Appendix 1. Analytical methods for quantification of nutrients in water

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UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
COLLEGE OF AGRICULTURAL SCIENCES

AGRICULTURAL EXPERIMENT STATION
SOIL AND WATER CHEMISTRY LABORATORY
STANDARD OPERATIONAL PROCEDURES FOR

APPENDIX 1. ANALYTICAL METHODS FOR QUANTIFICATION OF NUTRIENTS IN WATER

AGRICULTURAL EXPERIMENTAL STATION
SOIL AND WATER QUALITY LABORATORY

STANDARD OPERATING PROCEDURES FOR TOTAL PHOSPHORUS ANALYSIS IN WATER – SOP #011w
(Based in EPA method No 365.2)

1. Introduction

Dissolved orthophosphate is the form of P most readily available to aquatic plants, but numerous studies have shown that other forms of P can be hydrolyzed to the orthophosphate form in wastewater-treatment facilities and in natural waters. Therefore, when assessing the long-term potential for accelerated eutrophication of surface water due to P loading, many researchers and watershed managers want to know the total P concentration (regardless of P form) in water samples.

Polyphosphates and phosphates bound to organic substances do not react with the molybdate reagent used for colorimetric P analysis. Therefore, analysis for total P content of water samples requires that all condensed and organic P compounds, including particulate P, first be converted (hydrolyzed) to orthophosphate so they can be determined colorimetrically. This is accomplished by digesting the sample in strong acid at high temperature to oxidize the organic matter and release P as orthophosphate.

To determine total P (dissolved + particulate), an unfiltered sample is shaken (to suspend the particulate matter) just before submitting a subsample for digestion.

2. Summary of method

This method covers the determination of specified forms of phosphorus in drinking, surface, and saline waters, domestic and industrial wastes. The method is usable in the 0.01 to 0.5 mg P/L range.

The sample (50mL) is heated in the presence of sulfuric acid and potassium persulfate to reduce the sample to a volume of 10 mL of the initial volume, the sample is allowed to cool down and is brought back the original volume (50mL) with distilled-deionized water. An aliquot is then submitted for color developing. absorbance is measured at 870 nm, using an UV/VIS spectrophotometer (see procedure).

3. Conditions of sampling

3.1. General preparation of sampling containers.

This procedure should be used when preparing all sample containers and glassware for monitoring nitrates and phosphorus.

1) Wash each sample bottle or piece of glassware with a brush and phosphate-free detergent.
2) Rinse three times with cold tap water.
3) Rinse with 10 percent hydrochloric acid.
4) Rinse three times with distilled, deionized water.

3.2. Collection of the water sample

Sampling is performed following procedures delineated by USGS (Wilde et al., 1998) and others (Haygarth and Edwards, 2000). Please follow procedures as detailed in Appendix 2

4. Materials and equipment

4.1. Reagents

1. Sulfuric acid solution (H_2SO_4 - 11N). Transfer approximately 600 mL of distilled water to a 1000 mL volumetric flask. Slowly (and carefully) add 310 ml of concentrated H_2SO_4. After the solution has cooled, dilute to 1000 mL with distilled deionized water and mix.
2. Potassium persulfate, $K_2S_2O_8$ solid.
3. Ammonium molybdate solution. Dissolve 20 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 ml of distilled deionized water. Store in a plastic bottle at 4°C.
4. Ascorbic acid solution. Dissolve 26.4 g of L-ascorbic acid in 100 mL of distilled deionized water. The solution is stable for about a week if stored in an opaque plastic bottle at 4°C.
5. Antimony Potassium Tartrate solution. Using a 500 ml volumetric flask, dissolve 1.454 g of $K(SbO)C_4H_4O' 1/2\ H_2O$ in approximately 400 mL of distilled water, and dilute to volume. Store in a dark, glass-stoppered bottle.
6. Sulfuric acid solution (H_2SO_4 – 5N). Dissolve 70 mL of H_2SO_4 conc. in 500 ml of distilled water.
7. Sulfuric acid solution (H_2SO_4 – 10N). Dissolve 50 mL of H_2SO_4 conc. in 180 ml of distilled water.
8. Solution for color development, (Murphy-Riley). To make 500 ml of the solution:
    a. Transfer 250 ml of 2.5 M H_2SO_4 (7) to a plastic bottle.
    b. Add 75 mL of ammonium molybdate solution (4) to the bottle and mix.
    c. Add 50 mL of ascorbic acid solution (5) to the bottle and mix.
    d. Add 25 mL of potassium antimony tartrate (6) solution to the bottle and mix.
    e. Add 100 mL of distilled deionized water.

NOTE: If turbidity has formed in the combined reagent, shake and let stand for a few min until turbidity disappears before proceeding. Store in an opaque plastic bottle. The combined reagent is stable for less than 8 h, so it must be freshly prepared for each run.

9. Stock phosphorus solution: Use a 1000ppm (1mL=1mg P) Certified Phosphate (P) Standard solution.
    a. As an alternative standard dissolve 0.2197 g of potassium dihydrogen phosphate, KH_2PO_4, (previously dried at 105°C) in distilled deionized water. Dilute the solution to 1000 ml; 1.0 ml = 0.05 mg P.
10. Standard phosphorus solution: Dilute 1.0 ml of stock phosphorus certified solution to 1000 ml with distilled water; 1.0 mL = 1.0 µg P.

    Using the standard solution (10), prepare the following standards in 50.0 ml volumetric flasks:

Standard Phosphorus Solution (mL)	Concentration (mg L^{-1})
0	0.00
1.0	0.02
3.0	0.06
5.0	0.10
10.0	0.20
20.0	0.40
30.0	0.60
40.0	0.80

4.2. Equipment

1. Hot plate with adequate heating surface.
2. Acid-washed glassware and plastic bottles: graduated cylinders (5 ml to 100 ml measurements), volumetric flasks (100 ml, 500 ml, and 1000 ml), storage bottles (including dark glass-stoppered, and opaque plastic), pipets, and test tubes or flasks for reading sample absorbance
3. Photometer, spectrophotometer or filter photometer suitable for measurements at 650 or 880 nm with a light path of 1 cm or longer.

5. Laboratory Procedure

5.1. Analytical procedure

1. Thoroughly mix the sample, and measure a suitable portion (50 ml is recommended) into a 125 ml Erlenmeyer flask.
2. Add 1 ml of H_2SO_4 solution (1).
3. Add 0.4 g of solid Potassium Persulfate ($K_2S_2O_8$) and mix.
4. Boil the sample solution gently on the preheated hot plate until the volume is reduced to approximately 10 mL.
5. Cool the sample and dilute to 50 mL with distilled deionized water.

5.2. Color developing procedure

1. Mix 5ml of cooled diluted sample, 0.25mL of Sulfuric Acid Solution (7) and 5ml of color developing solution (8) into a 50mL volumetric flask, dilute to volume with distilled deionized water. Allow to react for a minimum hour but not longer than 8 hours before measuring color absorbance at 870 nm with a spectrophotometer.
2. To prepare the calibration curve, carry a series of standards through the digestion process. ***Do not use standards that have not been digested.***

Note: For samples with concentration levels lower than 0.2ppm use a BRAN+LUEBBE Auto Analyzer 3. See Manifold set up and flow system (Figure 1) for BRAN+LUEBBE Auto Analyzer 3, included in this SOP.

6. Instrument calibration

The equipment will be calibrated prior to the analysis of each sample batch, after each major equipment disruption, and whenever on-going calibration checks do not meet recommended control limit criteria. All calibration standards will be traceable to a recognized organization for the preparation and certification of QA/QC materials. Calibration curves will be established for each element and batch analysis from a calibration (analytical) blank and a minimum of three analytical standards of increasing concentration, covering the range of expected sample concentrations. The calibration curve will be well-characterized and will be established prior to the analysis of the samples. Only data which results from quantification within the demonstrated working calibration range may be reported by the laboratory. Samples outside the calibration range will be diluted or concentrated as appropriate and reanalyzed.

7. Quality assurance and quality control checks

Certified Reference Materials (CRMs) of the analyte of interest will be used to assess accuracy of a given analysis. At least one CRM fortified (spike) sample will be analyzed with each batch of 20 or fewer samples. Laboratory accuracy will be expressed in terms of percent recovery of the spiked sample. An accuracy goal of 70 – 130% must be met at all times. A CRM of the analyte of interest will also be used as an internal standard. The latter, which constitutes an analytical check point will be run 1 out of every ten samples. A deviation of more than 10% from the actual concentration would require establishment of a new calibration curve. All samples read between the before to last – and the last check point should be reanalyzed. Compliance with performing criteria (accuracy) will be checked prior to proceeding with the analysis. For every 20 samples analyzed triplicate analyses of an unknown sample will be performed to determine analytical precision. The acceptance criteria will be $\leq$ 20%RSD. If the laboratory fails to comply with either the precision or accuracy criteria the data for the entire batch will be considered suspect. Calculations and instrument will be checked, the CRM will be reanalyzed to confirm the results. If values are still outside the control limits in the repeat analysis, the laboratory is required to determine and correct the source of the problem and repeat the analysis until control limits are met. A field blank (trip blank) and a laboratory blank will be analyzed with every batch of 20 samples or less. The laboratory reagent blank (i.e. method blank) will be used to assess laboratory contamination during all stages of sample preparation and analysis. The reagent blank will be processed in a manner identical to the samples. A reagent blank concentration between the MDL and 3 times the MDL will require corrective action to identify and eliminate the source(s) of contamination before proceeding with sample analysis.

8. Calculations

Obtain concentration value of sample directly from prepared standard curve. Report results as P, mg/L.

9. References

USEPA, 1986. Quality criteria for water. Office of water regulation and standards. EPA-440/5-86-001. May 1986.

USEPA, 1999. EPA Methods and guidance for analysis of water. USEPA, Wash. D.C. CD-ROM ver. 2.0.

USEPA, 2004. Chapter 5. Water quality conditions. USEPA. [Online]. Available http://www.epa.gov/owow/monitoring/volunteer/stream/vms50.html (verified 22 February 2005).

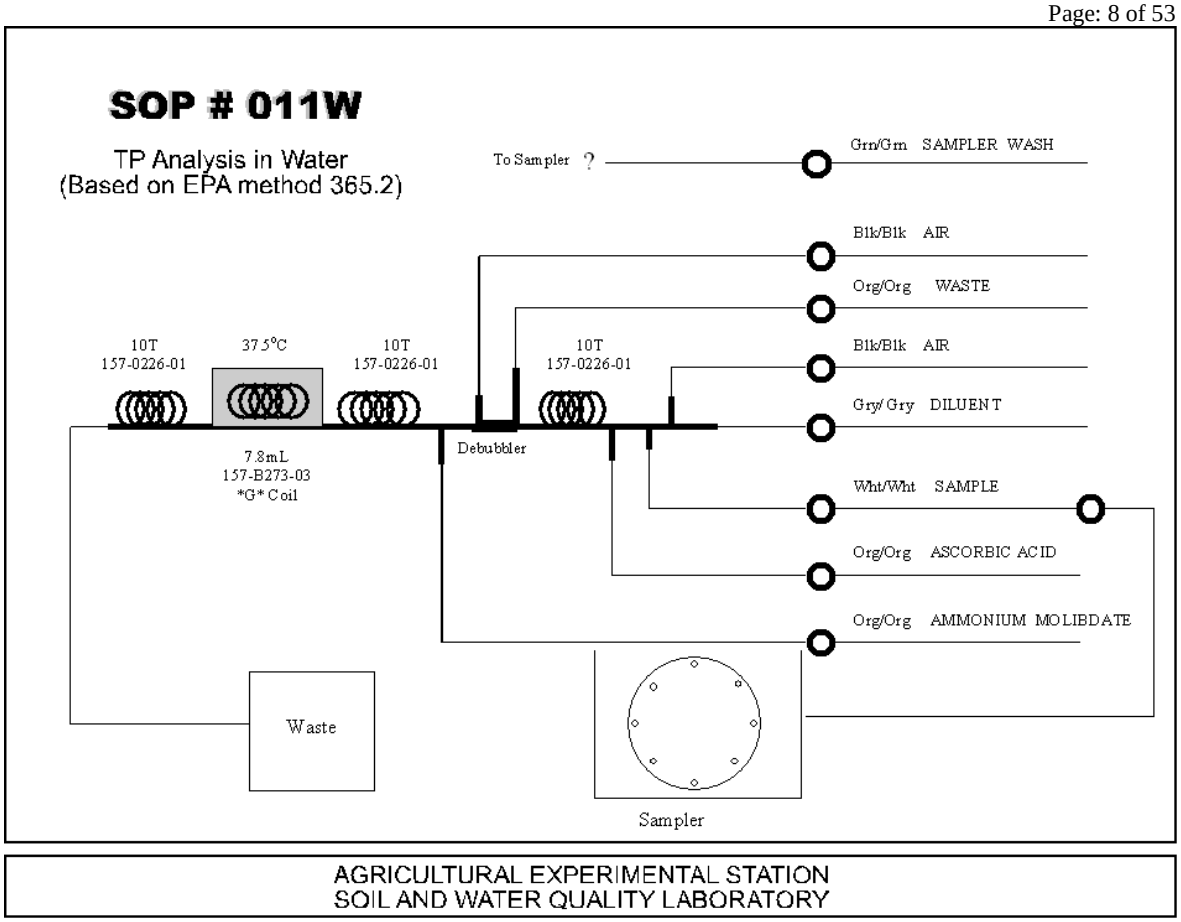

Figure 1: Manifold set up for TP determination in water

AGRICULTURAL EXPERIMENTAL STATION
SOIL AND WATER QUALITY LABORATORY

STANDARD OPERATING PROCEDURES FOR TKN ANALYSIS IN WATER – SOP #- 013W

PROCEDURE FOR DETERMINATION OF TOTAL KJELDAHL NITROGEN ANALYSIS BY BRAN + LUEBBE ION AUTO ANALYZER

(Based on EPA method No 351.2)

1. Introduction

Nitrogen is found in several different forms in terrestrial and aquatic ecosystems. These forms include ammonia (NH_3), nitrates (NO_3), and nitrites (NO_2). Nitrates are essential plant nutrients, but in excess amounts they can cause significant water quality problems. Together with phosphorus, nitrates in excess amounts can accelerate eutrophication, causing dramatic increases in aquatic plant growth and changes in the types of plants and animals that live in the stream. This, in turn, affects dissolved oxygen, temperature, and other indicators. Excess nitrates can cause hypoxia (low levels of dissolved oxygen) and can become toxic to warm-blooded animals at higher concentrations (10 mg/L) or higher) under certain conditions. The natural level of ammonia or nitrate in surface water is typically low (less than 1 mg/L); in the effluent of wastewater treatment plants, it can range up to 30 mg/L.

