			
Conc.	Reps		Range used:			
Blank			150 ppb:			
			Corr. 150 ppb:			
				Value	Passes QC	
					Yes	No
			Slope:			
			y-int:			
			r:			

STANDARD C			Cell size:			
Conc.	Reps		Range used:			
Blank			150 ppb:			
			Corr. 150 ppb:			
				Value	Passes QC	
					Yes	No
			Slope:			
			y-int:			
			r:			

SPIKE Recoveries
(1 spike/20 samples OR 1 spike/project, whichever is more)

QC Code	Percent Recovery	Passes QC	
		Yes	No
S1			
S2			
S3			
S4			
S5			

DUPLICATES
(1 duplicate/20 samples)

QC Code	Relative Percent Difference	Passes QC	
		Yes	No
D1			
D2			
D3			
D4			
D5			

STANDARD REFERENCE MATERIAL

QC Code	Reported value:	Passes QC	
	Percent Difference from Reported	Yes	No
R1			
R2			
R3			

QC Comments/Corrective Action:

Chain of Custody Record

Relinquished by:	Date:	Ship to:	Method of Shipment:
Received by:	Date:		Shipment ID:
Project:		Attn:	

Sample Description						Assay(s) Requested	# of bottles
Sampler	Date	Time	Description	Raw	Filtered		

Sample Preservation:	*FOR LABORATORY USE ONLY*	
	Laboratory batch ID:	
Sample Condition Upon Receipt:	Storage Refrigerator ID:	
	Storage Freezer ID:	

Special Instructions/Comments:

Sample Identification Label

NO3	LOCATION:			
NH4	STATION:		DEPTH:	
TKN	DATE:		TIME:	
TRP	COLLECTOR:			
TP	OTHER ASSAYS:			
THP				
BAFe	NOTES:			
Cations				
Raw				
Filtered				

1/1/93

Reagent Record

Reagent Mixture	Vol. Mixed	Date	Chem-ist	Reagent Inventory ID#	Replace-ment Date	Notes

Field Blank Record

Sample	Sample Source	Date Collected	Sam-pler	Raw/ Filt.	NH_4	NO_3	TRP/ SRP	TP/ PP	THP/ DHP	TKN/ DKN	BaFe/ DBaFe

Field Duplicate Record

Sample Source:_________________

Sample	Date Collected	Sam-pler	Raw/ Filt.	Dupli-cate #	NH_4	NO_3	TRP/ SRP	TP/ PP	THP/ DHP	TKN/ DKN	BaFe/ DBaFe
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											
				1							
				2							
Relative Percent Difference (RPD) between duplicates:											

Stock Standard Comparisons

Stock Standard	Chem-ist	New Standard			Previous Standard			Slopes differ > 5%
		Date Made	Std. Range	Slope	Date Made	Std. Range	Slope	
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N
								Y N

APPENDIX D

Shimadzu Spectrophotometer

Model:	UV-1700, UV-visible spectrophotometer
Use:	Colorimetric assays
Calibration Material:	Analyte Standards and NIST SRM-931e
Calibration Frequency:	Once per assay

Calibration Procedure: The instrument automatically initializes when the power is turned on. The initialization includes lighting of both lamps, optical alignment and energy check of both lamps, wavelength detection, and a baseline correction. Since the instrument is dual beam, both quartz cells are filled with distilled deionized water and placed in the cell holder. The instrument zeros and corrects for differences between cells. Any errors or abnormalities will appear as messages on the monitoring screen. The analyte standards are run first to measure the instrument response as a function of analyte concentration. A calibration curve is constructed. As a verification check, place a sealed cuvette containing the liquid SRM material into the instrument cell holder. Read the absorbances at 302, 395, 512 and 678 nm. These readings must not differ from the reported absorbances by > 5%.

Maintenance: The sample chamber and cells are cleaned weekly, the lenses cleaned once a year or as needed and baseline calibration done at least once a year.

Agilent 7890A Gas Chromatograph

Model:	Gas Chromatograph fitted with an FID detector and a multimode inlet.
Use:	fatty acid analysis
Calibration Material:	Supelco 37 mix of Fatty Acid Methyl Esters
Calibration Frequency:	once a month

Calibration Procedure: The machine is run with only the syringe and nothing else, not even washing hexane to get a clean baseline. Once the baseline is clean a hexane blank is run with the washing hexane to ensure the baseline is still blank. Fatty acid methyl ester standard is diluted in a series of a four-step set of standards and run on the GC. A calibration table is constructed and calibration curves are made for each FAME with their own unique slope.

Maintenance: MM inlet liner changed once a year, inlet septum changed once a month, column cut and new ferrules on inlet and outlet of column as needed, FID cleaned as needed.

Traceable Fish Scientific Electronic Thermometer

Model:	#15-077-8, Traceable Fisher Scientific.
Use:	temperature calibration for pipetter calibration, water bath calibration, air temperature check, cold room calibration, fridge and freezer calibration
Calibration Material:	none, use 2nd electronic thermometer to check temperature
Calibration Frequency:	only as needed, sent to factory
Calibration Procedure:	wipe off probe and check temperature.
Maintenance:	battery change as needed. Send to factory for calibration as needed.

Fisher Accumet pH Meter

Model:	915
Use:	Determine pH
Calibration Material:	Fisher buffers, pH = 4, 7, 10
Calibration Frequency:	Once per assay
Calibration Procedure:	The instrument is initially calibrated using the pH 7 and pH 4 buffers. The instrument automatically constructs a calibration curve and evaluates the probe efficiency. The pH 10 buffer is used as a calibration check. The reading must not differ from pH 10 by > 5%. Deviations from the theoretical calibration curve will indicate a stressed or faulty electrode.
Maintenance:	The reference probe is re-filled with KCl solution monthly. If the efficiency drops below .950 the probe should be rejuvenated or replaced.

Cole Parmer Conductivity Meter

Model:	1481-50
Use:	Determine conductivity
Calibration Material:	USGS Reference Standards (50 and 180 μS)
Calibration Frequency:	Once per assay
Calibration Procedure:	The instrument cell constant is determined using the USGS Reference Standards after manual temperature corrections. The conductivity readings of the reference standards should not differ from reported values by > 5%.
Maintenance:	Once per month the cover on the probe should be removed and the steel rings and PVC support should be cleaned with a cotton ball soaked in alcohol. Rinse with deionized water and dried.

Hach Turbidimeter

Model:	2100
Use:	Determine turbidity
Calibration Material:	Hach Reference Standards (0.61, 10, 100, & 1000 NTU)
Calibration Frequency:	Once per assay
Calibration Procedure:	The instrument scale is zeroed using the meter zeroing screw. Prepared standards are inserted into the instrument and the desired range is selected. The instrument reading is adjusted to the known NTU value.
Maintenance:	Periodically check connectors, cable attachments, indicating lamps, fuses, and circuit boards. Clean the sample cell thoroughly before each run. Discard the cell if it is scratched or etched.

Mettler Microbalance and Top Loader

Model:	PB602-S/FACT classic plus –TOP LOADER, AE240 analytical balance, Sartorius SE-2 microbalance
Use:	Gravimetric determinations
Calibration Material:	Class S Calibration Weights (0.01, 0.1, 1.0, & 10.0 g) (1mg, 500mg for microbalance)
Calibration Frequency:	Monthly for AE240, day of use for microbalance
Calibration Procedure:	Zero the balance, place one weight on the pan (handle these weights only with ivory-tipped forceps), take a reading. Repeat this procedure with the same weight for ten readings. Calculate the average, standard deviation, precision, and accuracy. Neither the precision nor the accuracy should exceed manufacturer tolerance limits. Repeat this procedure using another weight.
Maintenance:	Cleanliness is exceedingly important and any material spilled in the balance case must be cleaned up immediately. The balances are checked daily for level and zero point adjustments. Periodic calibrations will indicate malfunctions. **Balances are professionally calibrated and cleaned annually**.

YSI Dissolved Oxygen Meter

Model:	57 (Membrane Electrode)
Use:	Field measurements of dissolved oxygen
Calibration Frequency:	Once per assay
Calibration Procedure:	The instrument must be calibrated for current temperature and barometric pressure. The oxygen probe is calibrated by reading against air with 100% saturation. If the instrument reading of dissolved oxygen (DO) does not match the predicted DO (from calibration charts), the calibration knob is turned until the DO reading matches the predicted value.
Maintenance:	Store the electrode tip inside a moistened cap. Before instrument is calibrated it should be zeroed and the red line aligned with the needle at the 31EC position. Periodically check the integrity of the membrane if you get erratic readings. Check for air bubbles under the membrane that would interfere with gas diffusion across the membrane. Maintain the electrolyte solution level in the electrode.

THERMO-Precision Fisher Scientific Water Bath

Model:	50 liter
Use:	Nitrate assay, 20 degree C bath for all standards and samples
Calibration Frequency:	Monthly with electronic thermometers
Calibration Procedure:	Calibrate the temperature of the water bath by recording the stabilized readout with the actual temperature as measured by a thermometer. Measure the actual temperature at instrument readout of 15E, 25E, and 30EC. Create a calibration curve of instrument readout versus actual temperature.
Maintenance:	Water bath is periodically cleaned and refilled with deionized water.

Eppendorf and Rainin Automatic Pipettes

Models:	Variable Volume micro and macropipettes
Use:	Delivery of specified sample volume and reagents
Calibration Frequency	Before use, send out to factory get calibrated as needed
Calibration Procedure:	For each pipette: set the volume at mid-range of pipette delivery. Zero the Mettler microbalance, place a small plastic boat on the balance, tare. Pipette the specified volume of distilled deionized water (25EC) into the boat. Weigh the water. Repeat the procedure with the same pipette set at the same volume for ten weighings. Consult the chart Mass of Water Dispensed by Temperature and Volume. One ml of water @25EC should weigh 0.9970 g. Calculate the average weight, standard deviation, precision, and accuracy. The volume delivered should not exceed manufacturer tolerance limits.
Maintenance:	Each pipette should be periodically disassembled and checked for moisture, worn "O" rings, and re-lubricated.
Limitations:	Note that the accuracy of the variable volume pipettes varies with the volume delivered; because they are air displaced pipettes, they will need to be dialed UP in high elevation at the TERC. The pipettes should perform best at mid-range volume settings. Accuracy of delivery should be monitored especially for volumes < 100 µl and any volume of organic solvent.

Fisher Isotemp Muffle Furnace

Model:	550-126
Use:	Filter pre-combustion, and particulate phosphorus assays
Calibration Frequency:	Annually, with 500 degree C glass thermometer.
Calibration Procedure:	LED reading of temperature visible. Set temperature to 500 degrees C.
Maintenance:	Periodically check the ceramic furnace for cracks and cleanliness.

Fisher Scientific Isotemp Premium Drying Ovens

Models:	750G (large), gravity model, not forced air
Use:	Drying particulate samples, chemicals, and particulate phosphorus assays.
Calibration Frequency:	Annually
Calibration Procedure:	Ovens have LED temperature readings. One in the biology lab is usually set at 60 degrees C and one in the chemistry lab is usually set at 110 degrees C. Both can be calibrated with the electronic thermometer.
Maintenance:	Wipe out the ovens with a damp cloth once a week. Periodic re-calibration if the oven is used at high temperatures.