Ammonia (NH_3) is a colorless gas with a strong pungent odor. It is easily liquefied and solidified and is very soluble in water. One volume of water will dissolve 1,300 volumes of NH_3. Ammonia will react with water to form a weak base.

Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$.

2. Summary of Method

This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes.

The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. The applicable range of this method is 0.1 to 20 mg/L TKN. The range may be extended with sample dilution.

The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for 3.5 hours. The residue is diluted to 25 ml and placed on the Auto Analyzer for ammonia determination. The digested sample may also be used for phosphorus determination

3. Quality control definitions and interferences

3.1. Definitions

- **System Blank (SB):** A Volume of Matrix, same as the matrix used for the calibration standards but without the analytes.

- **System Standard (SS):** A known standard concentration which has been used for the standard calibration curve, using the same matrix and from the same cup. The **SS** is used to check the system performance.
- **Method Blank (MB):** An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents. The **MB** is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or the apparatus.
- **Check Point (CP):** A known standard concentration that is prepared separately from the one used for the calibration curve and treated exactly as all other samples including exposure to all glassware, equipment, solvents and reagents. The **CP** is used to determine if the concentrations of the standards used for the calibration curve are correct.
- **Spike (SPK):** A known concentration is added to replicate of a sample and treated exactly as all other samples including exposure to all glassware, equipment, solvents and reagents. The **SPK** is used to determine the % of recovery of analytes.
- **Precision (PRE):** A sample is tested five times and treated exactly as all other samples including exposure to all glassware, equipment, solvents and reagents. The **PRE** is used to determine the Relative Standard Deviation **(RSD)**.

3.2. Interferences

- High nitrate concentration (10X or more than the TKN level) results in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.
- Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus.

4. Safety

- The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be low as reasonably achievable. Cautions are included for known extremely hazardous material or procedures.
- Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in chemical analysis. The preparation of a formal safety plan is also advisable
- The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.
    - Sulfuric Acid
    - Sodium nitroprusside

5. Conditions of sampling

5.1. General preparation of sampling containers

This method should be used when preparing all sample containers and glassware for monitoring nitrates and phosphorus.

1. Wash each sample bottle or piece of glassware with a brush and phosphate-free detergent. Rinse three times with cold tap water.
2. Rinse with 10 percent hydrochloric acid.
3. Rinse three times with deionized water.

5.2. Sample collection

Sampling is performed following procedures delineated by USGS (Wilde et al., 1998) and others (Haygarth and Edwards, 2000). These procedures are summarized in Appendix 2.

5.3. Sample preservation

Samples are preserved at pH <2.0, by the addition of concentrated Sulfuric Acid and stored at 4^{o}C. Even when preserved in this manner, conversion of organic Nitrogen to Ammonia may occur therefore, samples should be analyzed as soon as possible.

6. Materials and equipment

6.1. Reagents

1. Digestion Solution: (Sulfuric acid-mercuric sulfate potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 700 ml of distilled, deionized water and 200 ml of conc. H_2SO_4.
2. Sulfuric Acid Solution 4%- (Sampler Wash Receptacle solution): Add 80 ml of conc. sulfuric acid to 1600 ml of ammonia free distilled, deionized water, cool and dilute to two liter with distilled, deionized water.
3. Stock Sodium Hydroxide solution (20%): To 600 ml of distilled, deionized water, add 500g of Sodium Hydroxide solution 40% w/w. Cool to room temperature and dilute to one liter with distilled, deionized water.
4. Stock Sodium Potassium Tartrate Solution (20%): Dissolve 200 g sodium potassium tartrate in about 600 ml of ammonia-free distilled water and dilute to one liter with distilled, deionized water and mix thoroughly.
5. Stock Buffer Solution: Dissolve 70g of sodium phosphate, dibasic Anhydrous (Na_2HPO_4) in about 800 ml of ammonia free distilled, deionized water. Add 50 g of sodium hydroxide solution 40% w/w and dilute to one liter with distilled, deionized water.
6. Working Buffer Solution 0.5M: Combine the reagents in the stated order; add 250 ml of stock sodium potassium tartrate solution (4) to 200 ml of stock buffer solution (5) with swirling. Slowly, with swirling, add 120ml of Sodium Hydroxide solution 20% dilute to one liter with distilled, deionized water. Add 1.0ml of Brij-35 30% and mix thoroughly.
7. Stock Sodium Nitroprusside: Dissolve 0.15g of Sodium Nitroprusisde in about 30ml of distilled, deionized water, mix well and dilute to 50ml with distilled, deionized water.
8. Sodium Salicylate / Sodim Nitroprusside: Dissolve 71.5g of Sodium Salicylate and 50ml of stock sodium Nitroprusside (7) 1n about 300ml of distilled, deionized water. Mix well and dilute to 500ml with distilled, deionized water. Add 1.0ml of Brij-35 30% and mix thoroughly. Store in a light resistant container. Make up fresh monthly.

9. Sodium Hypochlorite Solution: Dilute 6.0 ml sodium hypochlorite solution (clorox) to 100 ml with ammonia free distilled deionized water. Add 0.1ml of Brij-35 30% (two drops) and mix thoroughly, prepare fresh daily. Use any commercial bleach solution containing 5.25% of available chlorine.

6.2. Equipment

- Balance: Analytical, capable of accurately weighing to the nearest 0.0001g.
- Glassware: Class A volumetric flask and pipeting devises as required.
- Block Digestor BRAN + LUEEBE-20/40
- Digestion tubes: 1"x 8" heavy-walled 100ML pyrex tubes.
- Chemware PTFE (Teflon boiling chips)
- Analyzer in Autoanalizer 3 Brand and Lubbe.

7. Laboratory digestion procedure

7.1. Analytical Procedure

1. To 25 ml of sample, add 5 ml of digestion solution in the digestor tube (1) and mix (use a vortex mixer).
2. Add (4-8) Teflon boiling chips. **CAUTION:** too many boiling chips will cause the samples to boil over.
3. With Block Digester in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digester and switch to automatic mode. Set low temperature - timer for 1 hour. Reset high temperature to 380°C
4. and set timer for 2.5 hours.
5. Cool sample and dilute to 25ml with ammonia free distilled, deionized water.
6. Analyze in Autoanalizer 3 BRAN + LUEEBE.

8. Calibration and Standardization

1. Prepare a series of at least three standards for the Standard Curve covering the desired range, and a blank by diluting suitable volumes of standard solution with distilled, deionized water.
2. Process Standards Curve and blank as described in (Section **3.7**) procedure.
3. Set up Manifold and flow system as shown in Figure 1.
4. Place appropriate Standards Curve in sampler in order of decreasing concentration and perform analysis using (Section **3.9**) procedure.
5. After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Check Point (**CP**) sample. If measurements exceed ± 10% of the established (**CP**) value, the analysis should de terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the (**CP**) is recommended as a continuing calibration check.

9. Instrument Calibration

The equipment will be calibrated prior to the analysis of each sample batch, after each major equipment disruption, and whenever on-going calibration checks do not meet recommended control limit criteria. All calibration standards will be traceable to a

recognized organization for the preparation and certification of QA/QC materials. Calibration curves will be established for each element and batch analysis from a calibration (analytical) blank and a minimum of three analytical standards of increasing concentration, covering the range of expected sample concentrations. The calibration curve will be well-characterized and will be established prior to the analysis of the samples. Only data which results from quantification within the demonstrated working calibration range may be reported by the laboratory. Samples outside the calibration range will be diluted or concentrated as appropriate and reanalyzed.

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11. Colorimetric Analysis

1. Check the level of all reagents containers to ensure an adequate supply.
2. Flush all lines with 5N Sulfuric Acid for 30 minutes with proportioning pump in fast. Rinse for 30 minutes with distilled, deionized water.
3. Excluding the salicylate line, place all reagent lines in their respective containers, and start the proportioning pump.
4. When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump

and flush the coils with water using a syringe. Before restarting the system, check the concentration of the Sulfuric Acid solution and / or the working buffer solution.

5. After a stable baseline has been obtained start the sampler.

12. Calculations

Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve.

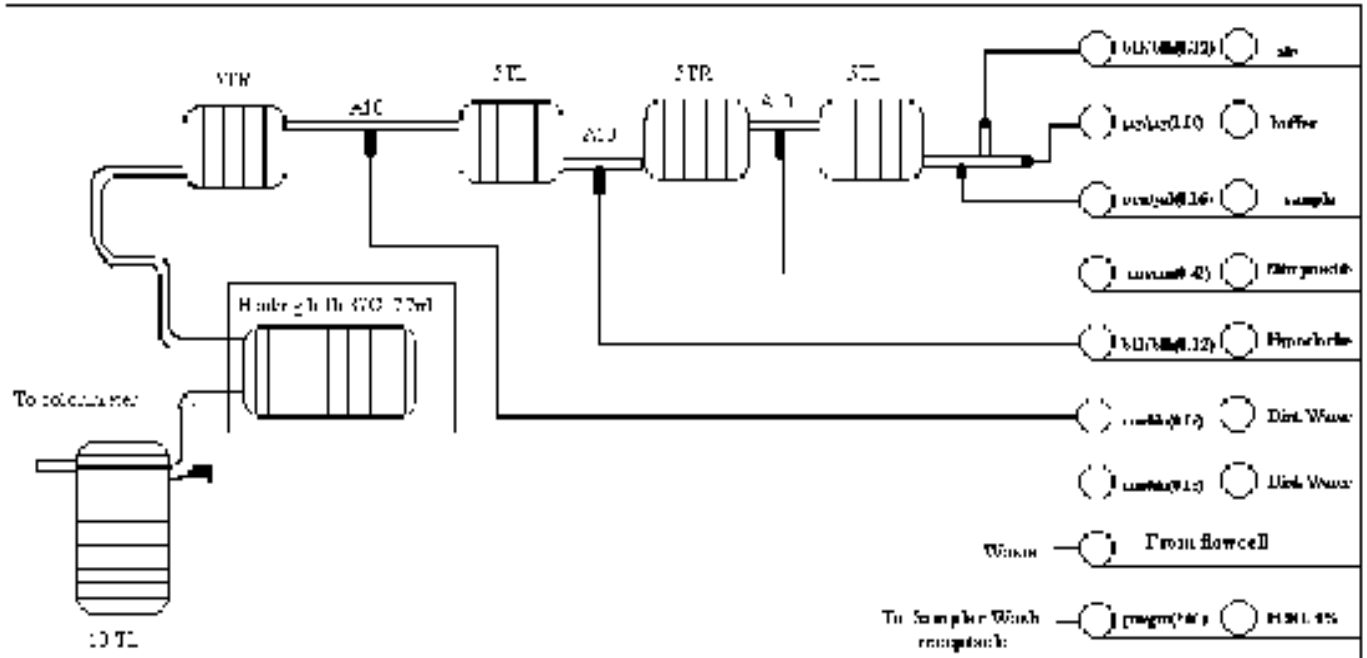
13. References

Haygarth, P.M., and A.C. Edwards. 2000. Sample collection, handling, preparation and storage. p. 84-90. In. G.M. Pierzinsky (ed.). Methods of phosphorus analysis for soils, sediments, residuals, and waters. Southern Cooperative Series Bulletin no. 396. available at: www.soil.ncsu.edu/sera17-2/pm_cover.htm

USEPA, 1999. EPA Methods and guidance for analysis of water. USEPA, Wash. D.C. CD-ROM ver. 2.0.

USEPA, 2004. Chapter 5. Water quality conditions. USEPA. [Online]. Available http://www.epa.gov/owow/monitoring/volunteer/stream/vms50.html (verified 22 February 2005).

Wilde, F.D., D.B. Radtke, J. Gibs, and R.T. Iwatsubo. 1998. National field manual for the collection of water-quality data. Techniques of water-resources investigations, Book 9, Handbooks for water-resources investigations. United States Geological Survey, Reston, VA.



MANIFORD SET UP AND FLOW SYSTEM

AGRICUTURAL EXPERIMENTAL STATION SOIL AND WATER QUALITY LABORATORY TOTAL KJELDAHL NITROGEN	**SOP - 013W**

AGRICULTURAL EXPERIMENTAL STATION
SOIL AND WATER QUALITY LABORATORY

STANDARD OPERATING PROCEDURES FOR NITRATE ANALYSIS IN WATER – SOP #AES- 021w
ANALYSIS BY BRAN + LUEBBE ION AUTO ANALYZER 3

(Based on EPA method No 353.1)

1. Introduction

Nitrates are a form of nitrogen, which is found in several different forms in terrestrial and aquatic ecosystems. These forms of nitrogen include ammonia (NH_3), nitrates (NO_3), and nitrites (NO_2). Nitrates are essential plant nutrients, but in excess amounts they can cause significant water quality problems. Together with phosphorus, nitrates in excess amounts can accelerate eutrophication, causing dramatic increases in aquatic plant growth and changes in the types of plants and animals that live in the stream. This, in turn, affects dissolved oxygen, temperature, and other indicators. Excess nitrates can cause hypoxia (low levels of dissolved oxygen) and can become toxic to warm-blooded animals at higher concentrations (10 mg/L or higher) under certain conditions. The natural level of ammonia or nitrate in surface water is typically low (less than 1 mg/L); in the effluent of wastewater treatment plants, it can range up to 30 mg/L. Concentrations that exceed the federal drinking water standard (10 mg of Nitrate as Nitrogen per liter of water) can result in methemoglobinemia, or "blue baby" syndrome.

Sources of nitrates include wastewater treatment plants, runoff from fertilized lawns and cropland, failing on-site septic systems, runoff from animal manure storage areas, and industrial discharges that contain corrosion inhibitors.

2. Summary of Method

This method covers the determination of nitrite singly, or nitrite and nitrate combined in drinking, ground, surface, domestic and industrial wastes. The applicable range is 0.05-10.0 mg L^{-1} nitrate-nitrite nitrogen. The range may be extended with sample dilution.

The automated procedure for the determination of nitrate utilizes the reaction whereby nitrate is reduced to nitrate by an alkaline solution of hydrazine sulfate containing a copper catalyst. The stream is then treated with sulfanilamide under acidic conditions to form a soluble dye which is measured colormetrically. The final product measured represents the nitrite ion originally present plus that formed from the nitrate. Chloride, sulfide, ferric ion and phosphate ion interfere.

In order to determine nitrate levels, the nitrate alone must be subtracted from the total (nitrate + nitrite). This can be achieved by substituting distilled water for the copper, hydrazine and NaOH lines on the manifold. A separate calibration curve should be determined for nitrate plus nitrite and for nitrite alone.