Refrigerators and Freezers

Models:	All brands of household models
Use:	Sample storage
Calibration Frequency:	Work days
Calibration Procedure:	Each refrigerator has a thermometer inserted through a stopper into a flask of water. The temperature of the refrigerator is recorded daily and plotted on a graph. The temperature should not deviate from 4EC by >1EC. The freezer temperatures are monitored with freezer thermometers. The temperatures should never exceed -5EC.

Maintenance: Periodic cleaning and temperature monitoring. Defrost the freezers once every 6 months.

Cold Room and variable temperature rooms

Models:	R.W. Smith Controlled Environment Rooms
Use:	Sample storage, experiments
Calibration Frequency:	Work days
Calibration Procedure:	Each room has a digital reading on the outside of the room and an audible alarm in case of failure. We also use our electronic thermometer daily to check room temperature.
Maintenance:	Periodic cleaning and temperature monitoring. NOTE: if the water is turned off in the building, the room's temperatures can fail.

APPENDIX E

Standard Operating Procedures

ORTHOPHOSPHORUS ANALYSIS

Total or Dissolved
Low level, Colorimetric

Introduction:

Orthophosphorus is converted to a phosphomolybdate complex by acidified ammonium molybdate. When the phosphomolybdate complex is reduced with ascorbic acid in the presence of antimony, an intense blue complex develops. The color intensity is proportional to the orthophosphorus concentration and is measured spectrophotometrically. Concentrations are reported as μg PO_4-P/L.

Interferences include arsenates (>100μg As/L) which react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Hexavalent chromium and nitrite have a negative interference.

The method is applicable in the range from 0-200 μg/L (ppb). the method detection limit (MDL) is 1.0 μg/L. the precision of the 30 ml method (99% confidence intervals) is ± 0.6 μg/L.

Pre-treatment of Water Samples:
Analysis is run on raw samples for total values or filtered samples for dissolved orthophosphate commonly referred to as SRP – soluble reactive phosphorus. Samples should be filtered on the day of collection. Keep filtered samples in tightly capped bottles, stored at 4°C. Assay should be performed within 10 days of collection. The orthophosphorus analysis is the third priority of all the nutrient assays.

Reagents:

1. Molybdenum-Antimony Solution – Store in glass bottle

Sulfuric Acid (H_2SO_2 36N)	244 ml
Ammonium Molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$	21.0 g
Antimony Potassium Tartrate $(C_8H_4K_2O_2Sb_2.3H_2O)$	0.60 g
Distilled Deionized Water (DDW)	Bring to 2000 ml

Cautiously add concentrated H_2SO_4 to about 1500 ml DDW in a volumetric flask. Mix, and allow solution to cool. Add ammonium molybdate and antimony potassium tartrate and mix to dissolve. Bring volume up to 2000 ml with DDW and store in glass.

2. Ascorbic Acid – Store in refrigerator, stable one month

Ascorbic Acid ($C_6H_8O_6$)	3.0 g
DDW	100 ml

An alternative is to dissolve ascorbic acid in 95% ethanol. This aqueous solution must be prepared fresh daily.

3. Standard Phosphate Solution (50 mg P/L)
Store in glass and refrigerate

Sodium Dihydrogen Phosphate (NaH_2PO_4)	0.1935 g
(Oven dried, 110°C)	
DDW	bring to 1000 ml

4. Working standards – Store in glass and refrigerate

Working standards are prepared form the 50 mg/L stock.. Typically 6 concentrations spanning the expected range of sample values plus a blank are run in duplicate. The standard range is 0.0, 5.0, 10.0, 20.0, 50.0 and 100.0 µg/L. A 200 µg/L can be added for samples in the range of 100-200 µg/L.

5. Spike Addition Stock (50 mg P/L) – Refrigerate

Intermediate spike solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. The final spiking concentration is 20 µg/L for samples in the range of 0-50 µg/L. Samples at concentrations >50 µg/L should be spiked at a level 0.5-10 times the sample concentration.

6. Standard Reference Material (100mg/L)
Store in glass and refrigerate

Potassium Dihydrogen Phosphate (KH_2PO_4)	0.2196 g
(Oven dried, 110°C)	
DDW	bring to 500 ml

The intermediate solutions should be prepared fresh for each assay. The final working concentration is 30 µg/L.

Procedure:
For expected phosphorus concentrations up to 100 µg/L, use a 30 ml sample and read in a 10 cm pathlength cell. Samples with higher concentrations can be read in a 4 or 1 cm cell.

1. Measure 30.0 ml of sample into clean test tube (25 × 175 mm, screwcap). The test tube, as well as all other glassware, must be immaculate since even a small amount of contaminate will affect the results.

2. Add 3.0 ml of the molybdenum-antimony reagent Vortex.

3. Add 0.3 ml (300 µl) ascorbic acid solution. Vortex

4. Allow samples to react for 20 to 30 minutes for the blue color to develop.

5. Read absorbance at 675 nm in chosen pathlength cell (10cm recommended). Samples can also be read at 880 nm.

Notes:

1. If the concentration of orthophosphorus is higher than the standard, the sample should be diluted and analyzed

.

2. Wear a lab coat. The molybdenum-antimony solution is notorious for destroying clothes.

Calculations:

To evaluate the phosphate concentration, generate a standard curve by plotting the absorbance versus the concentration of PO_4-P/L. If samples have been diluted, multiply the concentration by the appropriate dilution factor to calculate final concentration values.

QA/QC Procedures:

1. To determine the success and accuracy of each run, it is important to measure the percent recovery of orthophosphate. Run 5% of the samples as spikes. Calculate the percent recovery for each spiked sample.

2. Replicates should be run on 55 of the samples to assess precision of the analytical run. Calculate the relative percent difference (RPD) for each replicate.

3. Calculate the standard curve statistics: r^2, Y intercept, and slope.

4. Evaluate and check your quality control by answering the questions in the Q.C. Flow Chart (appendix A)

References:

Murphy, J. and Riley, J.P. 1962. A modified single-solution method for the determination of phosphate in natural waters. Analytica Chemica Acta 27:31-36

Revision Dates: Aug 23, 2007 Revision: UC Davis SOP

Phosphorus Standard Reference Solutions: SRP only

#1: KH_2PO_4

oven dry at 110 degree C
For a 100mg/L-P (100ppm) solution, weigh out 0.21954g and put in a
one liter volumetric flask. Fill to the mark with DDW.

(100,000ppb-P)(5ml) = (1000ppb-P intermediate)(500ml volumetric flask)
(1000ppb-P intermediate)(0.9ml) = (30ppb in test tube)(30ml test tube volume)

#2: ERA 505 lot # P147-505

For a 1030µg/L-P (1030ppb) solution, pipette 5 ml of the stock solution into a
one liter volumetric flask. Fill to the mark with DDW.

(1740ppb-P intermediate)(0.5ml) = (8.7ppb in test tube)(100ml volumetric flask)
Add 30 ml of the 5.15ppb solution into your test tube without further dilution

Note, the ERA 505 concentration changes EACH time you purchase a new one.
This concentration is just an example…

Phosphorus Spike: SRP only

(4000ppb)(0.15ml) = (20ppb in test tube)(30ml volume of test tube)

Phosphorus Standards:
TP, TDP, SRP

Sodium Dihydrogen Phosphate (NaH_2PO_4)
oven dry at 110 degree C for ONE HOUR
For a 50mg/L-P (50ppm) solution, weigh out 0.1935g and put in a one liter volumetric flask. Fill to the mark with DDW.

ppb (ug/L)	source (ppb)	volume (ml) to add to flask	volumetric flask size (in ml)
0	DDW from the day you make your standards		
5	10000	0.5	1000***
10	10000	0.5	500
20	10000	1	500
50	10000	2.5	500
100	10000	5	500
200	10000	10	500
400	50000	4	500
4000*	50000	40	500

10000**	50000	50	250

*4000ppb is the spike solution
**10000 ppb is the intermediate to make the other standards
*** larger volumetric flask for MDLs

test tube rack #2									

test tube rack #1									
today's water 0 ppb									
today's water 0 ppb									
0 ppb	5 ppb	10 ppb	20 ppb	50 ppb	100 ppb	200 ppb	blank spike: **20ppb** **29.85ml DI** 0.15ml 4000ppb-P	KH_2PO_4 **30ppb** 0.9ml 1000ppb, 29.1ml DI	ERA 505: 30ml of 5.15ppb
0 ppb	5 ppb	10 ppb	20 ppb	50 ppb	100 ppb	200 ppb	blank spike: **20ppb** **29.85ml DI** 0.15ml 4000ppb-P	KH_2PO_4 **30ppb** 0.9ml 1000ppb, 29.1ml DI	ERA 505: 30ml of 5.15ppb

PHOSPHORUS ANALYSIS (Persulfate Digestion)

Total or Dissolved
Low level, Colorimetric

Introduction:

All forms of phosphorus, including organic phosphorus, are converted to orthophosphate. The sample color intensity is proportional to the orthophosphate concentrations and is measured spectrophotometrically. The values are reported as μg P/L. The acid-persulfate digestion is more rigorous and complete than the sulfuric acid digestion, thus it is the best estimation of "total" phosphorus.

Interferences include arsenates (> 100 μg As/L) which react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Hexavalent chromium and nitrite have a negative interference.

The method is applicable for samples ranging from 0 – 500 μg/L. The method detection limit (MDL) is 2.0 μg/L. The precision of the 20 ml method (99% confidence intervals) is ± 0.5 μg/L.

Pre-treatment of Water Samples:

Analysis is run on either raw samples for total values or filtered samples for dissolved "total" phosphorus. If samples are to be filtered, the filtration should occur on the *day of collection*. Keep all samples in tightly capped bottles, stored at 4°C. Assay should be performed within one month of collection. The "total" phosphorus analysis is the fourth priority of all the nutrient assays.

Reagents:

1. Sulfuric Acid (0.45 M)

Sulfuric Acid (H_2SO_4 36N)	25.2 ml
Distilled Deionized Water (DDW)	dilute to 1000 ml

Add acid slowly to approximately 800 ml of DDW, with constant stirring. When the solution is cool, bring the volume to 1000 ml with DDW.

2. Potassium Persulfate Solution (Make this solution fresh daily)

Potassium Persulfate ($K_2S_2O_8$)	2.0 g
DDW	dilute to 500 ml

3. Sulfuric Acid-Persulfate Reagent – (1:1)

   Mix equal volumes of H_2SO_4 (0.45 M) and Potassium Persulfate Solution.

4. Sodium Hydroxide (10.8 N)

Sodium Hydroxide (NaOH pellets)	432.0 g
DDW	dilute to 1000 ml

   Add NaOH to about 500 ml DDW in a 1000 ml volumetric flask. Mix to dissolve NaOH and allow to cool. Bring volume up to 1000 ml. Mix and store in polyethylene bottle.

5. Molybdenum- Antimony Solution – Store in glass bottle

Sulfuric Acid (H_2SO_4) 36N	**244 ml**
Ammonium Molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$	21.0 g
Antimony Potassium Tartrate $(C_8H_4K2O_{12}Sb_2.3H_2O)$	0.60 g
DDW	bring to 2000 ml

   Cautiously add concentrated H_2SO_4 to about 1500 ml DDW in a volumetric flask. Mix, and allow solution to cool. Add ammonium molybdate and antimony potassium tartrate and mix **to dissolve**. Bring volume up to 2000 ml with DDW and store in glass.

6. Ascorbic Acid – Store in refrigerator, stable one month

Ascorbic Acid ($C_6H_8O_6$)	3.0 g
DDW	100 ml

   An alternative is to dissolve ascorbic acid in Ethanol (95%). This aqueous solution must be prepared fresh daily.

7. Standard Phosphate Solution (50 mg P/L)
   Store in glass and refrigerate

Sodium Dihydrogen Phosphate (NaH_2PO_4)	0.1935
(Oven dried, 110°C)	
DDW	bring to 1000 ml

8. Working Standards – Store in glass and refrigerate

Working standards are prepared from the 50 mg/L (50,000ppb) stock and stored in 1L glass volumetric flasks. Typically 6 concentrations spanning the expected range of sample values are run in duplicate. The standard range is 0.0, 5.0, 10.0, 20.0, 50.0, 100.0 and 200.0 µg/L (ppb).

9. Spike Addition Stock (50 mg P/L)

Sodium Dihydrogen Phosphate (NaH_2PO_4)	0.1935g
(Oven dried, 110°C)	
DDW	bring to 1000 ml

Intermediate spike solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. The routine spiking level for samples in the range of 0-100 ug/l is 50.0 ug/L. Samples at concentrations >100 ug/L should be spiked at a level 0.5-10 times the sample concentration

10. Standard Reference Material (50 mg P/L)

2-(aminoethyl) phosphonic acid ($C_2H_8NO_3P$)	0.1009g
(Oven dried, 110°C)	
DDW	bring to 500 ml

Intermediate reference solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. The final working concentration is 30 µg/L.

Procedure:

For expected phosphorus concentrations up to 100 µg/l, use a 20 ml sample and read in a 10 cm pathlength cell. For samples with higher concentrations, read in a 4 or 1 cm cell.

1. Measure 20.0 ml of sample into clean test tube (25 × 175 mm, screwcap). The test tube, as well as all other glassware, must be immaculate since even a small amount of contaminate will affect the results.

2. Add 8.0 ml of 1:1 acid-persulfate reagent to sample. Vortex.

3. Cap tightly and autoclave at 240°F for 45 minutes.

4. After cooling add 0.375 ml (375 µl) of 10.8 N NaOH solution. Vortex each tube for several seconds.

5. Add 2.0 ml of the molybdenum-antimony reagent. Vortex.

6. Add 0.2 ml (200 µl) ascorbic acid solution. Vortex.

7. Allow samples to react for 20 – 30 minutes for the blue color to develop.

8. Read absorbance at 675 nm in chosen pathlength cell (10cm cell recommended). Samples and standards can also be read at 880 nm.

Notes:

1. Due to interference by particulates, stream samples are filtered into the spectrophotometer cuvette **after** color development.

2. If the concentration of phosphorus is higher than the highest standard, the sample should be diluted and re-analyzed.

3. Wear a lab coat. The molybdenum-antimony solution is notorious for destroying clothes.

4. Be sure to purchase high purity potassium persulfate. The reagent can be cleaned by re-crystallization, if necessary. To re-crystallize, add approximately 300 g $K_2S_2O_8$ to 1500 ml of DDW. Heat slowly to 50°C (do not go over 5O°C or decomposition can occur) until the reagent is completely dissolved. Cool to 0°C and collect the reformed crystals on a pre-combusted glass fiber filter. Repeat the recrystallization steps, then dry a second set of crystals in a vacuum desiccators. Store the cleaned reagent in a labeled, sealed vial.

Calculations:

To calculate the phosphorus concentration, generate a standard curve by plotting the absorbance versus the concentration of the standards. Absorbance values of samples are then entered to give phosphorus concentration in µg P/L.

QA/QC Procedures:

1. To determine the success and accuracy of each run, it is important to measure the percent recovery of phosphorus. Run 5% of the samples as spikes. Calculate the percent recovery for each spiked sample.

2. Replicates should be run on 5% of the samples to assess precision of the analytical run.. Calculate the relative percent difference (RPD) for each replicate.

3. Calculate the standard curve statistics, r^2, Y intercept, and slope.

4. Evaluate and check your quality control by answering the questions in the Q.C. Flow chart (Appendix A).

References:

Goldman, C.R. 1974. Eutrophication of Lake Tahoe emphasizing water quality. EPA-600/3-74-034. U.S. Govt. Printing Office, Washington D.C. 408 pp.

Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments. M.J. Fishman and L.C. Friedman (eds.) 1985. Open-File Report 85-495. Denver Colorado.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. Bulletin 167. Fisheries Research Board of Canada, Ottawa, Ontario, Canada.

Revision Date: Aug 23, 2007 Revision: UC Davis SOP

PHOSPHORUS ANALYSIS (Sulfuric Acid Digestion)

Hydrolyzable Phosphate
Total or Dissolved
Low level, Colorimetric

Introduction:

Acid hydrolysis releases soluble orthophosphate from insoluble inorganic phosphates, organic phosphorus compounds, and pyrophosphates. After hydrolysis the samples are pH adjusted and analyzed for orthophosphate. The sample color intensity is proportional to the orthophosphate concentration and is measured spectrophotometrically. The values are reported as μg P/L

Interferences include arsenates (> 100μg As/L) which react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Hexavalent chromium and nitrite have a negative interference.

The method is applicable for samples ranging from 0-500 μg/l. the method detection limit (MDL) is 2.0 μg/L. The precision of the 20 ml method (99% confidence intervals) is ± <1 μg/L.

Pretreatment of Water Samples:

Analysis is run on raw samples for total values or filtered samples for dissolved hydrolysable phosphorus. If samples are to be filtered, the filtration should occur on the day of collection. Keep all samples in tightly capped bottles, stored at 4°C. Assay should be performed within one month of collection. The "total" phosphorus analysis is the fourth priority of all the nutrient assays.

Reagents:

1. Molybdenum-Antimony Solution – Store in glass bottle

Sulfuric Acid (H_2SO_4 36N)	244 ml
Ammonium Molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	21 g
Antimony Potassium Tartrate $(C_8H_4K_2O_2Sb_2.3H_2O)$	0.6 g
Distilled Deionized Water (DDW)	Bring to 2000 ml

Cautiously add concentrated H_2SO_4 to about 1500 ml DDW in a volumetric flask. Mix, and allow solution to cool. Add ammonium moylbdate and antimony

potassium tartrate and mix to dissolve. Bring volume up to 2000 ml with DDW and store in glass.

2. Ascorbic Acid – Store in refrigerator, stable one month

Ascorbic Acid ($C_6H_8O_6$)	3.0 g
DDW	100 ml

An alternative is to dissolve ascorbic acid in 95% Ethanol. This aqueous solution must be prepared fresh daily.

3. Sulfuric Acid (10.8 N)

Sulfuric Acid (H_2SO_4 36N)	300 ml
DDW	bring to 1000 ml

4. Sodium Hydroxide (10.8 N)

Sodium Hydroxide (NaOH, pellets)	432 g
DDW	bring to 1000 ml

Add NaOH to about 500 ml DDW in a 1000 ml volumetric flask. Mix to dissolve NaOH and allow to cool. Bring volume up to 1000 ml. Mix and store in polyethylene bottle.

5. Standard Phosphate Solution (50 mg/L elemental P)
Store in glass and refrigerate

Sodium Dihydrogen Phosphate (NaH_2PO_4) (Oven dried, 110°C)	0.1935 g
DDW	bring to 1000 ml

6. Working Standards – Store in glass and refrigerate

Working standards are prepared form the 50 mg/L stock. Typically 6 concentrations spanning the expected range of sample values plus a blank are run in duplicate. The standard range is from 0 – 200 µg/L.

7. Spike Addition Stock (50 mg P/L) – Refrigerate

Potassium Phosphate Monobasic KH_2PO_4 (Oven Dried, 110°C)	0.2149
DDW	bring to 1000 ml

Intermediate spike solutions are made form this stock. The intermediate solutions should be prepared fresh for each assay.

Acid Hydrolysis Procedures:

1. Measure 20.0 ml of sample into a clean test tube (25 × 175 mm, screwcap). The test tube, as well as all other glassware, must be immaculate since even a small amount of contaminate will affect the results.

2. Add 0.1 ml (100 µl) H_2SO_4 (10.8 N) Vortex.

3. Autoclave at 240°F for 15 minutes.

4. Allow samples to cool to room temperature.

5. Neutralize with 0.1 ml (100 µl) NaOH (10.8 N). Vortex.

Colorimetric Procedures:

6. Add 2.0 ml of the molybdenum-antimony reagent. Vortex

7. Add 0.2 ml (200 µl) ascorbic acid solution. Vortex.

8. Allow samples to react for 20 – 30 minutes for the blue color to develop.

9. Read absorbance at 675 nm in chosen pathlength cell*. Samples and standards can also be read at 880 nm.

   *For expected phosphorus concentrations up to 100 µg/L, use a 20 ml sample and read in a 10 cm pathlength cell. Samples with higher concentrations can be read in a 4 or 1 cm cell.

Notes:

1. Due to interference by particulates, stream samples are filtered into the spectrophotometer cuvette **after** color development.
2. If the concentration of phosphorus is higher than the highest standard, the sample should be diluted and re-analyzed.

3. Wear a lab coat. The molybdenum-antimony solution is notorious for destroying clothes.

Calculations:

To calculate the phosphorus concentration, generate a standard curve by plotting the absorbance versus the concentration of the standards. Absorbance values of samples

are then entered to give phosphorus concentration in μg P/L. If samples have been diluted, multiply the concentration by the appropriate dilution factor to calculate final concentration values.

QA/QC Procedures:

1. To determine the success and accuracy of each run, it is important to measure the percent recovery of phosphorus. Run 5% of the samples as spikes. Use the KH_2PO_4 solution for additions of phosphorus. Typical spike concentrations are 10 μg/L. Calculate the percent recovery for each spiked sample.

2. Replicates should be run on 5% of the samples to assess precision of the analytical run. Calculate the relative percent difference (RPD) for each replicate.

3. Calculate the standard curve statistics: r^2, Y intercept, and slope.

4. Evaluate and check your quality control by answering the questions in the Q.C. Flow Chart (Appendix A).

References:

Goldman, C.R. 1974. Eutrophication of Lake Tahoe emphasizing water quality. EPA-600/3-74-034. U.S. Govt. Printing Office, Washington, D.C. 408 pp/

Murphy, J. and Riley, J.P. 1962. A modified single-solution method for the determination of phosphate in natural waters. Analytica Chemica Acta 27:31-36.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. Bulletin 167. Fisheries Research Board of Canada, Ottawa, Ontario, Canada

TOTAL PARTICULATE PHOSPHORUS

Introduction:

Particulate matter is filtered from aqueous samples onto a Binder-Free Glass Microfiber filter (Whatman brand GF/C) to concentrate phosphorus. The filter is then heated in a muffle furnace, after which the organic residue is re-hydrated and total phosphorus is measured by the reactive phosphorus method. The method is applicable in any system since the volume of water filtered can be adjusted to collect the desired amount of particulate matter. The method detection limit is 0.02 μg P with a precision (99% confidence intervals) of ± 5%.