3. Sample preparation

Samples should be analyzed as soon as possible. For samples to be analyzed within 24 hours, preserve by refrigeration at 2˚C. If samples are to be preserved for more than 24 hours, acidify them with 2 mL of sulfuric acid per liter and refrigerate.

4. Materials and equipment

4.1. Reagents

Unless otherwise specified, all chemicals should be of ACS grade or equivalent.

1. Color reagent. To approximately 800 mL of distilled water add 185 mL concentrated phosphoric acid and 9.1 g of sulfanilamide. Dissolve completely. (Heat if necessary). Add 0.71 g of N-1 Naphthylethylenediamine dihydrochloride and dissolve. Dilute to 1000 mL. Add 1.0 mL Brij-35. Store in an amber container and keep refrigerated. Stability: one month.
2. Stock copper solution. Dissolve 3.2 g of cupric sulfate in distilled water and dilute to one liter.
3. Stock hydrazine sulfate. Dissolve 31.2 g of hydrazine sulfate in 800 mL of distilled water. Dilute to one liter. This solution is stable for six months if stored in a tightly stoppered amber bottle. CAUTION: TOXIC IF INGESTED. MARK THE CONTAINER WITH APPROPRIATE WARNING.
4. Working reductor solution. Dilute 25 mL of stock hydrazine sulfate [3] to about 900 mL wit distilled water. Add 10 mL of stock cupric sulfate solution [2]. Dilute to one liter with distilled water. Store in an amber bottle. Stability: one month. When first prepared, test the working reductor solution for efficient reduction capacity. Run both a nitrite and nitrate standard (2.0 mg L^{-1}) if the apparent nitrate concentration is much lower than the nitrite concentration, adjust the working reductor solution by 1 mL aliquot additions of the stock hydrazine solution is added, the nitrite peak will appear lower than the nitrate peak. When the proper concentration of hydrazine sulfate has been attained, no further adjustment is necessary.
5. Stock sodium hydroxide. Cautiously dissolve 130 g of 50% w/w sodium hydroxide in about 800 mL of distilled water. Cool and dilute to 1000 mL.
6. Working sodium hydroxide. Pipet 100 mL of stock sodium hydroxide [5] into 800 mL of distilled water. Dilute to one liter. Add 2 mL of Brij-35 and mix well.
7. Stock potassium nitrate. Dry about 1 g of potassium nitrate in an oven at 100 – 150 ˚C for 2 hours. Desiccate until cool. Dissolve 0.7218 g of dried and dessicated KNO_3 in about 800 mL of distilled water. Dilute to one liter, add 1 mL of chloroform to preserve the standard. Stability: six months.*
8. Working standards. Pipette the stock standard into a 100 mL volumetric flask. Dilute to volume with distilled water. Prepare the working standards daily.

mL of stock	mg NO_3 – N
0.5	0.5
1.0	1.0
2.0	2.0

*As an alternative a certified nitrate standard can be purchased from a recognized organization for the preparation and certification of QA/QC materials. Dilutions should be made accordingly to cover the range of concentrations expected in the samples.

4.2. Equipment

BRAN+LUEBBE Ion Analyzer equipped with 520nm and 660nm filters.

5. Calibration and standardization

1. Prepare a series of at least three standards for the Standard Curve covering the desired range, and a blank by diluting suitable volumes of standard stock solution with distilled, deionized water as specified in the previous section.
2. Allow the colorimeter to warm up for 30 minutes.
3. Set up Manifold and flow system as shown in Figure 1.
4. Place appropriate Standards Curve in sampler in order of decreasing concentration and perform the Instrument Calibration.
5. After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Check Point (**CP**) sample. If measurements exceed ± 10% of the established (**CP**) value, the analysis should de terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the (**CP**) is recommended as a continuing calibration check.

6. Instrument calibration

The equipment will be calibrated prior to the analysis of each sample batch, after each major equipment disruption, and whenever on-going calibration checks do not meet recommended control limit criteria. All calibration standards will be traceable to a recognized organization for the preparation and certification of QA/QC materials. Calibration curves will be established for each element and batch analysis from a calibration (analytical) blank and a minimum of three analytical standards of increasing concentration, covering the range of expected sample concentrations. The calibration curve will be well-characterized and will be established prior to the analysis of the samples. Only data which results from quantification within the demonstrated working calibration range may be reported by the laboratory. Samples outside the calibration range will be diluted or concentrated as appropriate and reanalyzed.

7. Quality assurance and quality control checks

Certified Reference Materials (CRMs) of the analyte of interest will be used to assess accuracy of a given analysis. At least one CRM fortified (spike) sample will be analyzed with each batch of 20 or fewer samples. Laboratory accuracy will be expressed in terms of percent recovery of the spiked sample. An accuracy goal of 80 – 120% must be met at all times. A CRM of the analyte of interest will also be used as an internal standard. The latter, which constitutes an analytical check point will be run 1 out of every ten samples. A deviation of more than 10% from the actual concentration would require establishment of a new calibration curve. All samples read between the before to last – and the last check point should be reanalyzed. Compliance with performing criteria (accuracy) will be checked prior to proceeding with the analysis. For every 20 samples

analyzed triplicate analyses of an unknown sample will be performed to determine analytical precision. The acceptance criteria will be ≤ 20%RSD. If the laboratory fails to comply with either the precision or accuracy criteria the data for the entire batch will be considered suspect. Calculations and instrument will be checked, the CRM will be reanalyzed to confirm the results. If values are still outside the control limits in the repeat analysis, the laboratory is required to determine and correct the source of the problem and repeat the analysis until control limits are met. A field blank (trip blank) and a laboratory blank will be analyzed with every batch of 20 samples or less. The laboratory reagent blank (i.e. method blank) will be used to assess laboratory contamination during all stages of sample preparation and analysis. The reagent blank will be processed in a manner identical to the samples. A reagent blank concentration between the MDL and 3 times the MDL will require corrective action to identify and eliminate the source(s) of contamination before proceeding with sample analysis.

8. Colorimetric Analysis

1. Check the level of all reagents containers to ensure an adequate supply.
2. Flush all lines with 0.1N Hydrochloric Acid for 30 minutes to clean the system. Rinse for 30 minutes with distilled, deionized water.
3. Place all reagent lines in their respective containers, start the proportioning pump, and allow the system to equilibrate.
4. Obtain a stable baseline with all reagents feeding distilled water through the sample line and start the sampler.

9. Calculations

Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve**.**

10. References

- Bran Luebbe. Autoanalyzer applications. Method No US-696F-82W.
- EPA method 353.1. www.nemi.gov

Figure 1.

AGRICULTURAL EXPERIMENTAL STATION
SOIL AND WATER QUALITY LABORATORY
Standard Operating Procedure For Nitrate
Analisis in Water-SOP#AES-021
(Based on EPA Method No 353.1
BRAN+LUEBBE
GRN/GRN (2.00) DISTILLED WATER
WHT/WHT (0.60) FROM F/C
BLK/BLK (0.32) AIR
WHT/WHT (0.60) SAMPLE
GRY/GYR (1.0) NaOH
ORN/WHT (0.23) SAMPLE
BLK/BLK (0.32) REDUCTOR SOLUTION
ORG/GRN (0.10) DISTILLED WATER
ORN/GRN (0.10) DISTILLED WATER
BLK/BLK (0.32) COLOR REAGENT
PT-27
TO F/C PUMP TUBE
WASTE
COLORIMETER
15 X 1.5mm ID
190-516-01
520nm
SAMPLER IV

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
COLLEGE OF AGRICULTURAL SCIENCES

AGRICULTURAL EXPERIMENT STATION
SOIL AND WATER CHEMISTRY LABORATORY
STANDARD OPERATIONAL PROCEDURES FOR

APPENDIX 2. STREAM WATER SAMPLING FOR NUTRIENTS AND MICROBIAL INDICATORS IN WATER
SOP #019W

1. Introduction

The way that samples are collected has important implications in the quality of the data. Thus, it is necessary that detailed sampling procedures be followed, that the equipment be in good working order and all materials used have been cleaned adequately. Sample containers must be free from contaminants and appropriate to the sample suite collected in them. The sampling technique must assure that samples are not contaminated during collection and processing. The following sections describe sampling conditions, equipment and norm of safety necessary for guaranteed good work.

2. Selection of sampling site

All sampling sites should be selected *a priori* prior to initiating any sampling activity. Details of the methodology used to select the sampling sites are in the Project QAPP. If previous sampling sites can be reactivated and used in the current project, they should be considered. Historical water quality data from these previous sites can provide useful data to the current data-collection effort. If new sampling sites are selected, the following factors should be considered:

- Maximum accessibility and safety
- Be sure that the site provides the information desired
- Make certain the site is far enough downstream from tributary inflow
- Locate at point of maximum turbulence

Any change in the location of pre-selected sampling stations should be made in the Project QAPP and submitted for approval prior to the use of a new sampling location. Changing of site locations will only be allowed under the following conditions:

- Lack of access to predetermined site
- Unsafe conditions
- Agreement of professional staff

The basic steps in establishing a new site include locating and describing the sampling site in the data records or database by physical positioning, determining site coordinates (latitude and longitude), and photographing the sampling site.

Sampling site coordinates in degrees, minutes, seconds, and fractions of seconds of latitude and longitude should be determined as accurately as practically feasible. Ways to determine coordinates, from least to most expensive, include: using topographic quadrangles, professional land surveying, and digitizing from maps using geographic information system (GIS) technology and/or using portable GPS devices. Once the site location has been established, a temporary marker (such as flag or pole) may be used.

3. Collection of water samples

The quality of water in streams and rivers commonly is determined by selected chemical, microbiological, or physical analysis of water samples collected to represent the water body.

3.1. Type of sampling.

Samples may consist of grab samples collected manually or storm events collected manually after the peak discharge has passed and is safe to collect the samples.

3.2. Guidelines for the sampling methods

Grab samples will be taken in the center of the channel of flow were the velocity is highest and the possibility that solids have settled is minimum. In order to avoid and excess of floating materials, the mouth of the collecting container should be placed a few inches below the water surface.

3.2.1. Grab sampling method

This method is developed in 3 steps:

Step 1. Prepare for sampling

1. Upon arrival at the field site, set out safety equipment such as traffic cones and signs. Park vehicle in a location and direction so as to prevent sample contamination from surrounding vehicle emissions.
2. Assemble sampling equipment and set up a clean work space.

Step 2. Collect sample

1. Put on disposable, powder-less gloves.
2. Select bottle to be used for sampling at the particular site and fill out any other missing information required in the label.
3. Wade to sampling location and face into the current. You will fill the sample bottle upstream of your body.
4. Open the sample bottle without touching the inside of the bottle cap; fill the bottle with a small amount of water to be sampled, cap, shake and discard the water downstream of your body. Rinse the bottle in this way three times. To collect the sample, plunge the rinse bottle mouth down into the stream and then tip the bottle into the current. Cap quickly and return to the shore-line or shore-bank to preserve the sample as necessary.
5. Take care not to disturb the streambed by bumping the sampler on it; bed material may enter the nozzle, resulting in erroneous data.
    - Do not overfill the sampler container. Overfilling results in a sample that is not isokinetic and that could be enriched with heavy particulates. This enrichment will result in an artificially increased sediment concentration and will bias particle-size distribution toward heavier and larger particulates.
6. Inspect each subsample as it is collected, looking for overfilling or under-filling of the sampler container and (or) the presence of anomalously large amounts of particulates that might have been captured because of excessive streambed disturbance during sample collection. **If you note any of these conditions, discard the sample, making sure there are no residual particulates left in the container, and resample.**
7. If a portion of the water sample will be analyzed for dissolved constituents (dissolved P or NH_4^+-N and NO_3^-) samples must be filtered in situ and then acidified with a few drops of concentrated H_2SO_4.

8. If the water sample will be analyzed for total nutrients, samples must be acidified with a few drops of concentrated H_2SO_4 (no filtering is needed).
9. Record all field observations and any deviations from standard sampling procedures after the samples are collected: Record start time and gage height (if applicable).

Step 3. Clean Equipment

Please refer to section 5.

3.3. Techniques to prevent sample contamination.

Contamination of water samples can be prevented by planning the order in which sites will be sampled and by recognizing potential sources of contamination. The most common causes of sample contamination during sample collection include poor sample-handling techniques, atmospheric input, inadequately cleaned equipment, and use of equipment constructed of materials inappropriate for the analyses targeted for study.

3.4. Cleaning sampling procedures.

Clean sampling procedures (sometimes called the parts per billion or ppb protocol) involve:

1. Using equipment that is constructed of non-contaminating materials and that has been cleaned rigorously before fieldwork and between field sites (section 5).
2. Handling equipment in a manner that minimizes the chance of altering ambient sample composition.
3. Handling samples in a manner that prevents contamination.
4. Routinely collecting quality control (QC) samples.

Clean Hands/Dirty Hands (*CH/DH*) techniques separate field duties and dedicate one individual (designated as Clean Hands) to tasks related to direct contact with the sample. Clean sampling procedures, including *CH/DH* techniques, were developed for collecting (and processing) samples vulnerable to contamination.

Clean Hands/Dirty Hands techniques for water quality sampling.

- Clean Hands/Dirty Hands techniques require two or more people working together.
- At the field site, one person is designated as Clean Hands (*CH*) and a second person as Dirty Hands (*DH*). Although specific tasks are assigned at the start to *CH* or *DH*, some tasks overlap and can be handled by either, as long as the prescribed care is taken to prevent contaminating the sample.
- Both *CH* and *DH* wear appropriate disposable, powderless gloves during the entire sampling operation and change gloves frequently, usually with each change in task. (Wearing multiple layers of gloves allows rapid glove changes). Gloves must be appropriate to withstand any acid, solvent, or other chemical substance that will be used or contacted.
- *CH* takes care of all operations involving equipment that contacts the sample; for example, *CH*
    - Handles the surface water sampler bottle.

  - Prepares a clean work space.
  - Works exclusively inside space work during collection/processing and preservation.
  - Sets up field-cleaning equipment and cleans equipment.
- *DH* takes care of all operations involving contact with potential sources of contamination; for example, *DH*
  - Works exclusively exterior to processing and preservation space.
  - Operates sampling equipment.
  - Operates cranes, tripods, vehicles, or other support equipment.
  - Handles stream gaging or water level equipment.
  - Sets up and calibrates field-measurement instruments.
  - Measures and records water levels and field measurements.

4. Selection of equipment for sampling.

Selection of equipment for collecting or processing water samples depends of data quality requirements, study objectives, and site conditions for water quality investigations. Criteria for selecting equipment for water sampling depend on:

1. The mechanical constraints of the equipment to perform adequately under given environmental conditions.
2. The adequacy of equipment operation to obtain water quality samples that represent the environmental conditions of the sample source.
3. The adequacy of the equipment materials and construction to maintain sample integrity and not to be a source of leaching and sorption of target analyses.

The following materials, equipment and reagents may be used

- YSI Model Professional Pro Plus
- Flow Probe Hand-held flow meter (or equivalent)
- Marking tape (tagline)
- Whirl-Pak® polyethylene bags.
- Nalgene 1L bottles
- pH buffer solution (4.0, 7.0, and 10)
- Conductivity Calibration Solution 1,000 µ/cm (or equivalent)
- HCl solutions
- Preservatives solutions
- Soap free-phosphorus
- Distilled and/or deionized water
- Field book
- Documentation necessary (Chain custody, site description, maps, etc…)
- Photographic camera
- First aid kit

5. Cleaning of materials for sampling

Procedures to remove contaminants to concentrations below the targeted method-detection levels can vary, depending on the cleaning supplies used, the type of equipment being cleaned, the solubility and concentration of contaminant(s), and the length of time

equipment is exposed to contaminant(s). The level of cleaning with extensive soaking and rinsing is dependent on the analytical precision level required in the sampling plan.

All sampling equipment will be made of non-contaminating materials and will be pre-cleaned and protectively packaged prior to entering the field.

5.1. General preparation of sampling containers.

Sample collection equipment and samples will only be handled by personnel wearing non-contaminating polyethylene gloves. Containers (plastic bottles) for water samples will be properly cleaned before contacting the sample.

5.2. Sampling bottle cleaning procedure for laboratory

- Wash each sample bottle or piece of glassware with a brush and phosphate-free detergent.
- Rinse three times with distilled water.
- Rinse with 10 percent hydrochloric acid by 10 minutes.
- Rinse three times with deionized water.

5.3. Field rinsing of bottles.

- Put on disposable, powderless gloves.
- Fill sample bottle about 1/10 full of rinse water. Cap bottle.
- Shake the bottle vigorously to rinse all interior surfaces.
- Discard rinse water by swirling the solution out of the bottle.

6. General preparation of sampling equipment.

Equipment should be cleaned periodically in the workplace laboratory, where complete disassembly is more practical and more thorough procedures are possible. Compared to cleaning at the field site, cleaning procedures carried out in the workplace laboratory involve longer exposure of equipment to cleaning solutions, more frequent changes of cleaning solution, and greater volumes of water.

The goal of equipment cleaning and decontamination is the removal from new equipment of residues from construction and machining and the removal of substances adhering to equipment from previous exposure to environmental and other media. Equipment used for sampling (sample collection, processing, and handling) must be cleaned before being used. To avoid cross-contamination, all equipment used in sample handling will be thoroughly cleaned before processing any sample or portion thereof.

NOTE: Waste generated during the cleaning procedure will be properly collected and transported to the lab for safe disposal.

6.1. Cleaning procedure of sampling equipment in laboratory.

- Prepare a contaminant-free space for cleaning and drying of the equipment.
- Clean the items used to clean the equipment.
    - Fill washbasins and (or) standpipes with the free-phosphate detergent solution. Put wash bottles, scrub brushes, and other small items used for cleaning into a washbasin (**Soak for 30 minutes).**
    - Rinse all items thoroughly with water to remove detergent residue.

    - Rinse with distilled water.
- Pour 10% HCl solution into washbasins, standpipes, and wash bottles. Soak for 15 minutes. **Do not** soak items with metal parts (exposed or hidden) in an acid solution.
- Disassemble sample-collection and sample-processing equipment.
- Place small equipment parts into washbasin labeled for detergent and fill with a 2 percent solution of free-phosphate laboratory detergent. The amount of detergent depends on the hardness of the tap water and the degree to which the equipment is dirty or contaminated.
- Soak equipment and tubing for 30 minutes: fill tubing with solution and keep submerged.
- Scrub exterior and interior of equipment surfaces to the extent possible, using a firm sponge or soft brush to remove any adhering material such as oil and grease, sediment, algae, and chemical deposits.
- Rinse equipment thoroughly with water to remove detergent residue.
- Rinse three times with deionized water.

6.2. Field rinsing of equipment.

- Rinse equipment and tubing with deionized water.
    - Rinse equipment with deionized water directly after contact with sample water and before the equipment have chance to dry.
    - Pay particular attention to removing material from grooves and crevices, O-rings, nozzles, and places where materials might be trapped.
    - Proceed to field detergent-wash option only for metal equipment components or for equipment that has become excessively contaminated.
- Rinse equipment and tubing with detergent. A field detergent wash is used for between-site cleaning of metal components of equipment, or for equipment that has become greasy or otherwise coated and requires detergent to remove foreign materials.
    - Place small equipment, tubing, and parts into basin labeled “detergent” and fill with a 0.2 percent detergent solution. Soak for about 10 minutes.
    - Scrub equipment surfaces with a firm sponge or soft brush to remove any adhering material such as oil and grease, sediment, algae, or chemical deposits. Pay particular attention to grooves and crevices, O-rings, nozzles, and other places where inorganic materials might be trapped.
    - Rinse equipment thoroughly with water to remove detergent residue. If nonmetal equipment has been detergent-washed, go to following step.
    - Rinse with 10% HCl solution to remove any remaining organic films and inorganic deposits.
    - Rinse equipment with deionized water.

7. Laboratory and field record

Sample collection in the field requires adequate documentation for quality assurance and control. The field record sampling station file may contain detailed written notes describing how samples were taken, field measurements, previous laboratory analyses, maps, photographs and correspondence.

7.1. Record Keeping

A designated person will be responsible for recording data in a waterproof field logbook. The field logbook may include:

- Data and time of start of sampling
- Name of personnel
- Location of station (latitude and longitude)
- Station description
- Field observations (weather, water conditions)
- Stations depth
- Station width
- Water velocity
- Number of grabs necessary and amount sampled
- Salinity, temperature, and pH
- Turbidity, and light penetration (PAR)
- Record all measured field parameters and their respective values
- Preservation methods
- Visual characteristics (accessibility, calibration results, divergence from protocols, safety hazard encountered, photographs, etc)
- Calibration equipment results

7.2. Chain of custody procedures.

A chain of records form will accompany every sample. Each person releasing a sample will sign and date the form and get the receiver's signature, with date and time, keeping one copy and giving one copy to the receiver. Chain of records documents will be maintained for each station.

A chain of custody sheet is initiated *in situ* and continued upon return to the laboratory (See Appendix 3).

7.2.1. Chain of custody for sample collection.

Attach chain of custody record tag to the sample container when the complete sample is collected. Ensure the container has the following information:

- Sample number
- Type sample
- Time take
- Date take
- Source of sample
- Preservative used
- Analyses required

- Volume of each sample
- Sample location and field measurements
- Other pertinent observations
- Name of person taking sample

Once the survey is complete, field logs will be retained by the project leader or his designated representative, as a part of the permanent record. This person is responsible for the care and custody of the collected samples until they are properly dispatched to the receiving laboratory.

NOTE: In case of visible water pollution or changes in water conditions, photographs (that include time, date, and site location) are taken, and the observations documented by writing. These photographs can be used as evidence, and are handled by chain of custody procedures to prevent alteration.

7.2.2. Laboratory custody procedures.

The laboratory shall set aside a sample storage security area. This should be a clean, dry, isolated space with sufficient refrigerator space that can be securely locked from the outside. Samples should be handled by the minimum possible number of person.

In the laboratory a person (custodian) must be designated to receive the samples. Only the custodian will distribute samples to personnel who are to perform analyses. A sample registration form and a sample analysis identification form will be filled out every time a sample batch is received for analysis (Appendix3). These forms will be completed by the AES laboratory manager and signed and accepted by both, the staff member handing the samples and the laboratory manager. The forms will contain all pertinent information necessary for the laboratory to process the samples. The analyst records in his laboratory notebook or analytical worksheet, identifying information describing the sample, the procedures performed, and the results of the testing. The notes shall be retained as a permanent record in the laboratory.

Once the sample analyses are completed, the unused portion of the sample, together with identifying label and other documentation, must be returned to the custodian.

8. Label or identification of sample

Labels will be fastened to outside each sample container. No jars will contain handwritten labels. As an extra check on proper labeling, all jars will be pre-labeled before samples are aliquoted. Labels will contain the following information:

- Project name
- Station number
- Station name
- Replicate number
- Date sampled
- Type of sample collected (grab or storm)
- Sequence number
- Leg sampled (sampling trip number)

8. Field quality control

Quality control validates whether what is being done is that required by the quality assurance program. Quality control describes those activities and procedures used to produce consistent and reliable data. Quality control checks are used to insure accuracy, precision, representativeness, comparability, and completeness of the data.

Field procedures used to assure Quality Control include:

- Quality Control blanks (lab blank, field blank).
- Field instrument standards and sample preservation standards (pH control, preservative volumes) are also used for Quality Control.
- Key aspects of quality control associated with sample collection are: field personnel will be thoroughly trained in the proper use of sample collection gear, sampling material that come in contact with the sample will be made of non-contaminating materials and will be thoroughly cleaned between sampling stations, sample containers will be of the recommended type and will be free of contaminants, preservation of holding times will be followed.

8.1. Field blank

The primary purpose of a blank sample is to identify potential sources of sample contamination and assess the magnitude of contamination with respect to concentration of target analyses. Field blanks are collected and processed at the field site in the same manner and using the same equipment as the environmental sample(s).

9. Field measurements

Field measurements should represent, as closely as possible, the natural condition of the surface water system at the time of sampling. The parameters measured in field are:

- Temperature, pH, dissolved oxygen and electrical conductivity measurements are taken *in situ* at midchannel (at a depth of 15 cm from the water surface), with an YSI-Professional Plus meter (Yellow Springs Instruments). Prior to sampling, the pre-calibrated instrument settings must be corroborated using buffers and standard solutions. The sampling team may carry an alternate equipment unit in case the main unit malfunctions.
- Water velocity is measured with a Flow Probe Hand-held flow meter (Forestry-Suppliers, Inc.), or equivalent, at each of the selected stream cross-sections. The stream at the sampling point must be inspected from bank to bank, observing water velocity, width, depth distribution, accumulation of sediments and other debris.
- Stream depth (water column depth), and stream width is measured with a marking tape (tagline) at selected increments depending on observed discharge, and channel conditions. Homogeneity of the bottom channel is corroborated by measuring the water column depth at selected intervals within the channel. Usually three to five increments are selected. A diagram is drawn in the field data sheet describing the cross section. The flowmeter is inserted at middepth of each of the width increments of the water column and slowly moved vertically for a period of two minutes. An average reading corresponding to the time the instrument is left *in situ* was recorded.

Stream flow for each of the increments is calculated from stream area (increment width x depth) and water velocity. Total stream flow ($ft^3\ s^{-1}$) is the weighted average of flow at each of the stream increments.

10. Equipment

Equipment used by physical measurements are expensive and relatively delicate and must be treated with care to produce the desired quality of data

10.1. YSI model Professional Plus

The YSI Model Professional Pro-Plus is a rugged, microprocessor based, digital meter with an attached sensors for dissolved oxygen, pH, temperature, specific conductance, oxidation-reduction. The multi-parameter probe is designed for use in field, lab, and process control applications as well as for environmental uses.

10.1.1. Cleaning

The single most important requirement for accurate and reproducible results in conductivity measurement is a clean cell. A dirty cell will change the conductivity of a solution by contaminating it.

NOTE: Always rinse the conductivity cell with clean water after each use.

To clean the conductivity cell:

- Dip the cell in cleaning solution and agitate for two to three minutes. Any one of the foaming acid tile cleaners, such as Dow Chemical Bathroom Cleaner, will clean the cell adequately. When a stronger cleaning preparation is required, use a solution of 1:1 isopropyl alcohol and 10N HCl. Remove the cell from the cleaning solution.
- Use the nylon brush (supplied) to dislodge any contaminants from inside the electrode chamber.
- Repeat steps one and two until the cell is completely clean. Rinse the cell thoroughly in deionized water.
- Store the conductivity cell in the meter storage chamber.

For dissolved oxygen measurement the cleaning of the electrodes are important for error reduction. Membrane life depends on usage. Membranes will last a long time if installed properly and treated with care.

To clean the dissolved oxygen cell:

- To clean the anode, remove the membrane and soak the probe overnight in 3% ammonium hydroxide.
- Rinse the sensor tip with deionized water.
- Add new KCl solution.
- Install a new membrane. Turn the instrument on and allow the system to stabilize for at least 30 minutes.

- To keep the electrolyte from drying out, store the probe in the calibration chamber with the small piece of sponge.

10.1.2. Calibration.

- **Calibration of electrical conductivity sensor**. Follow instructions of the User's manual or Quick Start guide. Calibration must be performed the within 24 hour of any sampling trip.
- **Calibration of pH sensor**. Follow instructions of the User's manual or Quick Start guide.
- **Calibraton of temperature sensor**. Follow instructions of the User's manual or Quick Start guide.
- **Calibration of oxidation-reduction sensor**. Follow instructions of the User's manual or Quick Start guide.
- **Calibration of dissolved oxygen.** Follow instructions of the User's manual or Quick Start guide. Calibration must be performed the within 24 hour of any sampling trip.

Each time the instrument is turned off, it may be necessary to re-calibrate before taking measurements. All calibrations should be completed at a temperature which is as close as possible to the sample temperature. Dissolved oxygen readings are only as good as the calibration.

11. Storage and sample preservatives

Sample preservation should be performed immediately upon sample collection (see QAPP for details). Samples will be filtered within 24-hr of collection. The filtrate will be transferred to acid-washed Nalgene bottles (Nalgene Co. Rochester, NY) and immediately stored frozen. Unfiltered samples will be stored frozen in the same bottles collected in the field.

12. Laboratory measurements

In the laboratory to be determined of following parameters:

- Nitrogen (Total Kjeldahl, nitrate) (see AES-SOP #013w)
- Phosphorus (Total P) (see AES-SOP # 011w)

13. Norms of safety

Fieldwork requires an awareness of potential hazards and knowledge of basic safety procedures. With proper planning and preparation, any recognized hazards should be able to be avoided. Individuals should remain alert to any changes in the surrounding environment throughout the sampling event that may require you to change or modify your sampling strategy.

13.1. General precautions

- Familiarize yourself with the sampling locations. Pay close attention to where you actually will be collecting the sample. Check for stability and slope if sampling from the bank. Avoid areas where obvious dumping of garbage or hazardous materials has occurred.