Pre-treatment of Water Samples:

Analysis is run on raw samples that are filtered onto pre-combusted (500°C) Whatman™ GF/C or GF/F filters. The samples should be filtered within 4 hours of collection. The filters can be frozen until analysis.

Reagents:

1. Sodium Sulfate (0.17 M) – Store at room temperature

Sodium Sulfate (Na_2SO_4)	6 g
Distilled Deionized Water (DDW)	bring to 250 ml

2. Magnesium Sulfate (0.017 M) – Store at room temperature

Magnesium Sulfate ($MgSO_4 7H_2O$)	4.2 g
DDW	bring to 1000 ml

   The magnesium sulfate solution is prone to phosphorus contamination, use the highest purity reagent grade chemical.

3. Hydrochloric Acid (0.20 M) – Store at room temperature

Hydrochloric Acid (HCL, 12 N)	16.7 ml
DDW	bring to 1000 ml

4. Molybdenum- Antimony Solution – Store in glass bottle

Sulfuric Acid (H_2SO_4 36N)	**244 ml**
Ammonium Molybdate (NH_4)Mo_7O_{24} $4H_2O$	21 g
Antimony Potassium Tartrate ($C_8H_4K_2O_{12}Sb_2.3H_2O$)	0.6 g

DDW	bring to 2000 ml

Cautiously add concentrated H_2SO_4 to about 1500 ml DDW in a volumetric flask. Mix, and allow solution to cool. Add ammonium molybdate and antimony potassium tartrate and mix **to dissolve**. Bring volume up to 2000 ml with DDW and store in glass.

5. Ascorbic Acid – Store in refrigerator, stable one month

Ascorbic Acid ($C_6H_8O_6$)	3.0 g
Ethanol (95%)	100 ml

An alternative is to dissolve ascorbic acid in DDW. This aqueous solution must be prepared fresh daily.

6. Standard Phosphate Solution (50 mg P/L)
Store in glass and refrigerate

Sodium Dihydrogen Phosphate (NaH_2PO_4)	0.1935g
(Oven dried, 110°C)	
DDW	bring to 1000 ml

7. Working Standards – Store in glass and refrigerate

Working standards are prepared from the 50 mg/L stock. Typically 5-6 concentrations spanning the expected range of sample values plus a bank are run in duplicate. Usually our standard concentrations range from 0 – 200 μg/L.

8. Spike Addition Stock (50 mg P/L)

Sodium Dihydrogen Phosphate (NaH_2PO_4)	0.1935g
(Oven dried, 110°C)	
DDW	bring to 1000 ml

Intermediate spike solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. Spike at a level appropriate to the sample concentration.

9. Standard Reference Material (50 mg P/L)

2-aminoethyl phosphonic acid	0.1009 g
DDW	bring to 500 ml

Intermediate SRM solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. Use a concentration appropriate for the range of the samples. The Lake Tahoe assays are spiked at 20-30 µg/L.

Procedure:

1. Filter the water sample through a pre-combusted glass fiber filter (2.4 cm, < 15 psi). Just before the last water passes through the filter, add two rinses of 2.0 ml Na_2SO_4 (0.17 M). (For solid samples, weigh out approximately 2-3 mg of sample into a 20 ml acid-cleaned borosilicate scintillation vial).

2. Record the volume of sample filtered.

3. The water filter can be folded in half, wrapped in foil, labeled, and frozen until the analysis.

4. Place the folded filter in a 20 ml acid-cleaned borosilicate scintillation vial.

5. For the standards, pipette 10 ml of each standard concentration (including a blank) into 20 ml acid-cleaned borosilicate scintillation vials.

6. Add 2.0 ml of $MgSO_4$ (0.017 M) to each vial and vortex or swirl to mix.

7. Dry the standards and filters in the scintillation vials (without caps) in a drying oven at 95°C, overnight. The samples can then be tightly capped and stored for later analysis.

8. Remove the caps from the vials and cover the vial opening with aluminum foil.

9. Bake the scintillation vials in a muffle furnace (500°C) for two hours.

10. After cooling, add 5.0 ml of HCl (0.20 M) and cap the vials. Vortex.

11. Heat the vials in the drying oven (80°C) for 30 minutes.

12. Cool. Vortex each sample. Filter the samples and standards through pre-combusted (500°C) Whatman™ GF/C or GF/F filters. Rinse the vial with three DDW rinses (approximately 2 ml aliquots of DDW for each rinse). Bring the final volume to 10 ml with DDW. Vortex to mix.

13. Add 1.0 ml of the molybdenum-antimony reagent. Vortex the tubes to mix.

14. Add 0.1 ml (100 µl) of ascorbic acid solution. Vortex the tubes to mix.

15. Read the absorbance at 675 nm in a 4 cm cell. Samples and standards can also be read at 880 nm.

16. An alternative to filtering (step 11) is to pour the supernatant from the vial into a graduated centrifuge tube. Rinse the vial with 5.0 ml DDW and add this to the respective centrifuge tubes. Bring the volume to 10.0 ml with DDW. Centrifuge the tubes at 2000 rpm for 5 minutes.

Notes:

1. The scintillation vials (Step 4) have the sample number scratched into the glass.

2. It is important to extract every drop of supernatant from the scintillation vials (steps 12 and 16).

3. Treatment blanks need to be run as samples for all runs.

4. Duplicate samples need to be collected for QA spikes and replicates.

5. If the concentration of phosphorus is higher than the highest standard, the sample value might be valid by extrapolation of the standard curve. Otherwise, the sample is lost since replicates rarely exist that can be re-analyzed. As a rule during filtration, when the filter starts to show some coloration, that means enough water has been filtered to obtain a value for particulate phosphorus.

Calculations:

To calculate the phosphorus concentration, generate a standard curve by plotting the absorbance versus the concentration of the standards. Absorbance values of samples are then entered to give phosphorus concentration in μg P/L. Particulate phosphorus concentration is calculated as:

$$PP_{\mu g/L} = ([P_{\mu g/L}] - [TreatmentBlank_{\mu g/L}]) \times \frac{\text{final sample volume (ml)}}{\text{Initial sample volume filtered (ml)}}$$

QA/QC Procedures:

1. To obtain a good average estimate of the blank value, for every run of particulate phosphorus 3 blank filters should be assayed as samples.

2. To determine the success and accuracy of each run, it is important to measure the percent recovery of phosphorus. Run 5% of the samples as spikes. Use the standard solution for additions of phosphorus. Typical spike concentrations are 50 μg/L. Calculate the percent recovery for each spiked sample.

3. Replicates should be run on 5% of the samples to assess precision of the analytical run. Calculate the relative percent difference (RPD) for each replicate.

4. Calculate the standard curve statistics: r, y intercept, and slope.

5. Evaluate and check your quality control by answering the questions in the Q.C. Flow Chart (Appendix A).

References:

Solorzano, L. and J.H. Sharp. 1980. Determination of total phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. 25:756-760

Revision Dates: Aug 27, 2007 Revision: UC Davis SOP

AMMONIUM ANALYSIS (Indophenol Method)

Low level, colorimetric

Introduction:

Most of the ammonia (NH_3) in fresh water exists in the ionic form (NH_4^+), except under very alkaline conditions (pH>9.0). Ammonium is an important source of nitrogen for bacteria, algae, and larger plants in lakes and streams. Concentrations are commonly low and the content of water samples can change quickly and markedly.

This method is a modifications of the method reported by Liddicoat et al. (1975) and Solorzano (1969). A blue indophenol reaction between ammonium, phenol, and hypocholorite takes place using potassium nitroferricyanide as a catalyst. The total ammonium present is then measured spectrophotometrically. The final concentration is reported as μg NH_4-N/L. Calcium and magnesium interference is eliminated by complexing with sodium citrate. Other advantages of this method are the eliminations of distillation or solvent extraction steps, improved sensitivity over nesslerization method, speed, stability of blue coloration, and absence of reaction with other forms of nitrogen. Interferences include matrices with high concentrations of Ca^+ and Mg^+, turbidity, and color in the samples that absorb in the photometric range being used. Mercuric chloride ($HgCl_2$) used as a preservative gives a negative interference by complexing with ammonia.

The method is applicable in the range from 0-500μg/L NH_4-N although it yields best results on samples containing less than 50μg/L. The method detection limit (MDL) is ±3.0 μg/L. The precision of the 10 ml method (95% confidence intervals is ±2 μg/L.

Pre-treatment of Water Samples:

Analysis is run on filtered samples. Samples should be filtered on the day of collection. Keep filtered samples in tightly capped bottles, stored at 4°C. Assay should be performed within one week of sample collection. The ammonium analysis is the first priority of all the nutrient assays.

Reagents:

1. Phenol-Ethanol Solution – Refrigerate

Phenol (C_6H_5OH)	10.0 g
Ethanol (95%)	bring to 100 ml

   Prepare this reagent in the fume hood and wear gloves. Phenol is toxic. Avoid inhalation and skin exposure.

2. Alkaline Citrate Solution – Store at room temperature

Sodium Citrate ($Na_3C_6H_5O_7.2H_2O$)	100.0 g
Sodium Hydroxide (NaOH, pellets)	5.0 g
Distilled Deionized Water (DDW)	bring to 500 ml

3. Oxidizing Solution – Prepare fresh daily

Alkaline Citrate Solution	80 ml
Sodium Hypochlorite (NaOCl) "Bleach"	20 ml

Use fresh sodium hypochlorite (shelf life < 1 month, refrigerated). Caution this is a strong oxidizer. Use a bleach with few additives. The cencentration should be ~ 5% bleach. Fisher brand is between 4-6%.

4. Catalyst – Prepare fresh daily

Potassium Ferrocyanide $K_4Fe(CN)_6.3H_2O$	0.50 g
DDW	bring to 100 ml

5. Standard Ammonium Solution (50 mg N/L) – Refrigerate

Ammonium Nitrate (NH_4NO_3) (Oven dried, 110°C)	0.2858 g
DDW	bring to 1000 ml

6. Working Standards – Prepare fresh daily

Working standards are prepared from the 50 mg/L stock. Typically 6 concentrations spanning the expected range of sample values plus a blank are run in duplicate. The standard are 0.0, 5.0, 10.0, 20.0, 50.0 and 100 µg/L. A 200 µg/l standard is used for higher range samples.

7. Spike Addition Stock (1000 mg N/L) – Refrigerate

Ammonium Nitrate (NH_4NO_3) (Oven dried, 110°C)	0.2858 g
DDW	bring to 1000 ml

Intermediate spike solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. The routine spiking level for samples in the range of 0-20 ug/l is 10 µg/L. Samples at concentrations >20 µg/L should be spiked at a level 0.5-10 times the sample concentration.

8. Standard Reference Material (1000 mg N/L) – Refrigerate

Ammonium chloride (NH_4CL)	3.821 g
(Oven dried, 110°C)	
DDW	bring 1000 ml

The intermediate solutions should be prepared fresh for each assay. The final working concentration is 30 µg/L.

Procedure:

1. Measure 10.0 ml of sample into clean test tube (2.0 cm × 12.5 cm, screwcap). The test tube as well as all other glassware, must be immaculate since even a small amount of contaminant will affect the results

2. Add 0.4 ml (400 µl) of the phenol-ethanol solution. Vortex samples thoroughly at high speed.

3. Add 0.4 ml (400 µl) of potassium ferrocyanide catalyst. Vortex samples thoroughly at high speed.

4. Add 1.0 ml of the oxidizing solution. Vortex samples thoroughly at high speed.

5. Place tubes under an ultraviolet light source for 45 minutes.

6. Read absorbance at 640 nm in a 4 cm cell.

Notes:

1. If analyzing samples with a concentration of 200 µg/L or higher, us a 1 cm cell.

2. If the concentration of ammonium is higher than the highest standard the sample should be diluted and re-analyzed.

3. This assay generates a regulated mixed waste solution. Pour all waste contents into the appropriately labeled waste jug.

Calculations:

To calculate the ammonium concentration, generate a standard curve by plotting the absorbance versus the concentration of the standards. Absorbance values of samples are then entered to give ammonium concentration in μg NH_4/L. If samples have been diluted, multiply the concentration by the appropriate dilution factor to calculate final concentration values.

QA/QC Procedures:

1. To determine the success and accuracy of each run, it is important to measure the percent recovery of ammonium. Run 5% of the samples as spikes. Calculate the percent recovery for each spiked sample.

2. Replicates should be run on 5% of the samples to assess precision of the analytical run. Calculate the relative percent difference (RPD) for each replicate.

3. Calculate the standard curve statistics: r^2, Y intercept, and slope.

4. Evaluate and check your quality control by answering the questions in the Q.C. Flow Chart (Appendix A)

References:

Liddicoat, M.I., S. Tibbits, and E.I. Butler. 1975. The determination of ammonia in seawater. Limnol. Oceanogr. 20:131-132.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypocholorite method. Limnol. Oceanogr. 14:799-801.

Brzezinsik, M.A. 1987. Colorimetric determination of nanomolar concentrations of ammonium in sea water using solvent extraction. Marine Chemistry 20:277-288.

Revision Dates: Aug 24, 2007 Revision: UC Davis SOP

NITRATE ANALYSIS (Hydrazine Method)

Low level, colorimetric

Introduction:

This method utilizes a hydrazine-copper reducing solution that reduces nitrate to nitrite followed by color development using a diazotization-coupling reaction. The method assumes a 1:1 stoichiometric reduction of nitrate to nitrite. The total nitrite present is then measure spectrophotometrically. The resulting concentration will be reported as μg NO_3+NO_2-N/L. To obtain concentration values for **only** nitrate, the nitrite concentration must be analyzed separately from an aliquot of the same sample by omitting the reduction steps. The nitrite concentration is then subtracted from the combined concentration value of [NO_3] + [NO_2].

Interferences include matrices with high concentrations of Ca^+ and Mg^+, turbidity, and sample color that absorbs in the photometric range being used. Concentrations of sulfide ion of < 10 mg/L will cause variations of NO_3^- and NO_2^- concentrations of ± 10%.

This method is applicable in the range from 0-500 μg/L. The method detection limit (MDL) is 2.0 μg/L. The precision of the 10 ml method (99% confidence level) is ± 0.3 μg/L.

Pre-treatment of Water Samples:

Analysis is run on filtered samples. Samples should be filtered with Whatman™ GF/C filters on the day of collection. Keep samples in tightly capped bottles, stored at 4°C. Assay should be performed within one week of sample collection. The nitrate analysis is the second priority of all the nutrient assays.

Reagents:

1. Stock Hydrazine – Prepare fresh daily

Hydrazine Sulfate ($NH_2NH_2.H_2SO_4$)	0.48 g
Distilled Deionized Water (DDW)	bring to 100 ml

Hydrazine is a regulated health hazard, please wear gloves to avoid skin exposure.

2. Copper Solution

Cupric Sulfate ($CuSO_4.5H_2O$)	0.040 g
DDW	bring to 100 ml

3. Reducing Solution – Prepare fresh daily

Stock Hydrazine	50 ml
Copper Solution	10 ml

DDW	bring to 100 ml

4. Sodium Hydroxide (1N)

Sodium Hydroxide (NaOH), pellets	20 g
DDW	bring to 500 ml

5. Sulfanilamide

Sulfanilamide (4-$NH_2C_6H_4SO_2NH_2$)	5.0 g
HCL (12N)	50 ml
DDW	bring to 500 ml

6. N-1-Naphthylethylenediamine Dihydrochloride (NED) – Refrigerate in dark bottle

NED	0.50 g
DDW	bring to 500 ml

This reagent should be remade monthly.

7. Phenate Buffer – Prepare fresh daily

Phenol (C_6H_5OH)	1.8 g
Sodium Hydroxide (NaOH, 1N)	15 ml
DDW	bring to 100 ml

8. Standard Nitrate Solution (50 mg N/L) – Refrigerate

Ammonium Nitrate (NH_4NO_3) (Oven dried, 110°C)	0.2858 g
DDW	bring 1000 ml

9. Working Standards – Prepare fresh daily

Working standards are prepared from the 50 mg/L stock. Typically 6 concentrations spanning the expected range of sample values plus a blank are run in duplicate. The standard are 0.0, 5.0, 10.0, 20.0, 50.0 and 100 µg/L. A 200 µg/L standard is used for higher range samples.

10. Spike Addition Stock (50 mg N/L) – Refrigerate

Ammonium Nitrate (NH_4NO_3) (Oven dried, 110°C)	0.2858 g
DDW	bring 1000 ml

The routine spiking level for samples in the range of 0-100 ug/l is 50 ug/L. Samples at concentrations >100 ug/L should be spiked at a level 0.5-10 times the sample concentration.

11. Standard Reference Material (50 mg N/L) – Refrigerate

Potassium Nitrate (KNO_3)	0.3607 g
(Oven dried, 110°°C)	
DDW	Bring to 1000 ml

Intermediate spike solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. The intermediate solutions should be prepared fresh for each assay. The final working concentration is 30 ug/L.

12. USGS Reference Sample – Store in refrigerator.

Precipitation Reference Sample

Most Probable Value (MPV) determined by comparison of results between three different labs that normally run low nitrate samples.

Procedure:

1. Measure 10.0 ml of samples, standards, SRMs into clean test tubes (2.0 cm.× 12.5 cm, screwcap). The test tube, as well as all other glassware, must be immaculately cleaned since even a small amount of contaminate will affect the results.

2. Place test tubes in rack in a 37°C water bath and allow them to equilibrate for at least 45 minutes. Cover the test tubes with Parafilm ™ strips.

3. While the test tubes are in the water bath add 0.4 ml (400 µl) phenate buffer immediately followed by 0.22 ml (200 µl) reducing solution to one row. Vortex samples in that row thoroughly at high speed. Cover the row that you just vortexed with Parafilm ™ so you don't accidently drip water from the water bath into your test tubes. Repeat row by row until you finish with one rack. Note the time. Continue on with any following racks.

4. Allow samples to incubate for **exactly** 30 minutes, taking into account the order and time of reagent addition.

5. Remove samples from water bath and let cool to room temperature (40 minutes) then add 0.4 ml (400 µl) of a 1:1 mixture of sulfanilamide and NED to each test tube. Vortex samples thoroughly at high speed.

6. Allow color to develop for at least 30 minutes but not longer than 2 hours.

7. Read absorbance at 543 nm in a 4 cm cell.

Notes:

1. If analyzing samples with a concentration of 200 μg/L or higher, use a 1 cm cell.

2. If the concentration of nitrate is higher than the highest standard, the sample should be diluted and re-analyzed.

3. This assay generates a regulated mixed waste solution. Please pour all waste contents into the appropriate waste jug.

Calculations:

To calculate the nitrate concentration, generate a standard curve by plotting the absorbance versus the concentration of the standards. Absorbance values of samples are then entered to give nitrate concentration in μg NO_3 + NO_2/L. If samples have been diluted, multiply the concentration by the appropriate dilution factor to calculate final concentration values.

QA/QC Procedures:

1. To determine the success and accuracy of each run, it is important to measure the percent recovery of nitrate. Run 5% of the samples as spikes. Calculate the percent recovery for each spiked sample.

2. Replicates should be run on 5% of the samples to assess precision of the analytical run. Calculate the relative percent difference (RPD) for each replicate.

3. Calculate the standard curve statistics: r^2, Y intercept, and slope.

4. Evaluate and check your quality control by answering the questions in the Q.C. flow chart (Appendix A).

References:

Kamphake, L.J., S.A. Hannah, and J.M. Cohen. 1967. Automated analysis for nitrate by hydrazine reduction. Water Research. 1:205-216.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. Bulletin 167. Fisheries Research Board of Canada, Ottawa, Ontario, Canada.

Revision Dates: Aug 24, 2007 Revision: UC Davis SOP

NITRATE ANALYSIS
(Hydrazine/Sodium Pyrophosphate Method)
Low level, colorimetric

Introduction:

This method utilizes a hydrazine-copper reducing solution which reduces nitrate to nitrite followed by color development using a diazotization-coupling reaction. The method assumes a 1:1 stoichiometric reduction of nitrate to nitrite. The total nitrite present is then measure spectrophotometrically. The resulting concentration will be reported as μg NO_3+NO_2-N/L. To obtain concentration values for **only** nitrate, the nitrite concentration must be analyzed separately from an aliquot of the same sample by omitting the reduction steps. The nitrite concentration is then subtracted from the combined concentration value of [NO_3] + [NO_2].

Interferences include matrices with high concentrations of Ca^+ and Mg^+, turbidity, and sample color that absorbs in the photometric range being used. Concentrations of sulfide ion of < 10 mg/L will cause variations of NO_3^- and NO_2^- concentrations of ± 10%.

The addition of the sodium pyrophosphate aids in reducing calcium and magnesium interferences.

This method is applicable in the range from 0-500 μg/L. The method detection limit (MDL) is 2 μg/L. The precision of the 10 ml method (99% confidence level) is ± 0.3 μg/L.

Pre-treatment of Water Samples:

Analysis is run on filtered samples. Samples should be filtered with Whatman™ GF/C filters on the day of collection. Keep samples in tightly capped bottles, stored at 4°C. Assay should be performed within one week of sample collection. The nitrate analysis is the second priority of all the nutrient assays.

Reagents:

10. Stock Hydrazine – Refrigerate in glass

Hydrazine Sulfate ($NH_2NH_2.H_2SO_4$)	1.20 g
Distilled Deionized Water (DDW)	bring to 250 ml

Hydrazine is a regulated health hazard, please wear gloves to avoid skin exposure.