- Take precautions against poisonous plants, rodents, sudden floods, heat exhaustion, sun exposure, and other environmental conditions that would negatively affect your health and safety (See appendix 3).
- Check the updated weather forecast for your area. Do not attempt to sample when thunderstorms or other severe weather is anticipated. Never enter a stream at high flow.
- Work in pairs whenever possible, especially if you will be sampling in remote locations. Additionally notify others of your itinerary and who to contact in case of an emergency.
- Be sure you have some sort of communication available, or nearby, if in remote areas. If possible, take a cellular telephone or two-way radio.
- Ideally, individuals should have the ability to swim and be familiar with life-saving techniques. Use of flotation devices should be considered if sampling in or near deeper waters.
- It is recommended that some sort of personal protective equipment be available and used during sampling.
- Be particularly careful if you notice any unusual characteristics in or around the water body being sampled, including discoloration, foul odors, fish or animal kills. Avoid contact with oily patches, sheens, discoloration, etc. If you should come in contact with any of these by accident, take immediate action to wash your hands and other affected parts of the body.
- Seek immediate medical attention if you notice any redness, rash, blisters, or irritation developing, especially after contact with anything unusual during the course of sample collection.
- Do not eat, drink, or smoke during the actual sample collection. Be sure your hands are washed before partaking in any of these activities.

13.2. Specific precautions

- Determine the location of the nearest hospital, clinic, or physician beforehand.
- If any preservatives or reagents are added to the sample collection bottles, you must be familiar with what has been added. You should have copies of the latest Material Safety Data Sheets (MSDSs) for those chemicals added. These are available from the Lab or from the chemical manufacturer. These MSDSs will inform you of any hazards and necessary precautionary measures that must be taken when handling these specific chemicals. Wear proper eye protection, if nitric acid (HNO_3) or sulfuric acid (H_2SO_4) is used as a preservative.
- Always consider the possibility that the water being sampled may be contaminated with pathogens or hazardous chemicals. Use caution and extra protection when working in or around water with known or suspected contamination. Sample tags should indicate the level of contamination so the laboratory can handle the sample appropriately.
- The temperature ideal comfort range for humans is between 10 to 32 ºC (60 to 90 ºF). If the temperature in the environmental or water exceed this range, it can happen Hypothermia and hyperthermia (See appendix 4).

- Receive prior training in first aid and cardiopulmonary resuscitation (CPR). This training is available in most cities from the Red Cross.

14. References

Lane, S.L., Flanagan, Sarah, and Wilde, F.D., March 2003, Selection of equipment for water sampling (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A2. Accessed August 2005. http://pubs.water.usgs.gov/twri9A2/

Lurry, D.L. and Kolbe C.M. 2000. Interagency Field Manual for the Collection of Water-Quality Data. U.S. Geological Survey in cooperation with the U.S. Environmental Protection Agency. Open-File Report 00–213. Austin, Texas.

OHIO – EPA, Division of surface water. General Safety and Health Precautions for the Collection of Water Samples from Rivers, Streams, and Creeks. Revised July 28 of 2005. [Online]. Available http://www.epa.state.oh.us/dsw/nps/HSAP_Checklist.html

Surface Water Quality Bureau, New Mexico Environment Department. 2004. Standard operating procedures for sample collection and handling. Revised July 29 of 2005. [Online] Available www.nmenv.state.nm.vs/swqb/SOP/standaroperatingprocedures_06-22-04.pdf

Surface Water Quality Bureau. New Mexico Environment Department. 2004. Standard operating procedures for sample collection and handling. Arizona Water Resources Research Center, 1995, p. 10–13.

Wilde, F.D., ed., April 2004, Cleaning of Equipment for water sampling (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A3. Accessed. August 2005. http://pubs.water.usgs.gov/twri9A3/

Wilde, F.D., January 2005, Preparations for water sampling: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A1. Accessed August 2005. http://pubs.water.usgs.gov/twri9A1/

Wilde, F.D., Radtke, D.B., eds., chapter sections variously dated, Field measurements: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6. Accessed August 2005. http://pubs.water.usgs.gov/twri9A6/

Wilde, F.D., Radtke, D.B., Gibs, Jacob, and Iwatsubo, R.T., eds., April 2004, Processing of water samples (version 2.1): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A5. Accessed August 2005. http://pubs.water.usgs.gov/twri9A5/

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AGRICULTURAL EXPERIMENT STATION
SOIL AND WATER CHEMISTRY LABORATORY
STANDARD OPERATIONAL PROCEDURES FOR

APPENDIX 3. CHAIN OF CUSTODY AND FIELD DATA FORMS

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CHAIN OF CUSTODY FOR SAMPLE COLLECTON

SAMPLING SITE DESCRIPTION	**SAMPLE DESCRIPTION**
Project: _____	Sample Number: _____
Sampling site: _____	Type sample: _____
Station number: _____	Time sample: _____
Station location: Latitude _____	Source sample: _____
Longitude _____	Preservative used: _____
Date (dd-mm-yy): _____	Volume sampled (mL): _____
Sampler's (name): _____	Quantity sample (# bottles or bag): _____
_____	Codification: _____
FIELD MEASUREMENT (Add diagrams in the Sheet 2)	**EQUIPMENT CALIBRATION**
	pH: 4.0_____ 7.0 _____ 10.0 _____
	Slope _____
Temperature: _____	Electrical conductivity: _____
Electrical Conductivity: _____	Dissolved oxygen: _____
pH: _____	**ANALYSIS REQUIRED**
Dissolved oxygen: _____	_____
Water velocity: _____	_____
Stream depth: _____	
Stream width: _____	
OBSERVATION:	

Sampler's (Signature): _____ Sheet ____ to _____

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LABORATORY CUSTODY PROCEDURES

SAMPLING SITE DESCRIPTION	**LABORATORY DESCRIPTION**
Project: _____	Laboratory name: _____
Sampling Site: _____	Received at laboratory by: _____
Source sample: _____	Date received: _____
Date sampling (dd-mm-yy): _____	Time received: _____
Type sample: _____	Number sample received: _____
Codification: _____	Temperature received: _____
Preservative used: _____	Sampler's (name): _____
	_____

ANALYSIS REQUIRED

Nitrogen (Total Kjeldahl, nitrate)	_____	Total organic carbon	_____
Phosphorus (Total P, dissolved P)	_____	Metals	_____
Chlorophyll a	_____	Total coliforms	_____
Total Suspended solids	_____	Other	_____

OBSERVATION:

Sampler's (signature): _____
Responsible of laboratory (signature) _____

Sheet ___ to _____

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AGRICULTURAL EXPERIMENT STATION (AES)
SOIL AND WATER CHEMISTRY LABORATORY
STANDARD OPERATIONAL PROCEDURES FOR

APPENDIX 4. AES LABORATORY SAMPLE DOCUMENTATION FORMS

Agricultural Experiment Station/ Soil and Water Chemistry Laboratory **Sample Registration Form**					
Date		Date		Date	
Hour		Hour		Hour	
Source of origin		Source of origin		Source of origin	
Project		Project		Project	
Sampling Date		Sampling Date		Sampling Date	
Temperature		Temperature		Temperature	
Number of Samples		Number of Samples		Number of Samples	
Sample Type		Sample Type		Sample Type	
Handed by		Handed by		Handed by	
Received by		Received by		Received by	
Commentaries and Observations		Commentaries and Observations		Commentaries and Observations	
Date		Date		Date	
Hour		Hour		Hour	
Source of origin		Source of origin		Source of origin	
Project		Project		Project	
Sampling Date		Sampling Date		Sampling Date	
Temperature		Temperature		Temperature	
Number of Samples		Number of Samples		Number of Samples	
Sample Type		Sample Type		Sample Type	
Handed by		Handed by		Handed by	
Received by		Received by		Received by	
Commentaries and Observations				Commentaries and Observations	

Agricultural Experiment Station Soil and Water Chemistry Laboratory								
Laboratory Sample Set ID number					Date			
General Sample Information								
Project:								
Sample Type:								
Source of Origin								
Sampling Date:								
Number of Samples:								
Date Received								
Additional Information								
Analyses to be Performed								
Water Samples					Soil Samples			
pH		CE			pH		CE	
DOC		TP			DOC		Olsen	
DP		TKN			Mechlich		Bray	
CHLa		TS			CaCL2		Exch.	
NH4		NO3			Others:			
Others:					Commentaries and observations			
Commentaries and observations								
Individual Sample Identification								
Commentaries and observations								

Approved by _____ Date_____

ANALYTICAL REPORT

Agricultural Experiment Station
Soil and Water Chemistry
Laboratory

Project:		**Analysis:**	
Experiment:		**Method:**	
Sampling date:		**Units:**	
Sample Type:		**Report Date:**	
Sample Number:		**Analyzed by:**	
Source of Origin:		**Approved by:**	
Sample	**Result**	**Sample**	**Result**
Commentaries/Observations:			
Spike/% Recovery		**Method Blank**	
Check Point		**System Blank**	
Precision		**System Standard (_____)**	

AGRICULTURAL EXPERIMENTAL STATION
SOIL AND WATER QUALITY LABORATORY

APPENDIX 5. STANDARD OPERATING PROCEDURES FOR MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS (ENTEROCOCCI ENUMERATION *AND BACTEROIDALES* HUMAN SPECIFIC MARKER HF183– SOP #024W

1. Enterococci enumeration

1.1. Cleaning of bottles to be used for sampling

1. Wash each sample bottle and cap with a brush and phosphate-free detergent.
2. Rinse three times with distilled water.
3. Immerse bottles for 10 min in 10% HCL.
4. Rinse bottles three times with deionized water.
5. Leave bottles to dry and cap after drying.
6. Seal bottles across the cap and the bottle with sterile indicator tape.
7. Autoclave bottles (American Sterilizer Corp) at 121^{o}C, 15 psi for 15 min. Do not remove "sterile" indicator tape after autoclave.
8. After autoclave, place bottles in plastic box or container for storage.

1.2. Grab sampling[1]

1.2.1 Laboratory

1. Identify bottles to be used for sampling during the incursion.
2. Adhere identification labels on bottles.
3. Fill out labels with all of the required information.

1.2.2. Field

10. Put on disposable, powder-less gloves.
11. Select bottle to be used for sampling at the particular site and fill out any other missing information required in the label.
12. Remove cap and immerse the bottle in the stream to a depth of 6 to 8 inches; fill the sample bottle about one-quarter full, cap bottle, shake gently.
13. Discard rinse water by swirling the solution out of the bottle.
14. Repeat the procedure (steps 3 and 4)
15. Remove cap and immerse the bottle in the stream to a depth of 6 to 8 inches, fill the sample bottle to the top and cap.
16. Place bottles in a cooler with ice, shielding from direct sunlight.
17. Transfer samples to UPRM-BNF laboratory
18. Process samples for fecal indicator bacteria within the allowed time limit. 6 hours is allowed for samples to reach the lab with an additional 2 hours is allowed for the analysis. The analysis must be <u>completed within 8 hours of collection</u>.

[1] See Appendix 2 (APPENDIX 2. STREAM WATER SAMPLING FOR NUTRIENTS AND MICROBIAL INDICATORS IN WATER, SOP #019W), Section 3.0. for details.

1.3. Storm sampling

1. Put on disposable, powder-less gloves.
2. Remove each bottle from the sampling rack and cap
3. Identify each bottle with all of the necessary information on the label.
4. Transfer samples to UPRM-BNF laboratory

1.4. Analysis of fecal enterococci[2]

1. Permit water sample in each sample bottle to reach room temperature
2. Transfer a 100 mL water-sample aliquot to sterile 100-mL manufacturer supplied polystyrene bottle; this will be the undiluted sample
3. Transfer 10 mL of water sample to a 90 mL sterile dilution tube (this will be the diluted samples and must be labeled as 10^{-1} D); if further dilution is needed the 10-1 D sample must be used and the procedure repeated.
4. Transfer the 10-1 D sample to 120-mL polystyrene bottles
5. Mix each sample with manufacturer-supplied growth medium until dissolved.
6. Pour the contents of each bottle into sterile Quanti-Tray® panel containing 97 wells and heat-seal.
7. Incubate Quanti-Tray® panels for fecal enterococci enumeration at 41 ± 0.5°C for 24 to 28 hours after sealing.
8. Determine the presence of fecal enterococci wells by detection of fluorescence with UV light at 365 nm.
9. Use a manufacturer-supplied table to convert the number of positive wells to most probable number (MPN) values. As needed use the proper dilution used in each subsample to quantify final MPN values.

2. *Bacteroidales* human specific marker HF183

2.1. Cleaning of bottles to be used for sampling – Follow procedures as in section 1.1 of this appendix.

2.2. Grab sampling – Follow procedures as in section 1.2 of this appendix.

2.3. Storm sampling – Follow procedures as in section 1.3 of this appendix.

2.4. Sample Processing

1. Each sample will be filtered twice and labeled as A and B.
2. Label tubes (MoBio DNA extraction).
3. Set up vacuum filtration unit. Unit consists of side-arm vacuum flask (500 – 1000 mL) fitted with a filter holder (for 25 mm filters) with capacity for > 100 mL water sample. Filter units must be washed, scrubbed, and well-rinsed prior to use.
4. Rinse filtration unit thoroughly (without filter) with 70% methanol and vacuum dry.

[2] Enumeratation of fecal enterococci with the Enterolert™ system (IDEXX Laboratories, Westbrook, ME).

5. Place filter membrane (nitrocellulose, 0.22-µm-pore-size GSWP, Millipore, Cat. # GSWP04700) on filter unit using flame-sterilized forceps. Do not touch filter with your hands/fingers. Preferably wear gloves.
6. Filter 100 mL of water sample. You may use less water if the sample is cloudy/high in suspended solids and refuses to go thru the filter, but you need to note how much water sample is filtered.
7. Remove filter membrane from unit using flame-sterilized forceps. Using a second set of flame-sterilized forceps, roll filter loosely and place it in labeled tube. The procedure is show in the following video: http://www.mobio.com/water-dna-isolation/powerwater-dna-isolation-kit.html . Try not to place lid of tube down on bench while doing this as you want it to remain sterile.
8. If you are going to reuse filter units, you need to wash, scrub, and rinse them before reusing them.
9. Run a blank extraction using ~100 ml of the purest water available (de-ionized/ distilled water or better quality). The blank should show the absence of human specific marker HF183 - be sure you use a clean/methanol-rinsed filtration unit and sterile forceps!
10. Freeze tubes at -20°C once filtration is finished.
11. Place tubes in a cooler with ice packs and send by overnight courier to GSU.

Notes:

- Aseptic techniques must be followed at all times.
- Manage all samples, glassware and materials in accordance with Good Laboratory Practices.
- Dispose and wash all materials in accord with laboratory SOPs and Good Laboratory Practices.