11. Copper Solution

Cupric Sulfate ($CuSO_4.5H_2O$)	0.040 g

DDW	bring to 100 ml

12. Reducing Solution – Prepare fresh daily

Stock Hydrazine	25 ml
Copper Solution	10 ml
DDW	bring to 50 ml

13. Sodium Hydroxide (1N)

Sodium Hydroxide (NaOH), pellets	40 g
DDW	bring to 1000 ml

14. Sulfanilamide

Sulfanilamide ($4\text{-}NH_2C_6H_4SO_2NH_2$)	5.0 g
HCL (12N)	50 ml
DDW	bring to 500 ml

15. N-1-Naphthylethylenediamine Dihydrochloride (NED) – Refrigerate in dark bottle

NED	0.5 g
DDW	bring to 500 ml

This reagent should be remade monthly.

16. Phenate Buffer – Prepare fresh daily

Phenol (C_6H_5OH)	1.8 g (add first)
Sodium Hydroxide (NaOH, 1N)	15 ml (add this second)
Sodium Pyrophosphate	3.18 g **(dissolve in a beaker and then add to phenol buffer)**
DDW	bring to 100 ml

17. Standard Nitrate Solution (50 mg N/L) – Refrigerate

Ammonium Nitrate (NH_4NO_3) (Oven dried, 110°C)	0.2858 g
DDW	bring 1000 ml

18. Working Standards – Prepare fresh daily

Working standards are prepared from the 50 mg/L stock. Typically 6 concentrations spanning the expected range of sample values plus a blank are run in duplicate. The standard range is from 0 – 200 μg/L.

19. Spike Addition Stock (50 mg N/L) – Refrigerate

Potassium Nitrate (KNO_3)	0.3607 g
(Oven dried, 110°°C)	
DDW	Bring to 1000 ml

Intermediate spike solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay.

20. USGS Reference Sample – Store in refrigerator.

Precipitation Reference Sample
Most Probable Value (MPV) determined by comparison of results between three different labs that normally run low nitrate samples.

Procedure:

1. Measure 10.0 ml of sample into clean test tube (2.0 cm.× 12.5 cm, screwcap). The test tube, as well as all other glassware, must be immaculate since even a small amount of contaminate will affect the results.

2. Place samples in a 37°C water bath and allow to equilibrate for at least 45 minutes. Parafilm ™ strips can be used to cover the test tubes.

3. While the samples are in the water bath add 0.4 ml (400 µl) phenate buffer immediately followed by 0.2 ml (200 µl) reducing solution. Vortex samples thoroughly at high speed.

4. Allow samples to incubate for **exactly** 30 minutes, taking into account the order and time of reagent addition.

5. Remove samples from water bath and let cool to room temperature (40 minutes) then add 0.4 ml (400 µl) of a 1:1 mixture of sulfanilamide and NED to each test tube. Vortex samples thoroughly at high speed.

6. Allow color to develop for at least 30 minutes but not longer than 2 hours.

7. Read absorbance at 543 nm in a 4 cm cell.

Notes:

1. If analyzing samples with a concentration of 200 µg/L or higher, use a 1 cm cell.

2. If the concentration of nitrate is higher than the highest standard, the sample should be diluted and re-analyzed.

3. This assay generates a regulated mixed waste solution. Please pour all waste contents into the appropriate waste jug.

Calculations:

To calculate the nitrate concentration, generate a standard curve by plotting the absorbance versus the concentration of the standards. Absorbance values of samples are then entered to give nitrate concentration in μg NO_3 + NO_2/L. If samples have been diluted, multiply the concentration by the appropriate dilution factor to calculate final concentration values.

QA/QC Procedures:

1. To determine the success and accuracy of each run, it is important to measure the percent recovery of nitrate. Run 5% of the samples as spikes. Use the KNO_3 solution for additions of nitrate. Typical spike concentrations are 50 μg/L. Calculate the percent recovery for each spiked sample.

2. Replicates should be run on 5% of the samples to assess precision of the analytical run. Calculate the relative percent difference (RPD) for each replicate.

3. Calculate the standard curve statistics: r^2, Y intercept, and slope.

4. Evaluate and check your quality control by answering the questions in the Q.C. flow chart (Appendix A).

References:

Kamphake, L.J., S.A. Hannah, and J.M. Cohen. 1967. Automated analysis for nitrate by hydrazine reduction. Water Research. 1:205-216.

Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis. Bulletin 167. Fisheries Research Board of Canada, Ottawa, Ontario, Canada.

KJELDAHL NITROGEN

Total or Dissolved
Low level, colorimetric

Introduction:

Organic nitrogen compounds are reduced to the ammonia by digestion with sulfuric acid in the presence of mercuric sulfate (catalyst) and potassium sulfate (increases digestion temperature). The ammonia produced by this digestion, as well as the ammonia originally present, is determined by reaction with phenol, hypochlorite, and potassium nitroferricyanide (catalyst) in an alkaline medium. The resulting blue indophenol color is directly proportional to the concentration of ammonia present and is measured spectrophotometrically. The concentration is reported as μg Kjeldahl – N/L. Values for organic nitrogen are calculated by subtraction of original nitrate and ammonia concentrations.

Mercuric chloride used as a preservative gives a negative interference by complexing with ammonia. Sulfide ions less than 10 mg/L will also interfere with color development. Turbidity may also cause problems during photometric readings.

This method may be used to analyze water and water-suspended sediment with 0 – 400 g Kjeldahl – N/L. Samples with concentrations greater than 400 μg/L need to be diluted. The method limit of detection is 35.0 μg/L. The precision of the 20 ml method (99% confidence intervals) is ±16 μg/L.

Pre-treatment of Water samples:

Analysis is run on raw samples for total values and filtered samples for dissolved Kjeldahl nitrogen. Samples should be stored in tightly capped bottles at 4°C. Analysis should be run within a month of the collection date. The Kjeldahl nitrogen analysis is the fifth priority of all the nutrient assays.

Reagents:

1. Stock Mercuric Sulfate Solution – Refrigerate

Mercuric Oxide (HgO)	8.0 g
Sulfuric Acid (H_2SO_4, 36N)	10.0 ml
Distilled Deionized Water (DDW)	bring to 100 ml

Mercuric oxide is a regulated health hazard. Avoid inhalation of powder and skin contact. Wear gloves and dispense reagent in the fume hood.

2. Digestion Solution – Store at room temperature

Potassium Sulfate (K_2SO_4)	133 g
Sulfuric Acid (H_2SO_4, 36N)	200 ml
Stock Mercuric Sulfate	25 ml
DDW	bring to 1000 ml

3. Phenol – Ethanol Solution – Refrigerate

Phenol (C_6H_5OH)	10.0 g
Ethanol (95%)	bring to 100 ml

Phenol is toxic. Avoid inhalation and skin contact. Dispense reagent in the fume hood and wear gloves.

4. Alkaline Citrate Solution – Store at room temperature

Sodium Citrate ($Na_3C_6H_5O_7.2H_20$)	100.0 g
Sodium Hydroxide (NaOH, pellets)	5.0 g
DDW	bring to 500 ml

5. Oxidizing Solution – Prepare fresh daily

Alkaline Citrate Solution	40 ml or 120 ml
Sodium Hypochlorite (bleach, NaOCl)	8.75 ml or 26.25 ml
Deionized water	1.25 ml or 3.75 ml

6. Catalyst – Prepare fresh daily

Potassium Ferrocyanide $K_4Fe(CN)_6$ 3 H_2O	0.50 g
DDW	bring to 100 ml

7. Buffer Solution – Prepare fresh daily

Sodium Phosphate Dibasic (Na_2HPO_4)	35.49 g
DDW	bring to 250 ml

The solubility of the sodium phosphate is aided by continuous stirring and low heat.

8. Sodium Hydroxide (10.8 N)

Sodium Hydroxide (NaOH, pellets)	432 g

DDW	bring to 1000 ml

This is a strong exothermic reaction, thus the reagent must be made several hours prior to usage. The volume changes when the solution temperature cools.

9. Sodium Hydroxide (0.1 N)

Sodium Hydroxide (NaOH, pellets)	2.0 g
DDW	bring to 500 ml

10. Stock Standard (1000 mg N/L) – Refrigerate

Ammonium Chloride (NH_4Cl)	3.821 g
(Oven dried, 110°C)	
DDW	bring to 1000 ml

11. Working Standards – Prepare fresh daily

Working standards are prepared from the 1000 mg N/L stock. Typically 4 concentrations spanning the expected range of sample values plus a blank are run in duplicate. The standards used are 0.0, 100.0, 200.0, 300.0 and 400.0 µg/L.

12. Spike Addition Stock (1000 mg N/L) – Refrigerate

Glycine (NH_2CH_2COOH)	5.360 g
(Oven dried, 110°C)	
DDW	bring to 1000 ml

Intermediate spike solutions are made from this stock. The intermediate solutions should be prepared fresh for each assay. The routine spiking level for samples in the range of 0-400 µg/l is 150.0 µg/L. Samples at concentrations >400 µg/L should be diluted into the analytical range of the calibration curve.

13. Standard Reference Material

Nicotinic Acid (4.743% N)	5.271 g
(Oven dried, 110°C)	
DDW	bring to 250 ml
Glycine	5.360 g
(Oven dried, 110°C)	
DDW	bring to 1000 ml

The intermediate solutions should be prepared fresh for each assay. The final working concentration is 200.0 µg/L.

Procedure:

1. Measure 25.0 ml of sample into acid-cleaned digestion tubes (25 × 198 mm).

2. Add acid-cleaned boiling chips (10-12).

3. Add 5.0 ml of digestion solution and mix

4. Place all tubes in block digester. Program the controller for two temperature ramps: 1) 160°C, 60 min. 2) 380°C, 90 minutes. The samples are extremely vulnerable to contamination by ammonia from the atmosphere during this process.

5. Remove tubes from digester, let sample cool slightly and rehydrate with 10 ml DDW. Vortex.

6. Transfer entire volume to a corresponding acid-cleaned screw top, calibrated 50 ml tube. Cap tightly.

7. Samples can now be stored for up to two days at 4°C.

8. Continue the assay by adjusting the pH of each sample (in fume hood).
    a. Add 5.0 ml buffer solution and vortex.
    b. Add 1.0 ml phenol-ethanol and vortex.
    c. Add an initial 3.3 ml of 10.8 N NaOH to each tube and vortex. Use a pH meter to adjust the final value to 10.20 ± 0.10 with 10.8N NaOH or concentrated H_2SO_4. Vortex.0

9. Bring volume to 25.0 ml with DDW.

10. Add 1.0 ml of potassium ferrocyanide catalyst.

11. Add 2.5 ml of oxidizing solution. Vortex samples.

12. Place tubes under an ultraviolet light source for 60 minutes.

13. Read absorbance at 640 nm in a 4 cm cell.

Notes:

1. Some block digesters do not evenly heat the samples. Standard duplicates should be run in different locations in the digester to compensate for uneven digestion.

2. The digestion process should be performed in a fume hood with acid – scrubber to remove toxic acid fumes.

3. Wear protective clothing and eye shields whenever working with hot digesting samples.

4. Samples must not consume more than half of the sulfuric acid during digestion. If samples go dry, they must be rerun.

5. Samples greater than 400 μg/L must be diluted and reanalyzed.

6. This assay generates a regulated mixed waste solution. Pour all waste contents into the appropriately labeled waste jug.

Calculations:

To calculate the nitrate concentration, generate a standard curve by plotting the absorbance versus the concentration of the standards. Absorbance values of samples are then entered to give TKN concentration in μg TKN – N/L. If samples have been diluted, multiply the concentration by the appropriate dilution factor to calculate the final values.

QA/QC Procedures:

1. To determine the success and accuracy of each run, it is important to measure the percent recovery of organic nitrogen. Run 5% of the samples as spikes. Calculate the percent recovery for each spiked sample.

2. Replicates should be run on 5% of the samples to assess precision of the analytical run. Calculate the relative percent difference (RPD) for each replicate.

3. Calculate the standard curve statistics: r^2, Y intercept, and slope.

4. Evaluate and check your quality control by answering the questions in the Q.C. Flow Chart (Appendix A).

References:

Liddicoat, M.I., S. Tibbits, and E.I. Butler. 1975. The determination of ammonia in seawater. Limnol. Oceanogr. 20:131-132.

Methods for the determination of Inorganic Substances in Water and Fluvial Sediments. M.J. Fishman and L.C. Friedman (eds.) 1985. Open-File Report 85-495. Denver, Colorado.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14:799-801.
State Water Resource Control Board. Quick Chem Method No. 10-107-06-2-F. Revision: D. Diamond. June 1986.

Revision Dates: March 7, 2002 Revision: UC Davis SOP

Lachat IL 500 TOC Analyzer
Lachat (800) 247-7613

Table of Contents

I. Read First!

DON'T TOUCH ANYTHING WITHOUT GLOVES
DO NOT USE CLEAR TYVEX TUBING
ONLY USE OPAQUE SILICONE TUBING

1. Before you use this tubing, rinse it with HCl following a 20 second rinse with DI water.

REMOVE TUBING FROM THE TAP AND USE WATER DIRECTLY FROM TAP WHEN POSSIBLE

II. Some Basic Tasks You Will Do Again and Again

Prepare Aluminum foil

1. Cut up 2" by 2" squares of aluminum foil
2. Wrap in foil package
3. Pre-combust (see below)

Pre-combustion

1. Pre-combust all test tubes, 2" by 2" squares of aluminum foil, metal tweezers, metal spatulas
2. Do not pre-combust volumetric flasks, volumetric pipettes or graduated cylinders
3. Rinse with HCl followed by 6 times of DI rinse.
4. Wrap or cover (beakers) with aluminum foil –vials must be completely wrapped in foil so that they never touch one another to prevent broken vials.
5. Pre-combust in muffle furnace (room 301) at 500^{o}C for 2 hours
6. Keep covered or wrapped until use
7. After BOD bottles cool, match numbered tops and bottles and store with caps

Autoclaving

1. Autoclave BOD bottles, volumetric flasks, volumetric pipettes and graduated cylinders
2. Rinse with DI
3. Fill with small amount HCl
4. Cover with aluminum foil
5. Place in autoclave
6. Turn knob at the base to 3 o'clock and fill with one plastic container full of water
7. Close door and jiggle to ensure it is close
8. Switch to "fast –instruments"
9. Turn clock to 15 minutes
10. When done, rinse 6 times with DI
11. Dry upside down on aluminum foil- or you can place in the drying oven
12. When dry, cover with pre-combusted aluminum foil squares to store

For items that are too tall to fit into the autoclave:

1. Rinse with HCl 3 times
2. Rinse with DI 6 times
3. Dry upside down on aluminum foil
4. When dry, cover with pre-combusted aluminum foil squares

III. Field Collection- Things you need

BOD bottles

1. Autoclave BOD bottles
2. Wash caps with acid and 6 times with HCl

Cooler

1. Put Ziplock^{TM} bags with ice in cooler
2. Make sure plastic separator is in cooler to prevent bottles from banging into each other

Ice
1. Place in Ziplock™ bags.

Gloves
2. Wear when dealing with bottles and when collecting samples

Data Sheet
3. Instead of labeling bottles, use data sheet so numbered bottles correspond to location on data sheet

Collecting samples
Bring cooler with ice and bottles into the field. Take capped bottle out of the cooler at the site. Rinse 3 times with the sample water. Fill so it is over flowing and then cap to insure there is no headspace in the sample. Take field duplicates of 20% of samples. Return to cooler and keep cool and dark (i.e. in the closed cooler) until you return to lab. DIC samples can be stored temporarily in the Cold Room, but should be moved to the bottom shelve on the refrigerator in room 304 as soon as possible to prevent contamination form volatiles in the cold room. **DOC samples should not be stored in the cold room.** DIC samples should be run within 3 days. DOC samples should be filtered then acidified within 3 days.

IV. Filtration-- Things you need:

Samples (obviously)
500 ml graduated cylinder
1. Graduated cylinder should be washed 3 times with HCl, rinsed 6 times with DI, dried upside down on aluminum foil and covered with pre-combusted aluminum foil until used.

Filtration set up
1. Before using rinse with HCl and 6 times with DI.
2. DO NOT wash with soap!!!!

Test tubes
1. Pre-combust vials, by washing with HCl and rinsing 6 times with DI. Then wrap in aluminum foil, by completely wrapping each tube so that no vial touches the next. Heat in muffle furnace to 500^{o} C for 2 hours.
2. Keep wrapped in aluminum foil packages until you are ready to use
3. When ready, always label vials with labeling tape so that you don't mess them up!!!

Pre-combusted aluminum foil

1. Cut 2” by2” squares of aluminum foil and enclose in a little aluminum foil package
2. Heat in muffle furnace for 2 hours
3. These are to place over vials after they are filled with sample

Aluminum foil to cover lab bench
GF/F filters

1. Whatman™ GF/F filters 2.5 cm
2. Wrap in a aluminum foil pouch
3. Pre-combusted @ 500° C for 2 hours
4. Placed grid side up

Data Sheet (for particulate carbon)
1 M H_2SO_4 in pre-combusted 25 ml beaker (for DOC only)
Pre-combusted Pasteur pipettes and bulb (for DOC only)

Filtering Samples

Put on gloves. After rinsing filtration set, use tweezers to place a pre-combusted GF/F filter on the mesh piece, grid side up. Put upside down beaker on filter and clamp. Fill graduated cylinder and record the volume of the sample on the data sheet (if you are interested in measuring particulate carbon, if not, it doesn’t matter how much liquid runs through filter). Pour liquid into filter setup. Turn on pump and run until all the liquid is gone. Pour this water into the clean, labeled vials and cover with pre-combusted aluminum foil.

Before filtering next sample, rinse the graduated cylinder, flask, filter and upside down beaker with DI 6 times. Do not place directly on lab bench. Instead, place rinsed equipment on aluminum foil surface.

If you are interested in particulate carbon, remove filter and place on labeled aluminum foil and place in 60°C drying oven (room 301). If not you can throw away the filter. After drying filters over night, then should be frozen or wrapped in tin capsules and stored in a desicator for later carbon analysis.

If you are filtering DOC samples, use Pasteur pipette to add one pipette full of 1M H_2SO_4 (See DOC) to the vials, as a preservative, before covering with aluminum foil.

The inorganic carbon samples should be run the same day (or the next if necessary). DOC samples can be stored in the refrigerator, **not the cold room,** for a month.

V. **Storage**

Before Filtration

1. **Avoid storing bottles in the cold room when possible- especially the DOC samples.**
2. Can be stored for 3 days until they need to be filtered and run (DIC) or preserved (DOC). But should be run as soon as possible.
3. Should remain in the dark. A closed cooler is sufficient. In the fridge, they should be in a covered box.

After Filtration

1. DIC samples should be run as soon as they are filtered.
2. DOC can be stored in the fridge in a dark box, after they are preserved, for 1 month.
3. PC carbon on filters should be dried, weighed, wrapped in aluminum foil and stored in the desicator in room until they can be analyzed. Alternately they can be frozen for storage.

VI. Reagents for the Machine

Phosphoric Acid (H_3PO_4)

1. Not used in DOC process, DIC only
2. Change out at least once a month
3. Fill a 250 ml volumetric flasks ¾ full with DI
4. Measure 17.25 ml of 85% Phosphoric Acid (cabinet in 304) to a graduate cylinder, after rinsing once with H_3PO_4 and add to the volumetric flask
5. Use a pre-combusted Pasteur pipette to finish filling the flask
6. Cover the flask with pre-combusted aluminum foil
7. Cover aluminum foil with Parafilm ™
8. Turn up side down 20 times to mix
9. Rinse out the beaker from machine with HCl and then 6 times with HCl
10. Re-fill beaker with new solution

Sodium Persulfate ($Na_2S_2O_8$)

1. Not used for DIC process, DOC only
2. Change out at least once a month
3. Weigh out 20 g of $Na_2S_2O_8$ (previously heated to 70°C for 2 hours, cooled and keep in desiccator) and add to 250 ml volumetric flask
4. Fill the volumetric flasks ¾ full with DI
5. Use a pre-combusted Pasteur pipette to add one pipette full of 1M H_2SO_4(See DOC)
6. Use a pre-combusted Pasteur pipette to finish filling the flask
7. Cover the flask with pre-combusted aluminum foil
8. Cover aluminum foil with Parafilm ™
9. Turn up side down 20 times to mix
10. Rinse out the beaker from machine with HCl and then 6 times with HCl
11. Re-fill beaker with new solution

Ultrapure water

1. Fill up before every run
2. Rinse large amber beaker with HCl and 6 times with DI
3. Fill with DI water (without using clear Tyvex tubing)

VII. DIC/TIC

What You Need:

Sodium Bicarbonate- dry in oven at 70ºC for 2 hours and store in desiccator

Sodium Carbonate- dry in oven at 70ºC for 2 hours and store in desiccator

Volumetric flasks-see making DIC standards chart

Volumetric pipettes- see making DIC standards charts

Small pieces of combusted foil- see page 4

Metal spatula

Small pieces of Parafilm ™

2, 250 ml beakers

1, small beaker

Pasteur pipette

Bulb

Making the standards

Standards should be made within 24 hours of the run and should not be stored in the fridge.

Autoclave all flasks, pipettes, beakers and Pasteur pipette- see page 4

Put on dry gloves. Make stock standard of the "standards" solution. Take a piece of the combusted foil, and use a metal spatula to weigh out $NaHCO_3$ (see Making DIC Standards Chart for values). Pour the $NaHCO_3$ into a 100 ml volumetric flask. Repeat with. Make a stock standard of the SRS by repeating above steps with Na_2CO_3 (see Making DIC Standards Chart for values). Fill small beaker with DI and use Pasteur pipette with DI to make sure all the substance is washed down into the volumetric flask. Fill the flasks of stock solution almost up to the line with DI from sink (without tyvex tubing). Use Pasteur pipette to top off. Cover with combusted aluminum foil. Cover foil with Parafilm ™. Mix by turning upside down at least 20 times.

The SRS stock solution is ready to be poured into a test tube and can be set off to the side. The "standards" stock solution is used as the highest concentration standard and is used to make the lower concentration standards.