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APPENDIX 6. STANDARD OPERATING PROCEDURES FOR OPTICAL BRIGHTENERS – SOP #25w

1. Optical brighteners (OB)

1.3. Cleaning of bottles to be used for sampling

9. Wash each sample bottle and cap with a brush and phosphate-free detergent.
10. Rinse three times with distilled water.
11. Immerse bottles for 10 min in 10% HCL.
12. Rinse bottles three times with deionized water.
13. Leave bottles to dry and cap after drying.
14. Seal bottles across the cap and the bottle with sterile indicator tape.
15. Autoclave bottles (American Sterilizer Corp) at 121ºC, 15 psi for 15 min. Do not remove "sterile" indicator tape after autoclave.
16. After autoclave, place bottles in plastic box or container for storage.

1.4. Grab sampling[3]

1.2.1 Laboratory

4. Identify bottles to be used for sampling during the incursion.
5. Adhere identification labels on bottles.
6. Fill out labels with all of the required information.

1.2.2. Field

19. Put on disposable, powder-less gloves.
20. Select bottle to be used for sampling at the particular site and fill out any other missing information required in the label.
21. Remove cap and immerse the bottle in the stream to a depth of 6 to 8 inches; fill the sample bottle about one-quarter full, cap bottle, shake gently.
22. Discard rinse water by swirling the solution out of the bottle.
23. Repeat the procedure (steps 3 and 4)
24. Remove cap and immerse the bottle in the stream to a depth of 6 to 8 inches, fill the sample bottle to the top and cap.
25. Place bottles in a cooler with ice, shielding from direct sunlight.
26. Transfer samples to UPRM-Soil and Water Quality Laboratory (Q-205 and Q-206).

1.3 Sample processing

1. Label 60 mL dark centrifuge tube.
2. Transfer a 50 mL water sample aliquot to a clean 60-mL dark centrifuge tube. Cap and label appropriately.

[3] See Appendix 2 (APPENDIX 2. STREAM WATER SAMPLING FOR NUTRIENTS AND MICROBIAL INDICATORS IN WATER, SOP #019W), Section 3.0. for details.

3. No special preservative is needed. Store in the dark at 4ºC until analysis (up to 15 days after sampling).

1.5. Analysis

1. Measure fluorescence of water samples with Turner Designs Model 10-AU-005 field fluorometer (Turner Designs, Sunnyvale, CA) fitted with filters for excitation (360 nm) and emission (436 nm), or equivalent.
2. To distinguish OB fluorescence from DOM fluorescence, measure fluorescence of sample before and after irradiance of sample with UV light.
    a. Place water samples in borosilicate glass cuvettes (25 mm i.d., 150 mm long).
    b. Irradiate for 60 min using a single UV (365 nm) light containing two, 25-Watt tubes (Model ULV-225D, Ultra Violet Products, Upland, CA).
3. Measure fluorescence as in step #1.
4. The loss of fluorescence (in percent) is used to separate samples with and without OB.

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STANDARD OPERATIONAL PROCEDURES FOR

APPENDIX 7. PLANTS AND ANIMALS PRECAUTIONS

Insects, reptiles and certain plants are always potential hazards for field personnel. Table 1 contains general information on the most common plant and animal hazards encountered by field staff (Lane and Fay 1997; Milne and Milne 1980; and Behler and King 1979). **SPIDERS, SCORPIONS and OTHER INSECTS** (Milne and Milne 1980; USGS 1997)

Table 1. Common and potentially hazardous animals encountered in the field.

Animals	Descriptions/Characteristics	Procedure
Black widow spiders	Female (only one that bites) is black with almost spherical abdomen, usually with red hour glass mark below or with 2 transverse red marks, separated by black. Inhabit fallen branches and under objects.	Take care when reaching into small dark spaces. If bitten by a black widow or brown recluse, seek medical attention as soon as possible. Scorpion stings may not require medical attention.
Brown recluse spiders	Orange-yellow thorax with dark violin pattern. Bases of legs orange-yellow, rest of legs grayish to dark brown. Abdomen grayish to dark brown with no obvious pattern. Frequent areas of human habitation and prefers dark spaces. Found outdoors in sheltered corners, among loose debris; indoors on the floor and behind furniture.	

Scorpions	Lobster-like pincers with a long up curved "tail" that ends in a poisonous stinger. Nocturnal, sensitive to vibrations. Frequent the desert. Not easily seen in the wild. Field boots are a favorite hiding place. Most scorpions are not dangerous and do not attack. Poison of most North American species is not lethal to humans but they do inflict a painful sting.	
Ticks	Small, less than 3 mm (< 1/8 inch). Clamps to host using dart-like anchor located just below the mouth. Wear long pants and tuck pants legs into socks and use a repellent containing the compound DEET (N-diethyl-meta- toluamide)	Check for ticks during and after field work. Remove with tweezers within 24-hours. Wash and disinfect the bite.
Bees	Bees vary in size from 2 mm (0.08 inches) to 4 cm (1.6 inches) long. Locations vary from ground nests to trees and manmade structures.	Avoid bee hives and wasp nests. Scrape off the stinger with a knife or other flat object (i.e., credit card). Wash well with soap and water. A cold pack may be used to reduce swelling. Use an over the counter sting ointment or solution of water and baking soda.

Wasps	Wasps vary in size from minute to 5 cm (2 inches) long. Adults have a narrow waist between the first and second abdominal segments. Locations vary from ground nests to trees and manmade structures.	If a member of a field team is allergic to insect bites or stings this should be made known to the rest of team. That person should carry a sting kit for use in emergencies. Symptoms of an allergic reaction include: pain, swelling of the throat, redness or discoloration in the area of the sting, itching, hives, decreased consciousness, or difficult or noisy breathing.

Table 2. Common and potentially hazardous plants encountered in the field.

PLANTS		
Plants	**Descriptions/Characteristics**	**Procedure**
Poison Ivy	Climbing poison ivy has alternate, trifoliate leaves with aerial roots that grow straight and are fuzzy. Found in most environments. Non-climbing poison ivy lacks aerial roots. The leaves are the same shape as the climbing poison ivy but are larger and broader. Vines without leaves can still cause a case of poison ivy. If a piece of vine is used as fire wood, the oily resins can be released into the air. The resin can also remain on unwashed clothing and equipment.	Do Flood the affected area with lots of cold water as soon as possible. Since the oily resin is only slightly soluble in water, a little water will only spread the poison. Use anti-itch cream. People allergic to poison ivy may require medical attention. Consider using pre-exposure lotion which creates a barrier against poison ivy, oak and sumac oils. Poison oak and ivy cleansers are available that can be used up to 8 hours after exposure. Do Not Use hot water or soap. These help increase the

		affects of poison ivy.

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STANDARD OPERATIONAL PROCEDURES FOR

APPENDIX 8. TEMPERATURE EXPOSURE

The ideal comfort range for humans is between 10 to 32 ºC (60 to 90 ºF). Hypothermia (cold) and hyperthermia (heat) normally occur outside this range.

1. Cold Emergencies: Hypothermia is a condition of reduced body temperature caused by exposure to cold, and aggravated by wet clothes, wind, hunger and exhaustion. Hypothermia can occur with air temperatures above 16 ºC (60 ºF) under wet and/or windy conditions.

• **Warning Signs.** Uncontrollable fits of shivering, incoherence, listlessness, fumbling hands, frequent stumbling, drowsiness, and the inability to get up after resting.
• **Treatment.** Remove person from cold and get to a dry warm place. Replace wet clothes with dry. Warm body slowly. Give warm non-alcoholic drinks. These are temporary measures until medical help is available.
• **Prevention.** The best way to prevent hypothermia is to stay warm and dry. Put on rain gear before it rains. Dress in layers and add more before getting cold. Find shelter before conditions become severe. During colder weather carry a complete change of dry clothes.

1. **Heat Emergencies:** Hyperthermia is caused by increasing body temperature due to exposure to extreme heat. Heat emergencies can be brought about by a combination of factors; physical exertion, clothing (waders), humidity, no breeze, air temperature and the rate of fluid intake. Working in the extreme summer heat creates a very real threat of suffering from some form of heat related stress.
• **Warning Signs.** Chilling, headache, unsteadiness, dizziness, nausea, dry skin (either hot and red (heat stroke) or cool and pale (heat exhaustion), rapid pulse and muscle pain/spasms.
• **Treatment.** General treatment for heat emergencies is cooling down and drinking plenty of fluids. A common symptom of dehydration is a head ache. Heat stroke requires medical attention and is considered to be life threatening.
• **Prevention.** Drink water in moderate amounts on a regular basis-do not wait until you are thirsty. Avoid alcohol, caffeine, and soda—these liquids are not water substitutes. Wear lightweight clothing and a wide-brimmed hat. Schedule activities that require the most exertion during early morning or late afternoon hours. Find some shade and take breaks during the day.

Appendix 9. Routine Analysis of Water and Wastewater for metals by ICP-AVOES. University of Georgia Environmental Services Laboratories.

Appendix 10. YSI Professional Pro Plus Quick Start Guide (as per manufacturer instructions).

THE UNIVERSITY OF GEORGIA
AGRICULTURAL and ENVIRONMENTAL SERVICES LABORATORIES
USDA Cooperative Extension Service
2300/2400 College Station Road
Athens, GA 30602 Telephones: (706) 542-5350 (SPW); 542-9023 (PHW); 542-7690 (FEW)

Standard Operating Procedure

Subject:

Routine Analysis of Water and Wastewater for Metals by ICP-AVOES

Revision 2.0
Effective Date: 2/3/2014

Credits:

Prepared and Revised by:	Jake Mowrer Research Professional III	Signature	2/3/14 Date
Reviewed by:	Jake Mowrer Research Professional III	Signature	2/3/14 Date
Reviewed and Verified at the Bench by:	Dr. Yuangen Yang Quality Assurance Manger	Signature	2/3/2014 Date
Approved by:	Jake Mowrer Program Coordinator	Signature	2/3/14 Date

1.0 SCOPE AND APPLICABILITY

Inductively coupled argon plasma axially viewed optical emission spectrometry (ICP-AVOES) is used to quantitatively determine the concentrations of various dissolved metals in waters and wastewaters. This standard operating procedure (SOP) is consistent with EPA method 200.7 'Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry' and is therefore approved for use in compliance monitoring programs.

2.0 METHOD SUMMARY

Water samples shall be suitably preserved by addition of HNO_3 to pH < 2.0 upon receipt by the lab and filtered prior to analysis when suspended solids appear excessive for passage through the ICP nebulizer. Samples containing more than 1% total solids should be considered for digestion prior to analysis by EPA method 3015A (microwave assisted digestion of aqueous samples).

Analysis of samples begins with introduction of the liquid into the nebulizer/spray chamber where uniform droplets are swept via an argon gas stream into a high temperature plasma torch. The power delivered by a radio frequency field is absorbed by atomic species in the sample inducing an electronic transition to higher orbital. Upon passage through this high energy field, the electrons 'relax' into more stable orbits releasing the previously absorbed energy. Much of this released energy is measurable as light in the ultraviolet to visible range of the electromagnetic spectrum. The wavelength of light emitted corresponds to a specific analyte while the intensity can be calibrated to indicate concentration.

This method is appropriate for the simultaneous analysis of any and all analytes specified in EPA method 200.7.

3.0 DEFINITIONS

3.1 Method Detection Limit (MDL) – the minimum concentration of analyte that can be determined with 99% confidence to indicate that the analyte concentration is greater than zero (this shall be documented annually according to SOP # 307, AESL Form 310).

3.2 Practical Quantitation Limit (PQL) – this limit describes the lowest concentration of analyte the lab will report and corresponds to a level at which the analyte can be reliably quantified to within +/- 20% of its true value (this shall be documented annually according to AESL Form 313).

3.3 Background Matrix – For waters the background matrix for standards and calibrants should consist of 2% HNO_3 and 1% HCl in deionized water. This same matrix should be used for blanks and lab fortified blanks.

3.4 Linear Dynamic Range – Describes an upper limit to which a calibration remains accurate to within +/- 90%. If reporting a value that significantly exceeds the uppermost calibrant concentration, a lab fortified blank at or above the determined sample concentration must be analyzed within these limits and included in the report.

4.0 EQUIPMENT, INSTRUMENTS, AND SUPPLIES

4.1 Polypropylene test tubes (20 mL)
4.2 Adjustable pipetters (Finpippette II; 0.02 – 0.1 mL, 0.1 - 1.0 mL, 1.0 - 5.0 mL)
4.3 Spectro Arcos ICP-AVOES Instrument
4.4 Cetac ASX-5200 Autosampler
4.5 LabTech H2150-1000 chiller unit
4.6 Peristaltic pump tubing (Orange-orange for sample, Green-blue for waste)
4.7 20 L carboy for waste storage.
4.8 Argon Gas

5.0 REAGENTS AND SOLUTIONS

5.1 HNO_3 ACS plus or Trace Metal Grade concentrated nitric acid (~ 69%)
5.2 HCl ACS plus or Trace Metal Grade concentrated hydrochloric acid (~37%)
5.3 Certified standards in solution for each analyte to be measured
5.4 Laboratory performance check solution (multi-element solution for initial calibration verification)
5.5 2% HNO_3 1% HCl solution for blanks, diluent, and rinse. (Prepare by placing approximately 300 mL DI water in a 500 mL volumetric flask. Add 10 mL conc HNO3 and 5 mL HCl and bring to volume with deionized water.
5.6 Mixed Calibration Standard Solutions – vary and should be made according to the pre-programmed method (i.e. LPC, EPD metals, etc.) Preprogrammed concentrations and analyte mixes can be found listed in the method section under standards definition in the software. In general, these will be prepared as dilutions from a pre made stock solution. Calibration standards not prepared from primary standards must be initially verified using a certified reference solution. For the recommended wavelenths listed in Table 1, some typical calibration standard combinations are given in Table 3.

6.0 HEALTH AND SAFETY

6.1 While handling samples and chemicals, wear personal protection equipment such as latex or nitrile gloves, safety goggles, and a lab coat.
6.2 Discard samples and standards containing acids only in sink with neutralizing trap.
6.3 Dispose all waste according to University guidelines.

7.0 PROCEDURES

7.1 SAMPLE HANDLING

7.1.1 When determining boron and silica in aqueous samples, only plastic, PTFE or quartz labware should be used from time of sample collection to completion of analysis. When possible, borosilicate glass should be avoided to prevent contamination of these analytes.

7.1.2 Acidify sample upon receipt (if not already done by client) with nitric acid to a pH<2.

7.1.3 Prepare water samples by warming to room temperature and then shaking the samples at least 5 times. With the exception of silver, samples may be analyzed for drinking water using this method as long as the sample was properly preserved with acid and has a turbidity of < 1NTU at the time of analysis. Drinking water samples with >1NTU should be digested and reported as total recoverable. For determination of total recoverable analytes in water, a digestion/extraction is required when elements are not in solution. Aqueous samples containing suspended or particulate material ≥1% (w/v) should be extracted as a solid type sample.