Rinse, then fill a 250 ml beaker with stock "standards solution. The second highest standard is made from the most concentrated standard aka the stock "standards" solution.

The lower concentration standards are made from one of these two solutions. Follow the Making DIC Standards Chart to determine the appropriate pipette used to measure the appropriate solution to deliver to the appropriate sized volumetric flasks to make all the standards. Cap each flask with foil and them Parafilm ™ and mix by turning upside down 20 times.

Preparing Rack

Start with a blank. Then pour standards from volumetric flasks in the tubes. Follow the first standards by a blank. Then pour SRS from volumetric flask into a test tube. Follow this by another blank. Then pour samples directly into test tubes. Follow by a blank. Then follow by another run of standards

VIII. DOC/TOC

What You Need:

Potasium Acid Phalate –KHP-to make a primary dilution standard that lasts for 4 months

Dry at 120^{o}C overnight then store in a desiccators

Ascorbic Acid

Dry at 120^{o}C overnight then store in a desiccator

Concentrated H_2SO_4 – to make a primary dilution standard only

Volumetric flasks-see Making DOC Standards Chart

Volumetric pipettes- see Making DOC Standards Chart

Small pieces of combusted foil- see page 4

Metal spatula

Small pieces of Parafilm ™

2, 250 ml beakers

1, small beaker

Pasteur pipette

Bulb

Making the standards

Standards should be made within 24 hours or the run and should be stored in the fridge if they will sit overnight.

Autoclave all flasks, pipettes, beakers and Pasteur pipette- see page 4

Put on dry gloves. Make a 500 ppm stock standard, or PDS (primary dilution standard) from dry KHP. Take a piece of the combusted foil, and use a metal spatula to weigh out 1.063 g of KHP (see Making DOC Standards Chart for values). Pour the KHP into a 100 ml volumetric flask. Fill small beaker with DI and use Pasteur pipette with DI to make sure all the substance is washed down into the volumetric flask. Fill the flasks of stock solution almost up to the line with DI from sink (**without tyvex tubing**). Add 1 ml of concentrated H_2SO_4. Use pasteur pipette to top off. Cover with combusted aluminum foil. Cover foil with Parafilm ™. Mix by turning upside down at least 20 times. Cover with foil to black out light. Store for up to 4 months.

Put on dry gloves. Make a SRS solution. Take a piece of the combusted foil, and use a metal spatula to weigh out ascorbic acid (see Making DOC Standards Chart for values). Pour the ascorbic acid into a 100 ml volumetric flask. Fill small beaker with DI and use Pasteur pipette with DI to make sure all the substance is washed down into the volumetric flask. Fill the flasks of stock solution almost up to the line with DI from sink (without tyvex tubing). Use Pasteur pipette to top off. Cover with combusted aluminum foil. Cover foil with Parafilm ™. Mix by turning upside down at least 20 times.The SRS stock solution is ready to be poured into a test tube and can be set off to the side.

Use the PDS solution to make a concentrated standard solution. Use a 20 volumetric pipette to add PDS to a 1L beaker. Then fill the flask up with DI from sink (**without tyvex tubing)** and top off with a Pasteur pipette. Cover with precombusted foil. Then cover with Parafilm ™. Mix by turning up side down 20 times. This concentrated solution will be used as the most concentrated standard and the solution from which the other standards are made.

Rinse, then fill a 250 ml beaker with stock “standards solution. The second highest standard is made from the most concentrated standard solution. The lower concentration standards are made from one of these two solutions. Follow the Making DOC Standards Chart to determine the appropriate pipette used to measure the appropriate solution to deliver to the appropriate sized volumetric flasks to make all the standards. Cap each flask with foil and them Parafilm ™ and mix by turning upside down 20 times.

Preparing Rack

Test tubes must be precombusted at 500^{o}C in the muffle furnace for 2 hours.
No test tubes can be placed in the far left column of the sample rack.
Start with a blank. Then pour standards from volumetric flasks in the test tubes. Follow the first standards by a blank. Then pour SRS from volumetric flask into a test tube.

Follow this by another blank. Then pour samples directly into test tubes. Follow by a blank. Then follow by another run of standards.

IX.Particulate Carbon

Filtering

Particulate Carbon is measured from C collected on GF/F filters-see **Filtration**

Storing

Filters are dried over night at 60^{o}C.

Then freeze filters for long term storage or store in a desicator for a long term storage. When you take them out of the freezer, you should place them in the drying oven (60^{o}C overnight).

Analysis

Carbon content and isotopic signature and be measured by sending them to the Stable Isotope Facility in Davis

Filters need to be wrapped in flat tin disks (Perkin Elmer N2411363), with combusted twezzers.

Samples should be stored in a desicator

http://stableisotopefacility.ucdavis.edu/13cand15n.html for more details

X.Using the Machine

Turning on the computer

1. User name and password are chemist

Filling up solutions and DI

1. Fill up rinse water container with DI water before each run. Acid wash and rinse 6 times with DDI.
2. Fill reagent container if they are less than half full or if they have been sitting for over a month-see Reagents for the Machine. Phosphoric acid is used in IC analysis. Sodium Persulfate is used in OC analysis.

Turning on the Machine

3. Turn on gas tank to 80 psi (left knob) and make sure the knob is open to the machine. If the right knob reads less than 250 psi you need to change out tank
4. Turn on the machine using the switch on the back, top, right corner
5. Turn on auto-sampler using toggle on back, top left
6. **Turn on Omni-TOC, user name and password are both Admin**
7. If you are going to run the machine you want to initialize analyzer, if you are just looking at data you do not need to initialize analyzer

Loading Method

1. Click on Method menu on top left
2. Click "load"
3. DOC/TOC the methods is "NPOC1"
4. DIC/TIC the method is "DIC_good"
5. If you want to edit method click "edit" in Method menu
6. Click okay

Starting Run

1. On bottom of screen click "Start Measurement"
2. Label as ex. DOC_Sep_MLTP, and save
3. Click Start

Rack Table

1. You will be asked if you would like to open an available rack table.
2. If you want to use a template click yes, and a list of templates will be shown. Choose one. Skip to 6.
3. If you want a new rack order, click no.
4. You will then be asked if all samples will be run with the current method. Click yes.
5. If you are making a new rack table, you will need to double click in the first cell under sample ID and type the sample name. Use down arrow to toggle through cells.
6. Once all sample names are listed, click on green triangle in the top menu, then the green triangles next to the sample names will turn into a red circle.
7. Once all green triangles have become red circles, click on the green check in the top right menu bar.
8. If this is a new rack table, the program will ask if you want to save the rack table. If you want to save it as a template, click yes.

The Run

1. Click start and the bottom right of the screen

2. The right tube purges and the left tube takes sample

Finish Run

1. The data should be saved automatically
2. Turn off program
3. Wait for sounds of machine rinsing to finish(2 mins)
4. Turn off auto-sampler
5. Turn off analyzer
6. Turn off gas

Looking at the Data

1. Open Omni-Toc
2. You do not need to initialize the analyzer
3. Click on Data Evaluation on the menu bar on the top of the screen
4. Select Analysis Table
5. Click on picture of a yellow folder and a list of the runs will appear, with the most recent run appearing at the top
6. Click on the run you want to access
7. The run will appear with the last sample on top and the first sample on the bottom. It is best to scroll down to the bottom and work back to the top
8. Double click on each row and an additional window will pop up with more info about the analysis
9. There should be 3 readings for AU (area units) for each sample, this is the value of interest to us, record these 3 replicates on the data sheet
10. Close each window by clicking on the red and yellow picture (I think it is suppose to be a door) on the top left

Making Standards and Reagents

Reagents

1.Phosphoric acid

69ml of 85% Phosphoric acid (acid in rm 302 acid cabinet) in 1000 ml of DI water (17.25 ml in 240 ml ml)

2. Sodium Persulfate Solution

80 g/L Sodium peroxodisulfate ($Na_2S_2O_8$) +5ml 1M H_2SO_4 (20g in 250 ml)

Preservatives

3. 1M H_2SO_4

55ml of 95-98%H2SO4(acid with yellow cap in acid cabinets) in 1000ml of DI water (13.75 ml in 250 ml) for TOC only, not TIC or DIC.

Add 2 drops of acid to a 40-1000ml sample to get the pH to less than 2.
This is for TOC or DOC only, NEVER for TIC or DIC!!!!!!!!

Standards

4. KHP –heated at 110 degrees C for 2 hrs and kept in desicator
Add 1M H2SO4 for preservation

5. NAHCO3- heated at 70 degrees C for 2 hrs and kept in desicator

Standards for Organic Carbon

Potassium Acid Phalate-KHP			
element	atomic weight	#	KHP
C	12.011	8	96.088
H	1.008	5	5.04
O	15.999	4	63.996
K	39.098	1	39.098
		sum molecular weight	204.222
		total/weightC	2.125364249

SO 2.28 mg of KHP has 1 mg carbon

Primary dilution standard in made every 4 months

PDS is 500 ppm

Add 1 ml of concentrated H2SO4 and 1.063 g KHP to 1L

Make Intermediate Dilution Standard 10 mg/L from PDS

Made by adding 20ml of PDS to 1 L

Making Standards from Intermediate Standard				
Stand. Conc.	Beaker Size	vol. pipette sizefrom 10ppm	vol. pipette size from 5ppm	source
0	250	x	0	tap
0.1	250	x	5	5 ppm
0.5	250	x	25	5 ppm
1	250	25	x	10 ppm
2	500	100	x	10 ppm
5	500	250	x	10 ppm
10	1000	x	x	PDS

Ascorbic Acid				
element	atomic weight	#	KHP	So 2.44 mg of acid = 1 mg carbon
C	12.011	6	72.066	To make 2ppm add 0.00488 g to 1 L
H	1.008	8	8.064	
O	15.999	6	95.994	0r 0.00122g to a 250 ml beaker
K	39.098	0	0	
		sum	176.124	

total/weightC	2.443926401

Standards for Inorganic Carbon

element	atomic weight	NaHCO3
Na	27.99	27.99
H	1.008	1.008
C	12.011	12.011
O	15.999	47.997
	sum	89.006
	total/weight C	7.410373824

So 7.41 mg of NaHCO3 has 1 mg C
Stock standard only good for one week. Make 50ppm solution in a 1 L volumetric beaker Add 0.371 g of NaHCO3 to 1L vol.flask. Use the 50ppm to make working standards.

Stand. Conc (ppm)	vol. flask size (ml)	vol. pipette size from 50ppm	source
0	250	none	DI
1	250	5.00	50 ppm
2	250	10.00	50 ppm
4	250	20.00	50 ppm
8	250	40.00	50 ppm
10	250	50.00	50 ppm
12	250	60.00	50 ppm
16	250	80.00	50 ppm
20	250	100.00	50 ppm

element	atomic weight	Na2CO3
Na	27.99	55.98
H	1.008	0
C	12.011	12.011
O	15.999	47.997
	sum	115.988
	total/weight C	9.656814587

So 9.66 mg of Na2CO3 has 1 mg of C

SRS only good for one week

Make 10 ppm solution in a 1L flask

Add 0.096 g of Na2CO3

0r 0.024 g to a 250 ml beaker