7.1.4 Samples requesting silver should be digested prior to analysis.

7.2 STARTING THE ICP-AVOES

7.2.1 With the instrument computer on, double click the 'Smart Analyzer Vision' icon on the desktop.

7.2.2 Once the software is loaded, turn on the chiller unit using the toggle switch located at the back of the unit and open the gas valve on the argon tank.

7.2.3 Verify that the peristaltic pump tubing is in good repair and lock down pump clamps.

7.2.4 At the top of the software screen, left click once on the 'flush' icon.  After this procedure is completed (~ 5 minutes) – repeat once more.

7.2.5 The ICP is now ready to be lit. Do this by left clicking on the torch icon at the top of the software screen  .

7.2.6 It is recommended that the technician lighting the plasma remain present for the entire procedure to observe whether or not the plasma successfully lights. It may be necessary to perform a hard shutdown by pressing the big blue button on the instrument above the peristaltic pump if there is a problem. There is a potential for damage to the torch in case of an ignition problem.

7.2.7 Following successful ignition of the plasma, allow at least 30 minutes for the instrument to 'warm up' before beginning any analyses.

7.3 CALIBRATING THE ICP

7.3.1 Prepare a series of calibrants containing a zero analyte concentration and 5 successive incremental concentrations of the metals to be analyzed. The concentrations may vary according to the expected concentration of the samples and application. Allowable concentrations are preprogrammed and method specific.

7.3.2 It is advisable to perform a calibration manually as most trace level applications require daily modifications to peak and background position.

7.3.3 Flush the system with the rinse blank for a minimum of 60 seconds between each standards.

7.3.4 Once the calibrants have been measured, perform peak and background adjustments. Observe the resulting calibration curve for each analyte to verify that all linear calibration models meet the following criteria: r^2 value > 0.999; no point exceeds +/- 20% residual value from calibration model. If this is not the case, the calibrants should be prepared and measured again.

7.3.5 Upon verification of acceptable calibration for all analytes of interest, the analyst should select the 'recalculate method' and begin analysis.

7.3.6 The r^2 value for the calibration curve should be ≥0.999. The software program for the ARCOS calculates the r^2.

7.4 PERFORMING ANALYSES BY ICP

7.4.1 Navigate to the 'Analysis' window in the Smart Analyzer software. Analysis may be performed manually or by programming the autosampler. It is advisable that the analyst only use the autosampler for large sets of 'like' samples with expected concentrations of analyte well above the PQL. For most trace level applications, the analyst should be prepared to review each spectral scan for appropriateness of background correction and absence of spectral interference before proceeding to the next sample.

7.4.2 Three separate measures by the ARCOS are preformed for one analysis.

7.4.3 Parameter settings on the ICP can be viewed on Table 2. A screen capture of a table is great.

7.4.4 All analytical runs shall include the quality assurance measures outlined in EPA method 200.7. Typical analytical runs for 20 and 40 samples are found in Tables 3 and 4, respectively. To summarize, the first sample analyzed following calibration is the Initial Calibration Blank (ICB), followed by the Initial Calibration Verification (ICV) standard prepared from a multi-element solution procured from a vender other than that used to prepare the calibration standards. The mid-level calibration standard should be measured next as a Continuing Calibration Verification (CCV) standard. Whenever possible, an additional QC sample that closely matches the nature of the material to be analyzed should be prepared in the same manner as the unknown sample. Additionally, one duplicate sample, laboratory spike, and matrix spike are included with every 20 samples or less. Use the same sample for each of these quality control checks where possible.

7.4.5 Following analysis of unknown and quality control samples, the results should be reviewed carefully to ensure that all QC criteria are met (Table 1; section 8.2). Additionally, spectra should be reviewed to ensure appropriateness of peak placement and background correction.

7.5 SPECTRAL INTERFERENCE

7.5.1 Run aluminum and iron for all water samples.
7.5.2 Any result for aluminum or iron in water greater than 50ppm Al or Fe should prompt the operator to view the spectral data in the software for interference.

7.6 REPORTING RESULTS

7.6.1 When results are reviewed and determined to be reportable, arrange all final results in the analysis window in the order in which they were run. This should match the example given in Table 1. Right click on the table in the analysis window and select copy.
7.6.2 Use the paste function to move the results to an excel template in the 2nd worksheet (C:\Documents and Settings\My Documents\Arcos Results\Report Templates)
7.6.3 Record the date and time of analysis. Record the r-squared values from each line from the smart analyzer regression window (method view). Paste screen prints of the calibration charts for each analyte reported into the worksheet.
7.6.4 Summarize the sample and QC results and perform all calculations for reporting the requested analytes to the client.
7.6.5 Report all values equal to or above the reporting limit.
7.6.6 Report all values as mg/L for water and wastewater samples.
7.6.7 For dissolved aqueous analytes, report the data generated directly from the instrument with allowance for sample dilution. If a dilution of the sample was necessary to bring analyte concentration into acceptable range, multiply the analyte concentration by the dilution factor. This may be done in the instrument software when entering the sample ID prior to analysis.
7.6.8 Enter the final results in the 1st worksheet and save the file onto the hard drive of the instrument computer in the 'ARCOS RESULTS' folder under the appropriate year and month. Then save the excel file in the J:\ drive folder labeled 'Jake to Rick'.

7.7 SHUTDOWN PROCEDURE

7.7.1 Place sample probe into a reservoir containing ~5% HNO_3. Let this solution flow through the system for ~ 10 min.
7.7.2 Remove probe from acid solution and place into DI water container. Run through system for ~ 5 min.
7.7.3 Remove probe from DI water and let air pump through system until drain tube shows no liquids to waste.
7.7.4 Click on pump icon to stop pump. Click on plasma torch icon to initiate plasma shutdown procedure.

7.7.5 When progress bar shows torch shut down procedure is complete, unclamp peristaltic pump tubing, turn off chiller, and shut off all gas valves.

8.0 CALCULATIONS

8.1 Dilutions in water

$$Final\ \mathrm{Re}sult = \frac{Test\ \mathrm{Re}sult \times Total\ Volume}{mL\ \ Sample}$$

9.0 QUALITY CONTROL/QUALITY ASSURANCE

9.1 Quality Control Procedures are described in Table 1. This table describes the typical QC completed for an analytical run and the QC standard for each parameter and the frequency at which they are required.

9.2 Matrix spike samples shall be spiked at a level between 20 and 100 x the MDL. This should be performed by pipetting a very small volume of highly concentrated standard into a much larger volume of sample (example: 0.05 mL 10 ppm std into 10 mL sample) For matrix spike samples, the amount used for each analyte must match the amount used for the laboratory spike. For total recoverable determinations, the spike must occur prior to the sample preparation. Over time, samples from all sources should be analyzed as matrix spike QCs to verify no matrix interferences.

9.3 It is necessary to clearly document precision and accuracy statistics specified in Table 5.

9.4 Holding time for metals analyzed in this SOP is 6 months. Samples can be held at room temperature or kept cool at 4 ^{o}C.

9.5 Storage of water samples should be in plastic or glass bottles.

9.6 Sample results must fall within the linear dynamic range for each analyte reported.

9.7 Acidify water samples with HNO_3 at at least 16 hours prior to analysis (~1 mL conc HNO_3 / 125 mL sample + 1 mL HCl for Ag or Sb analysis).

10.0 REFERENCE

A. USEPA (1994) Method 200.7 Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Rev. 4.4. U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

B. Arizona Department of Health Services, Office of Laboratory Licensure Certification and Training. Information Update #44. Requirements for the evaluation of interelement spectral interferences. March 16, 1998.

Revision Record

Sop 708.Routine Analysis of Water and Wastewater for Metals by ICP-AVOES

SOP Revision Number and Date:

SOP was reviewed by: _____ on _____ (date).

Was the SOP revised? _____ If the SOP was revised, then in the space below, describe the revised sections and the reason for the revision. Be specific as to the number of each section that was revised. (Example: "Section 3.0 was revised to include additional calculations because some were not included in the previous version.")

Version 1.0 dated August 1, 2011 was revised to include the following items:

1. Format modified to conform to new SOP template
2. MDL are modified as the latest test results.

SOP Revision Number and Date: _____

SOP was reviewed by: _____(name) on _____ (date).

Was the SOP revised? _____ If the SOP was revised, then in the space below, describe the revised sections and the reason for the revision. Be specific as to the number of each section that was revised. (Example: "Section 3.0 was revised to include additional calculations because some were not included in the previous version.")

TABLE 1 WAVELENGTHS, ESTIMATED MDLS, AND RECOMMENDED CALIBRATION CONCENTRATIONS.

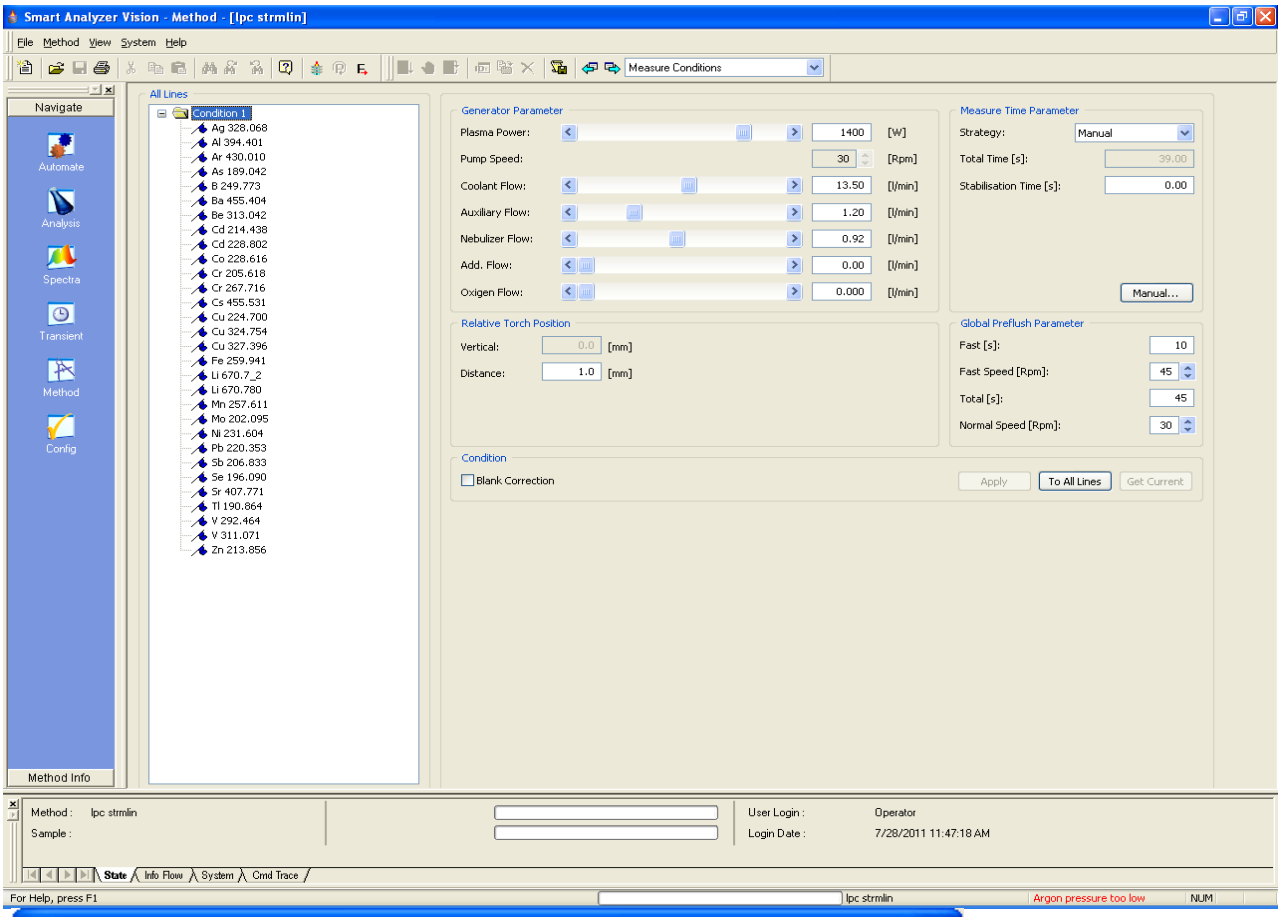
Analyte	Wavelength [a]	Method Detection Limits (ug/L) [b]	Reporting Limits (ug/L) [b]	Typical Calibration Range (mg/L) [c]
Aluminum	394.401	3.1	10	0.005-0.1
Antimony	206.833	1.92	8	0.005-0.1
Arsenic	189.042	2.46	5	0.005-0.1
Barium	455.404	0.034	0.5	0.0005-0.1
Beryllium	313.042	0.097	0.5	0.0005-0.1
Boron	249.773	1.01	2	0.001-0.1
Cadmium	214.438 228.802	0.192 0.265	1.0	0.001-0.1
Chromium	205.618	0.498	1	0.001-0.1
Copper	327.396	0.9	2.5	0.001-0.1
Iron	259.941	0.418	10	0.001-0.1
Lead	220.353	2.154	5	0.005-0.1
Manganese	257.611	0.055	0.5	0.001-0.1
Molybdenum	202.095	1.02	2.5	0.001-0.1
Nickel	231.604	1.10	2.5	0.001-0.1
Selenium	196.090	1.66	8	0.005-0.1
Silver	328.068	0.31	1	0.001-0.1
Thallium	190.864	4.76	10	0.005-0.1
Vanadium	311.071	0.42	1	0.001-0.1
Zinc	213.856	0.11	1	0.001-0.1

[a] The wavelengths listed are recommended because of their sensitivity and overall acceptability. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interferences.

[b] The method detection limits (MDLs) and reporting limits (RLs) are provided only as a guide to the actual current MDLs and RLs; which are updated annually. Additionally, detection limits may vary as the sample matrix varies. Detection limits for solids digested and then analyzed on the ICP are estimated using these values divided by grams extracted per liter, which depends upon the extraction procedure.

[c] Suggested concentration for instrument calibration. Other calibration limits in the linear ranges may be used.

TABLE 2 – ICP INSTRUMENT OPERATING CONDITIONS



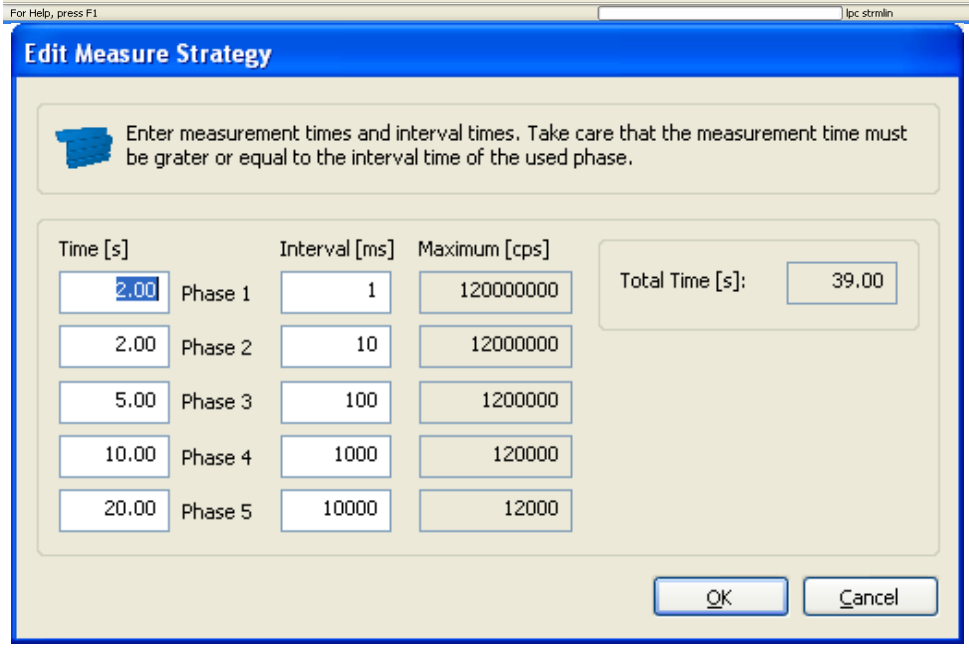


TABLE 3 ANALYTICAL RUN EXAMPLE (20 SAMPLES)

CALIBRATION – at least 5 points

ICB	Initial Calibration Blank
ICV	Initial Calibration Verification (also serves as QC Sample) (second source).
CCV	Continuing Calibration Verification

≤ 10 unknowns
Sample duplicate unknown

LCS	Laboratory Spike (DI spiked with standard)
MS	Matrix Spike (spiked field sample)
CCB	Continuing Calibration Blank
CCV	Continuing Calibration Verification

≤ 10 unknowns

CCB	Continuing Calibration Blank
CCV	Continuing Calibration Verification

TABLE 4 ANALYTICAL RUN EXAMPLE (40 SAMPLES)

CALIBRATION – at least 5 points
ICB Initial Calibration Blank
ICV Initial Calibration Verification (also serves as QC Sample) (second source).
CCV Continuing Calibration Verification
≤ 10 unknowns
Sample duplicate unknown
LCS Laboratory Spike (DI spiked with standard)
MS Matrix Spike (spiked field sample)
CCB Continuing Calibration Blank
CCV Continuing Calibration Verification
≤ 10 unknowns
CCB Continuing Calibration Blank
CCV Continuing Calibration Verification
≤ 10 unknowns
Sample duplicate unknown
LCS Laboratory Spike (DI spiked with standard)
MS Matrix Spike (spiked field sample)
CCB Continuing Calibration Blank
CCV Continuing Calibration Verification
≤ 10 unknowns
CCB Continuing Calibration Blank
CCV Continuing Calibration Verification

TABLE 5 QA/QC FOR ICP-ARCOS ANALYSIS

Parameter	QC Standard	Frequency
Samples analyzed within the holding time?	Samples should be analyzed within 6 months.	Per Batch
Laboratory Method Blank (CCB)	Must be less than 10% of the sample sample concentration or 2.2 x MDL (whichever is greater)	1 per 10 samples
Standard Curve (at least a 5-point curve/batch)	At least a 5-point calibration required; linearity with r≥0.995	Per Batch
ICV/QC Standard (This is supplied by a vendor such as NSI or ERA and is used as a second source to verify the instrument performance.)	Determined PPM must be within 15% of the label value. = 100 * [(label - determined)/label]	Per Batch
CCV (Midrange Standard on the standard curve. This is the Continuing Calibration Verification of Instrument Performance.)	Determined PPM should be within 15% of the label PPM. = 100 * [(label - determined)/label]	1 per 10 samples
LCS = Laboratory Spike (Deionized water spiked with a standard)	Accuracy (% Recovery) = 100* (Determined PPM/Calculated PPM) (Should be 80-120%.)	1 per 20 samples
MATRIX Spike (Spike an aliquot from one of the field samples.)	Accuracy (% Recovery) = 100* (A-B)/C Where: A = Spike Sample Result B = Unspiked sample Result C = Theoretical Spiking Value (Should be 80-120%)	1 per 20 samples
DUPLICATE ANALYSIS: Original Sample	Precision (RPD) = 100*(difference of the 2 values)/(average of the 2 values) = (Should be ≤15%.)	1 per 20 samples
DUPLICATE ANALYSIS: Sample Duplicate		

Professional Plus Quick-Start Guide

This Quick-Start Guide is meant to serve as a quick reference in operating the Professional Plus. It is not intended to replace the information found in the Operations Manual. For your convenience, this quick start guide will enable you to unpack your instrument and get to the field quickly.

GETTING STARTED

Unpack the instrument and install (2) C size batteries in the back of the instrument. Tighten the four screws of the battery plate on to the back of the instrument.

If necessary, install the sensors into the cable assembly by inserting the sensors into the ports and then hand tightening them. Do not use a tool and do not over tighten.

If using a 1010 cable, a sensor must be installed in port 1 for correct operation. If installing a pH/ORP combo sensor into a 1010 cable, ORP will not be measured. If using a 1020 cable, install a pH, ORP, pH/ORP, or an ISE sensor in port 1 and a DO sensor in port 2.

If using a Quatro cable, install a pH, ORP, or ISE sensor in ports label 1 and 2. A sensor must be installed in port 1 for port 2 to operate correctly. If you install a pH/ORP combo sensor into port 1 or port 2, ORP will not be measure. Install the Dissolved Oxygen sensor in the port labeled DO. Install the Conductivity/Temperature sensor in the port labeled CT following the instructions included with the sensor. For ease of installation, YSI recommends that you install a sensor into port 1 first; followed by DO installation, then port 2, and lastly C/T.

Please refer to the Getting Started Setup section of the Manual for a complete list of sensor/cable port configurations.

Install a port plug into any port that does not have an installed sensor. Attach the cable assembly to your instrument.

INSTALLING THE DO MEMBRANE

Note: The DO sensor is shipped with a red protective cap to protect the electrode. A new membrane cap must be installed before the first use.

1. Prepare the O_2 probe solution according to the instructions on the bottle. After mixing, allow the solution to sit for 1 hour. This will help prevent air bubbles from later developing under the membrane.
2. Remove, and discard or save the red protective cap.
3. Thoroughly rinse the sensor tip with distilled or deionized water.
4. Fill a new membrane cap with probe solution. Avoid touching the membrane portion of the cap.
5. Thread the membrane cap onto the sensor, moderately tight. A small amount of electrolyte will overflow.
6. Screw the probe sensor guard on moderately tight.

MENU FUNCTIONS

The Professional Plus has a menu-based interface. Press the "hot keys" to access the System, Sensor, Calibration, and File menus (from left to right at the top of the keypad). To navigate through the menus, use the up and down arrow keys to highlight a desired menu option with a highlight bar, and press the Enter key to activate the selection. Use the left arrow key to go back one screen. Press the Esc key to return to the run screen or to exit an alpha/numeric entry screen. The Pro Plus will automatically power on to the Run screen.

SETTING THE DATE AND TIME

1. Press the System key .
2. Highlight **Date/Time** and press Enter.
3. Highlight **Date Format** and press Enter. Highlight the correct format and press Enter.
4. Highlight **Date** and press Enter. Use the keypad to enter the correct date, then highlight ↵on the display keypad, and press Enter.
5. Highlight **Time Format** and press Enter. Highlight the correct format and press Enter.
6. Highlight **Time** and press Enter. Use the keypad to enter the correct time, then highlight ↵on the display keypad, and press Enter.
7. Press Esc to return to the Run screen.

SETTING UP SENSORS & REPORTING UNITS

A sensor must be enabled in the **Sensor** menu for it to operate. Once a sensor is enabled, the desired units for that sensor must be selected in the **Display** menu to determine what will be displayed.

1. Press the Sensor key.
2. Highlight **Setup** and press enter. Highlight the parameter of interest and press enter. Highlight **Enabled** and press enter to ensure a checkmark in the box. When enabling the ISE1 and ISE2 ports, you must select the correct sensor after enabling the port.
3. When Dissolved Oxygen is enabled, a submenu allows the user to select the sensor type (Polarographic or Galvanic) and membrane type being used. Highlight **Sensor Type** or **Membrane** and press **Enter** to modify these settings.
4. Press the left arrow key to return to the previous screen or press Esc to return to the Run screen.

Once changes to the Sensor menu have been completed, you must determine which units will be reported (i.e. %, mg/L, °C, °F, etc.).

1. Select the Sensor hot key on the keypad, highlight **Display**, and press enter.
2. Highlight the parameter you want to access and press the Enter.
3. A submenu will open allowing you to select the reporting units. Some parameters can be reported in multiple units. For example, DO can be reported in DO%, DO mg/L, and DO ppm. Other parameters, for example temperature, can only be reported in one unit. Make selections from the submenu, and then press the left arrow key to return to the Display menu or press Esc to return to the Run screen.

BAROMETER CALIBRATION

1. Determine your local barometric pressure (BP) in mmHg from a mercury barometer, an independent laboratory, or from a local weather service. If the

BP reading has been corrected to sea level, use the following equation to determine the true BP in mmHg for your altitude:

True BP = (Corrected BP in mmHG) – {2.5 * (Local Altitude in feet/100)}

2. Press the **Cal** key.
3. Highlight **Barometer** and press Enter. Use the arrow keys to highlight the desired units and press Enter to confirm.
4. Highlight **Calibration Value** and press enter to adjust.
5. Use the Alpha/Numeric screen to enter your True BP, then highlight <<<**ENTER**>>> and press enter.
6. Highlight **Accept Calibration** and press enter to finish the calibration.

CONDUCTIVITY, PH, AND ORP CALIBRATION

1. Press the **Cal** key.
2. Highlight the parameter you wish to calibrate and press enter. For Conductivity, a second menu will offer the option of calibrating **Specific Conductance**, **Conductivity**, or **Salinity**. Calibrating one automatically calibrates the other two. An additional sub-menu will require you to select the calibration units. For pH, auto-buffer recognition will determine which buffer the sensor is in and it will allow you to calibrate up to 6 points.
3. Place the correct amount of calibration standard into a clean, dry or pre-rinsed container.
4. Immerse the probe into the solution, making sure the sensor and thermistor are adequately immersed. Allow at least one minute for temperature to stabilize.
5. For any of parameters, enter the calibration solution value by highlighting **Calibration Value**, pressing enter, and then using the alpha/numeric keypad to enter the known value. Once you have entered the value of the calibration standard, highlight <<<**ENTER**>>> and press enter.
6. Wait for the readings to stabilize, highlight **Accept Calibration** and press enter to calibrate.
7. For pH, continue with the next point by placing the probe in a second buffer and following the on-screen instructions or press **Cal** to complete the calibration.

DO CALIBRATION

The Pro Plus offers four options for calibrating dissolved oxygen. The first is an air calibration method in % saturation. The second and third calibrates in mg/L or ppm to a solution with a known DO concentration (usually determined by a Winkler Titration). Calibration of any option (% or mg/L and ppm) will automatically calibrate the other. The fourth option is a zero calibration. If performing a zero calibration, you must perform a % or mg/L calibration following the zero calibration. For both ease of use and accuracy, YSI recommends performing the following 1-point DO % calibration:

1. Moisten the sponge in the cal/transport sleeve with a small amount of water and install it on the probe. The cal/transport sleeve ensures venting to the atmosphere. For dual port and Quatro cables, place a small amount of water (1/8 inch) in the calibration/transport cup and screw it on the probe. Disengage a thread or two to ensure atmospheric venting. **Make sure the DO and temperature sensors are <u>not</u> immersed in the water.**
2. Turn the instrument on. If using a polarographic sensor, wait 10 minutes for the DO sensor to stabilize. Galvanic sensors do not require a warm up time.
3. Press the **Cal** key, highlight **DO** and press enter.
4. Highlight DO%, then press Enter.
5. Verify the barometric pressure and salinity displayed are accurate. Once DO and temperature are stable, highlight **Accept Calibration** and press enter.

TAKING MEASUREMENTS AND STORING DATA

1. The instrument will be in Run mode when powered on.
2. To take readings, insert the probe into the sample. Move the probe in the sample until the readings stabilize. This releases any air bubbles and provides movement if measuring DO.
3. **Log One Sample** is already highlighted in Run mode. Press enter to open a submenu. Highlight **Sites** or **Folders** and press enter to select the site or folder to log the sample to.
4. If necessary, use the keypad to create a new Site or Folder name. If Site List and Folder List are disabled in the System menu, you will not see these options when logging a sample.
5. Once the Site and/or Folder name is selected, highlight **Log Now** and press enter. The instrument will confirm that the data point was logged successfully.
6. If you would like to log at a specific interval vs. logging one sample at a time, press the **System** key. Use the arrow keys to highlight **Logging** and press enter. Enable **Continuous Mode** and adjust the time **Interval** if necessary. On the Run screen, the option to log will change from **Log One Sample** to **Start Logging** based on the time interval entered.
7. During a continuous log, the **Start Logging** dialog box on the Run screen will change to **Stop Logging**.

UPLOADING DATA TO A PC WITH DATA MANAGER

1. Make sure Data Manager and the USB drivers are installed on the PC. The USB drivers will be installed during the Data Manager installation.
2. Connect the Communications Saddle to the back of the Pro Plus instrument and use the USB cable to connect the saddle to the USB port on the PC.
3. If connecting for the first time, Windows® may prompt you through two 'New Hardware Found' Wizard in order to complete the USB driver installation.
4. Open Data Manager on the PC and turn on the Pro Plus.
5. Click on the correct instrument in Data Manager under the **Select Instrument** heading. Once you've highlighted the correct instrument, click the **Retrieve Instrument Data** tab and check **Data, GLP, Site List, Configuration** or **Select All** options to retrieve data. Click **Start.**
6. After the file transfer is complete, the data is available for viewing, printing, and exporting from Data Manger and the data can be deleted from the Pro Plus if desired.
7. Press the File key and choose **Delete Data** if you no longer need the data on the Pro Plus.

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